BOOK OF ABSTRACTS OF THE
10TH INTERNATIONAL CHEMICAL AND
BIOLOGICAL ENGINEERING CONFERENCE
CHEMPOR 2008

BOOK OF ABSTRACTS OF THE 10TH INTERNATIONAL CHEMICAL AND BIOLOGICAL ENGINEERING CONFERENCE - CHEMPOR 2008

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BRAGA, PORTUGAL

This volume contains abstracts presented at the 10th International Chemical and Biological Engineering Conference - CHEMPOR 2008, held in Braga, Portugal, between September 4th and 6th, 2008.

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Foreword

This book contains the extended abstracts presented at the 10th International Chemical and Biological Engineering Conference - CHEMPOR 2008, held in Braga, Portugal, over 3 days, from the 4th to the 6th of September, 2008. Previous editions took place in Lisboa (1975, 1889, 1998), Braga (1978), Póvoa de Varzim (1981), Coimbra (1985, 2005), Porto (1993), and Aveiro (2001).

The conference was jointly organized by the University of Minho, "Ordem dos Engenheiros", and the IBB - Institute for Biotechnology and Bioengineering with the usual support of the "Sociedade Portuguesa de Química" and, by the first time, of the "Sociedade Portuguesa de Biotecnologia".

Thirty years elapsed since CHEMPOR was held at the University of Minho, organized by T.R. Bott, D. Allen, A. Bridgwater, J.J.B. Romero, L.J.S. Soares and J.D.R.S. Pinheiro. We are fortunate to have Profs. Bott, Soares and Pinheiro in the Honor Committee of this 10th edition, under the high Patronage of his Excellency the President of the Portuguese Republic, Prof. Aníbal Cavaco Silva. The opening ceremony will confer Prof. Bott with a "Long Term Achievement" award acknowledging the important contribution Prof. Bott brought along more than 30 years to the development of the Chemical Engineering science, to the launch of CHEMPOR series and specially to the University of Minho. Prof. Bott's inaugural lecture will address the importance of effective energy management in processing operations, particularly in the effectiveness of heat recovery and the associated reduction in greenhouse gas emission from combustion processes.

The CHEMPOR series traditionally brings together both young and established researchers and end users to discuss recent developments in different areas of Chemical Engineering. The scope of this edition is broadening out by including the Biological Engineering research. One of the major core areas of the conference program is life quality, due to the importance that Chemical and Biological Engineering plays in this area. "Integration of Life Sciences & Engineering" and "Sustainable Process-Product Development through Green Chemistry" are two of the leading themes with papers addressing such important issues. This is complemented with additional leading themes including "Advancing the Chemical and Biological Engineering Fundamentals", "Multi-Scale and/or Multi-Disciplinary Approach to Process-Product Innovation", "Systematic Methods and Tools for Managing the Complexity", and "Educating Chemical and Biological Engineers for Coming Challenges" which define the extended abstracts arrangements along this book.

A total of 516 extended abstracts are included in the book, consisting of 7 invited lecturers, 15 keynote, 105 short oral presentations given in 5 parallel sessions, along with 6 slots for viewing 389 poster presentations. Full papers are jointly included in the companion Proceedings in CD-ROM. All papers have been reviewed and we are grateful to the members of scientific and organizing committees for their evaluations. It was an intensive task since 610 submitted abstracts from 45 countries were received.

It has been an honor for us to contribute to setting up CHEMPOR 2008 during almost two years. We wish to thank the authors who have contributed to yield a high scientific standard to the program. We are thankful to the sponsors who have contributed decisively to this event. We also extend our gratefulness to all those who, through their dedicated efforts, have assisted us in this task.

On behalf of the Scientific and Organizing Committees we wish you that together with an interesting reading, the scientific program and the social moments organized will be memorable for all.

Braga, September 2008

Eugénio C. Ferreira

Manuel Mota

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Meeting the challenge

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The first CHEMPOR conference held in Lisbon in 1975 under the auspices of the Calouste Gulbenkian Foundation, heralded a regular wide ranging review of research and development in Portugal and the U.K. Progressively in later years the conferences have attracted contributions from other European countries and indeed, further afield. During this period there has been an increasing awareness of the problems for the environment, notably global warming, brought about by human activities. Recent predictions about the future are dire and represent a substantial challenge to the scientific and technical fraternity. The papers presented at the CHEMPOR conferences have made a contribution to knowledge and will continue to do so, that to a lesser or greater extent, will be helping to meet that challenge.

The paper will emphasise the importance of effective energy management in processing operations, particularly in the effectiveness of heat recovery and the associated reduction in greenhouse gas emission from combustion processes. It will be illustrated with a simple summary of data based opportunities in design and operation of heat exchange equipment to improve the utilisation of energy.

Green chemistry and the biorefinery

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An unprecedented set of legislative, economic and social drivers are forcing changes across the lifecycle for chemical products. More ambitious companies are seeing this as an opportunity to develop new, greener products, which are increasingly being sought by consumers. In order to obtain truly green and sustainable products, it is necessary to start with renewable raw materials, which for organic chemical products means biomass.

Future bio-resource derived chemicals will be based on the products of fermentation and controlled pyrolysis including biofuel production, from plant extractables, and from agro- and food wastes. We are already seeing a number of new bio-platform chemicals coming onto the market. It is very important that we build on these sustainable feedstocks with green chemical processing and, and green product design and using appropriate environmental impact metrics. In this way we can look forward to a future chemical industry that can *sustainably* meet the demands of a growing world population.

Adding value to chemical products

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Chemical engineering as an organized discipline is almost exactly 100 years old. For the first fifty years the discipline served many separate industries, including agriculture, metals, paints, and fuels. For the next fifty years – those just ended – the industry became focused on petrochemicals. It was enormously successful: for example, the industry made textile fibers which literally changed the clothes on human backs.

As it enters the next fifty years, chemical engineering is broadening its focus dramatically. This broader focus can be conveniently discussed around three headings. First, the petrochemically focused industry, which stopped growing about thirty years ago, remains an important commodity business. It has had two characteristics: an effective use of digital computation, and a deep level of science which sought small commercial advantages. While this part of the chemical enterprise will not grow dramatically, it will require continuing effort from chemical engineers.

Second, chemical engineering will focus on new energy sources, the result of increased demand for liquid fuels and an increased concern for the environment. Energy is certainly a major social problem. My concern is that any new research will be used as an excuse to postpone social changes needed for energy conservation. In the short term, any energy crisis can be eased not so much by scientific invention as by changes in public behavior.

Third, chemical engineering will focus on products where process adds significant value. Learning how to design such products, and teaching others how to undertake this design, will probably offer the greatest potential growth for chemical engineering. Adding value can be organized around a four-step template: identifying consumer needs, generating ideas which satisfy these needs, selecting the best idea, and manufacturing the product. This paper will detail how this third goal of adding product value can be effectively accomplished.

Innovation at interfaces

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The important problems and challenges in biochemical engineering occur at disciplinary interfaces. Innovative solutions to these problems require crossing multiple disciplinary boundaries at the interfaces of biology, chemistry and physics.

Lessons learned from innovation at interfaces will be drawn using diverse examples in biochemical engineering. Developing processes to convert lignocellulosic biomass into new fuels and chemicals requires facing the physical reality of converting a complex feedstock via novel biocatalytic routes to products that are difficult to recovery from dilute aqueous solution. Efficient optimization of cell culture processes for biotherapeutics production can be done through the use of microreactors in large scale experimental design, but one must address the uncertainty of scale up through an understanding of the physics of reactor design and the influence on biological response. Design of solid dosage forms of pharmaceutical products and processes for their manufacture requires an understanding of both the chemistry and physics of particulate materials and multiscale analysis to translate this understanding into process knowledge.

A process systems engineering approach for managing the complexity

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Process systems engineering (PSE) has been traditionally concerned with the understanding and development of systematic procedures for the design, control, and operation of chemical process systems. It emphasizes the development and use of systematic approaches to problem solution as well as understanding of the important issues. The oil & gas industry, the petrochemical industry and to some extent, the chemical industry have been the traditional users of methods and tools, including software, from the PSE community. Indeed, it is routine these days to perform a detailed mass and energy balance for a process through one of several process simulators that have been developed by the PSE community. Problems related to process optimization, process integration and process synthesis/design are currently routinely solved through knowledge based techniques as well as mathematical optimization techniques. Also, systematic methods and tools have been developed and applied to solve industrial problems in the area of planning and scheduling, on-line optimization, solvent selection/design and many more.

The above developments have been possible because of the availability of sufficient knowledge and data related to the relevant process engineering problems and an interest from the industry for collaboration with academia for their solution. This encouraged the development of reliable process simulation models that together with the available knowledge and data lead to the development of systematic (and/or computer aided) methods and tools for a wide range of problems for the chemical process industries. Most of the above developments can be linked to chemical processes involved with the manufacture of high volume bulk chemicals and the related industries. To a lesser extent, these methods and tools have also been applied to the manufacture of low volume specialty chemicals.

In the areas of chemicals based products, energy, sustainability, biosystems engineering, and enterprise-wide optimization all of which may be viewed as new opportunities for PSE, however, simply replacing experiment-based solution approaches with computer-aided systems will not provide the desired innovative solutions. Addressing these problems will require a systematic multidisciplinary approach where the model (including data) may come from different sources and multiple factors (process operation, product quality, cost, sustainability issues, time, etc.) may need to be considered to identity the truly innovative solutions. Here, a systematic model-based framework that can play the role of the “integrator” or “glue” is necessary. That is, a fast, efficient, reliable and robust model-based framework that is able to manage the complexities at different scales of size, time and problems. Such as a computer aided framework will generate and use multiscale models, design experiments to collect and analyze data, use in-house methods and tools for process-product monitoring systems (and their design), for optimization of the enterprise, for product-process development. The presentation will illustrate the main ideas through interesting problems in process-product engineering.

Development of crystallization processes for separating multicomponent mixtures

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In principle, crystallization, as distillation, is capable of separating a multicomponent mixture. In practice, while distillation is the workhorse for separating small molecules, crystallization is often used only for purifying high molecular weight compounds, leaving the burden of separations to liquid-liquid extraction, chromatography and other unit operations. There are a number of difficulties for further development. Crystallization is perceived to be a unit operation controlled by kinetics and, for a long time, relatively little was done to elucidate the conditions under which a pure product can be recovered from a multicomponent mixture from a thermodynamics point of view. Without such a foundation, rational process design is difficult. This is complicated by the fact that solids might exist in a variety of forms such as enantiomers, solvates, compounds and polymorphs, the crystals can have different habits and shapes, and the kinetic behaviors of electrolyte system, reactive system, and biological system can be very different in crystallization. Also, unlike distillation where a product from a distillation column is a pure liquid, the product from a crystallizer is actually a slurry consisting of the pure crystals and a mother liquor containing all the impurities in the original feed. There is, however, renewed motivation to advance the fundamentals of multicomponent crystallization in view of the shift of emphasis in the chemical processing industry from commodity chemicals to products such as pharmaceuticals, advanced materials and natural compounds which are normally in solid form.

This presentation describes efforts to address the aforementioned challenges in the past decade. High-dimensional phase diagrams provide a roadmap to recover the desirable component(s). Since solid-liquid equilibrium data are generally not available in thermodynamic databases, high throughput experimental techniques along with software tools have been developed for determining and representing the phase behavior. Kinetic issues are considered alongside process scaleup. Systematic methods and accompanying experiments are used for designing a plant for impurity management downstream of the crystallizer. Examples on monomers, pharmaceuticals, electrolytes and natural products will be discussed to illustrate this approach.

Educating chemical engineers for coming challenges

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Winds of changes have been blowing all over the world, in recent times, with visible effects in higher education.

Over the past twenty or so years we have been witnessing the bringing into our everyday life of the progress in science and technology accumulated during the second half of the XX Century, namely in life sciences and digital technology. At the same time, major political changes occurred in Europe, paving the way for the global market economy.

These changes can be seen and felt by all: (i) our individual and local universe is larger and larger; (ii) time and space concepts and dimensions have changed dramatically; (iii) the “economy and comfort” Society poses new demands to higher education; (iv) the reference of whatever (quality, competition, etc.) is now Europe and the World, not our City or our Country; (v) standards must be high, inflexibly high, attitude holistic, mind flexible; (vi) youngsters face new challenges in the managing of their individual carriers; (vi) lifelong learning is the key concept to have the edge.

The European countries started a major movement as a response to such changes, aiming at creating an European Area of Knowledge, the well known Bologna Process that is on the process of changing much of the face of both our higher education and our model of research.

In this framework, the structure and contents of (chemical) engineering degrees is under review as we look for new feasible curricula and methods of teaching/learning that answer the demands of Society.

Somewhere back in the fifties and sixties the second paradigm of chemical engineering emerged and swiftly gained recognition. With transport phenomena and studies based on elementary mechanisms it has been possible to transport chemical engineering away from the limited number of unit operations that dominated the conceptual approaches and in that way it has been possible to enlarge the frontiers of chemical engineering.

Today, maybe we do not speak of a third paradigm (or do we?), but we speak of life sciences and of biology as one of the four basic sciences, together with mathematics, physics and chemistry, we speak of environmental issues and of sustainability, of (nano) structures and of material science issues, we have to speak of an economy based on hydrogen and on other alternative energy resources, we have to speak of product development, still and always speaking of optimised, safe, simple to operate systems.

All this is part of chemical engineering, perceived necessarily on a multidisciplinary context. It is clear that there is not a single solution, ‘a single’ structure, in our search for new curricula

Also, new methods for learning have to be brought in and old concepts have to be revisited: (i) students should be brought nearer to the practice of chemical engineering; (ii) It is important that sufficient practical experience, both in the laboratory, pilot plant and industry should be included in the core curriculum; (iii) topics for promoting holistic thinking through integrated approaches and strengthening of horizontal issues should be part of the studies; (iv) cultural diversity in chemical engineering education is desirable, which means that student and educational staff exchanges between countries and cultures should be encouraged.

In the lecture I shall identify and examine both some of the ‘high pressures sources’ that are responsible for such ‘winds’ and the changes that are occurring. I shall also comment and give my views on some of the main tasks ahead for the building of the European Higher Education Area and on some specific implications in methods and curricula in the chemical engineering area.

Gas-liquid mass transfer in gas-liquid-liquid dispersions in a stirred tank: effect of spreading coefficient

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Keywords: mass transfer, gas-liquid-liquid, spreading coefficient, phase equilibria, multiphase reactors.

Interest in gas-liquid-liquid systems has recently been increasing due to its application in homogeneous catalysis systems [Cents 2001, Dumont 2003] and in the bioprocess industry. Aerobic fermentations [Nielsen 2003, Galaction 2004], VOC biodegradation [Daugulis 2003] and biotransformations using a gaseous substrate [Tecelão 2001] are examples of such bioprocesses involving an organic liquid finely dispersed in the aqueous medium.

In all these processes, mass transfer of one or more gases/vapours into/from the liquid-liquid emulsion occurs and is often rate determining. It is known that the presence of the second liquid phase dispersed in the continuous aqueous phase affects the gas-liquid mass transfer rate. An extensive review [Dumont 2003] of gas-liquid mass transfer to “oil-in water” systems reveals that, while most authors have found mass transfer enhancement due to the presence of the organic phase, both the extent of this enhancement and the mechanism by which it is achieved are not agreed upon by researchers. Gaps and contradictions in the experimental data do not allow definite choice between mathematical (and/or conceptual) models proposed for the phenomena involved.

The purpose of this work was to provide experimental evidence for the mechanisms at play. Gas-liquid-liquid mass transfer is studied by measuring evaporation of an organic liquid from an aerated stirred tank 0.232 m in diameter. The continuous liquid phase consisted of tap water, while the dispersed organic phase consisted of n-heptane, n-dodecane or solutions thereof. Exhaust air was sampled using 125 ml sampling bulbs and analysed by gas chromatography.

A simple measure of mass transfer effectiveness is the approach to equilibrium of the exit gas, i.e. the Murphree efficiency, *E*. In the present work, since there are no volatile organics in the inlet air, this is given by

$$E = \frac{p_{Gout}}{p_G^*} \tag{1}$$

where p_{Gout} is the partial pressure of the relevant organic compound at gas outlet, and p_G^* is its equilibrium partial pressure, which may be calculated assuming Raoult’s law for the liquid organic phase. The advantage of *E* is its simplicity and the fact that no gas phase mixing assumptions are required for its calculation. Thus, although mass transfer coefficients may be calculated from *E* under the aforementioned assumptions, the simpler concept of *E* is enough to draw conclusions from the data obtained in this work and will thus be used.

The figure presents E (for heptane) as a function of heptane mol fraction in the liquid, for an organic holdup of 2%, an air flow rate of 5.2 l/min, and three different stirrer speeds. Under these conditions, as well as for other holdups and air flow rates, *E* was found to be low at low concentrations of heptane in the organic liquid, and high (approaching unity) at high concentrations. In fact, under a given set of conditions, there appear to be two regions of approximately constant *E*, separated by a rather steep change at between 0.6 and 0.8 mole fraction of heptane in the liquid. This transition happens to coincide roughly with the concentration of hexane at which the spreading coefficient of the organic solution on the gas-liquid interphase changes from negative to positive. This concentration is calculated as 0.7. These results suggest that the mechanism controlling mass transfer changes depending on the value of the spreading coefficient: when it is negative, mass transfer from organic to gas is not direct; when it is positive, there is direct contact between the organic phase and the gas phase.

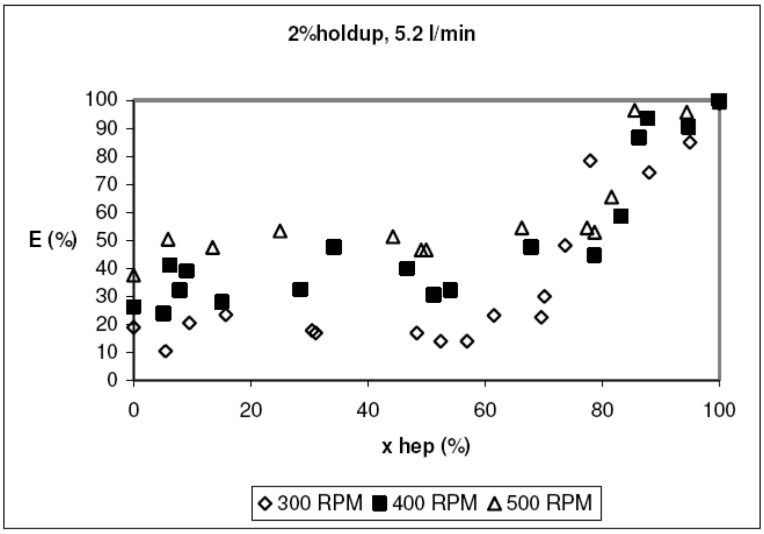

Figure 1 – Murphree efficiency as a function of mol fraction of heptane in the organic phase (stirrer speed: ◊ 300 rpm, ■ 400 rpm, ∆ 500 rpm)

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Oxygen transfer characterization in three-phase reactors using silicone oil as vector

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Keywords: vectors, transfer pathway, transfer enhancement, OTR.

The use of an organic phase as vector to enhance mass transfer in biological reactors has been applied since the 70's (Deziel et al., 1999). When the process implies the transfer of oxygen or other poorly water soluble compounds, the literature reports that the presence of an organic vector phase could improve significantly the transfer of the target compounds (Muñoz et al., 2007). Most of the works published have focused on the effect of vector on the global mass transfer coefficient (K_La) and to our knowledge, no published work have analyzed in detail, the transfer pathways from and to each of the three phases. Moreover, despite numerous published works on organic vectors and on three-phase bioreactors, it seems that no agreement on the positive or negative effect of organic vectors on K_La has been reached (Dumont and Delmas, 2003).

This work presents a mass transfer model that allows the characterization of gas/vector, vector/water and gas/water transfer, in three-phase reactors. The model was applied to an airlift and a stirred-tank reactor containing from 0 to 50% of silicone oil as vector. In these reactors, oxygen transfer was quantified through a modified sulfite method. Sulfite concentration in the water phase was measured by iodometric back-titration procedure according to Zhao et al. (1999). For measurement of the oxygen concentration in the vector phase, silicone oil samples were transferred to serological bottles with inert atmosphere containing an aqueous sulfite solution of known concentration. Bottles were incubated at 23 C and 160 rpm during 24 h. Final sulfite concentration was then measured and related to the correspondent dissolved oxygen concentration in silicone oil.

The results obtained confirm that the organic vector phase has a positive effect on oxygen transfer. Figure 1 shows the direct gas/water and the indirect gas/vector/water oxygen transfer rates (OTR) observed. In both reactor designs, the optimum oxygen transfer was observed with 10% silicone. Under these conditions, the overall transfer enhancement was about 65 and 84% for the airlift and stirred reactor, respectively, compared to reactors operated without silicone oil. It was also observed that the transfer enhancement was mainly due to a better air/water transfer. Indeed, with 10% silicone oil holdup, the direct air/water phase transfer contribution to the overall oxygen transfer was 94.7 and 93.0% for the airlift and stirred-tank reactor, respectively. The effect of vector on the air/water transfer has been previously reported by Galindo et al. (2000). They reported that the presence of vector decreased the bubble mean diameter and consequently increased the gas/water interfacial area. Our results confirm that the enhancement of mass transfer in three phase reactors is not due to the establishment of a high gas/vector/water transfer as commonly believed but to an increase of the direct gas/water transfer.

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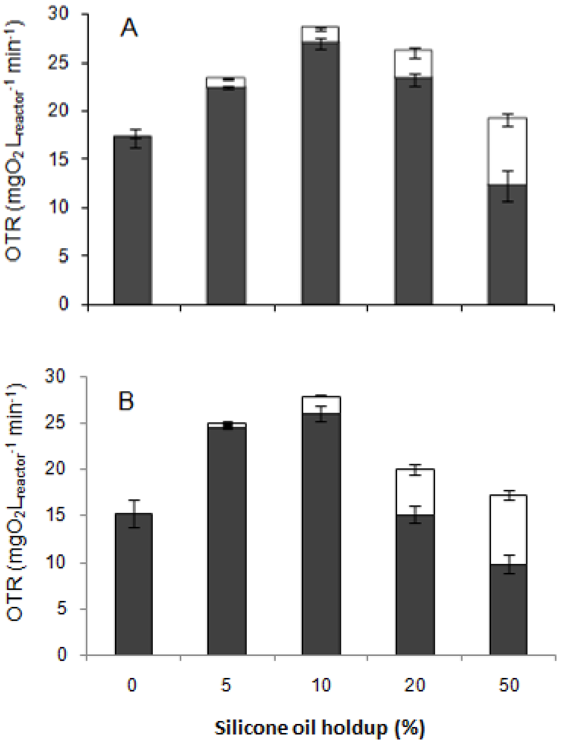

Figure 1. OTR values obtained in the airlift (A) and the stirred tank (B) reactors at different silicone oil holdups, where: (■) direct gas/water and (□) indirect gas/vector/water transfer.

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Fluidization behaviour of different shaped large particulate food materials: effect of moisture and shape

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Abstract

The use of *fluidization* is one of main applications in drying of agro-food materials. When an air stream is passed through a free flowing material resting on a permeable support, the bed starts to expand when a certain air velocity is reached. The *superficial velocity* of the air at this stage is the *minimum fluidization velocity*, with continual increase in air velocity, a stage is reached where the pressure across the fluidized bed drops rapidly, and the product is carried along the air stream. The velocity of air at this stage is called terminal velocity. During fluidizing operations the superficial velocity of the air should remain between *minimum fluidization velocity* and *terminal velocity*.
Fluidized bed drying has been recognized as a gentle, uniform drying, down to very low *residual moisture* content, with a high degree of efficiency (Borgotte et al., 1981). This is a very convenient method for heat sensitive food materials as it prevents them from overheating (Gibert et al., 1980, Giner and Calvelo, 1987). The fluidized bed drying for granular materials is now established (Butler, 1974, Masters, 1992) and many driers are operating throughout the world in the chemical and food industries. The properties of particulate materials relate to the type of fluidization technique (Shilton and Niranjan, 1993). The application of this technique is best suited to smaller and spherical particles. The disadvantages of this method include entrainment of friable solids by the gas and limited application to larger and poorly fluidized materials. Simultaneous moisture removal, shrinkage and structural changes are common in drying operations. These changes affect the physical properties of the agro-food materials and hence influence fluidization behaviour (Senadeera et al., 1998)
The Ergun equation (Ergun, 1952) is the widely accepted model to determine *minimum fluidization velocity* of a fluid to fluidize the particle (Kunii and Levenspiel, 1969; Zenz and Harbor, 1971; Michelis and Calvelo, 1994).

$$(1-\varepsilon_{mf})(\rho_s-\rho_f)\,\mathrm{g} = 150\frac{(1-\varepsilon_{mf})^2}{\varepsilon_{mf}{}^3}\frac{\mu u_{mf}}{(\phi d_p)^2} + 1.75\frac{(1-\varepsilon_{mf})}{\varepsilon_{mf}{}^3}\frac{\rho_f u_{mf}{}^2}{\phi d_p} \qquad (1)$$

where ε_{mf} – bed porosity at minimum fluidization velocity, ρ_s – particle density (kg/m^3), ρ_f – fluid density (kg/m^3), μ - viscosity (N s/m^2), u_{mf} – minimum fluidization velocity (m/s), d_p – particle equivalent diameter (m), ϕ - sphericity

The Ergun equation was used to calculate minimum fluidization velocity of baker's yeast (Egerer et al., 1985), peas (Rios et al., 1984) and diced potato and potato strips (Vazquez and Calvelo, 1980; Vazquez and Calvelo, 1983). An equation similar to Ergun was valid for peas (Michelis and Calvelo, 1994).
The values for velocity obtained by the Ergun equation are mostly reliable for spherical and relatively small particles. Most agro-food particulates however comprise of various shapes and sizes, and consist of larger particles. Therefore, the minimum fluidization values obtained from Ergun equation does not conform to the experimental values (Mclain and McKay, 1980, 1981a, 1981b; McKay et al., 1987)

The Ergun equation consists of *viscous* and *kinetic energy* terms. In the case of larger particles at higher Reynolds numbers (Re > 1000) the fluidization behaviour was mainly governed by the kinetic energy term in the Ergun equation. Hence the Ergun equation can be simplified for (Kunii and Levenspiel, 1969) wide variety of systems and a generalized equation can be applied to predict minimum fludisation velocity for larger particles when Reynolds number > 1000 using some modification.

$$u^2_{mf} = \frac{\phi\, d_p^{\,2}}{1.75} \frac{(\rho_s - \rho_f)}{\rho_f} g\, \varepsilon^3_{mf} \qquad (2)$$

where, ε_{mf} – bed porosity at minimum fluidization velocity, ρ_s – particle density (kg/m^3), ρ_f – fluid density (kg/m^3), u_{mf} – minimum fluidization velocity (m/s), d_p – particle equivalent diameter (m), ϕ - sphericity, g - acceleration due to gravity (m/s^2)

For wide variety of systems it was found that value $\frac{1}{\phi\, \varepsilon_{mf}^{\,3}} \cong 14$ (Wen and Yu, 1966) and a generalized equation can be applied to predict u_{mf} for larger particles when Re > 1000.

$$u^2_{mf} = \frac{d_p(\rho_s - \rho_f)}{24.5\, \rho_f} g \qquad (3)$$

where, ρ_s – particle density (kg/m^3), ρ_f – fluid density (kg/m^3), u_{mf} – minimum fluidization velocity (m/s), d_p – particle equivalent diameter (m), Re – Reynolds number

There is a continuous change in physical properties of the particulates during drying, which also changes the fluidization behaviour of the particles. It is important to understand these changes, so that the air-flow during drying can be controlled to achieve an optimum fluidization.

The objective of this study is to study the continuous change in minimum fluidization velocity for a given shape of food material during drying and relate this to moisture content by a suitable model, and compare the minimum fluidiztion velocity with the generalized model.

Changes in fluidization behaviour of three geometrical shapes of food particulates (cylindrical, parallelepiped and spherical) with change in moisture content during drying were investigated using a fluidized bed dryer. All drying experiments were conducted at 50 ^{0}C and 13 % RH using a heat pump dehumidifier system. Fluidization experiments were undertaken for the bed heights of 100, 80, 60 and 40 mm and at 10 moisture content levels.

Data were analysed using SAS, and an empirical relationship of the form $U_{mf} = A + B\, e^{-Cm}$ was developed for change of minimum fluidization velocity with moisture content during drying for cylindrical particulates and spherical behaviour was best fitted to the linear model of $U_{mf} = A + B\, m$. Due to irregularities in shape minimum fluidisation velocity of parallelepiped particulates (potato) could not fitted to any empirical model. Also a generalized equation was used to calculate minimum fluidization velocity.

Modeling of a novel photoreactor for multiphase applications

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Keywords: Photoreactor, Monolith, Optical Fiber, Model, Kinetics

Introduction

Here we present the modeling of a photocatalytic reactor system based on a novel design of the combination of side light emitting fibers in a ceramic monolith, i.e. an internally illuminated monolith reactor (IIMR). The tip-coated 'side-light fibers' are evenly distributed inside a ceramic monolith structure, on the inner walls of which titania photocatalyst is coated. The construction of the reaction system enables realization of the hydrodynamic regimes of Taylor flow and film flow. Since the catalyst is coated on the wall instead of on the fibers, the emitted light can reach the catalyst-reactant interface without being strongly attenuated by the catalytic layer. Compared with conventional Optical Fiber Reactors (OFRs), this unique configuration provides extra design flexibility since the light propagation process from the source to the catalyst-reactant interface is decoupled from the physical properties of the catalyst. Another advantage is that coating of a catalyst on a ceramic wall is easier than applying the catalyst on quartz fibers. Moreover, there is much less probability of catalyst deactivation due to strongly focused light emission as a result of fiber roughening, which may occur in the course of time. In this work we attempt to describe the reactor performance in relation with the light intensity profile in the channels and the reaction kinetics. This reactor model in combination with the observed reaction kinetics can be used to compare its performance with that of other reactors. We demonstrated its application in organic synthesis, namely the selective photo-oxidation of cyclohexane. Due to strong product adsorption it was decided to chose formic acid degradation as model reaction in future studies.

Coating

In our previous work it was concluded that the catalyst layer was very thick, which was deteriorating apparent reactor performance. A large amount of TiO_2 (~17 g) was present on the monolith, and hence a large amount of produced cyclohexanone was not quantified by just measuring the product concentration in solution. Also, from previous studies it followed that there is an optimal ratio of layer thickness and light intensity. Hence, the photon efficiency mainly determines the optimal amount of catalyst. Formenti et al. showed that 99% of the light absorption occurred within a 4.5 μm layer of TiO_2 powder, which indicated that the layer thickness applied in the last study (~80 μm) could be significantly reduced without losing any photo-activity, thus increasing the apparent photonic efficiency. Furthermore, it was shown that a significant amount of TiO2 did not contribute to the reaction, since it was present in the macropores of the cordierite. Based on this observation, silica was used to plug the cordierite monolith pores (~5 μm) avoiding loss of TiO_2 material. With this procedure a thinner, well defined coating of TiO_2 can be obtained.

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Light Intensity Model

Light intensity, light position and light profiles play an important role in modeling the performance of the IIMR. In this type of reactors the performance is expressed by the photonic efficiency, as defined in Eq 1. Modeling the reactor performance requires light of preferablu high intensity at all locations where there is catalyst. This intensity depends on the distance of the catalytic site to the light fiber (L_1), the incident angle (α) and the depth in the coating. Fig 1 shows our approach to estimate the light intensity at 21 wall sections of the monolithic channels. In each channel two fibers are located in diagonally opposite corners. It is assumed that the light is emitted from four point sources on each fiber. It is also assumed that the liquid in the corners where the fibers are located is stagnant, indicated by section p in Fig. 1, causing the light emitted from the two point sources located against the wall to be lost. These two point sources are therefore omitted in Fig. 1. Table 1 shows the lengths of the wall segments considered. The effective surface reaction rate, i.e. the rate per unit area of external coating surface, is obtained by integration of the reaction rate over the depth of the coating using the linear absorption coefficient *b* and assuming the chemical reaction rate to be proportional with the light intensity to the power *m*. Eq. (2) shows the resulting surface rate equation. The rate depends on the intensity of the light reaching the wall section (I_s''), the incident angle (α), the linear absorption coefficient (*b*), the proportionality power of the light intensity (*m*), the reaction order (*n*), and the concentrations of the reactant (C_A) and the concentration of the active sites in the coating (C_{sites}). For each photoreaction considered this model has to be adapted since a reaction may be limited by the *photo part* (interaction of the semiconducting material with light) or by the *catalysis part* (formation of radicals/active sites on the surface). Modeling results will be presented for a specific photoreaction.

Table 1. Length of the segments considered in the monolith walls.

S	L_S (m)
5	1.10^{-3}
4	1.10^{-3}
3	1.10^{-3}
2	6.10^{-4}
1	$2,5.10^{-4}$
p	$4,5.10^{-4}$

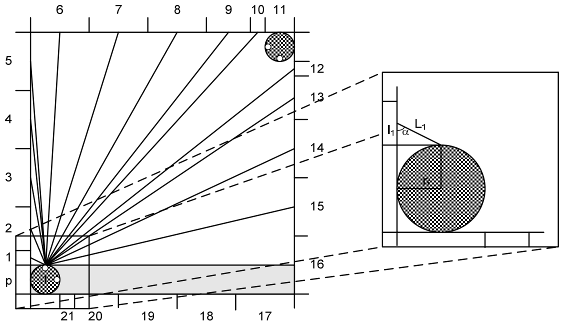

Fig. 1. Light directions in a monolith channel illuminated by two side-light fibers.

$$\xi = \frac{\text{Initial Rate (mol/s)}}{\text{Photon Flow (Einstein/s)}} = \frac{R^{in}}{\rho_p} = \frac{dn_{in}/dt}{d\left(N/N_{AV}\right)/dt} \tag{1}$$

$$xr_i = \left(I_s''\right)^m \frac{\sin\alpha}{mb} k_i C_A^n C_{sites} \tag{2}$$

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Evaluation of parameters and process conditions of the ethylbenzene dehydrogenation in a fixed bed reactor wrapped with permselective membrane

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Keywords: Membrane reactor; Permselective membrane; Ethylbenzene dehydrogenation; Mathematical modeling.

One of the most important raw materials of thermoplastic industry is styrene. Its demand grows considerably due its recyclable nature. The main chemical route for styrene production is the ethylbenzene dehydrogenization. However, the presence of produced hydrogen strongly affects the styrene productivity due to the reversibility of the main reaction and appearance of undesirable secondary reactions. Therefore, a hybrid reaction-separation process using a fixed-bed reactor wrapped with a permselective membrane (Hermann et al., 1997), to remove produced hydrogen as soon it is formed, is suggested to improve the process productivity.

In this context, the kinetic model proposed by Shell and Crowe (1969) presenting six series-parallel reactions, one of which reversible, was adopted to describe the reaction rate occurring in the fixed-bed reactor. This model is also applied in many literatures (Abdalla and Elnashaie, 1994; Hermann et al., 1997; Assabumrungrat et al., 2004; Kumar et al., 2006).

The membrane wrapping the catalytic tubular reactor is composed by three layers: a support macroporous layer; a microporous intermediate layer which increase the separation factor; and a dense metal Palladium layer which is responsible to promote the selectivity of hydrogen.

A mathematical model was developed taking account the different mass transport mechanisms occurring inside the membrane reactor. The adiabatic pseudo-homogeneous model neglecting radial dispersion was applied for fixed-bed reactor. To describe the multicomponent radial mass transport trough the various layers of the composite membrane, the following molels were used (Kumar et al., 2006): the Stefan-Maxwell equation (stagnate gas film near the membrane wall), the Dusty Gas model (macrospores support layer), Knudsen diffusion (microporous layer) and finally the Sievert law (dense metal layer).

There are many forms to promote the driving-force to remove hydrogen. In this work, the system simulation was carried out considering the application of vacuum at permeate side allowing the transmembrane hydrogen partial pressure difference.

The computational program was implemented according to the presented model and the simulation results showed the temperature, pressure and concentration profiles throughout the entire reactor, as well as the conversion of the ethylbenzene, selectivity and productivity of the styrene.

With these results, the effect of temperature, pressure and weight hourly space velocity (whsv) of the feed, length and diameter of the reactor on the styrene productivity were analyzed. Also, the influence of dense metal layer thickness was studied.

It was observed the increase of styrene productivity by increasing inlet temperature. Being endothermic reaction, this behavior was expected, although above 933.15K it did not continue.

The whsv analyses bring out maximum styrene productivity at low whsv values. However, this factor is related with the sizing parameters (diameter and length) of fixed bed, which was also analyzed.

Since the radial dispersion has neglected, the membrane inner diameter should be at least eight times greater than catalyst diameter. In such way, the smaller inner diameter analyzed that attends the restriction and shows better styrene productivity was 1”.

The effect of reactor length was also analyzed. The simulations were taken searching for small values to reduce process cost. The Analysis presented a small productivity peak at length of 0.6 m, recommended for better development and economy of process.

It can be seen that smaller thickness of dense metal layer results in higher styrene productivities. Thus, to attend the manufacturing restrictions, the recommended thickness of dense metal is 1.0 μm.

The process simulation applying all better conditions determined in this work for the industrial process presented an increase of 40.98% in the styrene productivity compared with the process developed with conventional fixed bed reactor.

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Solubility of multifunctional associating molecules: measurements and thermodynamic modeling

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Keywords: CPA EoS, Modeling, Phenolic compounds, Solubility, UNIQUAC

Introduction

Numerous molecules in the pharmaceutical, food and chemical industries present complex chemical structures with different functional group substitutions. Many of these groups such as amine, carboxyl and hydroxyl can form hydrogen bonds. To adequately describe the phase equilibria of these complex chemicals, models able to take into account association effects are required. Still, for model development and evaluation, experimental data on some representative systems are needed. In this work, new experimental measurements and literature data were combined to develop a methodology to model the phase equilibria of phenolic acids using the Cubic-plus-Association (CPA) equation of state. A comparison with the UNIQUAC activity coefficient model is also presented. Within the different classes of multifunctional associating molecules, phenolic compounds, due to their chemical and biological importance, are among the most important. Apart from being starting materials for many chemical syntheses, there is also huge evidence that some phenolic compounds have beneficial effects on human health.

Experimental

Solid-liquid equilibria of hydroxybenzoic acids such as gallic, salicylic, syringic and protocatechuic acids and phenilpropenoic acids such as trans-cinnamic, ferulic, coumaric and caffeic acids were measured from 288 K up to 323 K using the analytical shake-flask and a synthetic DSC methods. Particular attention was given to aqueous solubilities. Besides solubility data, the melting properties (fusion enthalpies and temperatures) were also determined by DSC. The corresponding pH of the saturated aqueous solutions as well as the acid dissociation constants were determined by potentiometry with a glass electrode.

Modeling

An activity coefficient model, the modified UNIQUAC proposed by Peres and Macedo (Peres and Macedo, 1996) and an equation of state, the CPA EoS (F. L. Mota, 2008) were evaluated for modeling the measured data, neglecting the heat capacity term in the general solid-liquid equilibria relation (Prausnitz et al., 1999).

As both the solutes and the solvent can associate (self and cross-association is present in these mixtures), the CPA EoS was also adopted in this work. As the studied phenolic acids are multifunctional associating molecules, a new methodology was proposed to take into account the different associating groups as well as their repetitions and ring positions in the chemical structure. Values for the associating energies and volumes of the acid groups will also be proposed. Different mixture results will be used to demonstrate the reliability of the proposed model.

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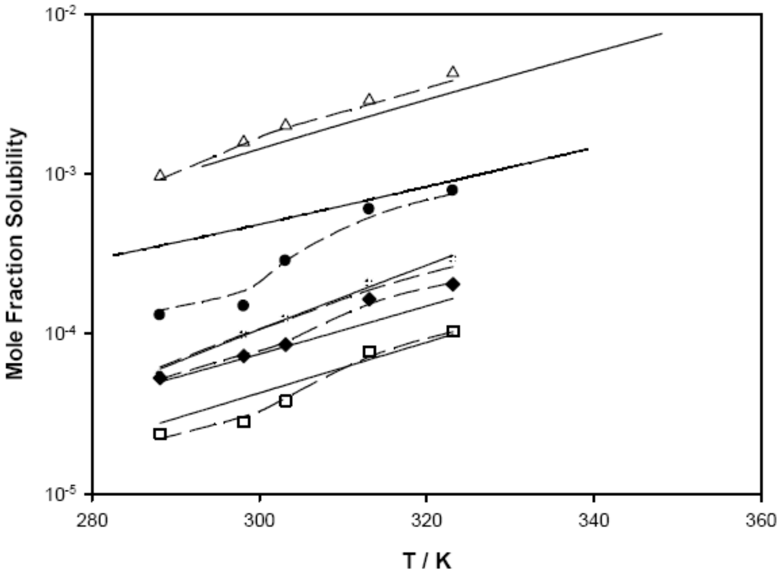

Figure 1: Experimental water solubility (●, salicylic acid; Δ, gallic acid; □, trans-cinnamic acid; ⊕, caffeic acid; ♦, ferulic acid) and modelling results by CPA (solid line) and UNIQUAC (dotted line).

Conclusion

In this work, aqueous solubility data were measured for some phenolic compounds. Besides solubility data, melting data and apparent acid dissociation constants were also determined, providing a broader knowledge about the solubilization processes of these molecules. From the measured melting data, pKa and solubility data, it can be concluded that the extent of hydrogen bonding with water is the dominant effect in determining the degree of solubilization.

Both CPA EoS and modified UNIQUAC model showed to be appropriate tools to represent the water solubility of these molecules.

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Diffusion coefficients and conductivities of 1-butyl-3-methylimidazolium-based ionic liquids

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Keywords: Diffusion coefficient, conductivity, ionic liquid, 1-butyl-3-methylimidazolium hexafluorophosphate, 1-butyl-3-methylimidazolium tetrafluoroborate, 1-butyl-3-ethylimidazolium methanesulfonate, 1-butyl-3-methylimidazolium trifluoromethanesulfonate

In this study, the mutual diffusion coefficients of [Bmin(1-butyl-3-methylimidazolium)][PF6], [Bmim][BF4], [Bmim][MeSO4], and [Bmim][CF3SO3] in water at infinite dilution for temperatures 30-50ºC were measured by the Taylor dispersion technique(Kao and Li, 2006, Snijder et al., 1993, Su et al., 2007). To correlate the diffusion coefficient data, the Wilke-Chang (1955) equation underestimated the diffusion coefficient, but a modified Wilke-Chang equation (Su et al., 2007) correlated diffusion coefficients well with molar volumes. The conductivities of these ion liquids were also measured for various aqueous ionic solutions and for the temperatures 30-50ºC. The infinite dilute conductances were derived from data. The Nernst-Haskell equation (Robinson and Stokes, 1959) accurately estimated diffusion coefficients using the infinite dilute conductance obtained.

The Taylor-dispersion apparatus used in this study was described previously by Kao and Li (2006). In the experiment, the mutual diffusion coefficients of ionic liquids in water were measured at infinite dilution. The samples of dilute solutions of ionic liquids in water were injected into a carrier fluid of pure deionized water. The concentration of the injection samples were kept as dilute as possible so that the diffusion coefficient measured was not influenced by concentrations of the samples, but a steady detectable signal could still be obtained. Figure 1 shows the measured mutual diffusion coefficients of ionic liquids in water at infinite dilution. This figure shows that the mutual diffusion coefficients at infinite dilution decrease with molecular weights. Video, patres conscripti, in me omnium vestrum ora atque oculos esse conversos, video vos non solunn de vestro ac rei publicae, verum etiam, si id depulsum sit, de meo periculo esse sollicitos (Doraiswami and Sharma, 1984). Est mihi iucunda in malis et grata in dolore vestra erga me voluntas, sed eam, per deos inmortales, deponite atque obliti salutis meae de vobis ac de vestris liberis cogitate. Wang et al. (1997) dice mihi si haec condicio consulatus data est, ut omnis acerbitates, onunis dolores cruciatusque perferrem, feram non solum fortiter, verum etiam lubenter, dum modo meis laboribus vobis populoque Romano dignitas salusque pariatur.

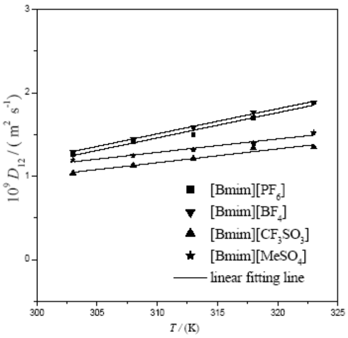

Figure 1. Diffusion coefficient of ionic liquids in water at infinite dilution.

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Diffusion coefficients of electrolytes are related to infinite dilution conductance by the Nernst-Haskell equation (Robinson and Stokes, 1959):

$$D^0 = \frac{RT}{F^2}\frac{|z_+|+|z_-|}{|z_+z_-|}\frac{\lambda_+^0\lambda_-^0}{\lambda_+^0+\lambda_-^0} \tag{1}$$

To obtain the infinite dilution conductances of the cations and anions, conductivities of ionic liquids in water at various concentrations were measured using the conductivity measurement apparatus model SC-170 supplied by Sunway Scientific Co. Figure 2 shows the conductivity of aqueous [Bmin][PF6] for temperature 30 to 50oC at various concentrations. The conductivity varies linearly with the square root of concentration:

$$\Lambda = \Lambda^0 - \left(\alpha\Lambda^0 + \beta\right)c^{1/2} \tag{2}$$

with α=0.229x10^{-4} (m^3/mol)$^{0.5}$, β=60.2x10^{-4} (S m^7/mol^3)$^{0.5}$ (Robinson and Stokes, 1959). The infinite dilution conductivity of the ionic liquids 0 Λ can be obtained by fitting the experimental data using the equation 2. The relation between the infinite dilution conductivity of an electrolyte and the infinite dilution conductances of the cation and anion is given by:

$$\Lambda^0 = \nu_+\lambda_+^0 + \nu_-\lambda_-^0 \tag{3}$$

From equation 3 and the infinite dilution conductivity data of ionic liquids, the infinite dilution conductances of the cations and the anions were determined using leastsquare method. The Nernst-Haskell equation can be used to predict the diffusivities of ionic liquids, the average absolute error of 4% was found. Considering the experimental error involved and that no adjustable parameter was used, the predictions are reasonably accurate.

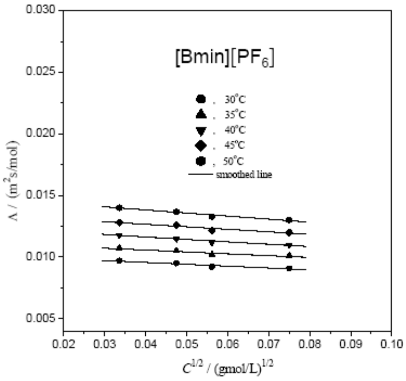

Figure 2. Conductivity of [Bmim][PF6].

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Inhibitor effect of selected anionic surfactants on the dissolution of calcium sulfate in aqueous brines

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Keywords: Calcium sulfate, Dissolution, Surfactants

Introduction

Although the prevention of calcium sulfate scale formation has received a lot of attention from industry and research, studies concerning the inhibition of dissolution of calcium minerals in aqueous brines are scarce. Effective inhibitors are usually good sequestering agents for the lattice cations and it is generally accepted that they exert their action through adsorption at cationic sites on the crystal surfaces. Additives may also adsorb upon crystal faces, reducing the rate of dissolution. Since the molecular interactions at the inorganic/organic interfaces depend on the size, ionic charge and structure of the admixture, Nancollas and Zawacki (1984), small molecules with high charge density will interact with charged crystal faces. The concentration and size of the inhibitor molecule are important parameters because the inhibition of dissolution requires a considerable coverage of the surfaces. Anionic surfactants with negative charged head groups seem to have a strong blocking effect upon the dissolution of calcium minerals, according to Mahmoud et al. (2004). The effectiveness of adsorption is inversely proportional to the minimum area demand per molecule, thus making of single-chained surfactants potential good inhibitors. It is not clear, however, to what extent other salts present in the solution affect the inhibitor performance.

Within the scope of this study, the effect of some selected anionic benzene sulfonate surfactants on the dissolution of different forms of calcium sulfate are here reported. Because the length of the chain determines the physicochemical behavior of surfactants in solution, representatives of C_8, C_{12} and C_{12}/C_{13} anionic surfactants were studied. Both the acid and the sodium salt forms of the polar head group were considered for some of these surfactants.

Materials and Methods

Three different forms of solid calcium sulfate were used in the study. Gypsum and anhydrite were commercial products supplied by Aldrich. The samples of anhydrite-rich sediments, $CaSO_4 \geq 42\%$ (w/w), were obtained from three salt dissolution caverns under leaching, belonging to the Porto de Mós diapir (Hetangian formation). The mineralogical composition of the sediments was confirmed by X-ray diffraction and FTIR spectroscopy (4000-400 cm^{-1}) and stereo and polarizing microscopy showing the presence of halite, anhydrite, low quartz, carbonate minerals (dolomite, ankerite) and minor contents of clay (clinochlore), organic and inorganic carbon, gypsum, muscovite and pyrite. Before use, the sediments retrieved from each of the leached salt caverns were air-dried, homogenized and sieved.

Four anionic surfactants with hydrophilic head group of the benzene sulfonate (BS) type were used: OBS (Na, octyl BS), DBS (Na, dodecyl-BS) and ADBS (dodecyl-BS acid). A commercial acid surfactant, a mixture of branched and straight chained alkyl, mainly C_{12} and C_{13}, here referred to as D550, was kindly supplied by InChemica, Portugal Analytical grade sodium chloride and deionized water were used to prepare

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the brines by mass to ± 0.01 g. The maximum overall uncertainty in the concentration of the surfactants' stock solutions was ± 0.3 mg L^{-1}.

Samples of solids were equilibrated in saturated NaCl solutions with surfactant concentrations ranging from 0 to 70 mg/kg of brine. The plastic flasks were shaken for 7 to 14 days in an orbital shaker, at room temperature. At the end of the run, the slurries were filtered and the remaining solids were air-dried and weighed. The residual solutions were analyzed for the content of calcium (S.M. 3500-Ca B. "EDTA Titrimetric Method"), sulfate (S.M. 4500 SO42- E. "Turbidimetric Method") and surfactant (MBAS standard method). The surfactant concentration in the solids was also determined.

Results and Conclusions

In what concerns the form of calcium sulfate used in the dissolution experiments, it was found that the presence of the surfactants did not affect the dissolution rate of pure gypsum or anhydrite, independent of the type or concentration of the admixture. However, when the source of the calcium salt was the anhydrite-rich sediments, reductions in solubility up to 70% could be obtained.

It was also found that the chain's length of the surfactant molecule correlate directly with the reduction in the solubility of calcium sulfate, being the effect of the C_8 surfactant, OBS, almost negligible. For the linear C_{12}-BS surfactants studied, the type of head group (acid for ADBS, and sodium salt for DBS) does not significantly influence the detergent performance as a dissolution inhibitor of calcium sulfate. The inhibitor effect of the C_{12} and C_{12}/C_{13} surfactants becomes clearer as the equilibration residence time increases: the dissolution of calcium sulfate in the blank run continues up to saturation, whereas the process is effectively blocked in the presence of the added surfactants. The mean diameter of the initial solids used is of importance in the dissolution process. Dissolution rates were quite similar for sediments 1 and 3, while solids from cavern 2 were slower to dissolve due to a coarser size distribution.

The results show that anionic surfactants with C_{12}-alkyl chains reduce the solubility of anhydrite-rich sediments in saturated brines, even for minute amounts of the admixtures. However, when solid halite is present, as in the salt caverns, the solubility of the calcium mineral is reduced only by as much as 50%, when the leaching water contains about 70 ppm of the surfactant. This distinct behavior can be explained by the existence of a larger solid surface area to which the organic molecules indistinctly adhere.

In spite of the negative charge of the sediments, anionic surfactants perform much better than cationic ones, thus showing that the dissolution inhibition cannot be attributed to electrostatic interactions between the solid surfaces and the charged head groups. The common assumption that high concentrations of sodium chloride enhance the tolerance of surfactants towards calcium ions in solution, due to the lowering of the critical micelle concentration, was not confirmed.

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Crosslinked analogues of ionic-liquids – a versatile medium and catalysts for organic reactions

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Keywords: Heterogenization, Synthesis of crosslinked polymers, Immobilization of ionic liquids

Ionic liquids (IL) are defined as salts with low melting points. In recent years IL, this new type of material, attracted a lot of attentions of chemists due to some unique properties such as negligible vapour pressure, good thermal stability and, what is especially important, high polarity. These features allowed the use of ILs as environmentaly friendly (no vapour pressure) and safe alternative to organic solvents. Developments in catalysis using IL were widely studied and reviewed (Gordon, 2001; Wilkes 2004). By a careful choice of the cation and anion it is possible to tune the properties of IL, for example its miscibility with water and ability to dissolve substrates and products of the reaction carried in such medium. However, sometimes it is not possible to achieve easy separation of the reaction products from the IL. In such case the obvious choice is the heterogenization of the ionic liquid by, for example, its immobilization in or on the solid support. One of the first examples of such work was immobilization of the imidazolium salts on the Merrifield type polymeric resin (Kim and Chi, 2004). They prepared an efficient catalytic system for the nucleophilic substitution reactions, which could be used many times with no loss of activity and easily removable from the reaction. The supported ionic liquid phases were reviewed by Mehnert (2005).

In this work we would like to present some results of research on solid polymeric analogues of ionic liquids (IL-analogues), in which polarity of the material is due to the presence of cations and anions known from the chemistry of ionic liquids: imidazolium and phosphonium and anions like trifluoromethylsulfonate, trifluoroacetate, p-toluenosulfonate. Such materials are obtained by modification of lightly crosslinked vinylbenzyl chloride-divinylbenzene copolymers with methyl substituted imidazole and of the same polymer with trialkylphosphines followed by subsequent ion-exchange of suitable anions. By an introduction of an additional function, such as for example sulfonic group, it was also possible to obtain a catalytically active polymer immobilized IL. Additionally, this material, as crosslinked ones, is totally insoluble in any solvent. The last feature makes possible a range of applications such as reactive chromatography, column-continous processes.

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Particle agglomeration in cyclones

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Keywords: Optimized cyclones; Recirculation; Turbulent Dispersion; Particle Agglomeration

The purpose of this work is to build a model to predict in a more realistic way the collection efficiency of a gas cyclone recirculating system [Chibante et al., 2007; Salcedo et al., 2007]. These systems consist in an optimized reverse-flow cyclone combined with partial recirculation of uncaptured particles via a straight-through cyclone concentrator. These systems can obtain very high collection efficiencies ($\eta >$ 90 %) for very fine particle distributions.

Collection efficiency models currently developed for gas cyclones, such as Mothes and Löffer's model [Mothes and Loffler, 1988], can predict with high accuracy the collection efficiencies of particles with diameters above 3-4 μm.

Experimentally, it was observed at laboratory, pilot and industrial-scales that recirculating cyclone systems have complete collection below about 250 nm, viz the grade-efficiency curves have a very pronounced hook-like shape, showing a minimum in collection at an intermediate particle size (from about 0.8 to 2 μm). However, no model, to the authors' knowledge, can correctly predict this behaviour. Muschelknautz's model [Hoffman and Stein, 2002] predicts, at high solid loadings, a fairly constant value of collection efficiency for the smaller particles, since it is postulated that a portion of the feed is separated unclassified, but it does not predict the abnormal high collection for very fine particles at low loadings.

This abnormal behaviour for very fine particles is attributed to agglomeration within the cyclone turbulent flow field, much as it happens in recirculating fluidized beds [Sommerfeld, 2001; Mao et al., 2002]. Thus, this work concentrates on modelling this phenomena, by considering the particles' trajectories inside the cyclone and the probability of interparticle collisions. It some of these collisions result in effective particle agglomeration, then one of the direct implications is that the particle size distribution processed by the gas cyclone differs from the feed size distribution to the cyclone.

In fact, upon agglomeration of fine particles by larger ones, the smaller particles will be captured as much larger particle, viz. with a much higher collection efficieny than the predicted by any of the currently available models. If the cyclone is highly efficient above about 4-5 _m, as it indeed happens with recirculating cyclone systems, then the finest particles will also be collected with these extremelly high efficiencies, and this could explain the minima observed in the grade-efficiency curves.

In this work, agglomeration was estimated using a stochastic Lagrangian approach of the inter-particle collisions in a turbulent field [Sommerfeld, 2001; Ho and Sommerfeld, 2002]. This is a complex method to predict the evolution of each particle inside a control volume, hence, its simplified structure is presented in Figure 1.

The model starts by solving the particle trajectory in a turbulent flow field, and by a set a parameters, determines if a collision occurs. In this case, the minimum of the relative velocity of the particles that collide to maintain them together is calculated. If that

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occurs, the outcome is an agglomerate with a different size and velocity from those of the original particles. In the next iteration, all the remaining particles and the new agglomerates are injected in the control volume and the process repeats until the available total time of interaction is reached.

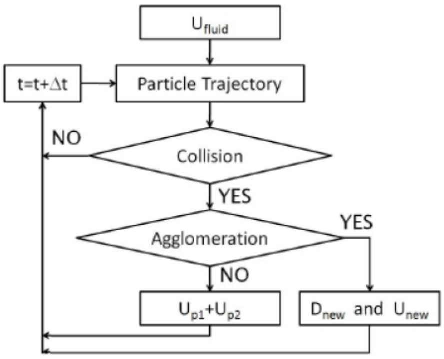
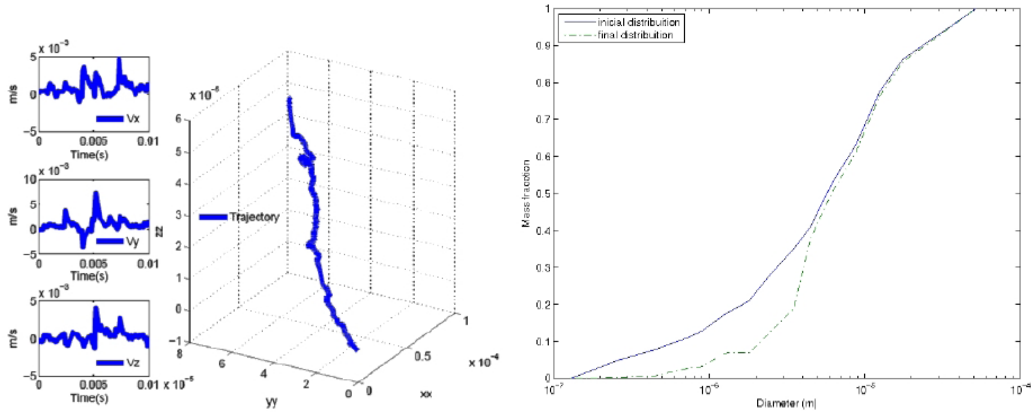


Figure 1: Scheme of the agglomeration model.

Some preliminary results are already available, and are presented in Figures 2a and 2b.



(a) Trajectory
(b) Cumulative Distribution

Figure 2: Examples of preliminary results

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Extending Kozeny-Carman permeability model to highly porous media

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Keywords: Permeability, Porosity, Tortuosity, Shape factor, Model

Highly porous media play a significant role in many practical applications including nanotechnology, biomedicine, fuel cells, catalysis etc. However, the interpretation of experimental permeability data is difficult whenever particle shape or particle arrangement become significantly different of conventional granular packing. In this case conventional Kozeny-Carman model (1) does not satisfy experimental conditions

$$k=\frac{\varepsilon^3}{K_0\tau^2(1-\varepsilon)^2S^2}=\frac{d^2\varepsilon^3}{36K_0\tau^2(1-\varepsilon)^2}=\frac{d^2\varepsilon^3}{72\tau^2(1-\varepsilon)^2} \qquad (1)$$

where k is the permeability, 1/m; S is the specific surface area based on the solid volume; $d=6/S$ is the equivalent spherical particle size; ε is the porosity; $K_0\tau^2$ includes tortuosity τ (ratio of pathway to the porous medium thickness) and K_0 - a coefficient dependent on the pore cross-section shape (for cylindrical pores K_0 = 2). Since Kozeny-Carman model is based on the geometrical models of a capillary tube, then the model adopting to high porous media with shaped particles (often with fractal properties) becomes a complex problem (see Figs. 1-2).

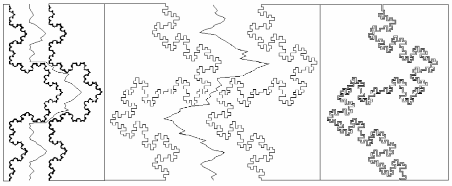
(a) (b) (c)

Fig. 1.

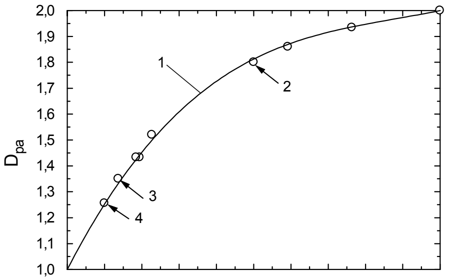

Fig. 2.

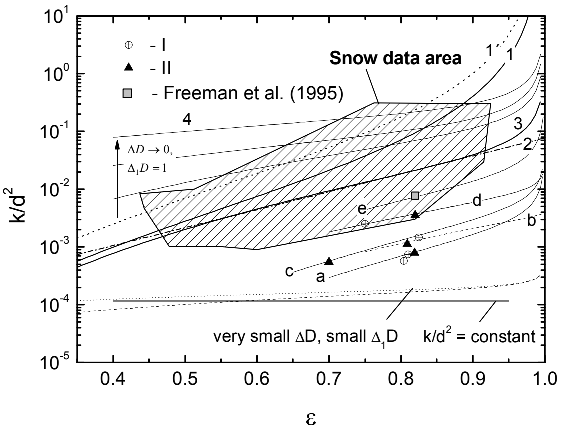

Fig. 3. Normalised permeability k/d^2 vs. porosity. See the text.

For instance (Fig. 1), non-symmetrical pore channels (a) and (b) built by fractal curves can have centreline tortuosity τ = 1.97 and 2.62 with a fractal dimension of 1.01 and 1.03. In turn, in case (c), tortuosity is much higher, τ = 8, and has a fractal dimension of pore channel walls of 1.5. Moreover, the porous medium fractal dimension is dependent on porosity and particle shape. A pore area fractal dimension D_{pa} vs two-dimensional porosity ε_{2D} for regular packing of discs is shown in Fig. 2, where 1 – correlation function; 2 – square packing; 3 – hexagonal packing; 4 – discs square arrangement of two sizes (space between large size square packing filled by inscribed discs). For non-spherical particle model (1) may be partially corrected by introducing

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$d = 6/(\Phi S)$, where the sphericity factor Φ of a particle is the ratio of the surface area of a sphere (with the same volume as the given particle) to the surface area of the particle. In this case, fractal behaviours may affect all or part of variables (Xu and Yu, 2008).

To show how the problem may be complex, two types of particles are presented in Fig. 3: snow (shaded area occupied by data of Jordan et al. (1999)) and diatomite (I), Yoon et al. (1992), kieselguhrs (II), Mota et al. (2000, 2003). Snow represents fractal particles, whereas diatomite and kieselguhr can form pores with fractal tortuosity. By ignoring dependence of τ on ε (see Fig. 3, curves 1, $k = (d^2\varepsilon^3)/[180(1-\varepsilon)^2]$, and 1′, $k = (d^2\varepsilon^3)/[72(1-\varepsilon)^2]$) as well as proposed for the snow layer the permeability (2), Jordan et al. (1999), we have

$$k = 0.077 d^2 \exp(-0.0078 \rho_i (1-\varepsilon)) \qquad (2)$$

where ρ_i = 917 is ice density, kg/m^3. However, in (2) we can speculate that $(1-\varepsilon)$ is accounted on fractal properties as well.

Based on performed theoretical investigation the following model is proposed

$$k/d^2 = \frac{\varepsilon^{3\Delta D}}{72(a/\varepsilon^n)^2(1-\varepsilon)^{2\Delta_1 D(\varepsilon)}} = \frac{\varepsilon^{3\Delta D + 2n}}{72A(1-\varepsilon)^{2\Delta_1 D(1+\varepsilon_0-\varepsilon)}} \qquad (3)$$

where n for granular bed is 0.4 – 0.5; $0 \le \Delta D \le 1$ and $0 \le \Delta_1 D \le 1$ are fractal measures for void and solid phases; ε_0 is the porosity when $1+\varepsilon_0-\varepsilon$ = 1; A is fitting coefficient.

The obtained results show that within model (3) we can describe a wide range of porous media, Fig. 3. In particular, correlation (2) fits well to curve 2, whereas by (3), curve 3 is well fitted, with the following parameters: ΔD= 0.69, n = 0.6, A = 2.5, and ε_0= 0.4. Example of fitting by (3) data of mineral porous media is given by curves a – e: a - Kieselguhr fine; b – Kieselgel; c - Radiolite 600; d - Dialite UND; e – Kieselguhr.

For snow (new snow, in particular) a tendence (Fig. 3, arrow) to increase the permeability was found, when $\Delta D \to 0$ and $\Delta_1 D = 1$. Curve 4 corresponds to the case $\Delta D = 0$, $\tau = 1$, when $A<1$: $k/d^2 = 1/[72 \cdot 0.5(1-\varepsilon)^{2(1.42-\varepsilon)}]$. Below curve 2 conventionally $\Delta D = 1$ and $\Delta_1 D < 1$ that explains by pore structure of type Fig. 1c. With decreasing ΔD below 0.1 and $\Delta_1 D$ below 0.3 we observe insignificant linear changes in k/d^2 and in limited case of ΔD and $\Delta_1 D$ equal zero dependence (3) becomes $k/d^2 = 1/(72A)$, see Fig 3, bottom part. Finally we can conclude that further experimental investigation should be useful to improve the model and validate the application range.

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Optimal operating conditions for the separation of lactobionic acid, sorbitol, fructose and lactose using a 5-section pseudo-SMB

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Keywords: lactobionic acid; adsorption; multi-component separation; JO technology

Lactobionic acid and sorbitol have been widely applied in pharmaceutical and food industries. Recently, several studies for the development of processes to produce lactobionic acid have been made (Miyamoto *et al.*, 2000; Nordkvist *et al.*, 2007). One important pathway was the use of glucose-fructose oxidoreductase (GFOR) and glucono-δ-lactonase (GL) enzymes from cells of *Zymomonas mobilis* bacteria, to convert lactose and fructose to lactobionic acid and sorbitol (Satory et al., 1997). This bioprocess, however, has a high Michaeli's constant (K_m) for lactose and, so, high concentration of substrates is needed to achieve maximum reaction velocity. In order to increase the productivity of the process, the produced quaternary mixture should be separated to allow the recycle of unconverted substrates to the reaction media and the recovery of lactobionic acid and sorbitol. This could be achieved using the Simulated Moving Bed (SMB) technology.

Some SMB configurations have been proposed to achieve the separation of multi-component mixtures. The use of two SMB in series, which can be either separated or combined in a single device (eight zone SMB or nine zone SMB) has been discussed for ternary separation. Cascade of different SMB systems has been reported for ternary and quaternary separation. Five zone SMB system was also investigated, with an additional side-stream besides products of extract or raffinate. Commercially, ternary separations have been carried out with two SMB cascades using direct or indirect connections. Another alternative technology for ternary separation is a device called JO chromatographic separator commercialized by *Organo Corporation* (before *Japan Organo Corporation*) (Masuda *et al.*, 1993). In this technique, there is a switch in the operating mode throughout a process cycle: the system operates in batch chromatography mode alternating with SMB mode with no feed. The JO system (pseudo-simulated moving bed) concept has been discussed in a number of publications: Mata and Rodrigues (2001) have developed a pseudo-SMB model for such process, Kurup et *al.* (2006) have considered multi-objective optimization of these units, Borges da Silva and Rodrigues (2006) analyzed JO performance and suggested an extended JO for quaternary separation; and Sayama *et al.* (1992) have experimentally tested this technology for separation of raffinose from beet molasses.

In this work, we analyze the feasibility of the JO technology to separate the mixture of products, lactobionic acid and sorbitol, and of unreacted substrates, lactose and fructose, obtained from the enzymatic conversion catalyzed by GFOR/GL enzymes. A 5-section JO is an alternative technology to allow this quaternary separation. The separation of the mixture of lactobionic acid, lactose, sorbitol and fructose using a gel type ion exchange resin loaded with K^+ ions has been investigated through fixed bed experiments (Pedruzzi *et al.*, 2008). Measurement of single-component equilibrium isotherms for all four compounds is carried out in a preparative column packed with the resin at 313K. Mass transfer coefficients for the compounds have been estimated by solving the dynamic model of the chromatographic column. With these equilibrium and kinectics adsorption data, equilibrium theory and detailed process model taking into

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account axial dispersion and mass transfer resistance are used to determine the operating conditions for the 5-section JO. The design and optimization of a pseudo-SMB unit is appreciably harder than that of a classical SMB unit, since the number of operating variables to determine appropriate pseudo-SMB operation is larger. Here, a optimization process is developed to find the optimal operating conditions in separating the quaternary mixture from the JO unit.

The 5-section JO model is numerically solved using the commercial package gPROMS v.3.0.3 using the Orthogonal Collocation on Finite Elements discretization methods for the axial domain. For the optimization procedure it was used one of the optimization algorithms within the gPROMS package, namely the CVP_SS, a control vector parameterisation (CVP) approach which assumes that the time-varying control variables are piecewise-constant functions of time over a specified number of control intervals, with a "single-shooting" dynamic optimisation algorithm (SS). The objective function is based on the maximization of unit productivity under a constant desorbent flow rate, constant adsorbent amount and constrained by the purity and recovery of the products.

The 5-JO system performs the separation of the quaternary mixture of sugars and derivatives quite well. At 313 K, all compounds are adsorbed linearly on the K^+ resin (except LBA with slightly unfavourable equilibrium behaviour) and with high mass-transfer rates. In this case, the performance of the system measured in terms of purity and recovery of the four components is quite good. By this optimization study, optimal operation conditions can be determined for this JO configuration such that one can simultaneously recover all the four components with high-purity and allow important improvements on the studied bioprocess: the recycle of the substrates and higher productivities.

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Observation of inertial particle motion in laminar flow in a stirred vessel

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Keywords: Stirred Vessel, Laminar Flow, Particle Motion, Solid-Liquid Flow, Nonlinear Dynamics

Mixing is one of the most important unit operations in chemical and biochemical industries. Although turbulent flow condition is efficient for mixing, some situations require laminar mixing condition. Koiranen *et al.* (1995) proposed specific principles for effective mixing, in laminar flow mixing regimes for highly viscous liquids or shear-sensitive materials. In the laminar flow mixing regimes, however, global mixing is inefficient due to the existence of isolated mixing regions (IMRs). Much attention has been paid to how to eliminate IMRs at Low Reynolds numbers. Nishioka *et al.* (2007) found that particles released at the liquid surface were captured by the recirculation of IMRs in pahse-locked orbit. This Phenomenon may conceivably affect laminar mixing characteristics in a stirred vessel. In this study, inertial particle motion in a stirred vessel was investigated experimentally and numerically at low Reynolds Numbers (*Re* = 10, 30). The mixing system consists of a cylindrical flat-bottom vessel without baffle and a 4-bladed Rushton turbine, as shown in Figure 1. The trajectories of resin particles having the density of 1377–1663 kg/m^3 and the diameter of 5 mm were analyzed using an image processing. Numerical simulation using a commercial CFD code (R-FLOW) was also performed to elucidate the mechanism of particle motin in laminar flow in the stirred vessel. The trajectories of particles having the density of 1200–1450 kg/m^3 and the diameter of 5, 8 and 10 mm were analyzed. The geometry and and boundary conditions for the simulation were the same as those for the experiment.

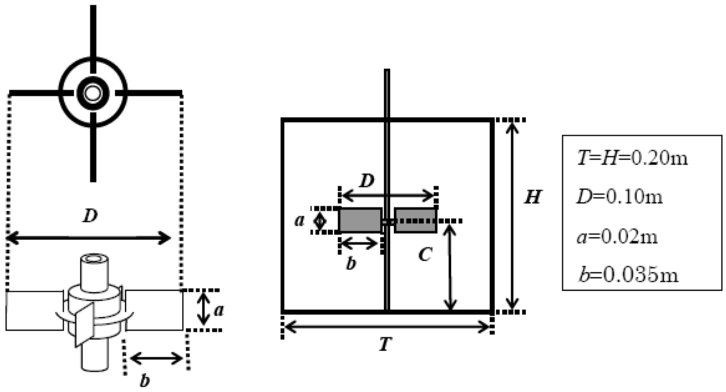

Fig. 1 Schematic of stirred vessel

In this study, the primary and secondary circulation flow directions were defined φ- and θ-direction respectively. The period for one round of a particle in φ-and θ- direction is also defined *P*φ and *P*θ. The relation between *P*θ and *P*φ of the particle captured into the upper IMR was investigated in the case of *C/T* = 0.5 at *Re* = 10. When 30 minutes after injection, Poincaré section obtained particle obit shows that the particle motion covers the full surface of the torus orbit and Pθ /Pφ of the particle is irrational, as shown in Figure 2 a). On the other hand, when 120 minutes after injection, Figure 2 b) shows that the circular orbit on the Poincaré section converges on three discrete points. This indicates that Pθ /Pφ is rational and the particle motion become phase locked on the torus. Ohmura et al. (2003) revealed that the ratio of the time period for one round of a filament around the core IMR in θ-direction to the time for one round of a tracer particle in the filament in φ-direction is rational. It can be, therefore, considered that particles initially move on the surface of the core torus and finally move in a filament within IMR. The particle trajectories obtained by numerical simulation show good agreement with the results of the experiment, as shown in Figure 2 c). Figure 3 shows that the drag force on particle in r-direction has drastically changed after particles are captured by the recirculation of IMRs. This result indicates that the drag force in r-direction is important factor for particle capture in the IMRs.

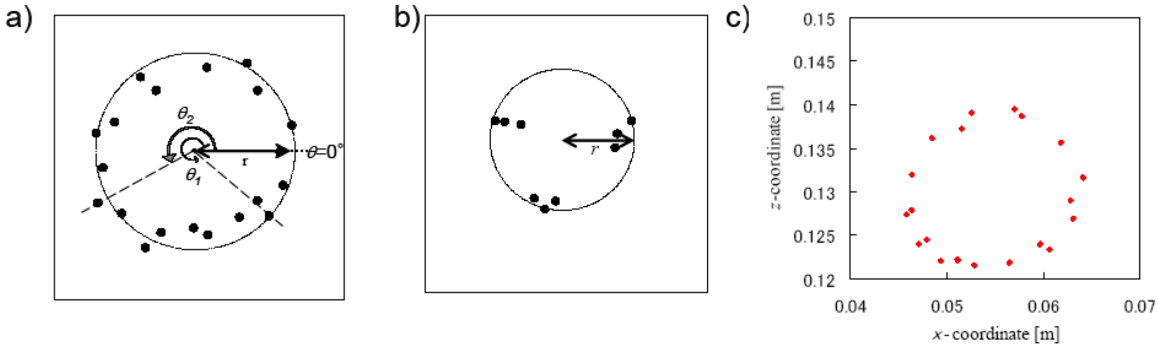

Fig. 2 Poincaré sections of particle orbit; a) experimental (30 min), b) experimental (120 min) and c) numerical (30 min)

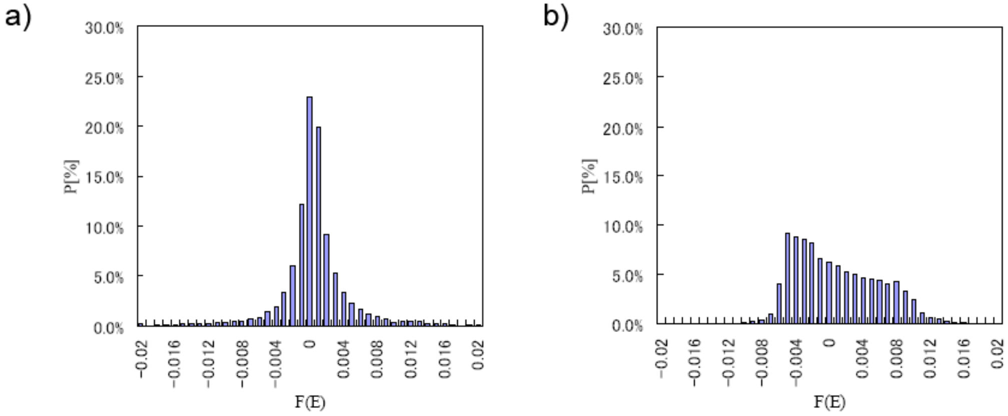

Fig. 3 Distribution of drag force on particle in r-direction; a) 0–25 min (before the convergence) and b) 25–60 min (after the convergence)

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Assessment of the tartaric stability of wines deionised by electrodialysis

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Keywords: electrodialysis, wine, potassium hydrogen tartrate, tartaric stabilization.

The tartaric stability of wine and the control of the potassium hydrogen tartrate (KHT) at low temperature is an issue of concern for the wine industries and many of the wine producers assess that through the use of a very robust control quality test, the refrigerator test (Ribéreau-Gayon, et al.). The electrodialysis, as a technology of tartaric stabilization, has been extensively used instead of the conventional technologies based on the cold treatment, because electrodialysis can ensure an easier, more reliable and precise control of the wine final KHT concentration. The aim of work is to review different methods to evaluate the stability degree of wine partially deionised by electrodialysis and to verify if they give equivalent results to those obtained by refrigerator test.

The electrodialysis experiments were carried out with wine samples that were supplied by José Maria da Fonseca Vinhos SA, Azeitão, Portugal The red, white and liqueur wines were made from grapes harvested in 2005, 2006 and 2001, respectively. Wine samples of 3 L were processed in a bench scale electrodialysis apparatus, model EUR2C-7P18 from Eurodia Industrie, S.A, France. The ion exchange membranes were supplied by TOKUYAMA SODA, Japan and the ED stack had seven cells constituted by 7 anionic membranes (type CMXSb) and 9 cationic membranes (type CMXSb). Each membrane had an effective area of 2 dm2. Two cation exchange membranes were placed in the stack extremities in contact with the electrode rinsing solutions. A sample of 2.96 L of wine was processed at room temperature by ED using a constant electric potential difference of 1.5 Volt/cell. The conductivity and pH of the wine was monitorized during the experiment.

The assessment of the wine stability by the refrigerator test consisted in freezing the wine samples of 100 ml in a refrigerator at -20 ºC. The wine samples were first filtered through a 2.5 µm Whatman filter paper, grade 42. After freezing, the wine samples were placed inside a recipient with tepid water and the potential KHT precipitation was observed during the unfreeze process at naked eye. The absence of KHT precipitation indicates a stable wine, otherwise the wine is classified as unstable. For red wine, a less severe procedure was applied, once the samples were placed at -4ºC during 6 hours, after the previous filtration.

In the mini-contact test (Benítez el al. 2003) a sample of wine with 4 g/L of added KHT (pro analysis grade from Riedel-de Haën) is maintained at a temperature of -4ºC for 65 hours or at -8ºC for the same time for liqueur wines, and continuously agitated, by a magnetic stirrer (AGIMATIC-N model at 700 rpm). The amount of KHT precipitated was assessed by the conductivity decrease (CRISON GPL32 model conductivity meter) and the temperature control was assured by a thermostatic bath with ethylene glycol as the circulating fluid (pro analysis grade from LANOLAB and commercial ethylene glycol from ELF). The experimental saturation temperature of the wines was determined by measuring electrical conductivity in a two-step experiment described by Santos et al. 2002.

The refrigerator test showed that the red and liqueur wines were stable without deionization by electrodialysis, while the white and the rose wines were only stable after 25% and 30% of deionization, respectively. The mini-contact test during 65 h predicted that the required degree of deionization was 20%, 28%, 7% and 20% for white, rose, red and liqueur wine, respectively. The discrepancy between the deionization degree estimated at 24 h and at 65h may have a relative deviation of 5% for rose wine and of 48% for red wines. The analysis of the saturation temperature for the white and rose wines show that these wines were stable (based on the criteria of Maujean et al. (1985)) at deionization degrees of 25% and 20%, respectively. For the white wine, both the mini-contact test at 64 h and the saturation temperature measurement give the same results as those of the refrigerator test. For the rose wine, this is only true for the mini-contact test. In fact, the analysis of the saturation temperature predicts that the wine is stable at a deionization degree of 15% while the refrigerator test indicates a stable wine only at a deionization degree of 25%. For the red and liqueur wines the mini-contact test at 0.3 h is compatible with the refrigerator test, but the mini-contact test at 65 h is only compatible for the red wine. Despite of its simplicity and of the wine freezing, the refrigerator test exhibits good reproducibility and shows also a good agreement with the more time-consuming mini-contact test at 65 h, for all the wines, except the liqueur wine. Even in this case, the wine is considered stable by the refrigerator test and unstable by the other method.

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Characterization of a microporous titanosilicate AM-3 membrane – pure gas permeation

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Keywords: AM-3, Titanosilicates, Microporous Membranes, Gas permeation

Membrane processes are very attractive methods to separate a variety of gas mixtures due to their relatively low energetic consumption and high selectivities observed in comparison with more conventional processes (Xomeritakis and Tsapatsis, 2001). Titanosilicate membranes belong to the microporous inorganic group; these materials exhibit a crystalline structure with molecular dimensions and are capable to separate mixtures based on differences on affinity and sieving effect (Poshusta et al., 1998). In addition, they offer benefits over classical zeolite membranes (Lin et al., 2004), namely: they can be synthesized without organic templates, avoiding subsequent calcination treatments which often causes irreversible defects and/or loss of active surface groups; they are prepared under moderate pH conditions, reducing chemical attack of the support surfaces; and they exhibit novel possibilities of isomorphous framework substitution, allowing for fine-tuning of catalytic and adsorption properties.

AM-3 is a synthetic microporous titanosilicate analogue of mineral penkvilksite $Na_4Ti_2Si_8O_{22}.5H_2O$ (Lin et al., 1997, 2006; Rocha and Anderson, 2000). Its structure consists of SiO_4 tetrahedra connected by individual TiO_6 octahedra forming a three-dimensional framework of 6-ring channels and contrarily to most titanosilicates it does not contain Ti–O–Ti linkages.

In this work, a microporous AM-3 membrane is studied. A thin AM-3 film was hydrothermally crystallized on a porous α-alumina support by *in situ* direct crystallization technique, and was subsequently characterized by measuring pure N_2 permeation flux. A heating/cooling cycle up to *ca.* 200 ºC was performed in order to investigate the presence of adsorbed compounds (e.g., water) in the titanosilicate crystals. Figure 1 shows experimental and calculated results for the permeation flux as function of transmembrane pressure drop.

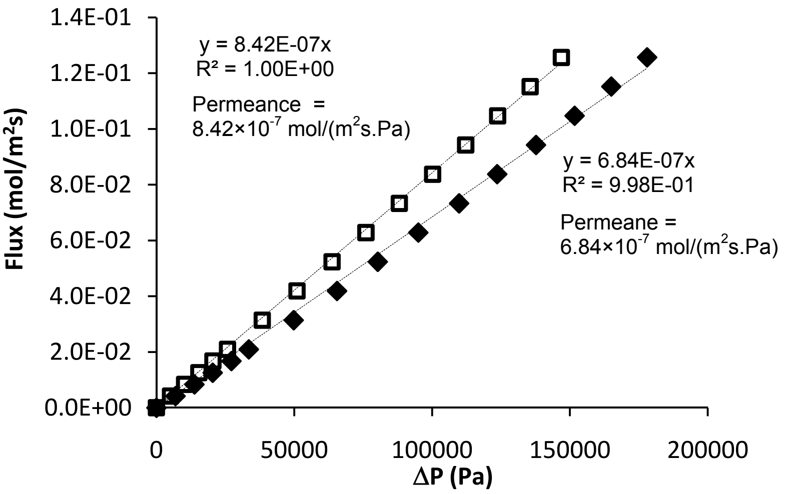

Figure 1: N_2 permeation flux plotted as function of transmembrane pressure drop before (♦) and after (□) the heating/cooling cycle (lines: correlations).

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The enhancement in the N_2 flux after the heating/cooling cycle confirms the hydrophilic character of AM-3 crystals. Permeances found were 6.84×10^{-7} mol/(m^{2}sPa) and 8.42×10^{-7} mol/(m^{2}sPa), respectively. Generally, pure N_2 permeances have values in the range of 1×10^{-7} and 1×10^{-6} mol/(m^2.s.Pa); over-enhanced values denunciate the existence of rough defects in the membrane. Accordingly, results suggest a good quality membrane.

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Pure solvent solubility of some pharmaceutical molecules

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Keywords: Solubility, Drugs, Pure solvents, Modelling.

Introduction

During the search for novel or improved therapies, new drugs are proposed. Solubility of drug-candidates is important both for drug production and its therapeutic use. Many separation processes in the pharmaceutical industry are based on the solubilities in different solvents. Solvation plays an important role in the organism in each stage of drug transport and delivery. Properties like lipophilicity, hydrophilicity, the ability to establish hydrogen bonds and other interactions of the molecules with the surrounding media play an important role in the solvation process. Although some predictive thermodynamic tools can be used to determine drug solubility, the availability of experimental data is still fundamental for an appropriate model development and evaluation.

In this work, solubilities of some drugs, such as paracetamol, budesonide, furosemide and allopurinol, were measured in the temperature range between 25 ºC and 42 ºC, in pure solvents (water, ethanol, acetone, n-hexane, ethyl acetate and carbon tetrachloride). The Non-random Two-Liquid Segment Activity Coefficient (NRTL-SAC) equation (Chen and Song 2004), one of the most successful models for the representation of drug solubility, was used to model the data. The obtained agreement is very satisfactory (root mean square deviation of 0.051).

Experimental

All the solubility experiments were carried out using the isothermal saturation shake-flask method at five different temperatures: 25, 30, 37, 40 and 42ºC. Samples from the saturated liquid phase were removed with isothermal syringe filtration and its drug composition determined by liquid chromatography (HPLC). Melting data (melting temperature and enthalpy of fusion) of the pure drugs were also obtained by differential scanning calorimetry (DSC).

Modelling

The temperature dependence of solubility in pure solvents can be described by the following general equation, considering that the heat capacity difference between the liquid and the solid phase is negligible (Prausnitz et al. 1999):

$$\ln x_2 = -\frac{\Delta_{fus}H}{R}\left(\frac{1}{T}-\frac{1}{T_m}\right)-\ln\gamma_2$$

where x_2 is the solubility, $\Delta_{fus}H$ is the enthalpy of fusion, R the gas constant, T the absolute temperature, T_m the melting temperature and γ_2 is the activity coefficient of the solute calculated by the NRTL-SAC model.

The NRTL-SAC model is a modification of the polymer NRTL equation, where the combinatorial term is calculated from the Flory-Huggins approximation for the

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combinatorial entropy of mixing and the residual term is set equal to the sum of the local composition interaction contribution for each segment. For each solute and solvent molecule, it describes their effective surface interactions in terms of three types of conceptual segments.

Results and Discussion

Among the studied drugs, only for paracetamol there is data available in the open literature (Bustamante et al. 1998; Granberg and Rasmuson 1999; Hojjati and Rohani 2006). In general, the agreement between paracetamol data measured in this work and published in the literature is good, showing the adequacy of the method for drug solubility measurements.

Experimental and modelling results in water are presented in Figure 1 where it can be seen that NRTL-SAC is an appropriate tool for the solubility modelling of aqueous solutions of these complex molecules.

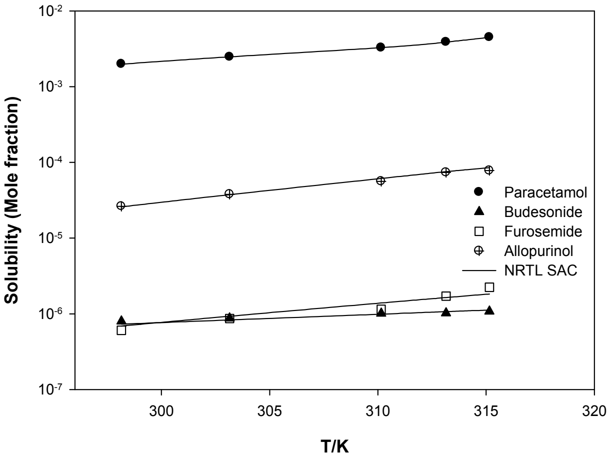

Figure 1 – Experimental aqueous solubility data of the studied compounds.

Conclusions

Solubilities of some pharmaceutical compounds in pure solvents were measured, at five temperatures, in the range 25ºC - 42ºC, using the shake-flask method. The NRTL-SAC model showed to be an appropriate tool to represent the solubility of these molecules.

Ackowledgments

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Basicity promotion and mechanism aspect of liquid selective oxidation of alcohols on Au/Actived-Carbon catalyst

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Keywords: Selective Oxidation, alcohols, Molecular Oxygen, Au/AC, Alkali

Liquid phase oxidation of alcohols with molecular oxygen is an attractive route for the synthesis of fine chemicals, [1] Carbon supported metal catalysts are particularly useful in this context, since carbon materials are stable in acidic and basic media.[2, 3] Gold seems to be a more promising catalyst than other noble metals (such as Pt and Pd), as it is more resistant to deactivation. [4-6]

It is known that gold catalysts used for liquid phase oxidation of alcohols require strongly basic medium. 7 However, to the best of our knowledge, no work regarding the role of basicity and the detailed mechanism has been reported up to date. So, we think it is worthwhile to investigate the role of basicity and the mechanism of gold catalyzed alcohol oxidation. Herein, we used 1wt. % Au/AC as catalyst and benzyl alcohol as substrate, and NaOH as promoter for investigation. It was found that H-abstraction proceeds easily in the presence of NaOH, while oxygen dissociation, which has close relation to the oxygen partial pressure, seems to play a crucial role in the reaction.

Fig.1 shows the results of alcohol oxidation obtained under different conditions: At point (a), both selectivity and conversion are low. The selectivity increased abruptly when NaOH was added, no matter there is oxygen or not – points (b) and (c), indicating that NaOH plays a crucial role in the reaction. Only low conversion was observed at point (b), where oxygen was absent, suggesting that oxygen must have taken part in the reaction and it is not a stoichiometric reaction between benzyl alcohol and NaOH. Interestingly, a moderate result is observed at point (c), which implies that molecular oxygen could oxidize alcohol directly in the presence of alkali. Expectably, both selectivity and conversion increased greatly when catalyst, oxygen, and alkali are all present - point (d). Comparing the data at points (a) and (d), one can easily conclude that basicity is necessary for the reaction to be carried out. The reason is that the H-abstraction from alcohol is easy in the presence of alkali.
The effect of oxygen partial pressure on the activity was also investigated and the results are shown in Fig.2. It is seen that the activity increased greatly with the oxygen partial pressure. On the other, it is known that the rate of oxygen dissociation relates closely to the oxygen partial pressure. So, we considered that oxygen dissociation (i.e. rupture of O-O bond) is a crucial step of the reaction.

$$\mathrm{Au/C} + (\mathrm{OH}^-)_{\mathrm{bulk}} \rightleftarrows [\mathrm{Au\text{-}OH}^-]/\mathrm{C} \qquad (1)$$

$$(\mathrm{PhCH_2OH})_{\mathrm{bulk}} \rightleftarrows [\mathrm{PhCH_2OH\text{-}Au\text{-}OH}^-]/\mathrm{C} \qquad (2)$$

$$\left[\mathrm{PhCH_2OH - Au - OH^-}\right]/\mathrm{C} \xrightarrow{O_2} \left[\mathrm{PhCHO - Au}\right]/\mathrm{C} + \mathrm{H_2O} + (\mathrm{OH^-})_{\mathrm{bulk}} \qquad (3)$$

$$[\mathrm{PhCHO\text{-}Au}]/\mathrm{C} \rightleftarrows (\mathrm{PhCHO})_{\mathrm{bulk}} + \mathrm{Au/C} \qquad (4)$$

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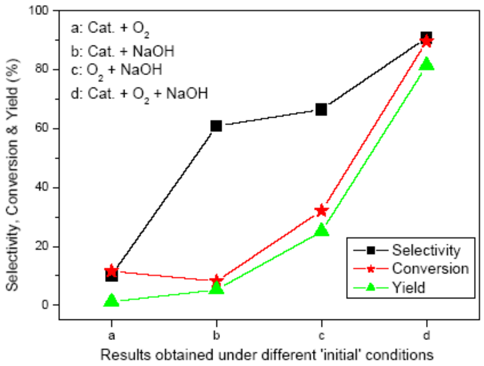

Fig.1: Results of selective oxidation of benzyl alcohol on 1wt.%Au/AC catalyst obtained under different initial conditions (molar ratio of NaOH/alcohol = 1)

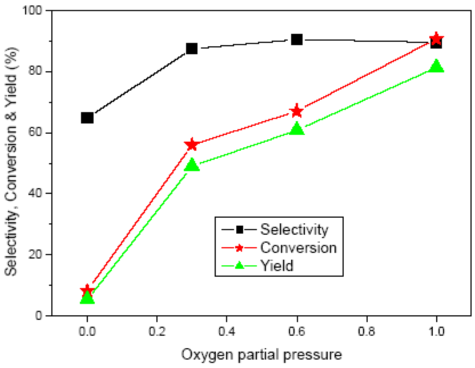

Fig.2: Results of selective oxidation of benzyl alcohol on 1wt.%Au/AC catalyst under different oxygen partial pressure (molar ratio of NaOH/alcohol = 1)

In fact, we have mentioned above that although molecular oxygen could oxidize alcohol directly in the presence of NaOH, the results are not satisfactory. While based on the fact that H-abstraction is easy and oxygen dissociation is a crucial step in the present reaction, we therefore advanced the idea that rupture of O-O bond is the rate-determining step of the reaction, as shown in reactions (1) – (4).

In conclusion, this work indicates that for gold catalyzed liquid alcohol oxidation, H-abstraction from alcohol can proceed easily in the presence of basicity and therefore, rupture of O-O bond turns to be the rate-determining step of the reaction

Acknowledgement:

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Computer-aided phase equilibria prediction for organic systems: UNIFAC-CI. Analysis, revision and extension

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Properties of chemicals are fundamental for the design and analysis of chemical, pharmaceutical, food, agrochemical and related industries. In order to meet the increased demands with respect to complexity of the chemical molecular structures, wider range of chemicals and accuracy, further development of current estimation methods and techniques and/or development of new models are necessary. The objective of this paper is to develop new features of the current methods. At the same time, the extended estimation methods need to be computationally simple and efficient, so they can be used routinely for process-product engineering calculations. Also, the extensions need to consider the coupling of molecular modelling with "engineering" end-use models used in process simulators. Therefore, the group contribution approach, which has been finding increased use in both pure component and mixture property calculations, is considered as the basic model to improve. The main challenge here is how to increase the applicability, accuracy and versatility of the group contribution (GC) models without requiring additional experimental data? The result is the development of a hybrid model that combines molecular descriptors theory and group contribution theory pure component properties and for mixture properties prediction using a GC^{plus} approach[1]. In this paper only the UNIFAC based mixture property prediction for phase equilibria calculations is highlighted.

The main idea of this methodology is the use of connectivity indices (CI) to describe the molecular fragmentation that is characteristic for the UNIFAC group contribution method and that relates properties (molecular interactions in this case) with molecular structure. The result is the automatic generation of group interaction parameters (GIPs) for the UNIFAC group contribution method. Derivation and use of the method has been reported in a recent publication[2]. These include: 1) the comparison of the perfomance of UNIFAC for vapor-liquid equilibria (VLE) calculations using GIPs generated through CI (UNIFAC-CI) vs original UNIFAC, 2) the evaluation of the performance of UNIFAC-CI vs experimental data when the gaps into the UNIFAC matrix are filled, and 3) the performance of UNIFAC-CI vs experimental data when a totally new group for original UNIFAC is proposed. The current version of the UNIFAC-CI method includes groups formed by C, H, O, N, Cl, F and S atoms. This feature of the method implies the potential generation of a very large number of GIPs as well as filling-up the original GIPs matrix without additional experimental data. In this work, an analysis of the strenghts and limitations of the UNIFAC-CI method will be discussed, together with guidelines and recommendations for the user.

The paper will highlight, with several examples, the use and performance of UNIFAC-CI method for mixtures including heteroatoms, compare it against other models, analyze the correlation errors and evaluate the prediction ranges. Also, a framework to extend the methodology to different group contribution models (e.g. other versions of UNIFAC, pure compound property prediction models) and different classes of phase equilibria calculations (LLE, SLE) will be presented.

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Molecular weight distribution in free-radical polymerisation – model development, experimental validation and process optimisation

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Keywords: Mathematical modelling, MWD, Free-radical polymerisation, Optimisation

Victims of a growing and increasingly demanding market, the polymer industries face different challenges in order to offer the best product at the lowest cost possible or, to come up with innovative solutions that meet clients necessities. Many of the variables that directly affect relevant product quality indices are difficult (sometimes impossible) to measure along the process, within a reasonable time. This often leads to a poor product quality monitoring and control. The costumer usually specifies values for end-use properties that have to be met by the producer. This is achieved by relating the non-molecular parameters (e.g. tensile strength, impact strength, colour, crack resistance, thermal stability, melt index, density, etc.) to fundamental polymer properties such as the molecular weight distribution (MWD), copolymer composition and its distribution, particle size distribution (PSD), etc. The latter variables, particularly MWD, PSD and copolymer composition are more easily measurable than the former variables, leading to inferential control of the former through feedback on the latter. Modelling of polymerisation processes is of great industrial importance since the polymer manufacturers find a better understanding of their existing polymerisation reactions and process behaviours, enabling the design of more efficient technology and the development of new products (Yoon et al., 2004).

The MWD is regarded as one of the most important polymer architectural parameters to be controlled in any industrial polymerisation process since it is directly related to some important polymer properties such as stiffness, impact strength, rheology, softening point, gloss, shrinkage, etc. (Yoon et al., 2004). It is not enough to control only the molecular weight averages or even the polydispersity index (PI). The latter is a measure of the width of molecular weight distributions, theoretically very important. However in the past it alone has been used to characterise the polymer MWD. This is not correct since when considering real polymers, the importance of PI is partly lost, as stated by Rogošić et al. (1996). The authors present a few examples of simulated distributions having the same polydispersity index and various widths, and conclude that it is completely unacceptable to draw conclusions on the absolute width of the molecular weight distributions of polymers uniquely on the basis of PI.

It is therefore useful to have a model that comprises an effective methodology for calculation of the complete MWD, improving both operation and control of processes as well as the end-product properties of polymeric materials. Always bearing in mind final control purposes, this work aims for the development of a detailed model that describes the entire MWD resulting from free-radical solution polymerisation, taking place in batch and semi-batch reactors.

In this work two different homopolymerisation systems are studied, namely methyl methacrylate and styrene. The dynamic process models consider a detailed kinetic scheme for this particular type of polymerisation, taking into account chain transfer to monomer to solvent and to one (or more) chain transfer agents, and termination both by disproportionation and combination (coupling). The model also features thermal initiation and the possibility of using an arbitrary number of initiators that can react simultaneously. To account for diffusional limitations that take place during polymerisation, the model simulates gel and glass effects, by using the strategy proposed by Chiu et al. (1983).

We make use of the methodology developed by Crowley and Choi (1997a) for the representation of the entire MWD through discrete chain length intervals. The method consists in choosing a finite chain length domain and dividing it into a desired number of intervals. For each interval, the amount of "dead'" polymer in that chain length range is calculated and divided by the total amount of "dead" polymer on the overall domain. The MWD resulting from the application of this technique are compared with those based on the direct simulation of the dynamic mass balance equations for species of the various chain lengths (from 1 to an arbitrary large number). The results are found to be comparable. Further validation of the model numerical results against results from other established polymerisation modelling packages are also considered, with very favourable results. Finally, comparison with experimental data takes place for both polymerisation systems. The empirical values were obtained from sets of experiments performed in a lab-scale polymerisation apparatus and provide a means for validation of the proposed mathematical model.

The model is further extended to copolymerisation systems, by applying the pseudo-homopolymerisation approximation. By defining a pseudo-homopolymer probability variable (Storti et al., 1989), polymer chains ending with different monomer units are lumped into a single chain type, allowing for a significant simplification of the model equations. MWD (Crowley and Choi, 1997a) and copolymer composition (Mayo and Lewis, 1944) are calculated along reaction time.

From an industrial point of view, it is desirable to have a model that retrieves optimal operation conditions (initiator design, temperature and initiator concentration profiles, etc.) when given a target MWD, with appropriate constraints on the monomer conversion. A model of such kind also enables the development of reliable control strategies to run this type of processes and attain desired MWDs. For that purpose, an approach to dynamic optimisation is developed. Several formulations of the optimisation problem are explored. The one showing better solutions considers the minimisation of reaction time, with constraints set for the monomer conversion and for the errors in the matching of the target MWD (Crowley and Choi, 1997b). Both initiator feed rate and temperature are considered as control variables, presenting different sensitivities towards the MWD.

All simulations and optimisations were performed in the *gPROMS*™ platform, sometimes making use of the interaction with other software applications.

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PVC paste rheology: polymer properties and viscoelastic behaviour

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Keywords: PVC plastisol, rheology, viscosity aging, particle size, polymer properties.

Considering the several types of poly(ethylene) and poly(vinyl chloride) (PVC), PVC is the second world consumed thermoplastic. Although the vast majority of homopolymer and copolymer products are produced in a suspension system, a particular type is produced by the emulsion or microsuspension processes, making the so called dispersion grades (Saeki and Emura, 2002). These types of PVC polymers are normally fine powders of spherical particles (0.1-15 µm) that are mixed with a plasticizer to produce a dispersion (suspension), normally referred as paste or plastisol. Industrially, the said dispersion is applied over a substrate, by spreading, coating or molding, before the gelification and fusion into the final commercial products, such as: films, sheets, paper coatings, mats, flooring, life preservers and roof tops (Sarvetnick, 1972; Nakajima and Harrel, 2001).

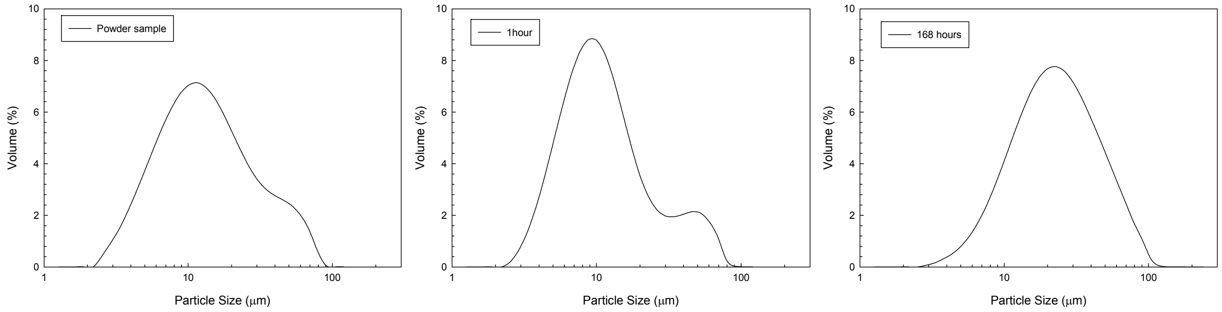
Depending on each application type, the shear stress applied over the plastisol during the production stage makes the study of the paste's rheological behaviour a very important factor to determine and fine tune all process conditions and formulations (Marcilla *et al*, 1997). Also, in the particular case of the emulsion PVC polymers, the conditions during the free radical polymerization, as well as the process conditions of the industrial spray-dryer and subsequent milling of the powder, can have a great influence over the final properties (Nakajima and Harrel, 2001).

The emulsion polymers are normally made up in the presence of a surfactant and a free radical initiator, to produce a polymer that can have different particle sizes and surface characteristics. Also, depending on the production process, an aggregation effect is normally observed, affecting the size and size distribution of the final powder. Nevertheless, during the plastisol formation, these agglomerated particles are not sufficiently strong to resist to the high shearing mixing conditions, together with the solvating plasticizer effect over the polymer. This effect is normally translated by an increase of the plastisol viscosity with time, in a process normally called “viscosity aging” (Nakajima and Harrel, 2005). The aging rate and the rheological behaviour of the plastisols are also sensitive to the type of surfactants used during the polymerization process, type and quantity of plasticizer and to other additives used in the final formulation, such as fillers, pigments and thermal stabilizers (Sarvetnick, 1972, Alsopp and Vianello, 2003).

The application of a developed laser diffraction spectroscopy's technique (LDS) to evaluate the particle size distribution of the initial polymer's powder, as well as his evolution on the plastisols, allows very significant information of the aggregates dimension during the aging period, correlating with the paste's viscoelastic response (Rasteiro and Antunes, 2005) . This relationship, together with the auxiliary analysis of the zeta potential, fractal dimension and morphological observations of the powder, can contribute for a better understanding of all parameters affecting the overall rheological behaviour.

Although less studied, the intrinsic properties of the emulsion polymers, such as molecular weight distribution and thermal stability, which are closely related with the polymerization's conditions, can provide a valuable insight to tune and/or modify the production process, namely, the selection of surfactants and the type of free radical initiator used in several reaction steps of a common semi-batch industrial reactor.

On figure 1, the PSD evolution from the original powder to a given plastisol formulation, since it was prepared (1h) until 24h after, clearly demonstrates the aggregates destruction over time and the correspondent modifications on the newtonian viscosity (figure 2 a), b)).

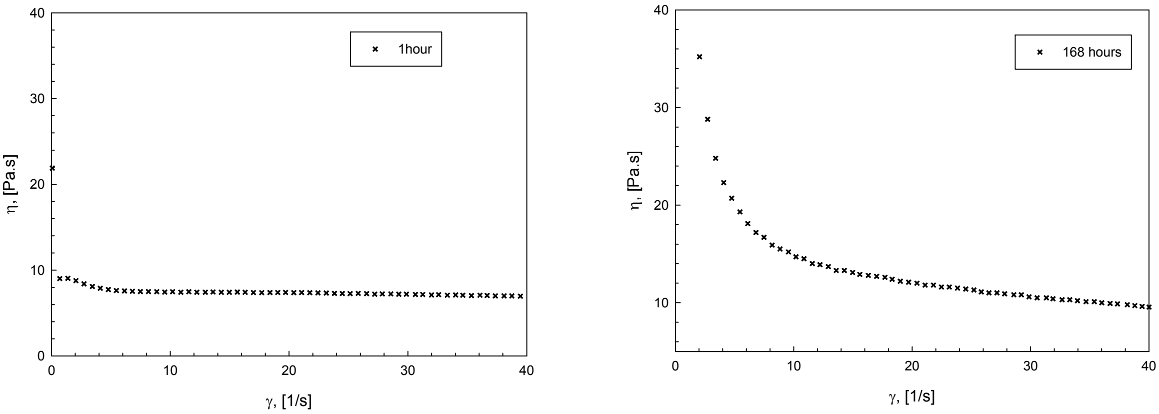


a) Powder

b) Plastisol 1h

c) Plastisol 168hours

Figure 1: PSD distributions (from LDS) of powder sample (a), plastisol sample at 1h (b) to 168h (c).



a) Plastisol at 1h

b) Plastisol at 168h

Figure 2: Viscosity (η) *vs* shear rate (γ) for plastisol sample at 1h (a) and 168h (b).

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Optimization of fluidized bed PVC drying

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Keywords: PVC, fluidized bed, drying

All the major PVC production processes include a step of drying of the granular material. This operation is generally realized in fluidized bed (Saeki and Emura (2002)). The huge worldwide PVC production combined to the fact that drying is highly energy consuming leads to a need for optimization of process conditions to minimize energy consumption without degrading production rate and quality.

In this work, fluidized bed drying of PVC is optimized using a model validated on an experimental laboratory pilot fluidized bed. The experimental setup used includes a fluidized bed equipped with a mechanical stirrer to avoid agglomeration of particles in the beginning of the drying.

These experiments are used to validate a model based on a simplified approach of the transport phenomena in the dryer and in the grains (Debaste *et al.* (2008)): gas phase in the reactor is assumed to behave like a continuous stirred tank reactor. Evaporation from the grain is divided in two successive period: a constant rate period characterized by a constant mass transfer coefficient and a falling rate period in which evaporation is assumed to take place from a linearly decreasing fraction of the surface of the grain. This Heat losses to the surrounding are also taken into account. This model has one fitting parameters: the critical humidity defining the transition between the two zones. Parameter value are obtained on a typical experiment and then validated on other sets of data. Typical results (air humidity and temperature at the dryer's outlet) for the model (continuous line) and experimental (points) are presented on figure 1.

Cross-validation gives satisfactory results for air humidity and total drying time. Thus, this model is used for the process conditions optimization.

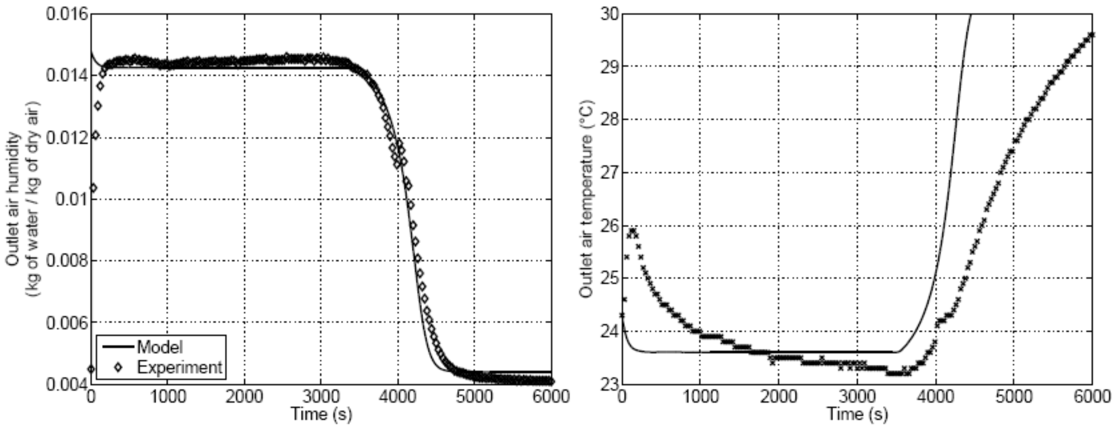

Figure 1: Comparison between model (continuous lines) and experimental datas (circles) for outlet air humidity and temperature

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Identification of the conditions to minimize the drying time doesn't require use of a model: inlet temperature and air flow rate have to be maximized and inlet air humidity has to be minimized. However, the model is useful to predict what set of conditions allows to achieve a given drying time. Choosing a cost function, taking into account energy costs of inlet air drying, inlet air warming and flow rate, and combining it to the model allows to determine which conditions leads, for a given drying time, to the minimal energy consumption. A typical result is presented of figure 2 for a lab scale pilot dryer: dashed line present curves of constant production rate for different inlet temperatures and flow rates. The continuous line gives the minimal cost as a function of process time.

Unsurprisingly is the temperature the most sensitive parameter of this study. Therefore impact of a better thermal insulation on the cost function is studied. Also, to assess the usefulness of the model for design modifications of the dryer, air recirculation impact on the cost function is tested independently of potential practical problem arising from such a recirculation.

Thus, the developed and cross-validated model presented here is useful for operational condition optimization and small design modification. Future work will include continuous feed of solid particles.

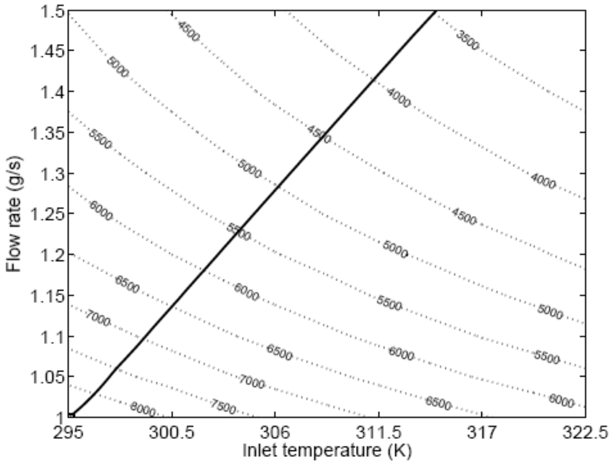

Figure 2: Simulated iso-time of production (dashed line) and optimal conditions curve (continuous line).

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SEC/MALLS measurement of the branched structure of methyl methacrylate + ethylene glycol dimethacrylate copolymers synthesized by atom transfer radical polymerization

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Keywords: Branching, Crosslinking, Atom-Transfer, Molecular Weight, Radius of Gyration.

In the last few years, atom transfer radical polymerization (ATRP) of acrylate and methacrylate monomers in the presence of a small amount of cross-linker has been investigated in order to produce branched polymers and gels with applications in microelectronics, coating industries or biomedicine (Wang and Zhu, 2005; Gao et al., 2007). In this context, it is explored the production by ATRP of branched/crosslinked polymers with lower degree of heterogeneity than that obtained by conventional free radical polymerization (FRP). This work reports the ATRP of methyl methacrylate (MMA) + ethylene glycol dimethacrylate (EGDMA) initiated by methyl α-bromophenylacetate (MBPA) and mediated by copper bromide (CuBr) ligated with 1,1,4,7,10,10-hexamethyltriethylenetetramine (HMTETA). The synthesized copolymers are analyzed using size exclusion chromatography (SEC) with simultaneous detection of refractive index (RI) and multi-angle laser light scattering (MALLS) signals. Important details of the molecular architecture of these materials can therefore be measured, namely absolute molecular weights and *z*-average radius of gyration. Different experimental runs are performed in order to investigate the influence of the operating conditions (e.g. temperature and initial molar ratios MA/EGDMA/MBPA/CuBr/HMTETA) on the structure of the products. Experimental observations are accompanied by modeling studies considering a kinetic scheme comprising a total of 22 species and 44 different chemical reactions. These studies are carried out in the framework of a general kinetic approach for describing non-linear irreversible polymerizations (Costa and Dias, 2007, 2005; Dias and Costa, 2007, 2006).

Figure 1(a) shows the RI chromatograms of ATRP synthesized polimethylmethacrylate (PMMA) samples with different polymerization times. The living character of this polymerization is confirmed by the observed growth of the molecular weight (MW) without a concomitant raise of polydispersity index (PDI). Figure 1(b) shows the good control of the molecular architecture achieved with ATRP. Figure 2(a) shows the formation of a polymer population at a low concentration but high molecular weight in ATRP of MMA/EGDMA. This observation is only possible using a SEC/RI/MALLS system. Time evolutions of the molecular architectures of crosslinked samples are compared in Figure 2(b).

It is expected that these results can be used to design soluble hyperbranched polymers with an improved structure control relatively to those obtained by conventional radical polymerization (FRP).

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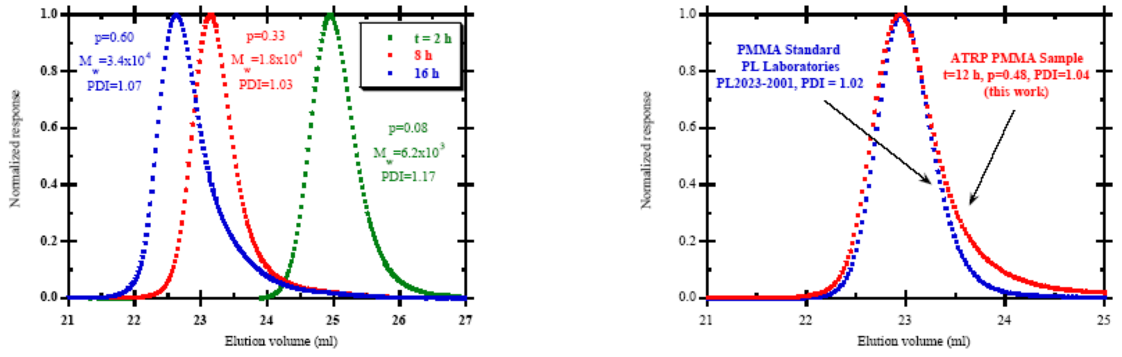
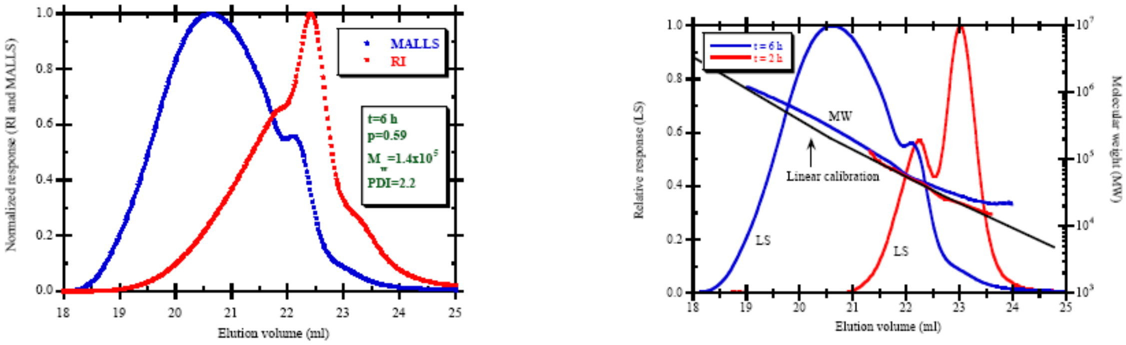


(a) (b)

Figure 1: (a) RI chromatograms of ATRP synthesized PMMA at 80 °C. Samples with different times of polymerization showing the living character of the polymerization (increase of molecular weight MW without a concomitant increase of PDI). Polymerizations in MMA/Toluene solution (35 % v/v) with initial molar ratios MMA/MBPA=501 and MBPA/CuBr/HMTETA=1/1.02/0.99. (b) Comparison of the RI chromatograms of PMMA samples: commercial standard and ATRP synthesized.



(a) (b)

Figure 2: (a) MALLS and RI chromatograms of a MMA/EGDMA sample synthesized using ATRP at 90°C. Copolymerization MMA/EGDMA was performed with 0.5% mole fraction of EGDMA. (b) Molecular weight along SEC chromatogram of MMA/EGDMA samples synthesized using ATRP at 90°C.

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Thermal degradation kinetics of polyethylene in dynamic conditions using simultaneous DSC/TG analysis

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Keywords: Polyethylene, Kinetic modeling, thermal degradation, differential scanning calorimetry (DSC), thermogravimetry (TG)

Introduction

Plastics comprise a major fraction of urban solid wastes. Pyrolysis (thermal degradation at 400-600 °C in an inert atmosphere) [1]. is being considered as a sustainable solution for the recycling of urban polymer wastes [2]. In this work, we present a kinetic model that permits the analysis of polyethylene pyrolysis using the combination of signals obtained from TG and DSC at four different heating rates.

Experimental

Pure high density polyethylene (HDPE, Mw~300 000, Mw/Mn~20) and low density polyethylene (LDPE, Mw~390 000, Mw/Mn~21) samples were kindly given by Borealis. Pyrolysis experiments were carried out in a TA Instruments SDT 2960 simultaneous DSC-TGA apparatus under 80 mL/min of N_2 with heating rates of 5, 10, 20 and 40 ºC/min up to 600 ºC. The gas phase products of the thermal degradation of the polymers were collected and analysed by gas chromatography (GC).

Results and Discussion

Fig 1 show the results obtained in the DSC-TGA run on HDPE at four different heating rates and as it can be seen, the DSC signal (Fig. 1b) shows two endothermic peaks. The first peak corresponds to the melting of the HDPE, and, thus, has no accompanying weight change, as it can be confirmed in Fig. 1a .A second endothermic peak occurs at higher temperatures and corresponds to the degradation of the polymer and, as such, it is accompanied by a weight loss corresponding to the volatilisation of the sample. Moreover, the effect of heat transfer becomes more important at higher heating rates [3]. Consequently, degradation temperature and melting temperature increases when working with the higher heating rates.

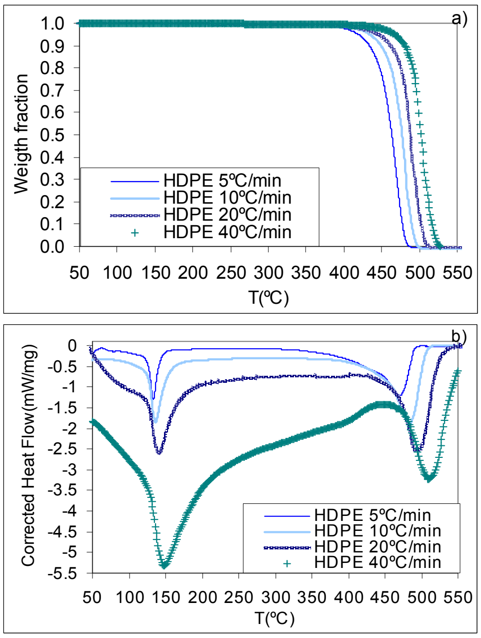

Fig. 1.TG (a) and Heat Flow (b) curves obtained in the degradation of HDPE at different heating rates.

The degradation process takes place by a

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succession of bond breakages. The initial bond breakages will lead to quite large molecules which will not be volatile and will not lead to any measurable weight change, thus, only the combination of TG and DSC analysis will be able to describe adequately the degradation processes.

The kinetic model developed was based on the material and energy balances to the number of bonds in the sample at each time. By performing an energy balance to the pan:

$$\text{Heat Flow} = -mCp\frac{dT}{dt} - k(T)N\Delta H_{\text{C-C}} + \Delta H_{vap}\frac{dm}{dt} \qquad \text{(Eq. 4)}$$

where m is the weight of the sample at any given time, which is obtained experimentally, Cp is the average heat capacity, $\Delta H_{C\text{-}C}$ is the average C-C bond enthalpy, ΔH_{vap} is the average vaporization enthalpy per unit mass, k is the rate constant for the bond breaking reaction and N is the number of unbroken bonds. This model was fitted to the experimental Heat Flow curve, within the range of temperatures where degradation occurs, with very good results (see figure 2). This fitting to the experimental data allowed us to estimate kinetic and thermodynamic parameters.

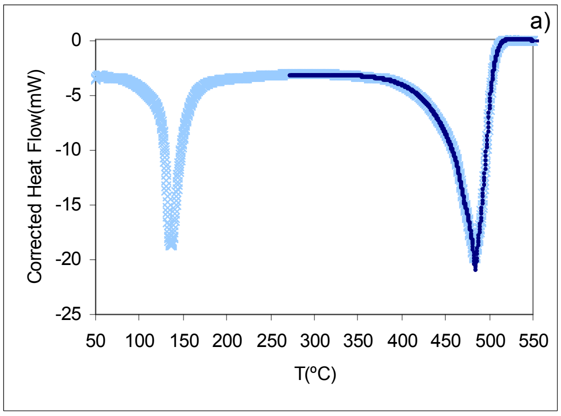

Fig. 2 Experimental (×) and calculated (—) heat flow curves - (a) HDPE 10ºC/min

The gas phase products were analysed by gas chromatography and their distribution, for the four different heating rates, in terms of %molar, are shown in fig. 3. The products consisted mainly of hydrocarbons, both alkanes and alkenes in the C1 to C8 range. As a general observation the amount of lighter products increases as the heating rate increases, in accordance with an observed increase in the fitted apparent activation energy. If the goal is to optimize the production of a gasoline fraction lower heating rates should be used whilst to obtain lighter fractions higher heating rates are more favourable.

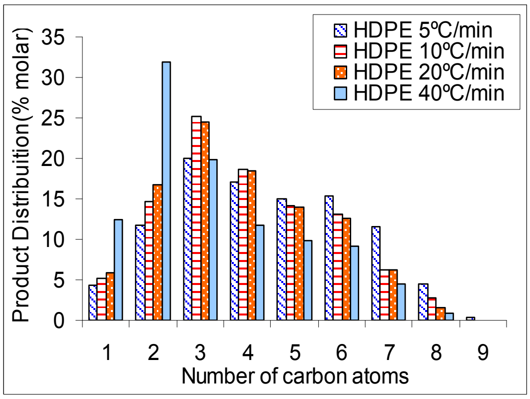

Fig. 3 Distribution of evolved products at different heating rates with HDPE.

The results obtained in the case of the degradation of LDPE lead to similar conclusions.

Acknowledgements

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Decyl acetate synthesis by enzyme catalysis in *sc*CO2

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Keywords: Supercritical CO2; immobilized lipase B of *Candida antarctica*; Transesterification; Ping-pong bi-bi mechanism; Decyl acetate.

The growing interest in industrial biocatalysis and in the research concerning enzymatic catalysis opened a wide range of applications in the field of pharmaceuticals, (fine-)chemicals and intermediates, leading to an increasing number of industrial biotransformations (Rozzell, 1999). Simultaneously, increased concern for the environment led to awareness for environmental friendly production methods. In this field, supercritical fluids may play a major role as they can be used for the reduction of organic waste, with the additional benefit that separation after reaction is relatively simple, which offers several process advantages. Among the four most extensively studied supercritical, *sc*, fluids (CO_2, ethane, ethene and water), there is a wide interest in the use of *sc*CO_2 since it has several advantages like non-toxicity, non-flammability, availability in high purity and solubilities of solutes can be tuned by changing temperature and pressure. As *sc*CO2 has the GRAS (generally regarded as safe) status, it can be used in food and pharmaceutical processes without major regulatory issues. The possibility to combine two sustainable technologies – biocatalysis and supercritical technology using green/natural solvents – allows to establish processes without environmental costs, and whose products are considered natural, resulting in a significant increase of their market value due to the present trends of such products.

In this work, the production of decyl acetate was studied. It is an ester with applications in the industry of fragrances where its floral odour is greatly appreciated. Since it mainly appears in nature in expensive oils, enzyme catalysis in a green solvent, such as CO2, can be a valuable process for production of natural decyl acetate. The reaction studied was the transesterification of vinyl acetate with decanol, obtaining decyl acetate as the main product. Lipase B of *Candida antarctica* (CALB), immobilized in the macroporous resin Lewatit B (Novozym 435®), was chosen as catalyst.

The synthesis of decyl acetate was studied in a high-pressure experimental set-up, equipped with a variable volume batch reactor, operating isotermally at 35 ºC and 100 bar, using as solvent CO2 in supercritical conditions.

The enzymatic content was determined for each particle size of the catalyst. It was shown that the smallest particles have a larger specific amount of enzyme, with an inversely proportional relation between the enzymatic content and the particle size (Table 1). It seems that the enzyme is located in an external shell of the particle, following an “egg-shell” model type, with a thickness of 60 μm (assuming a homogeneous distribution), independent of the particle size.

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Table 1: Enzymatic content for each particle size.

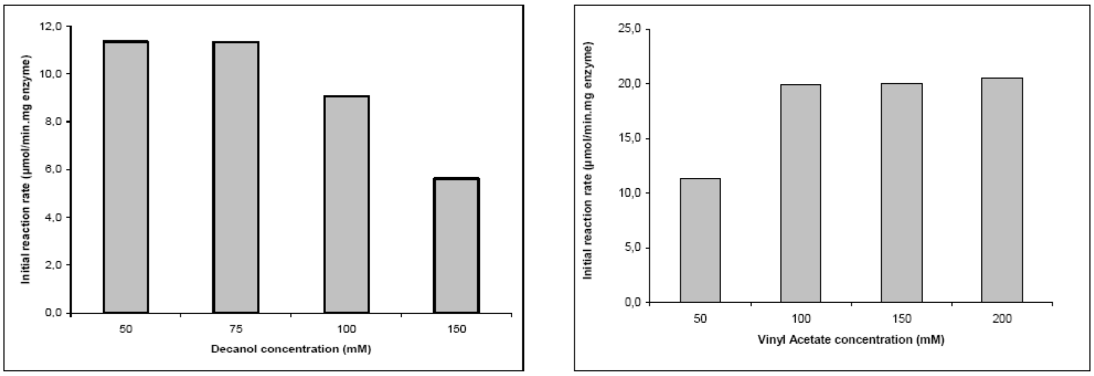
Particle diameter (µm)	Enzymatic content (mg enzyme/g particle)
>598	54,09
425-500	61,94
297-350	84,13
250-297	89,18

The mass transfer resistances were evaluated, both external and internal. For the first ones, the reactor stirring speed was varied, keeping all the other conditions constant (temperature, pressure, amount of enzyme, particle size, initial substrate concentrations). The experimental results showed that external mass transfer resistances are eliminated using an agitation of 950 r.p.m..

To access internal mass transfer resistances, several reactions were performed using different particle sizes, but with the same amount of enzyme, the same initial substrate concentrations, the same stirring speed, temperature and pressure. The results show that the size of the catalyst particles does not have a significant effect on the initial reaction rate. Therefore, the internal diffusional limitations can be considered as negligible.

The effect of the feed concentration of substrates on the initial reaction rate was also studied. It was observed that, from a certain concentration of decanol in excess relatively to vinyl acetate, the reaction is inhibited by the alcohol (Figure 1a). On the contrary, the reaction is favored when the reactor is fed with excess of vinyl acetate, with a significant enhancement of its initial rate (Figure 1b). These results are consistent with a Ping-pong bi-bi type mechanism with competitive inhibition by the alcohol, commonly used in the description of enzymatic reactions of esterification/transesterification (Yadav and Devi, 2004).

Intrinsic kinetic parameters will be determined using an activity based Ping-pong bi-bi kinetic equation to account for the thermodynamic non-ideality of the reaction mixture.



(a) (b)

Figure 1: Effect of the feed concentration of substrates on the initial reaction rate: (a) excess of decanol; (b) excess of vinyl acetate.

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Improving propylene streams purity using catalytic membrane reactors

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Keywords: catalytic membrane reactors, hydrogenation, propylene purification

The selective removal of impurities such as propyne and propadiene from industrial propylene streams is an important process in the petrochemical industry (Fajardo *et al.*, 1996). For the synthesis of polypropylene, propylene should contain less than 10 ppm of propadiene and 5 ppm of propyne (Liu *et al.*, 1997). However, typical industrial propylene produced by steam cracking contains about 5 % of alkynes and dienes (Fajardo *et al.*, 1996). Usually, this separation is performed by a selective hydrogenation process in a fixed-bed catalytic reactor (Liu *et al.*, 1997).

An alternative approach for propylene purification is the use of a sterically-based separation membrane such as a carbon molecular sieve or zeolite membrane (Steel and Koros, 2003). However, this approach yields low separation factors when the mixture components have similar kinetic diameters. In such cases, the separation can be improved by functionalizing the membranes with transition metals (Padin *et al.*, 2000, Stoitsas *et al.*, 2005). These facilitated transport membranes should be highly selective towards propyne and propadiene due to the presence of unsaturated bonds in such hydrocarbons.

In this work, we propose combining the two strategies mentioned before: using a membrane-based separation process and the selective hydrogenation of propyne and propadiene impurities to propylene at the permeate side, taking advantage of a synergic effect. The main objective is to analyze theoretically the effect of the catalytic hydrogenation of such impurities on the enhancement of the performance of a functionalized zeolite membrane. The proposed approach focuses on the use of a tubular membrane selective to dienes and alkynes with a catalytic layer on the permeate side (Fig. 1).

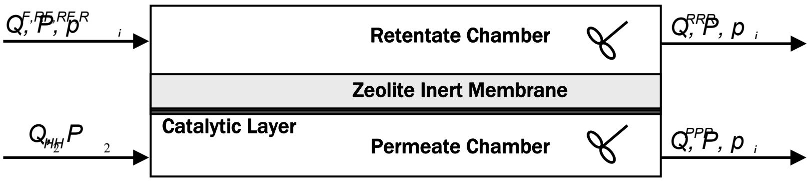

Figure 1 – Schematic diagram of the catalytic membrane reactor.

The propylene mixture is fed at higher pressure to the feed side (retentate chamber) while a low-pressure hydrogen stream is fed to the permeate side. The selective hydrogenation of the propyne and propadiene impurities on the permeate side should increase greatly the driving force for the permeance of such species, consequently increasing the overall separation factor. On the other hand, the deeper hydrogenation of propene to propane must be minimized.

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It is assumed a perfectly mixed flow-pattern in both retentate and permeate chambers, as well as isothermal operation. The study will focus on the effect of several design and operation variables on the overall separation/reaction, namely the selectivity of the zeolite membrane, the reaction selectivity, stage cut, ratio of retentate and permeate total pressures, kinetic activity (defined by the Thiele modulus).

Acknowledgments

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Improvement by alkaline treatment of HZSM-5 zeolite performance in DME steam reforming

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Keywords: dimethyl ether, steam reforming, HZSM-5 zeolite, alkaline treatment

Introduction

Dimethyl ether (DME) steam reforming is a promising alternative for hydrogen production, given that DME synthesis from syngas is more profitable than methanol synthesis and, moreover, CO_2 can be added in the feed together with the syngas (Aguayo et al., 2007). Furthermore, DME is relatively inert, non-corrosive, non-carcinogenic and can be stored and handled as LPGs. Steam reforming of DME is carried out upon bifunctional catalysts, and it consists of two steps in series: DME hydrolysis upon an acid function and methanol steam reforming upon a metallic function (Tanaka et al., 2005). The selection of the acid function is essential for the development of catalysts for DME steam reforming, given that hydrolysis is the process limiting step. γ-Al_2O_3 is the more commonly used acid function in literature for DME steam reforming (Takeishi and Suzuki, 2004), but due to its very low acidity, high temperatures (> 400 ºC) are required for attaining high conversion, and sintering of the metallic function (based usually on Cu) is considerable at these temperatures. As an alternative, the use of zeolites such as ZSM-5 has been proposed (Semelsberger et al., 2006), but due to their excessive acidity, they give way to hydrocarbon formation.

In this paper, a HZSM-5 zeolite has been subjected to an alkaline treatment in order to moderate its acidity (Alonso et al., 2007), with the aim of minimizing the formation of hydrocarbons in DME steam reforming. The severity of the treatment with alkali for maximizing the selectivity and the yield of hydrogen have been determined.

Experimental

The bifunctional catalysts have been prepared by physical mixture of equal mass proportions of the metallic function (a commercial catalyst for methanol steam reforming supplied by Süd-Chemie) and different acid functions, which are a pure HZSM-5 zeolite (SiO_2/Al_2O_3= 30 supplied by Zeolyst) and zeolites modified by different alkaline treatments. These consist of mixing at 80 ºC the ammonium ZSM-5 zeolite with a NaOH solution of different concentration for different times. All the zeolites have been calcined at 575 ºC for 2 h. The porous structure of the zeolites has been measured by N_2 adsorption and the acid structure by differential adsorption and TPD of NH_3.

The kinetic runs have been carried out in automated reaction equipment provided with an isothermal fluidized-bed reactor connected on-line to a MicroGC Agilent 3000. The hydrodynamic properties of the bed have been improved by mixing the catalyst (particle size between 150 and 250 μm) with inert alumina (particle size between 60 and 90 μm) in a catalyst/inert ratio of 1/4. The operating conditions used are: atmospheric pressure, steam/DME/He ratio= 3/1/0.85, space time up to 0.60 $g_{catalyst}h/g_{DME}$, temperature in the 225-325 ºC range.

Results and discussion

Table 1 shows the results of total acidity and prevailing acid strenght of the acid function. It is observed that the treatment with alkali moderates acidity, affecting more

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noticeably to total amount of acid sites than to the acid strength of the sites. The results of TPD of ammonia confirm these results.

Figure 2 shows the results of DME conversion (left) and yields of hydrogen and CO (right) for different operating conditions at zero time on stream. The conversion and yield of H_2 with the Z30 zeolite catalyst are slightly higher than with the treated zeolites, as would be expected taking into account its higher acidity. Nevertheless, the yield of CO with this catalyst is significantly higher than with the modified zeolites. For the catalysts of pure zeolite or zeolite subjected to a moderated alkali treatment, the maximum yield of H_2 is obtained at 300 ºC, and the subsequent decrease in H_2 production at 325 ºC is due to the rapid formation of hydrocarbons from methanol and DME (MTG process). At 300 ºC, an induction period is needed for the formation of hydrocarbons, and the duration of the period significantly increases with the alkali treatment. The A0.4-300 zeolite catalyst (severe alkaline treatment), avoids hydrocarbons formation in the whole range of temperature studied. Nevertheless, the higher temperature required for attaining high DME conversion favours a higher formation of CO by reverse water-gas shift reaction. Moreover, catalyst deactivation by Cu sintering will also be higher.

Consequently, a moderate alkaline treatment is suitable for modifying the HZSM-5 zeolite in order to use it as an acid function for DME steam reforming. A moderate alkaline treatment allows for obtaining high H_2 selectivity and yield at temperatures that are low enough to minimize the formation of byproducts (hydrocarbons), formation of CO and deactivation by Cu sintering.

Table 1. Total acidity and acid strength of the zeolites for different alkaline treatments.

		Z30	A0.2-10	A0.2-300	A0.4-300
Alkaline treatment	NaOH concentration, mol/l	--	0.2	0.2	0.4
	Time of treatment, min	--	10	300	300
Adic properties	Total acidity, $mmol_{NH3}/g_{zeolite}$	0.92	0.74	0.69	0.69
	Acid strenght, kJ/mol_{NH3}	130	125	120	110

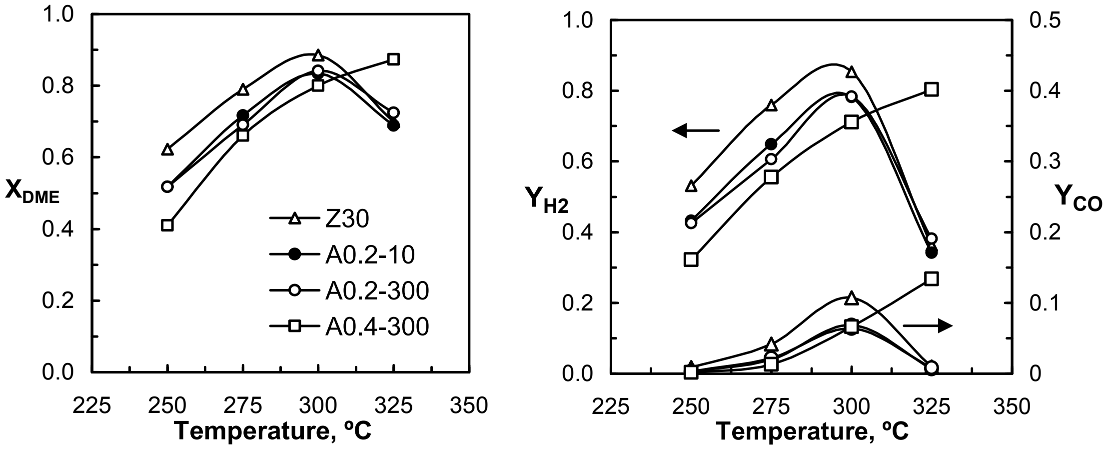

Figure 1. Conversion of DME and yield of H_2 and CO in DME steam reforming for the different acid functions. Space time= 0.60 $g_{catalyst}h/g_{DME}$; steam/DME= 3/1; 1 atm.

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Application of a group contribution CPA EoS to the modeling of biodiesel production

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Keywords: Biodiesel, Group contribution method, CPA EoS, Water solubility

Biodiesel, a blend of fatty acid alkyl esters that can be mixed with regular diesel, has become an attractive alternative to conventional fuels as is made from renewable sources and presents several environmental and economical benefits.

Being able to describe the various types of phase equilibria occurring in a biodiesel production and purification facility is of utmost importance for the design and optimization of the process and to insure fuel quality.

In terms of thermodynamic behaviour, the change from petroleum based diesel to biodiesel represents a change from simply van der Walls intermolecular interactions to a more complex combination of van der Waals, polar and association interactions that considerably increase the non-ideality of the involved mixtures.

In the biodiesel production process the ester-rich current leaving the transesterification reactor is saturated with glycerol, catalyst and unreacted alcohol and soaps. This current is washed in counter current with acidified water in a liquid-liquid extractor to neutralize the catalyst and to convert soaps to free fatty acids. The raffinate current is composed of water saturated biodiesel while the extract is a low pH aqueous solution containing the polar compounds. In the following drying section, the biodiesel is then refined in order to meet the standard specification developed by the European Committee for Standardization (CEN) of a maximum water content of 0.05% (w/w).

Due to the lack of data of the water solubility in fatty acids, esters and biodiesel mixtures in the open literature, measurements for 6 fatty acids, 11 esters and 6 commercial biodiesels in the temperature range of 288.15-323.15 K were carried out using a Metrohm 831 Karl Fischer (KF) coulometer. The results presented in this work are in agreement with the available literature data, showing the ability of the experimental methodology used for measuring the water solubility in fatty acids, heavy esters and commercial biodiesels.

Several phase equilibria involving associating molecules are involved in the production of biodiesel. In the petrochemical industry, cubic equations of state such as SRK and PR have been traditionally employed for phase equilibria calculations, but in the case of biodiesel production, an equation of state explicitly dealing with association interactions is required.

The Cubic-Plus-Association (CPA) EoS was here applied to the description of the water solubility in fatty acids, esters and biodiesel mixtures, required for a correct dimensioning of the biodiesel washing and purification processes. The CPA-EoS had already shown to be an accurate model to correlate the mutual solubilities of aqueous systems with alkanes, aromatics, amines and alcohols. The CPA equation of state can be expressed as the sum of two contributions: one accounting for physical interactions,

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that in the current work is taken as the SRK EoS, and another accounting for association, the Wertheim association term:

$$Z^{assoc} = -\frac{1}{2}\left(1+\rho\frac{\partial \ln g}{\partial \rho}\right)\sum_i x_i \sum_{A_i}(1-X_{Ai}) \quad \textbf{(1)} \qquad X_{Ai} = \frac{1}{1+\rho\sum_j x_j \sum_{B_j} X_{Bj}\Delta^{A_iB_j}} \quad \textbf{(2)}$$

where ρ is the density, *g* a simplified radial distribution function and X_{Ai} the fraction of pure component i not bonded at site A. Δ_{AB} is the associating strength between sites A and B on the associating molecules calculated from the pure component association energy and volume, respectively, ε_{AB} and β_{AB}.

In order to obtain the pure component CPA parameters, liquid density and vapour pressure data are necessary. Unfortunately, for some of the compounds of interest for the biodiesel industry, the absence of data strongly limits the use of the CPA EoS. To overcome this problem, in this work, a new group contribution method was also proposed for four different chemical families (n-alkanes, n-alcohols, n-esters and nacids) and used to describe the solubilities mentioned above.

Given the families of compounds under study, the chemical groups adopted as suitable for the description of these compounds were: (CH3), (CH2), (OH), (HCOO), (COO), (COOH) and (CH=). The CPA EoS parameters are then obtained from the following relations:

$$a_0 = k_1 \times \left(\ln\left(k_2 + \sum_{i=1}^{n_{groups}} n_i a_{0i}\right)\right)^{k_3} \quad \textbf{(3)} \qquad c_1 = k_1 \times \left(\ln\left(k_2 + \sum_{i=1}^{n_{groups}} n_i c_{1i}\right)\right)^{k_3} \quad \textbf{(4)}$$

$$b = \sum_{i=1}^{n_{groups}} n_i b_i \quad \textbf{(5)} \qquad \beta = k_1 + k_2 \exp(-k_3 \sum_{i=1}^{n_{groups}} n_i \beta_i) \quad \textbf{(6)}$$

$$\varepsilon = \sum_{i=1}^{n_{groups}} n_i \varepsilon_i \quad \textbf{(7)}$$

The GC-CPA EoS, as well as the usual approach for computing the pure compounds parameters, can accurately describe the water solubility in binary ester systems and in biodiesel mixtures and the mutual solubilities of aqueous fatty acid systems.

For both approaches, a single, small, and temperature-independent *kij* was enough to describe the water solubility. The binary interaction parameters are chain length dependent for the families studied.

The GC-CPA EoS can even provide better results for the water solubility than the approach using the CPA parameters adjusted from pure component experimental data. The proposed model thus seems to be a promising tool for the biodiesel industry and future work will focus in extending this approach to other systems of interest for biodiesel production and purification.

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Sequential batch reactor and plug flow reactor network comparison under dynamic conditions for wastewater treatment

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Keywords: SBR, PFR, Reactor Network, Wastewater Modeling and Simulation, Dynamic Modeling and Simulation, Wastewater Treatment.

Introduction

The Sequential Batch Reactor (SBR) technology is widely used for biological wastewater (WW) treatments with carbon and nutrients removal purposes. This kind of technology can apply aerobic, anoxic, as well as anaerobic processes (Artan and Orhon, 2005; Benitez et al., 2006). A SBR process can be analyzed as composed of two stages: a Batch Reactor Network ("sequencing") followed by a Feeding Pattern ("wastewater management"), as illustrated in Figure 1(a). Under steady state conditions, SBR compositions at the beginning of the cycle are equal to the final ones and the wastewater composition and flow rate are both kept constant. The main purpose of this contribution is to model a real multiple parallel SBR plant by a single Plug Flow Reactor Network (PFRN), as presented in Figure 1(b) (Artan and Orhon, 2005). Under steady-state conditions, it can be shown that mathematical models of both systems, expressed in terms of dimensionless variables, are exactly the same.

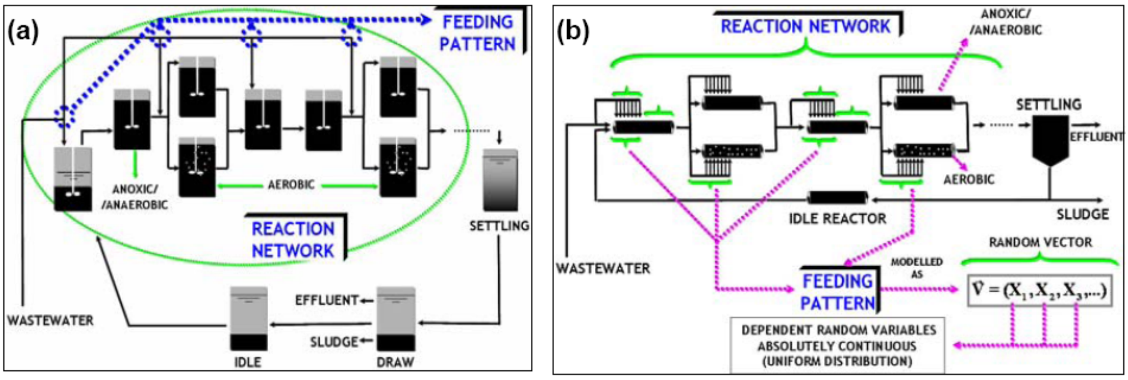

Figure 1: SBR representations: Batch Reactor Network/Feeding Pattern (a) and PFRN (b).

The much more simplified structure of the PFRN in comparison with the multiple parallel SBR representation makes that approach very suitable for stationary analysis and design purposes. New insights of both approaches can be obtained comparing their dynamic simulations. Actual research on this area is practically negligible. The comparison of dynamic responses of SBR and PFRN representations, under step changes in wastewater flowrate and composition, is the main contribution of this paper. This kind of perturbation is common in real industries.

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Mathematical Modeling and Numerical Methods

The SBR and PFRN transient mass balances (equations 1 and 2 respectively) are presented below. It should be pointed out that the SBR model is a lumped model; otherwise the PFRN model is a distributed model.

$$(1)\quad \frac{\partial C_i(t)}{\partial t} = Fe_{(SBR)}.\frac{Q(t).[C_{f,i}(t) - C_i(t)]}{V(t)} - Da_i.r_i(t)$$

$$(2)\quad Fe_{(PFRN)}.Q(z,t).[C_{f,i}(t) - C_i(z,t)] - Da_i.r_i(z,t) = \frac{\partial[C_i(z,t)]}{\partial t} + v(z,t).\frac{\partial C_i(z,t)}{\partial z}$$

$$Fe_{(PFRN)} = \frac{Q^*}{v^*} \qquad Fe_{(SBR)} = \frac{T^*.Q^*}{V^*} \qquad Da_i = \frac{T^*.r_i^*}{C_i^*}$$

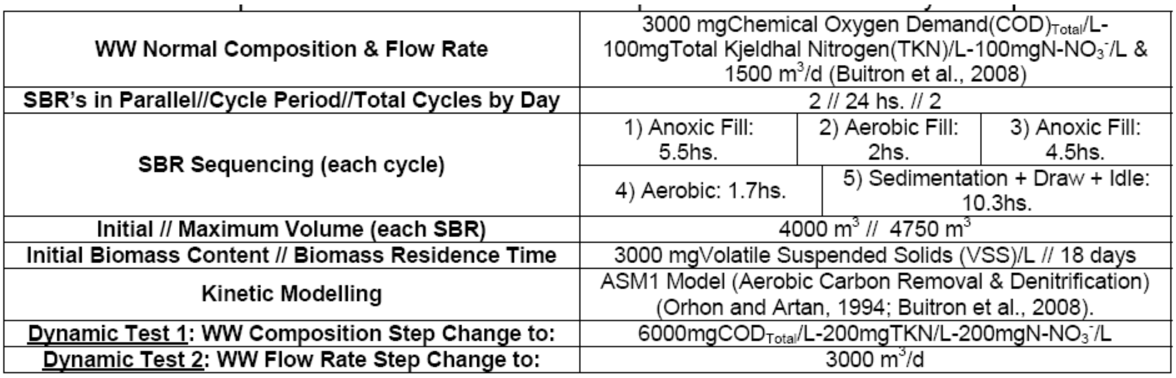
Where:

- **Ci**: Dimensionless concentration for component "i" [Cf: in Feed/C: in Reactor].
- **z,t**: Dimensionless position (only for PFRN), and time.
- **Q,v**: Dimensionless wastewater flow rate, and internal PFRN flow rate.
- **V,ri**: Dimensionless SBR volume, and "i" component consumption rate.
- **Fe,Dai**: Dimensionless Feeding Number, and Damköhler Number for "i" component.
- ***Ci*,T*,Q*,v*,V*,ri****: Reference values kept constant.

Ordinary differential equations were solved through a 4th order Adaptive Runge-Kutta, and partial differential equations were also solved, after discretization in space variable by a Finite Differences method (400 discretization nodes), by the same Runge-Kutta method.

The Table 1 summarizes the plant conditions adopted in the simulations. These conditions were obtained from a typical real dairy SBR plant (milk powder plant).

Table 1: Operational data and simulations parameters for the dairy SBR plant



<table>
<tr><td>WW Normal Composition & Flow Rate</td><td colspan="6">3000 mgChemical Oxygen Demand(COD)$_{Total}$/L-100mgTotal Kjeldhal Nitrogen(TKN)/L-100mgN-NO$_3^-$/L & 1500 m^3/d (Buitron et al., 2008)</td></tr>
<tr><td>SBR's in Parallel//Cycle Period//Total Cycles by Day</td><td colspan="6">2 // 24 hs. // 2</td></tr>
<tr><td rowspan="2">SBR Sequencing (each cycle)</td><td colspan="2">1) Anoxic Fill: 5.5hs.</td><td colspan="2">2) Aerobic Fill: 2hs.</td><td colspan="2">3) Anoxic Fill: 4.5hs.</td></tr>
<tr><td colspan="3">4) Aerobic: 1.7hs.</td><td colspan="3">5) Sedimentation + Draw + Idle: 10.3hs.</td></tr>
<tr><td>Initial // Maximum Volume (each SBR)</td><td colspan="6">4000 m^3 // 4750 m^3</td></tr>
<tr><td>Initial Biomass Content // Biomass Residence Time</td><td colspan="6">3000 mgVolatile Suspended Solids (VSS)/L // 18 days</td></tr>
<tr><td>Kinetic Modelling</td><td colspan="6">ASM1 Model (Aerobic Carbon Removal & Denitrification) (Orhon and Artan, 1994; Buitron et al., 2008).</td></tr>
<tr><td><u>Dynamic Test 1</u>: WW Composition Step Change to:</td><td colspan="6">6000mgCOD$_{Total}$/L-200mgTKN/L-200mgN-NO$_3^-$/L</td></tr>
<tr><td><u>Dynamic Test 2</u>: WW Flow Rate Step Change to:</td><td colspan="6">3000 m^3/d</td></tr>
</table>

Results and discussion

The dynamic response solutions for effluent composition in both tests are shown in Figure 3 and Figure 4. These results demonstrate that, despite the different nature of SBR and PFRN dynamic models, the qualitative dynamic evolutions are quite similar, and even their quantitative values are not so different.

Conclusions

The well known analogy of the SBR and PFRN stationary models were also verified in this contribution. New insights were obtained through the comparison of transient responses to typical perturbations of both models. Qualitative and quantitative aspects of both dynamic responses were analysed. The similitudes of the dynamic responses encourage the use of PFRN models to describe confidently the stationary and dynamic behavior of real SBR plants. Dynamic optimization of real SBR plants using the PFRN approach is a work in progress by the authors.

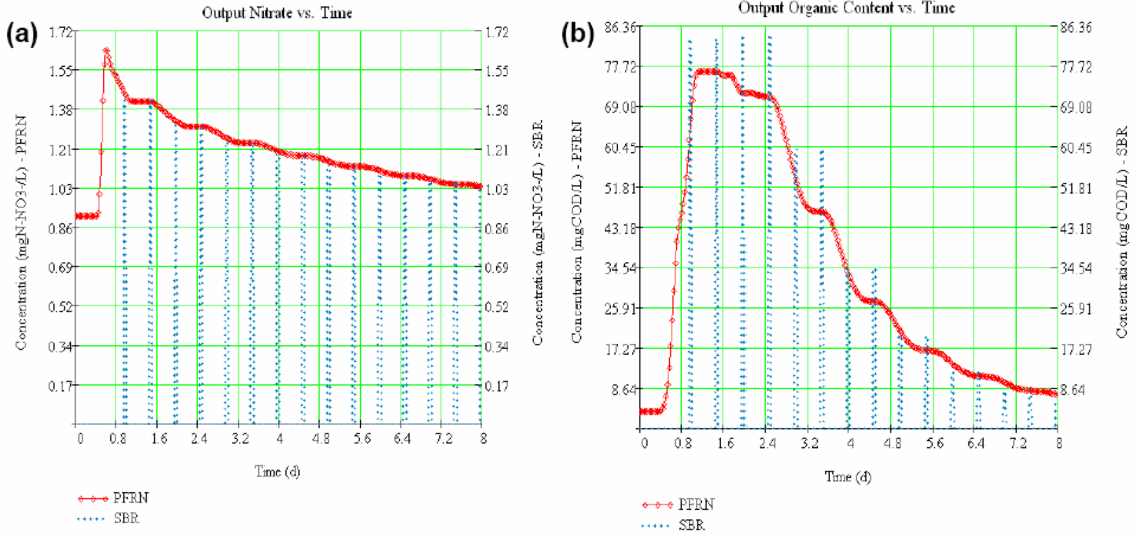


Figure 3: Simulation results for effluent composition during Test 1: Nitrate (a) and Organic Content (b) evolution

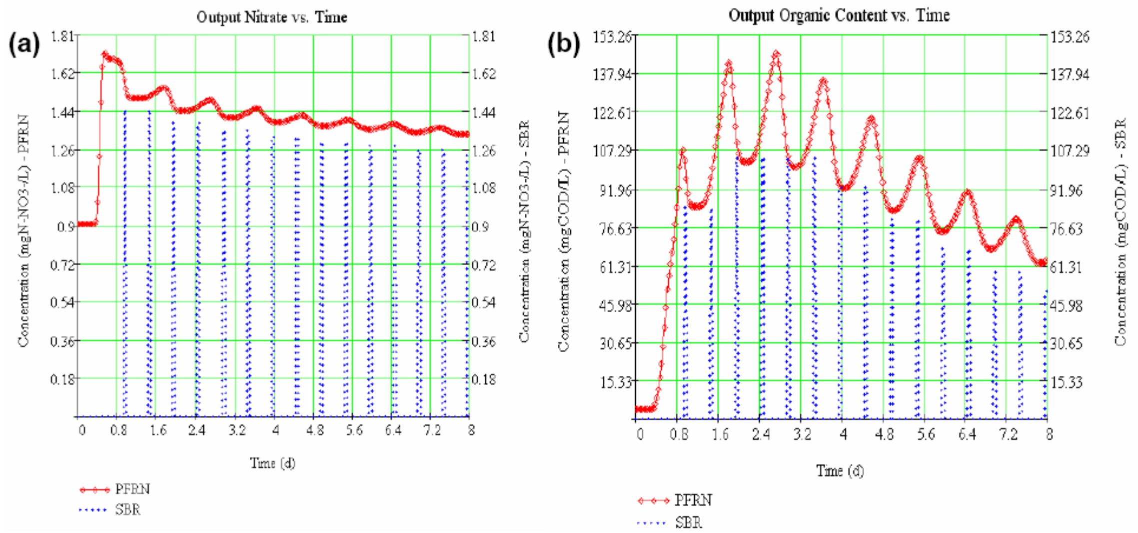


Figure 4: Simulation results for effluent composition during Test 2: Nitrate (a) and Organic Content (b) evolution

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Design and Engineering of Structured Molecular Matter

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Keywords: Chemical Product Design, Innovation, Product Engineering

Abstract

Product innovation processes have been studied extensively by new product development managers, marketers and researchers, and many references can be found in the literature describing the topic [1-3]. Most of these references however focus on discrete product industries that manufacture products in which mechanical action or signal processing (or combinations thereof) determine the principle end-use performance. In industries that deliver products in which parameters like chemical and/or physical properties of the constituting materials, their processing and their ultimate product morphology determine product performance, the use of systematic design methods and tools is less familiar.

“Chemical product design and engineering” now rapidly develops as a new branch within the chemical engineering discipline and may be considered as the implementation of an integrated and structured product innovation process to be used in processing industries that manufacture complex, formulated and structured chemicals-based products.

The paper will highlight how lessons learnt from the tools used in the discrete industries can be applied to speed-up and structure the design and development of new chemical products.

Innovation of formulated chemicals-based products from the design stage to the commercialization stage is often described as an activity that requires thinking in different dimensions and the use of different scientific disciplines in the various stages of the design and development process. The overall product creation activity can be split into different stages that however have to be integrated into a concerted integrated activity to enable short time-to-market periods. The stages are:

- Idea or product concept description
- Definition of product requirements
- Product option generation and process option generation
- Option selection and option evaluation
- Product engineering and development
- Pilot production and test-marketing
- Upscaling of product manufacturing processes
- Implementation and sales

Each stage requires a mindset to think in different dimensions and to integrate different scientific disciplines. In the smallest dimension, in the literature often referred to as the “nano” level, the designer has to consider the chemical and physical properties of the materials that are to be selected to achieve the endproduct performance. At this stage also the chemistry at the interfaces and the interactions between the different constituents needs to be considered. Within the same nano-dimensional area, molecular engineering (also often referred to as “product engineering”), may be required to design or select materials that are foreseen to deliver the desired end-

performance. Likewise, the material characteristics need to be considered in relation to the product manufacturing technology that can or will be applied.

At the next dimensional level, referred to as the “micro-level”, product physics play an important role in both product and process considerations. For example polymer rheology, material phase transitions, or particle size, shaping and dynamics in liquid or solids-based product structures, needs to be considered in the processing and handling of intermediate or final formulated and shaped products.

Finally, “meso- and macro-level” dimensions like unit operatons, manufacturing facilities and markets for the products have to be considered. These dimensions appear to be closest to the classic chemical engineering discipline, but also require a minset to think in terms of products, customers and product economics.

For chemical engineering students that will become involved (as more and more will do) in the design of structured chemicals-based products it is important to be educated in these multidisciplinary aspects. The paper will give an overview of the basic design phases for an actual product, using familiar tools and highlighting the multi-dimensional and multi-disciplinary nature of designing, engineering and developing structured molecular matter. It will be shown how a structured design and engineering methodology can aid in reducing the “time to market” for new product concepts, and how this can be applied to the wide range of chemical product industries.

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Systematic modelling framework in product-process design and development

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Keywords: Product Design, process design, work-flow, multi-scale modelling.

The design, development and manufacture of a product and its process need to be consistent with the end-use characteristics of the desired product. One of the common ways to achieve the desired characteristics of the product-process is through trial and error based experiments. Although it is the most commonly used practice, it can be expensive and time consuming. An alternative approach is the introduction of a systematic model-based work-flow in product-process design which replaces some of the time consuming and/or repetitive experimental steps with computational tools.

The appropriate models for product-process design needs to have multi-scale features as the properties defining the chemical structure and end-use characteristics of the product are dependent on parameters of different size and time scale. The advantages of the use of multi-scale modelling approach is that the design, development and/or manufacturing of a product-process can be described at different scales of length and time, providing thereby the knowledge of the applied phenomena at diverse degrees of abstractions and details. Charpentier, 2002; Gani, 2004; Klatt and Marquardt, 2007 and Charpentier, 2007 have highlighted the importance of multi-scale approach in product-process design and also the multi relationship among different disciplines specialized in different fields at different scales of size, time and complexity.

In this paper, we propose a framework for systematic model-based work-flow in product-process design (see figure 1). This framework will allow the design of the product-process assisted by computer-aided modelling tools to guide the user through the development of the desired product-process (related to the formulated chemical products). The framework includes the product design and process design components; modelling tools; and templates (work-flow) for guiding the user through the design steps. The work-flow starts with a specification of the characteristics and properties of the product to be designed. Based on this information, alternatives are generated, which are then tested and evaluated to identify the chemicals and/or their mixtures that satisfy the desired product specifications (qualities, needs and properties). Next, modelling tools are employed to generate the appropriate models for simulation of the product behaviour and end-use characteristics. Additionally, production of the product may also be considered to evaluate the process performance criteria and verify if the desired product-process targets are matched. Otherwise, it is possible to return to the product design part and revise the product design problem or even generate new models until the desired formulation (product) is found.

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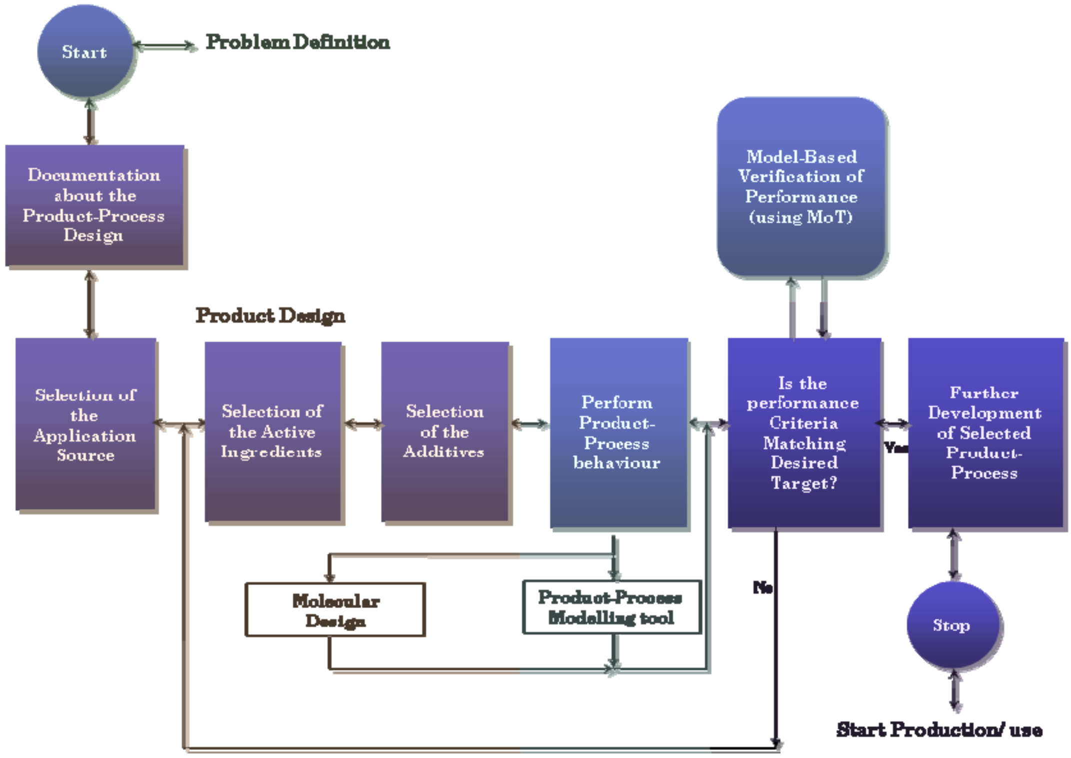

Figure 1. Work-Flow for systematic product-process design

The performance of the systematic model-based work-flow in product-process design will be illustrated through a case study embedding the modelling and design of the release of a formulated pesticide product. Product design, in this case, involves the design of microcapsules and its contents (the active compound, the membrane used for the microcapsule, solvents, additives, release medium, etc.). With respect to process design and modelling, the simulation of the release of the active ingredient through the microcapsule is performed, highlighting the use of multi-scale models. Through this case study, the advantages of the use of the model-based framework in formulated chemical product design will be illustrated.

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Combining predictions at multiple scales for process modelling

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Keywords: Multiscale modelling, Wavelets, Data-driven modelling, Time series

Introduction

Process modelling plays a central role in Chemical Engineering, as virtually any activity in this field requires a simplified description of reality, i.e., a model. Multiscale modelling, is a class of modelling approaches that has recently gained momentum with new and relevant applications emerging in engineering, where the phenomena to be simultaneously addressed appear spread through different scales of length and operating at different scales of time. In fact, this is not a new problem for chemical engineers. For instance, the dynamic analysis of fixed bed catalytic reactors does require the simultaneous modelling of phenomena going on in the catalytic particle and in the bulk phases of the reactor. However, such traditional analysis usually led, after some simplifications, to modelling at a single scale, with appropriate boundary conditions linking phenomena at different scales. Current applications are however more demanding in the way the vertical integration of all relevant scales for the problem at hand is accomplished. For instance, as we move down to the micro- and nano- scales, in order to analyze, design, control and monitor systems and operations at such fine scales, the whole hierarchy of phenomena occurring at the different, intermediate, scales of length and time must be necessarily addressed and properly combined. It is also becoming increasingly evident that some class of systems do exhibit very well defined scale dependent associations and dynamics, notably biologic complex systems (Henzler-Wildman et al., 2007).

Significant advances have been achieved in modelling such type of systems, using first principles based approaches (Charpentier,McKenna, 2004), as we can attest by looking to the number of publications appearing in the Chemical Engineering literature. However, another paradigm of multiscale modelling should also be developed, in order to properly address those situations where large amounts of data are available, generated from phenomena occurring at different scales, but where fundamental knowledge of the underlying mechanisms is incomplete or even inexistent. Such data-driven multiscale modelling of systems, is therefore an area demanding for new developments, to keep the pace of the challenges raised by modern science, namely by the large amounts of information currently being made available with the new measurement techniques, that now require proper processing and integration in order to generate useful information about the whole system.

In this communication, we present an approach for data-driven modelling, where predictions computed from models estimated at different scales are properly combined, after scale selection. Such an approach is focused on prediction ability and is implemented according to a well defined and coherent sequence of steps, which can be performed quite efficiently, leading to potential improvements in the time and efforts spent in complex system identification tasks.

Multiscale modelling approach

The multiscale data-driven modelling approach essentially consists on independently estimating models at different scales, using the wavelet transform coefficients of a receding horizon sliding window that always includes the last measured values, in order to make the resulting model adequate for on-line use. Then, following a well

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defined sequence of steps, the relevant scales to include in such a multiscale modelling are selected (those with predictive information power) and modelled with rather simple model structures. According to the approximate decorrelation ability of the wavelet transform, such set of parallel system identification tasks is rather efficient to carry out, requiring simple model structures for describing well the observed dynamic trends of the outputs.

Illustrative example

In order to have the required complete knowledge about the true values of the output variables, for assessing and comparing our methodology, the multiscale approach was applied to a simulated industrial non-isothermal CSTR operating under feedback control, where an irreversible and exothermic first order reaction (A→B) takes place (Luyben, 1990). There are 10 variables involved in this process, collected every 10 s, and the main purpose is to identify and estimate a proper model for predicting the composition of reactant A (CA) in the outlet stream, using all variables available (soft sensor for CA). The data set used for the estimation tasks is composed by 10000 observations (~28h of continuous operation), while a second group of data, also with 10000 observations, constitutes a test set, based on which the prediction performance relative to each model structure is computed. The results obtained (RMSEP, computed using the real and the observed values of CA), show the superiority of the proposed approach regarding the single-scale counterparts.

Table 1. Prediction results obtained (RMSEP).

RMSEP	**MS-ARX(1,1)**	**ARX(1,1)**	**ARX(2,2)**
Observed	0.0820	0.1065	0.0900
Real	0.0422	0.0806	0.0570

Conclusions

We propose a model structure consisting of assemblies of black-box models describing system evolution at different scales of time (or frequency bands), the result of which, once combined by the inverse wavelet transform, give rise to the predicted response at the finest scale. In the illustrative example presented, better predictive results were obtained when comparing to the classical single-scale approaches. The proposed systematic methodology also allows for an effective reduction in the analysis time of complex data sets, as well as brings important concepts to the core of the analysis, such as those leading to the selection of scales with predictive power, something that is absent from other current approaches.

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Integration of traditional and supercritical fluid techniques to design drug delivery systems

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Keywords: controlled release formulations, supercritical techniques, drug delivery

The use of biocompatible and biodegradable polymers as drug-carrier systems is increasingly more popular in pharmaceutical applications. Although several controlled drug delivery systems are available, the use of micro/ nanoparticles appears to be the most interesting way, as these systems facilitate the diffusion through biological barriers.

Several processes are commonly used for fabrication of polymer micro and nanospheres. The emulsion-solvent extraction/ evaporation technique is one of the most common traditional methods employed in the production of particles from preformed polymers due to its simplicity and compatibility with various polymers. To overcome some limitations of the conventional methods other technology has been investigated to produce particles for drug delivery applications. Production of micro or nanoparticles using supercritical fluid technology is very attractive since it provides an alternative solution to the various problems encountered in traditional techniques. The possibility of producing very small particles with a narrow size distribution using mild and inert conditions represents a major improvement over the conventional processes. However, for some specific drug delivery systems none of these particle production techniques - emulsification solvent evaporation in the traditional methods and RESS, SAS or PGSS in the supercritical fluid technology - seem to be in fact effective in terms of encapsulation efficiency. The major problems in these systems are due to the drug and polymer solubility and swellability.

As an alternative, the combination of traditional methods to produce empty microspheres and supercritical fluid impregnation for drug loading in these particles can promote an improvement for some drug delivery systems. Supercritical fluid impregnation can be a useful method for the preparation of pharmaceutical forms when the active compound is soluble in carbon dioxide and the polymer can be swollen by the supercritical fluid.

The aim of this research is to compare microspheres produced by traditional methods, by supercritical fluid techniques and by integrating supercritical fluid impregnation with traditional methods. Biocompatible polymers (as drug carries) and anti-cancer and anti-inflammatory compounds (as model drugs) were used. Fundamentals studies are required for the design of adequate process, hence, the solubility of the drug in carbon dioxide as well as the sorption degree of polymeric matrix in the presence of carbon dioxide were studied and results are presented and discussed.

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Thermoresponsive hydrogels for drug delivery systems

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Keywords: Dextran, Chitosan, N-Isopropylacrylamide, Drug Delivery Systems

Introduction

Controlled release from biocompatible materials has received recently much attention for its biomedical applications. Due to their biocompatibility and biodegradability, gluocopyranosides such as chitosan and dextran appears as promising polymer materials if one is able to regulate their rheological properties and the encapsulation/release efficiency (Ferreira et al, 2002). N-Isopropylacrylamide has also attracted many attentions due to its thermalsensitive behaviour (Mathews et al, 2006). In this work we prepared graft polymer hydrogels from chitosan and dextran.

Materials and Methods

The starting polymers were dissolved in the appropriate aqueous media (simple water for dextran, and 5% acetic acid for chitosan) in the suitable semi-dilute concentration range (1-4% w/v) and as graft polymer we used N-isopropylacrylamide (NIPAAm). There were used different molecular weights for dextran, 144000 and 5000000. The reaction was initiated either using ammoniumcerium (IV)–nitrate radical initiator, an isocyanate with one double bond and a biocompatible photoinitiator, Irgacure 2959 for the photopolymerization or γ radiation. Swelling capacity and cycles of incorporation/release of the drug were evaluated with essays where PBS solution's pH and temperature were varied in the physiological range of interest (withdrawing of aliquots and UV-spectrophotometer analysis). The swelling capacity was also evaluated using PBS solutions with different ionic forces. The transition temperature of each solution was analysed by visible spectroscopy. The structure of each gel was evaluated by using different techniques, such as FTIR and ^{1}H-NMR. As model drug we used a potent antiemetic substance used in the prevention of chemotherapy-induced nausea and vomiting, Ondansetron®, which was incorporated in the gel both before and after the co-polymerization.

Results

FT-IR data for the grafted Dex-NIPAAm systems revealed the presence of two peaks around ~1650 and ~1550 cm^{-1} which can be ascertained with the typical amide I and II bands of NIPAAm, revealing the success of the grafting reaction; this was also confirmed by ^{1}H-NMR. Elementary analysis also showed an increase in the Nitrogen proportion in all systems with the increase of the amount of co-polymer used or the reaction time. A grafting yield was determined for all systems, with results being higher for dextran systems. The transition temperature was determined for all gels, with results varying from 33 to 35ºC. The higher temperatures were determined for gels with the highest concentration in to co-polymer. Swelling results showed that, irrespective of

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the polymers, there is a positive correlation between co-polymer concentration and swelling, as well as with drug incorporation. Release essays show an initial burst followed by slow release but no significant pH dependence (in the range of interest) and a moderate dependence with temperature. The systems with less concentration in co-polymer were the ones showing the fastest release pattern. As for the dependence in temperature we verified that all systems at higher temperature, 40ºC, were the ones showing the fastest release times when compared with the others at room temperature, 25ºC. The next figure shows the release pattern for dextran systems with chemical initiator at different temperatures.

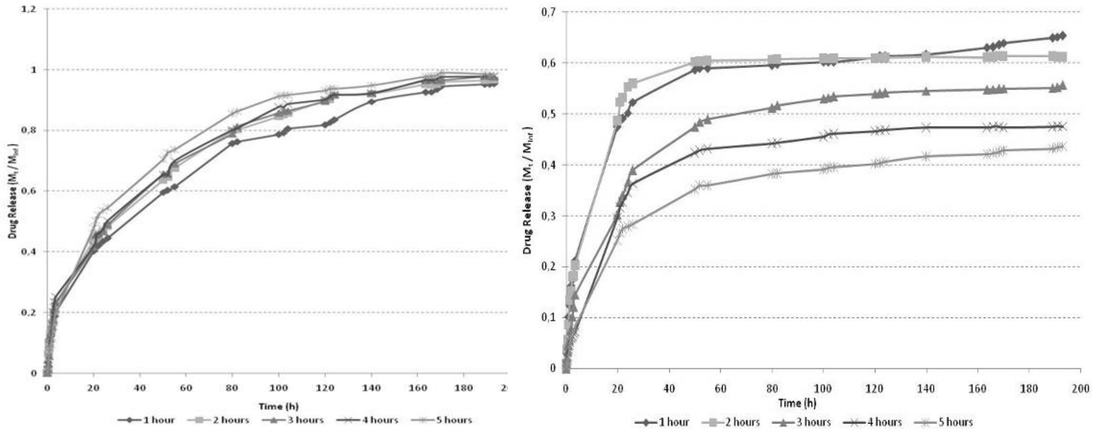

Figure 1 – Release patter of dextran based systems at 25ºC and 37ºC.

Conclusions

As conclusion we can say that there is a positive correlation between the concentration of the co-polymer and some properties studied in this work. All the different methods used in this work were successful with results showing the introduction of the co-polymer in the final systems. All gels presented thermal behaviour, with all the properties studied showing significant differences at 25ºC and 37ºC. Globally we can say that the most promising gels studied were the gels made using photopolymerization.

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CFD simulation of an oxidation ditch

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Keywords: Activated Sludge, CFD, Oxidation Ditch

Abstract

The oxidation ditch technology was developed in Netherlands by A. Pasveer in 1950s (Pasveer 1962) and the first full scale plant was set working in 1954 (EPA 2000). Nowadays, oxidation ditches found application in USA in over 9200 wastewater treatment plants (EPA 2000). The process is also commonly used in Portugal (Vila Nova de Gaia - Febros, Viana do Castelo – Areosa, Golegã ...). A survey of 14 activated sludge (AS) processes in USA showed that oxidation ditches have more robust/reliable operation than other AS processes (Benefield and Randall 1980), particularly in enduring unfavorable temperature conditions and with regard to nutrient removal.

An oxidation ditch is a modified activated sludge biological treatment processes used in Waste Water Treatment Pants (WWTP) having a ditch system with a single or multi - channel configuration, within a ring-, oval- or horseshoe-shaped basin, equipped with aeration and mixing devices, generally surface brush aerators. The process runs with long solid retention times (SRTs) resulting in high BOD removal efficiency, average 75 – 95 % up to 99% (Benefield and Randall 1980, EPA 2000). The oxidation ditches, besides BOD removal, can also perform denitrification, due to presence of zones with high and low dissolved oxygen (DO) concentrations.

The oxidation ditches are usually regarded as complete mix systems (EPA 2000), although this process is still scarcely studied in what regards to the flow regime. Simulation for AS has been mostly based on ideal reactor models: Continuous Stirred Tank Reactor (CSTR) or in a cascade of CSTRs.

The use of Computational Fluid Dynamics (CFD) techniques allows detailed analysis of the overall transport mechanisms in the oxidation ditch: macromixing. The flow inside an oxidation ditch is here simulated using Reynolds Averaged Navier-Stokes equation (RANS) and Large Eddy Simulation (LES) with the CFD commercial code Fluent®. From the simulations of the oxidation ditches hydrodynamics the Residence Time Distributions (RTD), i.e. macromixing, are computed and compared with some of the ideal models use in design approaches to this process: Plug Flow Reactor with Recirculation (PFRR), CSTR and CSTRs cascade. The RTD is obtained from a Lagrangian approach, i.e. particles are introduced to the oxidation ditch from the inlet with a Dirac pulse and then the outlet particle flux is recorded.

Here the CFD simulation considers the system consists of oval tank and a jet aeration device that simultaneously forces the fluid circulation throughout the ditch and injects a stream enriched with oxygen. The average residence time of fluid in the ditch is 4h. A momentum source was considered imposing a flow velocity with value of 0.3 m/s (typical for oxidation ditch aerated by conventional devices, e.g. commonly used surface brush-type aerators). In Figure 1 are shown the pathlines from the RANS of the oxidation ditch.

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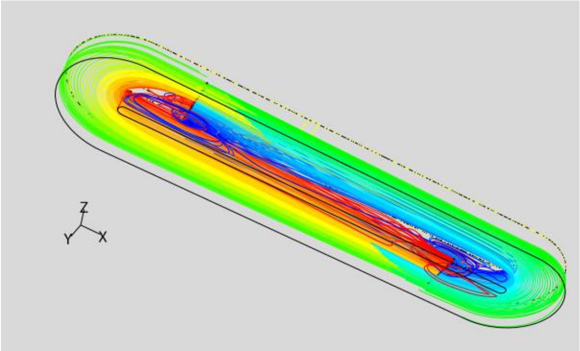

Figure1. Pathlines inside the oxidation ditch

The AS simulation is made using the RTD from the CFD simulation and the ideal models, using Zwitering maximum segregation model (Zwietering 1959) and a simplified kinetic model for the AS (Substrate + Microorganisms + Dissolved Oxygen). Although the typical kinetics in the AS process are slow, and the concentration profiles of substrate and microorganisms do not vary significantly throughout the oxidation ditch, the process shows strong dependence on the macromixing, as shown in Figure 2 plot. The plot of Figure 2 shows the substrate concentration values at the oxidation ditch outlet for a range of:

- cells residence time, 5d $\leq \tau_c \leq$ 15d;
- and a macromixing parameter that represents the average number of turns a fluid element travels around the oxidation ditch before it exits, 0.1 $\leq \beta \leq$ 100.

When β tends to zero the oxidation ditch approaches a plug flow reactor (PFR) and when it tends to infinite it tends to a CSTR. Further work regarding the macromixing effect on the oxidation ditch AS process will be shown in this communication. From this work some procedures will be pointed to base the AS processes design on CFD simulations, namely on the study of the design effects on macromixing.

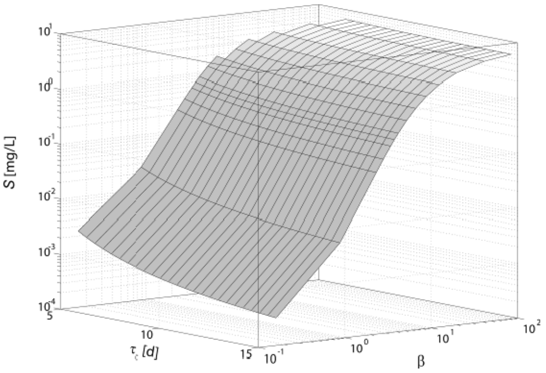

Figure2. Substrate concentration in an oxidation ditch outlet for several circulation values (β) and cells residence time (τ_c).

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CFD modelling of trickle-bed reactor in the catalytic wet air oxidation of syringic acid

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Keywords: Computational Fluid Dynamics, Multiphase flow, Trickle-bed reactor.

Multiphase flows are often encountered in environmental reaction engineering and the knowledge of computational transport phenomena models for the practical analysis of such complex chemical processes is far from being complete. In fact, three-phase systems related with catalytic wet air oxidation (CWAO) in the treatment of effluents containing a high content of organic matter are emerging as a new application of trickle-bed reactors (TBR). TBR models reported in the literature considered isothermal operation and used either a pseudo-homogeneous approach or a heterogeneous model with plug-flow for gas and liquid phase whereas others accounted for liquid flow non-uniformity and maldistribution by using an axial dispersion model (Al-Dahhan *et al.*, 1997). To improve the capability of multiphase reactor models, one has to solve the complete multidimensional flow equations coupled with chemical species transport, reaction kinetics, and kinetics of phase change. Therefore, multicomponent reacting flows in which kinetics models are experimentally determined have to be modeled with additional phase interaction terms. This study aims to assess an Eulerian multiphase CFD model in order to investigate the phases interaction coupled with turbulence models to make use of large-scale CFD computations that can be helpful in pointing out the cause of incomplete catalyst utilization in particle-scale level.

Taking into account the numerical power available nowadays to address complex chemical process operational and design issues, our case study outlines an alternative CFD modeling method to investigate the oxidation behaviour of a TBR in terms of total organic carbon profiles taking into account the kinetic expressions obtained elsewhere (Lopes *et al.*, 2007). Transport phenomena such as mass and heat transfer are integrated in an Eulerian *k*-fluid model, resulting from the volume averaging of the continuity and momentum equations and solved for a 3D representation of the bed at unsteady state and the catalytic performance is investigated in terms of TOC (total organic carbon) depletion of a model phenolic pollutant encountered in olive mill wastewater processing – syringic acid.

The representative computational grid for the experimental system investigated in this work is shown in Fig. 1. It consists of a cylindrical vessel with 5 cm internal diameter and 1 m bed height packed with catalyst spherical particles 2 mm diameter. The catalytic bed is meshed in tetrahedral cells by means of the FLUENT preprocessor GAMBIT 2. The mesh quality was analyzed using the skewness criteria and the results were made to be grid independent. Numerical simulations were compared previously to experimental data in order to validate the predicted hydrodynamic parameters pressure drop and liquid holdup. The simulated operating conditions were 10-30 bar pressure and temperatures from 290 to 500 K. Gas and liquid mass flow rates were in the range 0.10 – 0.70 and 0.05 – 15 kg/m^{2}s, respectively.

The system domain is discretized by an unstructured finite volume method using the CFD solver FLUENT 6.1. The gas flow rate at the distributor is defined via inlet-velocity-type boundary condition with gas volume fraction charging according to the

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specifications made in the simulations. The flow model is based on solving Navier-Stokes equations for the Eulerian-Eulerian multiphase model along with multiphase *k-ε* turbulence model. The governing differential equations are solved using iterative solution to the discrete form of the mathematical model using a PISO algorithm for pressure-velocity coupling with first order implicit formulation for unsteady integration and first order upwind scheme discretization for spatial derivatives. The gas and liquid are described as interpenetrating continua and equations for conservation of mass and momentum are solved for each phase. The transient calculations were made for two-phase flow starting with different time steps and the converged solution is assumed when the scaled residuals of all variables were smaller than 10^{-4}.

In Fig. 2 it is shown the TOC degradation in steady-state for an isometric view of twenty-five catalytic layers, radially. According to Fig. 2, it is possible to detect different TOC concentration profiles in the catalyst surface corresponding to different oxidation rates. This fact is related to the liquid holdup distribution achieved in the trickle-flow regime so that different wetting levels can be detected, which can cause insufficient reactant or pollutant feeding to the catalyst surface. This fact is evaluated in terms of liquid and gas distribution with interface tracking methods. In a broad range of gas and liquid flows studied at different operation conditions, CFD demonstrated the considerable effect of temperature, pressure, gas-liquid flows in the catalytic wet air oxidation of syring acid solution.

After our hydrodynamic studies, one can conclude that the Eulerian k-fluid model is a rational choice for flow simulation in packed beds, if good closures for fluid/fluid and fluid/particle interactions is incorporated in the CFD model used. The CFD approach can state that external catalyst wetting can be investigated in downflow flow mode performing a benchmark of hydrodynamic parameters encountered in the commercial-scale TBR deployment for CWAO technology.

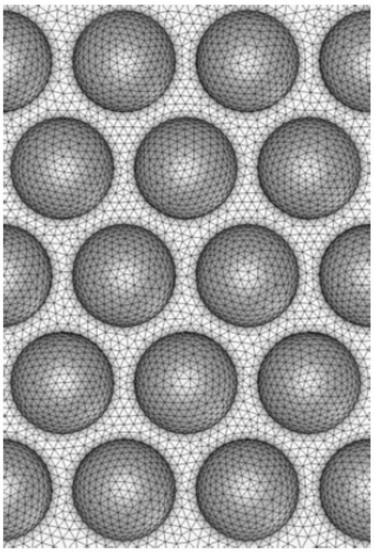

Fig.1 – Tetrahedral mesh used in TBR simulations

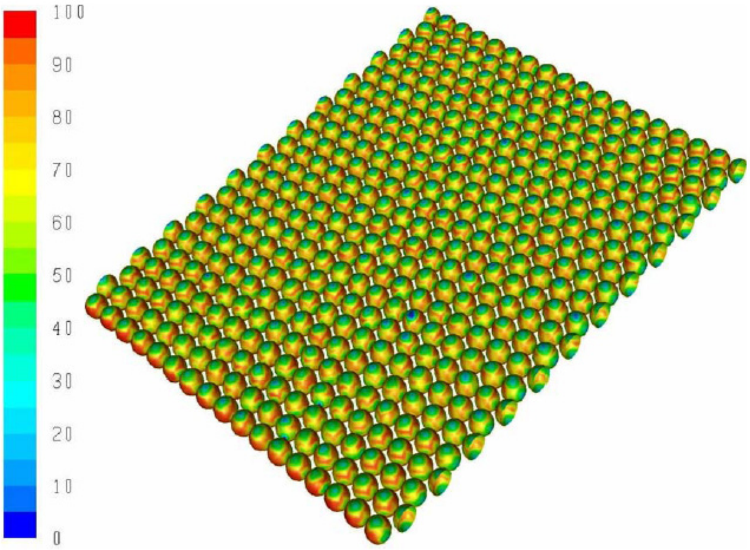

Fig. 2 – Total Organic Carbon degradation map for 25 catalytic layers in steady-state

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Characterization of water species revealed in the drying operation of squid by using water proton NMR analysis

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Keywords: water species, reproducibility of the water species, peak temperature of TPD, correlation time, moisture diffusivity

For the discrimination of water molecules during the drying process of a squid, multifunctional water molecules were separately characterized by water proton-NMR, temperature-programed desorption (TPD) of moisture, and moisture diffusivity (*De*) analysis methods as a function of the moisture content (W_0). The proton-NMR spectrum showed the appearance of three peaks at the specified chemical shifts with different ppm's, indicating three different species (species-A, -B, and -C) distributed in the squid muscle, each of which had a characteristic behavior of the correlation time (τ_C) as a function of the W_0. The modified moisture content linearly increased in the whole range of W_0 for species-A suggesting that it can be attributed to water species. Species-C showed no change without depending on W_0, indicating a characteristic species different from the water species. Species-B was difficult to be assigned to any species without a presumption as some hydrogen species binding with protein molecules.

The τ_C of species-A was drastically varied at W_0 = 120%-d.b., indicating two further categories, i.e., species-A_1 and -A_2, depending on the dehydration degree. For species-A_1 at W_0 > 120%-d.b. (region I), the τ_C was $4.8\times10_{-9}$s independently of the W_0, whereas, for species-A_2 at W_0 < 120%-d.b. (region II), the τ_C decreased from 1.2×10^{-7} to 1.0×10^{-8} s with increasing the W_0. Species-A_1 and -A_2 were further examined by the TPD method and *De* analysis. For species-A_1, *De*, the activation energy of moisture diffusivity (E_D), and the peak temperature of the TPD profiles (T_P), respectively, indicated identical values of $1.8\times10_{-6}$ m_2/h, 17 kJ/mol, and 110ºC without depending on the W_0 in the range of W_0 =120 ~ 360%-d.b. (region I). For species-A_2, the three parameters clearly varied as $De = 1.7\times10_{-6} \sim 0.6\times10_{-6}$ m^2/h, E_D = 25 ~ 35 kJ/mol, and T_P = 117 ~ 150ºC, with decreasing the W_0 from 120 to 30%-d.b. (region II). Species-A_1 and -A_2 were assigned as free water and slightly restricted water, respectively. Species-C was accurately assigned as a lipid by relating to the spectra of proton NMR spectroscopy of squid-liver lipid.

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Mixed convective heat transfer augmentation in a backward-facing step utilizing nanofluids

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Keywords: Heat transfer Enhancement, Cu-Water nanofluid, Backward-facing Step, Mixed convection

Two-dimensional mixed convection in a backward-facing step utilizing nanofluids is investigated numerically. The top wall (cold) is is maintained at a constant temperature T_C while the bottom wall & step (hot) are kept at constant temperaure T_H. The mixed-convection heat transfer in a backward-facing step has been examined numerically using suspensions of copper nanoparticles in water. Richardson number, $Ri = Gr / Re^2$ emerges as a measure of relative importance of natural and forced convection modes on the heat transfer. The governing parameters were $0.01 \leq Ri \geq 100$, Prandtl number, Grashoff number, $Gr = 104$, $Pr = 6.2$, for nanoparticle volume fractions, χ = 5%, 10%, 15% and 20% of base fluid. A finite volume method SIMPLE has been used to solve the governing equations of nanofluid flow and heat transfer numerically on a staggered grid system using a higher order upwind scheme (QUICK).

The flow pattern doesnot vary significantly when $Ri << 1$ while it differs significantly when $Ri >> 1$ with the use of nanofluids compared to conventioned fluids. These are shown by contours of streamlines velocity vectors and isotherms for different Ri for different values of χ. The plots of total Nusselt number ($\overline{Nu}$) shows that the heat trasfer increases with increase in particle volume fraction χ. A drastic increase in heat transfer occurs while utilizing nanofluids compared to conventional fluids.

In recent years, there has been an extensive research on the heat transfer in nanofluids. The forced convective flow and heat transfer in a backward-facing step utilizing different nanofluids has been studied by (?). The problem of 'mixed convection heat transfer enhancement utilizing nanofluids in a backward-facing step' has not yet been investigated to the best knowledge of the author. Separated flow as encountered in a backward-facing step occurs in various engineering applications such as combustors, heat exchangers, gas turbine blade and micro-electronic circuit boards.

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Electric swing adsorption process for gas purification

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Keywords: Adsorption, carbon dioxide, VOCs, modeling, process design

The Electric Swing Adsorption (ESA) process for cleaning gas streams is revised. In particular, we show results indicating its potential for application in the removal of VOCs (Volatile Organic Compounds) from pharmaceutical industry and also CO_2 from flue gases.

Electric Swing Adsorption (ESA) is the term given to the processes where the Joule effect generated by passing electricity through a conductor is employed to heat a column filled with adsorbent. This heat increases the temperature of the adsorbent releasing previously adsorbed gas and acting as the regeneration step in a cyclic scheme (Ettlili et al., 2006). To start a new cycle, the temperature of the adsorbent should be reduced using part of the purified gas (purge). To maximize the performance of ESA, the difference in capacity between adsorption and regeneration temperatures should be high. This is contrary to the desired properties of the materials to be employed in Pressure Swing Adsorption technology. In Figure 1 two adsorption equilibrium isotherms of a generic adsorbent at two different temperatures (Tf feed temperature, where adsorption takes place and Td desorption temperature where regeneration proceeds). If this adsorbent is employed in Pressure Swing Adsorption (PSA) processes, the amount desorbed is (qf – qb) where qb is the amount of gas adsorbed at the blowdown pressure. If the same material is employed in ESA process, the effective capacity is (qf – qd), which is, in this case, much higher.

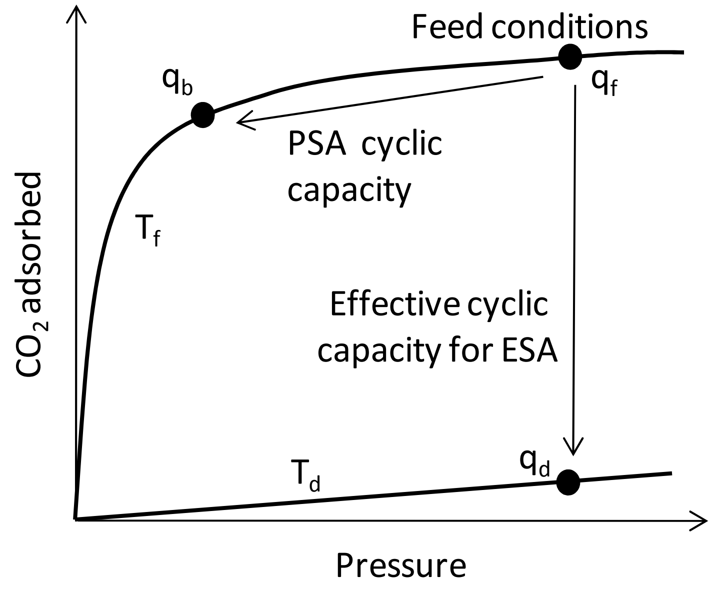

Figure 1. Comparison of effective capacity achieved in ESA and PSA ($T_f < T_d$) on a generic adsorbent.

The innovation of ESA process is that it employs electricity to regenerate the adsorbent. In terms of adsorption phenomena, the ESA process operates like a Temperature Swing Adsorption (TSA) process but much higher unit productivities (less amount of adsorbent to purify a desired stream per unit time) can be achieved. The ESA process is better suited for removal of small amounts of contaminants (<10%) or specially for polishing. In this context we evaluate the use of ESA for two different purposes: carbon dioxide removal from natural gas fueled power stations and VOCs from pharmaceutical industries.

The removal of CO_2 from power stations is required to reduce risks of undesired irreversible climate change. The removed CO_2 should be concentrated, compressed and stored in safe places (saline aquifers or unmineable coal seams). Natural gas fueled stations have a CO_2 content close to 4% and amine scrubbing is not suitable (amine degradation with high oxygen contents). ESA process can be an interesting process to remove CO_2 operating at high productivities and thus reducing the size of the capture plant. Our initial results of CO_2 capture employed a four step cycle and resulted in an enrichment of 4 times (Grande and Rodrigues, 2008). In this work we show how process modeling and also more selective materials can improve both performance and power consumption of the process.

Another interesting application of ESA process is in the removal of small concentrations of volatile organic compounds, VOCs. In this work we will show how ESA technology can be employed in the removal of ethanol from air streams. In this work we will provide an ESA cycle to remove ethanol using an activated carbon honeycomb monolith resulting in a process with reduced pressure drop and operating at high productivities.

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Cholesterol removal by nanofiltration in organic solution

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Keywords: Cholesterol; Triacylglycerides; Phospholipids; Nanofiltration

The lipids contained in egg yolk are principally triacylglycerides (65%, w/w), glycerophospholipids (28%, w/w) and cholesterol (5%, w/w). A more or less selective extraction of these lipids can be performed using various organic solvents such as diethyl ether, methyl chloride, ethyl acetate, propane/butane, hexane, benzene, ethanol (Tokarska, and Clandinin, 1985) or supercritical CO_2 in the presence or the absence of ethanol (Rossi et al., 1990). In our case, after extraction, these products were recovered in 95% ethanol. The lipid concentration in ethanol ranged from 3 to 15%, w/w. The lipids present in the ethanol were glycerophospholipids (760 $g.mol^{-1}$), 80%, and a mixture of cholesterol (386.7 $g.mol^{-1}$) and triacylglycerides (860 $g.mol^{-1}$), 20%.

Glycerophospholipids contain fatty acids that can be highly polyunsaturated. They can thus be considered as a source of essential fatty acids in C18 or conditionally essential fatty acids in C20 or C22 for the families of ω6 (molecules composed of a polyunsaturated carbon chain with two or more double bonds, the first double bond being located on the 6th carbon atom from the terminal methyl) and ω3 (the first double bond is located on the 3rd carbon atom).

Thanks to their surface-active properties, glycerophospholipids, once completely purified, can be used in the preparation of emulsions of lipids in water used in the fabrication of food additives or even pharmaceuticals. For these applications, the amount of cholesterol in egg yolk is a problem. Cholesterol ingestion can increase cholesterol concentration in the blood, which is a marker linked to the risk of arteriosclerosis and coronary diseases. It is therefore of interest to eliminate the cholesterol from the lipids extracted from egg yolk. No information was found in the literature that would help estimate the performances of filtration or even of membrane processes for the separation of this type of mixture. In view of the size of the compounds, we decided to use a process of nanofiltration. However, the initial mixture was complex and the treatment was made difficult by the following: (i) The molecular weights of the compounds were quite similar: 386.7 $g.mol_{-1}$ for cholesterol, between 850 and 1020 $g.mol_{-1}$ for triacylglycerides and between 760 and 878 $g.mol_{-1}$ for phospholipids, (ii) The solution was ethanolic. The stability of a membrane in an organic solvent such as ethanol depends on the physicochemical characteristics of the solvent and of the membrane. The membrane-solvent interaction may result in swelling, plasticization or dissolution of the membrane material and subsequent loosening of the membrane structure, leading to modified separation properties and/or loss of mechanical strength under pressure. Besides, the cut-off of a membrane determined in aqueous solutions (e.g., as provided by the manufacturer) is insufficient to characterize its MWCO in organic solutions. The MWCO of a membrane varies depending on the solute and the solvent. Tsui et al. (2004) reported that low lipid rejection could be due to lipid-membrane interactions that cause plasticization of the polymer, leading to membrane swelling and reduced lipid rejection.

Our objective was the fractionation of a complex ethanolic solution. The membrane cutoff could not be taken as the main criterion for the selection of the best membrane, and this for several reasons: the molecular weights of the different compounds were similar and the membrane cut-offs were defined by each manufacturer according to a specific protocol. Moreover even if these cut-offs had been more precisely defined, extrapolating to organic solutions would have been hazardous. It is very difficult to take into account the effects of the electric charges in an ethanolic medium, and no information is available concerning the membrane behaviour with regard to an alcoholic medium. However, we clearly showed that fractionation was possible with a cellulose acetate membrane. We studied the influence of some operating parameters on the permeate flux and defined the optimum conditions of transmembrane pressure and flow rate. First, we were able to remove the cholesterol (75%) from the ternary mixture. With only a longer diafiltration step, we limited the losses in phospholipids (5%) but the removal of cholesterol was smaller (61%) and slightly lower than the objective of 80% of cholesterol removed. This discrepancy is explained by the difficulty to optimise a process for which no reliable concentration determination can be performed on line. However, in view of the results obtained, increasing the diafiltration time will enable us to reach our objective. The membrane was totally regenerated after a wash with ethanol. The variation of the retention coefficient as a function of the concentration leads us to consider that the upstream solution has a kind of “organisation” which constitutes an important resistance to mass transfer. The cholesterol being the problem, the possibility to separate this compound by an appropriate membrane is an important breakthrough in the domain of nutraceutics and nutritional supplements.

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Beer aroma recovery by pervaporation

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Keywords: Beer, Aroma Compounds, Pervaporation, Response Surface Methods

Increasingly used treatment processes of drinks, such as pasteurization or beverages dealcoholization, submit the beverages to conditions that cause the loss of important aroma components. The aroma recovery from beverages is becoming then a critical food processing operation (Catarino et al., 2007). A possible strategy for recovering the loss of aroma profile considers the extraction of aromas before the beverage being submitted to the processing operation (Mendes et al., 2007). Presently there are available suitable processes for aroma extraction, being membrane-based processes like pervaporation the most promising. Pervaporation is a highly selective separation process that can be carried out at low temperatures, which is advantageous for removing thermosensitive compounds (Karlsson and Trägårdh, 1997).

In this work the extraction of a beer aroma profile by pervaporation is studied. A design of experiments (JMP5.1, SAS), using a response surface methodology (RSM), was applied in order to obtain the hyper-surface of the process response to the main operation conditions. The operating conditions selected were the feed temperature, feed velocity and permeate pressure, while the process responses were the permeate flux, the selectivity towards selected aroma compounds and the relationship between the concentration of the aromas in the permeate. The most important beer aroma compounds are high alcohols and esters. The chosen selected aroma compounds for quality control were ethyl acetate and isoamyl acetate (esters) and propanol, isobutanol and amyl alcohols (high alcohols). The equilibrium between high alcohols and esters should be similar to the original beer for obtaining a balanced aroma profile.

Pervaporation experiments were carried out using a laboratory unit as well an industrial one (Mendes et al., 2007). Second order polynomial fitting models were obtained describing the effect of all selected independent variables on the process responses (includes cross-terms). A good agreement between experimental and predicted values was obtained.

Figures 1 and 2 show the surface response plots of the permeate flux and of the ratio between high alcohols and esters concentrations as a function of the independent variables. One can observe that the permeate flux is positively affected by the temperature of the membrane module, as expected, with a small influence of the feed velocity. On the other hand, the smaller the permeate pressure, the higher the flux (Fig. 1). The effect of operation conditions on the aromas selectivity's assumes different trends according to the compounds nature. As a result, the ratio between high alcohols and esters concentrations increases with temperature, decreases with feed velocity and decreases with permeate pressure (Fig. 2).

The developed models were then used in order to solve the trade-off between all the responses and to optimize the process. Additional runs were performed in order to confirm the optimal conditions within the errors range predicted by the RSM models.

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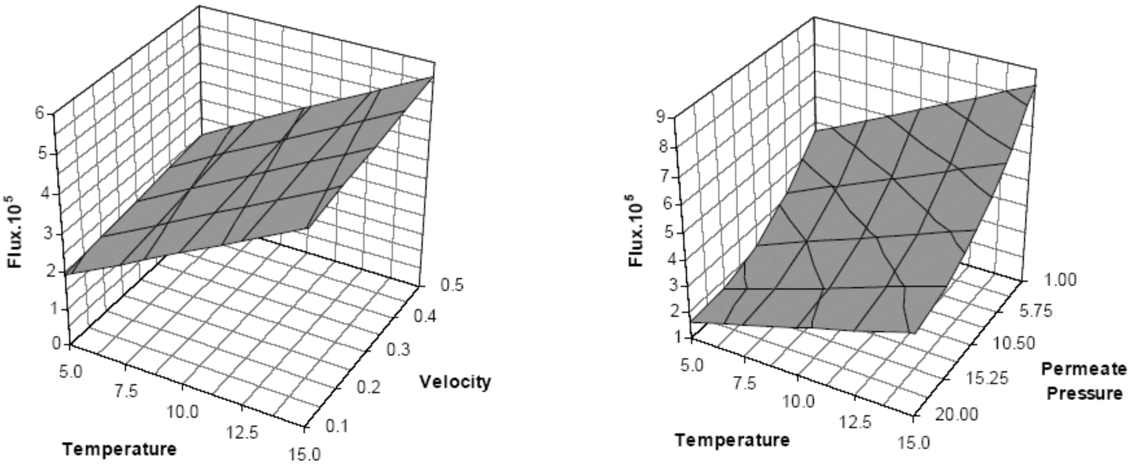

Figure 1 – Effect of the temperature (ºC), feed velocity (m.s^{-1}) and permeate pressure (mbara) on the permeate flux (kg.m^{-2}.s^{-1}).

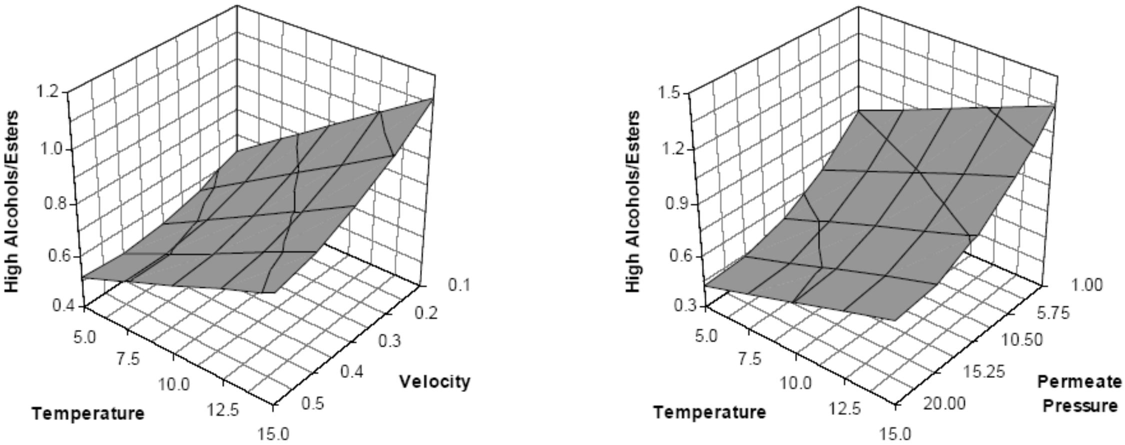

Figure 2 – Effect of the temperature (ºC), feed velocity (m.s^{-1}) and permeate pressure (mbara) on the high alcohols/esters ratio.

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Determination of the wall shear stress by numerical simulation: membrane process applications

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Keywords: Computational fluids dynamics, wall shear stress, channel, geometry, ceramic membrane, fouling

Membrane processes have been intensely developing during the last decades, and mainly in dairy industries. Thus, microfiltration is more and more used for milk sterilizing and casein separation. Ultrafiltration is currently the most used process for applications as protein separations or milk composition standardization.

Considering the feed effluent complexity, concentration polarization (CP) phenomenon and fouling are accentuated limitations for the development of membrane dairy filtration processes. The accumulation of materials near the membrane, with the deposition of colloids and suspended solids triggers the reduction of its performances. Fouling strongly depends on the wall shear stress that occurs from radial velocity gradient during the filtration step. So, better knowing the wall shear stress is fundamental to reduce the fouling phenomena. Enhancing turbulence to the membrane surface: Dean vortices (Moulin et al., 1999), dynamic filtration (Koutsou et al., 2004) and unsteady flows (Krstic et al., 2002) enables indeed to increase the mass transfer coefficient and sweep the deposition at the surface (Noriatsu et al., 2001).

In our work we decided to study the repartition of wall shear stress on cylindrical, square, triangular and hybrid channels by numerical simulation. We aim at establishing predictions for different commercial ceramic membrane geometries and confirm those numerical results with experiments. On the basis of these results, we determine the channel geometry that reduces uttermost the fouling.

For this aim, numerical modelling tool appears as the most appropriate (Ghidossi et al. 2006). We used a commercial Computational Fluid Dynamics package FLUENT. Numerical simulations were made for high Reynolds numbers in complex geometries. A large range of case was studied in this paper for a more accuracy prediction of the wall shear stress. The problem was studied in 3D. The grid was refined near the wall with the intention of resolving the laminar sub-layer. We used RNG k-ε model for it appears in literature as preferentially used in turbulent flow. We consider the wall as impermeable. This assumption could be validated: (i) in ceramic membrane, the permeate flow is often lower than 2% of the circulating flow; (ii) it was shown that the wall shear stress can be calculated in permeable tube with the value obtained in no permeable tube and the value of permeate flow. No mass transfer model was used. This simplified approach was: "the higher the shear stress, the higher the permeate flux is whatever the effluent". The advantage of this approach is the global view of the system, disadvantages are: predicting permeate flux is impossible and only comparisons are acceptable. An experimental campaign of milk ultrafiltration validated this approach.

As expected, the cylindrical geometry develops constant wall shear stresses on the entire perimeter. For other geometries, the results prove that this distribution has a

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maximum located in the middle of the side and a null value at the angle of the square for any rate of flow. The geometry of the channel has an influence on the ratio (τ_{local}/τ_{max}) and on the dead zones created but there is no influence of the velocity.

The highest wall shear stress values are obtained for lowest channel sections and for the cylindrical geometry. The triangular geometry has a light advantage for the largest sections but it shows an important heterogeneity on the wall shear stress distribution. The angles are subjected to a rapid fouling, which affects the process performances during the filtration step and in the regeneration step.

Experiments were realised to study the effect of wall shear stress on membrane performances. To better apprehend the effect of the reversible and irreversible fouling as a function of the membrane geometry, filtration of milk was realised. Commercial ceramic membranes with 7 or 19 cylindrical channels and 27 triangular channels are used and a 52 square channels membrane and a very last and innovative geometry, named Evolution are compared. The experimental results fit well our modelling predictions.

The Evolution membrane was designed according to the whole CFD results consistent with experimental results. It has a hybrid form between triangular and cylindrical shape and aims at optimizing membrane processes applied to milk filtration.

A good agreement is obtained between the experimental and numerical results and the performances of the commercial membranes are studied and compared.

Our experimental studies clearly show that the wall shear stress influences the reversible resistance whatever the transmembrane pressure or the channels geometry. Its increase reduces the CP phenomenon. For all geometries, irreversible fouling also decreases with the increase of the wall shear stress: the assumption was validated. However, the evolution is less important than for reversible resistances. Indeed, irreversible fouling develops in the form of deposit of cake of filtration but also in the form of absorption in the porous matrix, not directly influenced by the wall shear stress. For chosen channel geometry, the results show that the higher the section size, the lower the shear stress is and the higher the fouling is. For a fixed channel section, a weak difference for the geometries was noticed considering the evolution of the reversible resistances. The slight difference is explained by an influence of the angles that lower the shear stress intensity. The results enable to optimize those processes, the membranes geometry and their implantation in the industrial module.

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Active mixing strategies for confined impinging jets

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Keywords: Mixing, CFD, Reaction Injection Moulding, Confined Impinging Jets

Abstract

Confined impinging jets (CIJ), where two opposed jet streams impinge each other in a confined mixing chamber have a wide range of applications, mainly for the production of polyurethanes in Reaction Injection Molding, RIM, and in precipitators. The flow regime inside the chamber can be *oscillatory* or *self sustainable chaotic*. In *self sustainable chaotic* flow regimes, the jet streams engulf each other in evolving vortices throughout the mixing chamber. In *oscillatory* flow regimes both streams flow segregatedly throughout the mixing chamber. In self sustainable chaotic flow regimes the vortices formation is associated with strong jets oscillations (Teixeira *et al.*, 2005) that present frequencies around typical values, while the oscillatory flow regimes present low amplitude oscillations with very well defined frequency. Erkoç *et al.*(2007) reported the usage of dynamic measurements of static differential pressure signal between the two impinging jets for the determination of oscillations typical frequencies at both flow regimes.

The flow field structures occurring at self sustainable chaotic flow regimes are the main mixing mechanisms in the RIM process and other CIJ mixers, as shown in Santos *et al*. (2005). The efficiency of mixing in such kind of processes is critical for the quality of the product as shown from the polymerization works of Kolodziej *et al*. (1982 and 1986). When fast reactions are present and short mixing times are required it is of outmost importance to operate the CIJ mixers at self sustainable chaotic flow regimes. In case of RIM the process wide spreading has been seriously compromised due to failure of operation at chaotic flow regimes, either self-sustained or imposed.

From the very early works on RIM of Malguarnera and Suh (1977) it was reported that to establish a self sustainable chaotic flow regime, the Reynolds number has to be above a certain value and the momentum ratio of the jets has to be equal to one. Erkoç *et al.* (2007) showed that even if these conditions are satisfied, the continuous operation of RIM at self sustainable chaotic regime is not assured, as for the same conditions, both oscillatory or self sustainable chaotic regime can be observed.

The modulation/pulsation of the inlet flow streams is called active mixing (Nguyen and Wu, 2005) and is used to promote/enhance mixing in static mixers. Here seven different strategies of the inlet flow streams modulation will be studied computationally: a single jet oscillation, the two opposed jets oscillation in-phase or out-of-phase and at different frequencies and amplitudes. The imposed oscillations can interact with the natural/non-forced frequencies of oscillation of the flow enhancing mixing: by increasing the amplitude of the natural frequency; or inducing multiple frequencies, which would break the typical flow field structures; or at least aid/forcing the natural system oscillation in order to prevent transient situations where convective mixing

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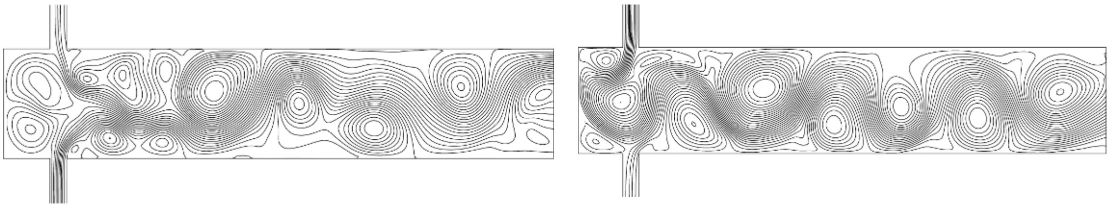
patterns may not occur under short periods. Figure 1 shows two cases of the simulated cases, both are out-of-phase flow oscillations imposed to the opposed jets with amplitude 50% of the jets flow rate. In Case a the oscillation frequency, *f*, is twice the jets non-forced oscillation frequencies, ϕ, and in Case b the *f* is half of ϕ. In Case b a remarkably well ordered sequence of vortices that occupy the whole chamber diameter is observed. In Case a there are some smaller vortices in the jets entering region that will generate a sequence of distorted vortices downstream. The active mixing strategy can completely change the mixing patterns and thus in mixing sensitive systems, such as RIM or nanoparticles precipitation, different material properties can be obtained.



Case a Case b

Figure 1 Streamlines of flow field in a CIJ mixing chamber from dynamic simulations at average Re=300, using different active mixing strategies.

The active mixing strategies are studied from 2D Computational Fluid Dynamics (CFD) simulations. The effect of the opposed jets modulation will be assessed from the flow maps and from the flow dynamics and respective frequency analysis. Effect of flow dynamics on mixing is assessed from the relation between local dynamic velocity data and mixing mechanisms and scales established by Santos (2003). This work proved the concepts patented for RIM robust operation (Lopes *et al.* 2005) with Control of Oscillation and Pulsation, a novel RIM technology registered under the name RIMCOP®.

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Ultrasound – assisted extraction of active principles from *Arctium lappa L.* (Burdock) roots, *Stevia rebaudiana Bert.* leaves and *Cynara scolymus L.* (Artichoke) leaves

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Keywords: Polyphenolic acids, Flavonoids, Stevioside, Ultrasound – assisted extraction, HPLC

The intensification of the extraction process in ultrasonic fields is addressed, emphasizing the advantages of obtaining hypoglycemic extracts from medicinal plants with great economical potential: flavonoids and polyphenol acids from *Cynara scolymus L.* (artichoke) leaves and *Arctium lappa L.* (burdock) roots and sweet diterpenic glycosides from *Stevia rebaudiana Bert.* leaves.

The objective of this work was to isolate an optimum extraction domain for a fast and reliable protocol of ultrasound-assisted extraction (UAE). Compared with classic methods like maceration, heat and/or stirred extraction, the UAE has the highest productivity, defined as the quantity of the extracted product with respect to time and volume of mixture. The efficiency of the classic extraction was investigated with respect to the solvent used, the ratio of sample quantity to solvent volume, the plant material granulation size and the time of extraction.

The quantification of flavonoids and poliphenolic acids from autochthonous medicinal plants was performed with a spectrofotometry method, while the diterpenic glycosides of paraguayan plant were measured by HPLC.

The optimum ultrasound - assisted extraction parameters for diterpenic glycosides of *Stevia rebaudiana* are: water as solvent, 1/5 (w/v) as ratio of sample to solvent and 10 min as extraction time.

The highest total flavonoids quantity was extracted in ultrasonic field from the *Cynara scolymus L.* leaves using a solution of 55^0 ethanol solution, a 1/10 (w/v) ratio of sample to solvent and in 10 min time of extraction. The highest total polyphenol acids amount was extracted ultrasound-assisted from the Arctium lappa L. roots using 70^0 ethanol solution, a 1/5 (w/v) ratio of sample to solvent and in 15 min time of extraction.

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Monitoring multi-component liquid reaction systems containing highly dispersible heterogeneous catalysts using *in situ* diode array spectrophotometry and band-fitting techniques

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Keywords: Diode array spectrophotometry, band-fitting, kinetic modelling, zeolite NaY, cutinase

Introduction

Process analytical technologies may provide outstanding contributions for the quality control of various production processes, namely in the food and pharmaceutical industries (Lopes et al., 2004) and they will also have a significant impact in laboratory experimental data interpretation.

Herein, the reaction kinetics of the hydrolysis of *p*-nitrophenyl butyrate (PNPB) catalysed by a single-site mutant of *Fusarium solani pisi* cutinase, supported on zeolite NaY, was followed using UV/Vis *in situ* diode array spectrophotometry. In aqueous media, the highly dispersible solid catalyst particles produce very significant interference with the absorption measurements. This effect is further aggravated by the dynamic behaviour of the light scattering phenomena caused by the random breakdown of catalyst particle aggregates over the course of the reaction. Combining a high-throughput data acquisition technique, such as diode array spectrophotometry, with spectral band-fitting methods allowed the retrieval of quantitative data on the concentrations of the various species present in the reaction medium, thus enabling us to perform a kinetic analysis of the reaction.

Experimental

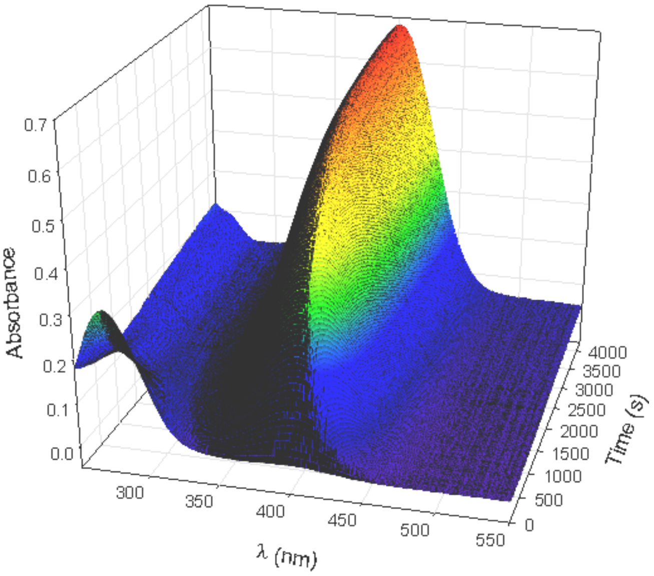

Fig. 1 Graph of the absorbance spectra captured over the course of the hydrolysis of PNPB catalyzed by zeolite NaY-supported cutinase T179C.

A *F. solani pisi* cutinase single site mutant T179C was selected from a complete saturation mutagenesis library (Brissos et al., 2008). Zeolite NaY (Union Carbide, Si:Al = 2.5) was used as a support after calcination and the enzyme was immobilized by adding 400 $mg_{zeo}.mL^{-1}$ to a 20 $mg_{enz}.mL^{-1}$ solution in 50 mM potassium phosphate buffer pH 8.5 for 30 min, subsequently washed and vacuum dried for 6 h. The hydrolysis of PNPB was followed *in situ* using an Ocean Optics USB2000® UV/Vis diode array spectrophotometer equipped with a Mikropack® DH-2000-BAL deuterium-halogen light source. The reactions took place in a cell with a reaction volume of 1 mL, at 30 ºC and with

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magnetic stirring. A stock solution of PNPB (Sigma, 98 %) 3.5 mM in acetonitrile (Merck, HPLC grade) was prepared and kept at 0 °C. For each assay, 0.25 mg of catalyst and 990 μL of phosphate buffer were placed in the cell and the reaction was started by the addition of 10 μL of the PNPB solution.

Reference spectra (molar absorptivities, ε) in the $250<\lambda<550$ nm range were obtained from an orthogonal calibration set (Brereton, 2000) prepared with standard solutions of PNPB and its product *p*-nitrophenol (PNP). No absorption was detected with the other product, butyric acid. A reference spectrum of the zeolite suspension was obtained using a weighed average of the spectra collected during a blank assay.

Results and discussion

Throughout each assay, the spectrophotometer collected light intensity spectra that were converted to absorbance. A 3D plot of these spectra is shown in Fig. 1. From this graph, if we focus on the λ_{max} values for PNPB (273.52 nm) and PNP (402.06 nm), it is apparent that the absorbance of both species increases simultaneously after ~2000 s, when the sum of PNPB and PNP concentrations was expected to remain constant. Zeolite particles are clusters of small crystallites and change their aggregation state during the assay, increasing light scattering effects and preventing accurate absorbance measurements. To obtain the various species concentration profiles, each spectrum (at a given time) was decomposed into a linear combination of the reference spectra, with and without the zeolite contribution (Fig. 2).

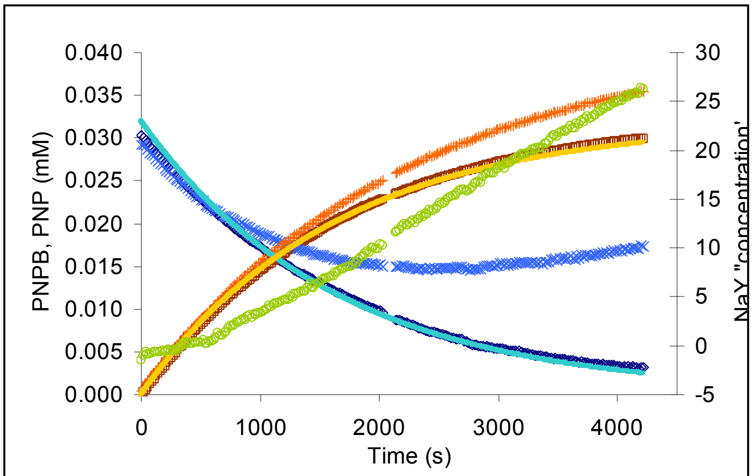

Fig. 2 Hydrolysis of PNPB catalysed by zeolite NaY-supported cutinase T179C. Concentrations determined with $\varepsilon(\lambda_{max})$ (PNPB ×, PNP +) and by using $\varepsilon(\lambda)$ and considering the effect of NaY (PNPB ◊, PNP □, “NaY” ○). The lines are the result of kinetic modelling.

Accounting for the zeolite effect enabled the kinetic modelling of the reaction, using a first order, reversible mechanism. The differential equations corresponding to the material balances were solved using the Euler method and the kinetic rate constants were estimated by least-squares. The forward and backward reaction rate constants were, respectively, $k_1 = (6.130 \pm 0.004) \times 10^{-4}$ s^{-1} and $k_{-1} = (1.1 \pm 0.2) \times 10^{-4}$ mM^{-1}s^{-1}.

Acknowledgments

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High-throughput pharmaceutical process monitoring: integrating the hardware and software components

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Keywords: Process Analytical Technology, High-Throughput Monitoring, hemometrics, Spectroscopy, Industrial Investment

The Process Analytical Technology (PAT) initiative issued by the Food and Drug Administration (FDA) in 2003 recommends to the pharmaceutical industry the development and employment of new technologies in the production and qualification of pharmaceuticals products (US FDA, 2003). The PAT framework states that the monitoring efforts should be focussed directly on the process and not after each production step (Herkert et al., 2001). Spectroscopic methods such as on near-infrared (NIR), Raman or acoustic coupled with reflection/immersion probes are among the PAT tools that have been providing the best results (Cooley and Egan, 2004).

PAT implementations are now seen by the industry with a more global perspective (Lopes et al., 2004). From the raw-materials reception until the final product release, PAT applications can be implemented with high benefits not only for quality process improvement but also for maximizing production and reducing the time-to-market period (acting on R&D sectors). A key feature of a successful and economic viable PAT implementation is the integration of the hardware component (e.g., spectrometers, fibbers, probes) and the software component which should be compliant with the industry requirements (Bakeev, 2005). This communication addresses practical aspects of PAT solutions implementations (hardware and software) in the pharmaceutical industry and related economic issues. Specific areas of implementation such as raw-materials characterization, granulation, drying, mixing, compression and product packing, in terms of practical PAT methodologies implementation and economic revenues were discussed. A comparison between the expected revenues versus cost and risk of implementation for different production stages was provided. A survey of the main methods to apply with chemometrics multivariate resolution methods to extract meaningful chemical composition and to provide uncertainty estimations is provided and supported with different pharmaceutical industry data (Martens and Næs, 1989). The chemometrics complementary procedures include resampling strategies to estimate confidence and prediction intervals, rotational methods to adjust models to chemical meaningful patterns and using constraints to prevent unreal model solutions.

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Pharmaceutical Engineering

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Keywords: pharmaceutical engineering, PSE, quality control, quality assurance

Process analytical technologies (PAT) are “systems for analysis & control of manufacturing processes based on timely measurements of critical quality parameters and performance attributes of raw-materials and in-process products, to assure acceptable end-product quality at the completion of the process”, i.e., quality by design (FDA, 2004).

PATs are a landmark in the acceptance of process systems engineering (PSE) tools in modern pharmaceutical manufacturing and quality assurance of food and drug processes in general. PATs involve the application of process analytical chemistry (i.e., in-process monitoring techniques), chemometrics (e.g., data-based modeling techniques) and process control techniques (viz., intelligent use of process data with multivariate supervision and diagnosis strategies).

In this talk we present a prospective view on how this new field made up from integrating established engineering approaches and other scientific areas into pharmaceutical and biologics production will play a decisive role in leading to better, safer and less expensive medicines, and eventually to a “pharmaceutical engineering” discipline itself.

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Production of oligo-galacturonic acids by pectin hydrolysis using sub- and supercritical water

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Keywords: Supercritical water, SCWO, hydrolysis, pectin

Sub- and supercritical water has gained increasing attention in green chemistry, offering various advantages in comparison to conventional, organic solvents or extraction media. The critical point (CP) of water is at 22,1 MPa and 374°C. By variation of pressure and temperature from ambient conditions above the CP the dielectric constant ε be selected in a range of 3 to 80, causing an increase of solvation power. Most organic substances and biolpolymers are dissolved in supercritical water. The ion product is increased up to three orders of magnitude, accelerating degradation of polymers by hydrolysis. Our work was focussed on hydrolysis of pectin from waste of food processing to investigate the potential to produce oligo-galacturonic acids (OGA). OGA with a grade of polymerization (PG) of 3 to 8 can be used as highly valuable, functional food ingredient with benefits on consumers health by preventing diarrhea. At present enzymatic or thermal hydrolysis with low conversion rates are used for OGA production.

A continuous pilot scale reactor was developed for hydrolyis of up to 10 l/h of pectin solution at a maximum pressure of 30 MPa, a maximum temperature of 500°C and a reaction time of 1 to 60 s. To ellaborate kinetic data a defined residence time was required, beeing obtained by injecting the pectine solution into supercritcal water. The reaction can be terminated after passing the reactor by cold water injection. A solution of citrus pectin (1 to 4 % concentration) was used to determine reaction rates, characterization of reaction products was performed by Size Exclusion Chromatography (SEC) and Mass Spectrometry (MS). In a pressure range of 12 to 25 MPa the reaction rate was found to be dependent on temperature mainly, above a temperature of 270°C predominantly mono-galacturonic acid was formed, wheras at lower temperatures a mixture of OGA with PG of 3 to 8 was obtained after reaction times in a range of 5 to 10 s. It was found that saturated and unsaturaded OGA can be produced, dependent on degree of esterification of citrus pectin. To fractionate hydrolysates and win specific OGA Ion exchange chromatography (IEC) and Gel Permeation Chromatoghraphy (GPC) have been applied. Kinetic data and processing conditions will be discussed along with the techniques feasibility to produce valuable substances by sub- and supercritical water hydolysis.

Synthesis of triproprionin from crude glycerol the by-product of biodiesel production

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Keywords: glycerol, tripropionin, fuel additives

Biodiesel, which is a mixture of methyl esters of fatty acids, is produced from vegetable oils by transesterification with methanol. As a by-product 1 mol of glycerol is produced for every 3 mol of methyl esters, which is equivalent to approximately 10 wt. % of the total product.

Glycerol markets have reacted strongly to the increasing availability of glycerol: although the global production of biodiesel is still very limited, the market price of glycerol has dropped rapidly. If the production of biodiesel increases as predicted, the supply of glycerol will create a glut on the market. Therefore, new uses for glycerol need to be found. Although glycerol could be burnt as a fuel, there is a tremendous potential to develop a variety of new processes and product lines from glycerol, taking advantage of its unique structure and properties.

As glycerol is a nontoxic, edible, biodegradable compound, it will provide important environmental benefits to the new platform products. Lower cost glycerol could open significant markets in polymers, ethers, esters and other compounds. From a technical standpoint, glycerol's multifunctional structure can be exploited by several different means.

One alternative is to esterify glycerol with organic acids (e.g. acetic acid) or anhydrides (e.g. acetic anhydride) and produce oxygen-containing components, which could have suitable properties for use for example in fuels or solvents.

Our aim was to study the esterification of glycerol by propionic anhydride (Fig. 1).

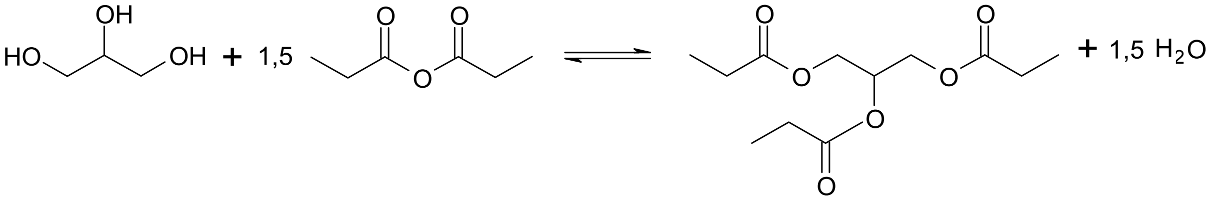

Fig. 1. Reaction scheme for the esterification of glycerol with propionic anhydride

The most difficult problem in connection with crude glycerol refining is removal of salt which is formed during neutralization of the catalyst (KOH, NaOH). Due to the fact that presence of salt does not disturb the reaction and it is insoluble in tripropionin, this problem can be solved by simple filtration.

Propionic anhydride was used in excess to shift reaction equilibrium towards the product. This reaction can be carried out by help of entraining solvents to remove the water azeotropically or by straightforward distillation in absence of entraining solvents. Effect of the reaction conditions was studied and kinetics was determined.

The unreacted propionic anhydride and the formed propionic acid and residual water were distilled off at 120°C under reduced pressure.

Effect of tripropionin blending on engine performance characteristics and environmental repercussions were studied (Table 1).

VW-AUDI 1.9 TDI engine was used for the measurements. Blended fuel contained 5 wt% tripropionin.

Table 1. Comparison of reference fuel and blended fuel

	Reference fuel	**Blended fuel**	**Change [%]**
THC [ppm]	35,6	37	+3,9
NO_X	767	768	+0,1
CO (V/V%)	0,0174	0,0163	-6,3
Smoke [FSN]	1,48	1,29	-12,8
Specific fuel consumption [g/kWh]	251	260	+3,6
$T_{exhaust}$ [°C]	524	520	-0,8

Due to the fact that the European biodiesel production releases about 600 kt glycerol per year, new outlets for glycerol are urgently needed.

Tripropionin represents a promising material, which can be used as fuel additive to improve engine performance characteristics and environmental repercussions. Further studies need to be done to find the optimal ratio of tripropionin in Diesel oil and to examine other fields of application of this relatively new product.

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The future sustainability of biodiesel

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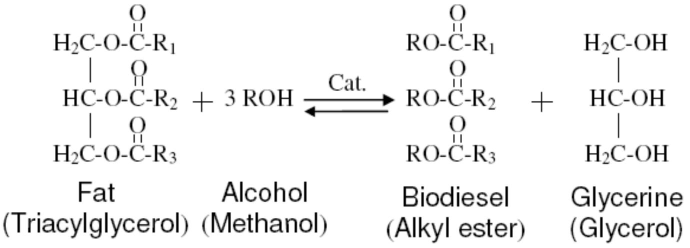
Keywords: Biodiesel, Soybean oil, Non-edible oil, Renewable Energy, CO_2 Reduction

The biodiesel is a renewable fuel that can be produced by transesterification, in which a vegetable oil (edible and non-edible) or animal fat reacts with a short-chain alcohol in the presence of a catalyst, to form esters and glycerol (Marchetti et al., 2007).



Because the growing of plants consumes CO2, biodiesel has a closed carbon cycle dramatically reducing the CO2. This allied with the desire to reduce dependence on foreign energy sources, manifesting itself through a variety of public policies over the years, have served to increase biodiesel use in the European Union (EU) (Knothe et al., 2005).

EU imports more than 20 million Tons of biodiesel, so must be by far the biggest and most important biofuel, representing more than 80% of total biofuels production. The EU is the worldwide leader in biodiesel production, both in terms of biodiesel capacities and production. In 2006 it was produced 77% of world-wide biodiesel production. An alternative fuel like biodiesel can be technically feasible, environmentally acceptable and readily available but it is not until now economically competitive, due to the price of raw materials (EBB, 2006).

Since the economic growth is always accompanied by commensurate increase in the transports sector, this type of energy is gaining more and more importance due to the depleting of fossil fuel resources and also to avoid the monopoly of some oil-exporting countries. As a consequence of this depletion, the fuel price grows exponentially and the vegetable oils prices follow it. Moreover, the limited oleaginous production implies limitations in vegetable oils production and thus, the demand is higher than the offer.

Because of these mentioned situations, the biodiesel has to be considered as a complement, or adjuvant to push ahead, the end of fossil diesel in a medium term, and never a total substitute.

Industrially, the biodiesel is made mostly with rapeseed oil, palm olein, soybean oil and in small cases with animal fats. The parameters given by the specific European norm (EN 14214) are very restricted since they were based mostly on a biodiesel produced with rapeseed oil, thus it is not possible to produce an extreme biodiesel with only one type of oil/fat other than rapeseed oil of the referred ones.

In a global macro-economic point of view, the selection of the vegetable oil is very important, not only because of its cost but also due to the repercussions that this

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selection has in the world food structure. The competitiveness between biodiesel and fossil diesel can only be equalised if the non-edible raw materials are used.

Non-edible oil as raw materials, with enormous potential but still in development production, are obtained from plant species such as Jatropha curcas (Sarin et al., 2007), Pongamia pinnata (Meher et al., 2006) or microalgaes. Nevertheless, extreme biodiesel produced with these oils could not also fulfil totally the necessary specification of the EN 14214. These are indeed the expected raw material for the future biodiesel production, with enormous potentials due to the yield/crop and content of oil of the oleaginous. However, their massive production for the biodiesel industry is under development and thus cannot be at short time the answer to the biodiesel demand.

From all the mentioned above, only the soybean oil is each time more a non-edible oil (less than 5% is used in alimentation). We can consider that in this case the principal product of the oil extraction is the soybean mill, very rich in proteins, that is used in every animal feed, since soybean is considered a proteoleaginous. In addition, the soybean oil utilisation in the animal and human feed has very limitations. Given its economic value and availability, the soybean oil is nowadays a preferential raw material in the manufacture of biodiesel. However, as it was mentioned before, for example, the soybean oil presents an iodine index between 128 and 132, too high to meet the European specification of biodiesel in this parameter (equal to 120). Because of this fact, it is not currently possible in all EU countries that have transposed these specifications, manufacture biodiesel entirely from soybean oil, it becomes necessary to incorporate other vegetable oils of higher costs, such as rapeseed oil from north of Europe and palm olein from tropical countries.

The south of Europe has the appropriate edaphoclimatic conditions to produce soybean, which could have a significant contribution as endogenous production, in a similar comparison with the north appropriate edaphoclimatic conditions to produce rapeseed.

It can be conclude that soybean oil is in short time the answer since most protein comes from this proteoleaginous, increasing mill for the food chain in which results a subproduct that can be used to decrease the fossil fuels, producing biodiesel.

Other way to promote the biodiesel and its future viability is the creation of conditions by governments to impose the biodiesel blending in every diesel, as well as, the removal of biofuels subsidies with the concomitant increasing of the fossil fuels taxes.

In which respect to the international context, the EU market is disturbed by the continuing negative effects of “B99” (blending 1 part of diesel and 99 of biodiesel) United States (US) export subsidy. With this unfair scheme the US producers can access the EU markets with a remarkable advantage, selling the biodiesel at the same or even at a lower price than the cost of EU industry’s raw materials (EuropaBio, 2007).

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Construction and bioproduction of a "green" synthetic protein-based polymer exhibiting a smart behaviour

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Keywords: protein-based polymers, elastin-like, silk-elastin-like, microparticles, biodegradable, transition temperature, biocompatible, thermoplastic

Natural occurring elastomeric proteins occur in a wide range of biological systems, fulfilling precise functional roles [Tatham and Shewry, 2000]. Their properties are due to the presence of short repeating oligopeptide sequences contained in fibrous proteins, such as silk fibroin (GAGAGS) and mammalian elastin (VPGVG). Elastin is widely distributed in vertebrate tissues, acting statically in dermis to resist long-term forces and dynamically in arteries to store and release energy rapidly. Natural silk from *Bombyx mori* (silkworm) has been used for centuries either in textile industry or as biomedical suture material, exhibiting impressive mechanical properties as well as high biocompatibility [Kim *et al*, 2004].

With the development of protein engineering and nano(bio)technologies in general, it is now possible to use amino acids to design and produce genetically engineered Protein-Based Polymers (PBPs) fully biodegradable that simulate the properties of natural occurring proteins . With the advance in recombinant DNA technology it is possible to precisely control the composition, sequence and length of large molecular weight PBPs [Haider *et al*, 2000]. Recombinant Elastin-Like Polymers (ELPs) are biopolymers based on the aminoacid sequence VPGXG (V-valine, P-proline, G-glycine), where X, termed the guest residue, is any naturally occurring aminoacid except proline. The most striking feature of the ELPs is their Inverse Temperature Transition (ITT) behaviour. Below a specific critical temperature (T_t) and in the presence of water they are soluble, with the polymer chains relatively extended in a disordered state and fully hydrated mainly by hydrophobic hydration. Above the T_t, the polymer chains hydrophobically fold and adopt a dynamic structure, called β-spiral, stabilized by hydrophobic contacts . The ability of ELPs to self-assemble into nanostructures in response to environmental changes allows their utilization in many devices such as microparticles for controlled drug delivery systems or nanosensors.

The polymer poly(VPAVG), a ELP where the central glycine (G) is substituted by a L-alanine (A), was chemically synthesized by Rodríguez-Cabello and co-workers and described by Urry as having thermoplastic properties. These groups reported its characterization, demonstrating its extreme biocompatibility both *in vitro* and *in vivo,* as well as the ability to self-assemble, forming microparticles that can entrap active substances during the self-assembling process [Herrero-Vanrell *et al*, 2005; Rincón *et al*, 2006].

In the present work a new thermal responsive, biologically synthesized ELP based on the $(VPAVG)_{220}$ sequence was produced, by recurring to standard molecular genetic tools and, as expected, the polymer displayed an inverse temperature transition (T_t)

which could be explored as a purification step. Additionally, the purified polymer $(VPAVG)_{220}$ showed the ability to self-associate at physiological temperature forming aggregates.

The culture media and fermentation conditions were optimized using a Central Composite Design (CCD) approach while exploring the use of low cost carbon sources like lactose and glycerol. Sequence and purity of $(VPAVG)_{220}$ was confirmed by MALDI TOF analysis and purified polymer was subjected to thermal and physical characterization. Due to its self-assembling behaviour near 34 ºC stable spherical microparticles of a ~1µm diameter were obtained, ready solubilized when a strong undercooling was achieved.

Moreover, we have constructed and produced a new set of copolymers (Silk-ElastinLike Polymers – SELPs) consisting of flexible ELP and crystalline silk-like blocks (GAGAGS) at different proportions. By this strategy it was possible to produce a variety of biomaterials with diverse physical properties, such as viscosity and gelation time depending on the number of elastin-blocks and silk-like blocks, respectively [Megeed *et al*, 2000]. The stability of these SELPs in combination with their biocompatibility and unique mechanical properties, provide the basis of their exploitation for biomedical applications.

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Lignin-based polyurethane materials

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Keywords: Lignin, Polyurethanes, Bio-based, Added-value

Lignin is defined as a random, amorphous three-dimensional polymeric network, which does not possess a uniform, homogenous, well defined structure with well established repeating units. Commercially available lignins are, most often, Kraft or lignosulfonates associated to the corresponding most commonly used industrial processes for wood delignification and fibres isolation (Kraft and sulphite, respectively). These lignins are largely used in dispersing and binding applications and a small part is employed in the production of specially chemicals like vanillin and dimethylsulfoxide (Gosselink et al., 2004). Recently, sulphur-free lignins are becoming an emerging class of lignin products, partly motivated by environmental policies, but also, by less capital intensive associated technologies. They could be obtained from three main sources: (i) biomass conversion technologies – biorefineries; (ii) solvent pulping – organosolv processes; and (iii) soda pulping of alternative biomass resources, such as agricultural harvesting residues and non-wood fibre crops (Lora and Glasser, 2002).

The utilization of lignin as a macromonomer in polyurethane synthesis often follows two global approaches: (i) the direct utilization of lignin without any preliminary chemical modification, alone or in combination with other polyols (Thring et al., 1997, Evtuguin et al., 1998, Cateto et al., 2008) or, (ii) by making hydroxyl functions more readily available by chemical modification, such as esterification and etherification reactions (Glasser, 1989, Gandini et al., 2002, Nadji et al., 2005).

The four technical lignins used in this work represent three different pulping processes (Kraft, Soda and Organosolv) and different botanic origins, i.e., softwood, hardwood and non-wood lignin types. Indulin AT and Curan 27-11P (commercialized in the alkali form) are softwood lignins obtained by the Kraft pulping process and were supplied by MeadWestvaco and BorregaardLignoTech, respectively, whereas Sarkanda lignin was supplied by Granit SA. The latter is a non-wood lignin obtained from a soda pulping-precipitation process, patented by Granit SA. Alcell lignin of Repap Enterprises Inc. is extracted from mixed hardwoods (maple, birch and poplar) by an organosolv process using aqueous ethanol. A summary of the performed characterization is shown in Table 1.

In the present work we have studied the two previously described global approaches. The first one involved the direct use lignin as a co-monomer in combination with a linear polycaprolactone (PCL). PCL was introduced into the formulation to provide flexibility and enable polymerization in bulk. The produced polyurethanes have properties that vary from very flexible to very hard materials depending on the amount of lignin effectively incorporated, and according to the molecular weight of the used PCL. For the formulations studied, the success of lignin incorporation by chemical reaction with the isocyanates (formation of urethane linkages) was noticeable. In the second approach lignin was oxypropylated and the ensuing polyols used for rigid polyurethane foams preparation, alone or in combination with other commercial

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polyether-polyols. The produced foams have shown mechanical strength and conductivity similar, or even superior, to those of foams produced with commercial polyols alone. Examples of the produced materials are shown in Figure 1.

Table 1. Properties of the technical lignins used in this work

Lignin Sample	Total OH (mmol/g)	Phenolic OH (mmol/g)	COOH (mmol/g)	Ash content (% w/w)
Alcell	5.26	3.81	0.23	0.05
Sarkanda	5.26	2.41	0.62	3.26
Indulin AT	6.99	3.95	0.39	3.06
Curan 27-11P	6.21	3.63	0.47	17.0

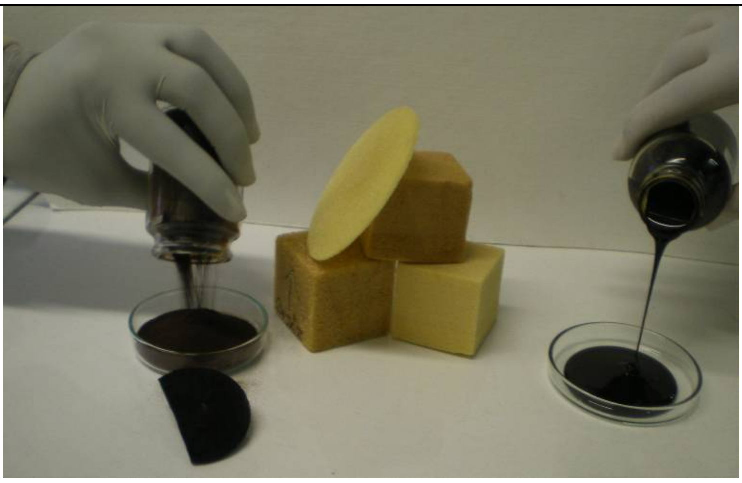

Figure 1. Original lignin and examples of the produced materials.

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Catalytic wet air oxidation plus biological treatment to deal with a high-strength o-cresol wastewater

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Keywords: Activated carbon, catalytic wet air oxidation, biodegradability enhancement, respirometry, biological treatment, inhibition and toxicity.

Wastewater reduction and treatment is one of the challenges faced by our society. As an example, in the EU, 2700 tons/year of phenolic compounds wastewater are released having both, toxic and bactericide effect (EPER, 2008). Several technologies have shown their potential for treating a wide variety of wastewater. Nearly all of them are based on the oxidation of the organic pollutants, which are converted into carbon dioxide and water or into harmless intermediate products, more suitable for a biological treatment (Hancock, 1999; Mishra et al., 1995 and Bhargava et al., 2006). Therefore, Catalytic Wet Air Oxidation (CWAO), H_2O_2 promoted CWAO and Fenton process have become attractive techniques to efficiently treat organic wastewater that is either too concentrated or toxic to be biologically restored.

However, complete mineralisation of pollutants is extremely costly and the bactericide effect of some often found pollutants prevents industrial wastewater from being directly treated in a biological wastewater treatment plant (WWTP). Coupling of an initial oxidative step with a biological treatment can solve these pollution problems in a rational and less expensive way (Scott and Ollis, 1995; Otal et al., 1996; Mantzavinos et al., 1999; Patterson et al., 2002 and Bankian and Mehrvar, 2004).

Hence, this study examines the feasibility of coupling a Catalytic Wet Air Oxidation (CWAO), with activated carbon (AC) as catalyst, and an aerobic biological treatment to treat a high strength *o*-cresol wastewater. Two goals are pursued: a) To determine the effect of the main AC/CWAO intermediates on the activated sludge of a municipal WasteWater Treatment Plant (WWTP) and b) To demonstrate the feasibility of coupling the AC/CWAO effluent as a part of the influent of a municipal WWTP. Also, it is established the AC/CWAO intermediates distribution and the biodegradability enhancement. The main challenge in the coupling is to achieve the right balance between the oxidation deepness (economic cost) and the effluent biodegradability (distribution of oxidation products) after the oxidation step, which ensures the success of a subsequent biological treatment.

First of all, several CWAO tests were completed (140ºC-160ºC, 2-9 bar of oxygen partial pressure, at a space time of 0.12 h) for a high-strength o-cresol wastewater. All the experiments were done in a fixed bed reactor, operating in trickle flow regime, and using activated carbon (AC) as catalyst. The results of this part (Suárez-Ojeda et al., 2007a) show that o-cresol disappearance, chemical oxygen demand (COD) removal and total organic carbon (TOC) abatement are very sensible to temperature change but almost independent of oxygen partial pressure. For instance, in CWAO of o-cresol at 2 bar of oxygen partial pressure, as temperature increases from 140 to 160ºC, o-cresol conversion increases from 30% to 85%, COD removal from 15 to 50% and TOC abatement from 18% and 47%.

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Secondly, biological parameters were measured using respirometric tests that were completed before and after CWAO and independently, for each one of the identified intermediates (Suárez-Ojeda et al., 2007a). In the case of CWAO effluents, these tests have enabled the determination of the biodegradability, toxicity and inhibition over a non-acclimated activated sludge. For CWAO intermediates, these tests have allowed to obtain the biomass yield coefficient for biodegradable carboxylic acids and to detect some co-metabolic effects, which serve to explain the results obtained for CWAO effluents. Taking into account these results, it was possible to establish whether or not the CWAO effluents are suitable for a non adapted sludge and to develop suitable acclimatisation procedures to couple the CWAO step with a biological pilot plant.

Finally, the results of coupling the AC/CWAO effluent as a part of the influent of a municipal WWTP are presented (Suárez-Ojeda et al. 2007b). For instance, using 30%, as COD, of AC/CWAO effluent in the inlet to the pilot scale WWTP, the integrated AC/CWAO-biological treatment achieved a 98% of total COD removal and, particularly, a 91% of AC/CWAO effluent COD removal without any undesirable effect on the biomass.

The whole study shows a promising way for the development of efficient coupled processes for the treatment of wastewater containing toxic or biologically non-degradable compounds.

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Two-aqueous phase extraction for the removal of organic pollutants and metal ions

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Abstract

The respect of environment is nowadays a world-wide requirement which implies the development of new curative processes and the design of intrinsically clean processes. These processes apply mainly to wastewater treatment (water polluted by organics or heavy metal). The criteria of water quality (respect of legal norms) and economy are decisive; in order to take into account the physical and chemical specificities of the effluents, essays with real wastewaters are necessary.

Surfactant adsorption at interfaces makes them useful auxiliaries in several types of extraction processes. On the other hand, volatile organic solvents, commonly used in liquid-liquid extraction, are subject to more and more severe regulations, due to their toxicity and/or ecotoxicity. Since the phase diagrams of many polyethoxylated surfactant/water binary systems show a lower consolute point curve, the existence of the corresponding two-phase region can be turned to account to extract metal ions, small organic molecules or biological macromolecules from their dilute aqueous solutions. This operation, known as liquid-coacervate or cloud-point extraction (CPE), consists of two steps: (1) organic solute solubilization into the extractant (aqueous solution of nonionic surfactant) and (2) cloud-point phase separation of this solution into a dilute phase and a surfactant-rich one, called coacervate,. Whether the dilute phase or the coacervate is of interest, two main types of applications may be considered: removal of pollutants from aqueous effluents and concentration, recovery and, possibly, separation of high added-value substances [1-6].

Since the CPE technique has been proposed, there a lot applications in the wastewater treatment. However, previous studies on the removal of dissolved contaminants by CPE were mainly based on the use of single surfactant systems and emphasized on either organic solutes [4-6]. Few studies have been conducted on the applications of mixed surfactants or the simultaneous removal of metal ions and organic solutes in a CPE process [7]. One of the most important properties of these organised structures is their good capacity to solubilise solutes of different character and nature. These solutes may interact electrostatically, hydrophobically or by a combination of both effects.

This is therefore a “Green Chemistry” process, i.e. environmentally friendly and energy saving. Cloud point extraction of metal ions (Ni^{2+}, Cd^{2+}, Pb^{2+}, Cr^{3+}) with mixed micelles of polyethoxylated alcohols (CiEj) and anionic surfactants (sodium dodecylsulfate “SDS” or dodecylbenzenesulfonates “SDBS”) is achieved from model solutions of metal nitrates, possibly mixed with organics. The nonionic surfactant, along with its cloud point property, is responsible for phase separation; the anionic species plays the role of a complexing agent. The major interest is the absence of classical but expensive chelating agents. Moreover, CPE technique thus offers a convenient alternative to conventional liquid-liquid extraction that uses organic solvents. The polluted aqueous phase and the surfactant solution were mixed together and separated at room temperature or after heating, according to the cloud point curve of the surfactant. The results are promising, due to the extraction percentages lying between 50 and 90%, the best performances being obtained for Pb2+, whose hydration enthalpy is the lowest.

Moreover, the ability of those mixed micelles to extract both organic (phenol and 2,4-dimethylaniline) and heavy metal pollutants from model solutions is shown. The compromise to be found between E% (maximum) and coacervate volume fraction (minimum). These results suggest that mixed micelles can form hydrogen bonds between hydroxyl or amino groups and the most available ether oxygen atoms of the polyoxyethylene chain (reducing surfactant solubility)[8]. The rate of recovery decrease in such systems could be correlated to the log P (octanol/H2O partition coefficient) of the aromatic solutes but not to their aqueous solubility [9-10]. However, metal ions bind to the surface of negatively charged micelles of anionic surfactants by Coulombic interactions. In summary, metal ions bind or adsorb on the surface of the oppositely micelles, while organic solutes solubilize within the micelles.

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Biosorption of Cr^{VI} supported on mordenite zeolite

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Keywords: Zeolite, Chromium, Biosorption, Mordenite.

Introduction

Zeolites are widely known to be versatile materials, having found industrial application in various fields, such as catalysis, adsorption, molecular sieving and ion exchange. In the pollution control field, the adsorption and ion exchange capacities have been exploited in the treatment of industrial effluents. However, zeolites can only be used as ionic exchangers for the removal of cationic species, due to the proprieties of their structure [1]. Chromium contaminated effluents are commonly found where industries such as tanneries, metallurgy, plating or textile finishing are present. The most common Cr compounds in these effluents are the dichromate and chromate anions, where Cr is present in the Cr^{VI} state. Since these are negatively charged molecules, zeolites are unable to remove these compounds alone. Synthetic FAU zeolites have been found to be effective supports for bacterial biosorbents in the treatment of aqueous Cr^{VI} [1, 2]. The combined system is constituted by *Arthrobacter viscosus* bacterium supported on NaY or NaX zeolites. This bacterium is able to reduce Cr^{VI} to the lower oxidation state Cr^{III}, whose species are readily ion exchanged by the zeolitic support. Later, the Cr containing supports were found to be active catalysts for the oxidation of VOCs [1].

This work presents the usage of a different zeolitic structure as support, the mordenite zeolite (MOR). Two MOR zeolites, H-form (HMOR) and Na-form (NaMOR), were tested as supports for the biosorption of aqueous Cr^{VI} species.

Materials and Methods

HMOR and NaMOR (Zeolyst, Inc.) were previously calcined at 500 ºC during 8 h under dry air flow prior to use. *Arthrobacter viscosus* culture medium was prepared 24 h before usage [1]. The Cr^{VI} removal essays were performed in batch conditions, where 150 mL of each aqueous dichromate solution were added to 1.0 g of NaMOR or HMOR zeolites and 15 mL of *Arthrobacter viscosus* suspension. Cr^{VI} concentrations, ranging from 20 to 120 mg_{Cr}/L, were tested for each zeolite and each solution was tested in triplicate. Total experimental time was 9 days, previously determined as enough to reach equilibrium. Samples were collected at the initial and final stages. The collected slurry was centrifuged and the supernatant was recovered and tested for Cr^{VI} concentration using the 1,5-diphenylcarbazide colorimetric method [3].

Results

Cr^{VI} removal was observed for both NaMOR and HMOR based biosorption systems. Figure 1 presents the Cr^{VI} removal for both supports.

The total removal was greater in the HMOR-*Arthrobacter viscosus* system, in every tested Cr^{VI} concentration. The final pH values of the solutions were found to be in the 3.1 to 3.4 range for the HMOR system, and in the 6.0 to 7.0 range for the NaMOR based system. The more acidic conditions found in the HMOR system are favourable to the reduction of Cr^{VI} by the *Arthrobacter viscosus* bacterium. It is expected that the HMOR might retain a higher quantity of Cr than its NaMOR counterpart.

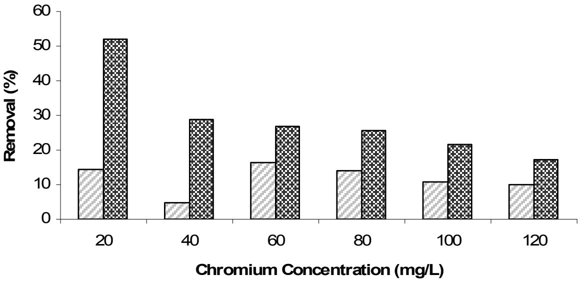

Figure 1: Cr^{VI} removal for NaMOR (light grey) and HMOR (dark grey), for the different chromium concentrations.

The Langmuir adsorption isotherm model was applied to each set of experimental data, and the plotted result is presented as shown in figure 2:

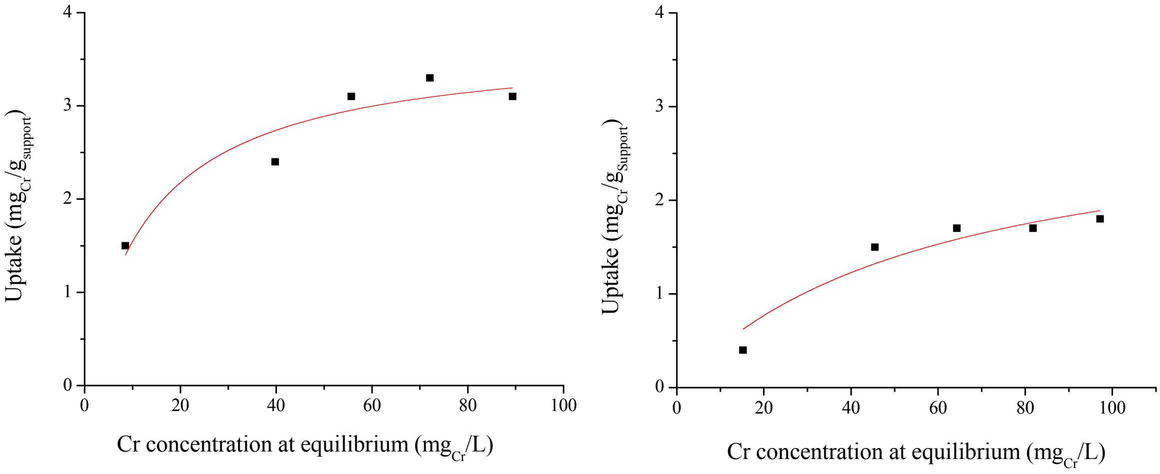

Figure 2: Uptake vs. equilibrium concentration for HMOR – bacteria (left) and NaMOR – bacteria (right), with plotted Langmuir fitting to experimental data for both systems.

The Langmuir adsorption model presented good fitting to experimental data, thus indicating that the Cr^{VI} removal performed by both systems is similar to an adsorption process. The calculated maximum uptakes (q_{max}) were 3.5 $mg_{Cr}/g_{support}$ for the HMOR system and 2.0 $mg_{Cr}/g_{support}$ for the NaMOR system.

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Phosphorus removal from an industrial wastewater by struvite crystallization into an airlift reactor

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Keywords: Crystallization, Phosphorus recovery, Struvite, Airlift reactor.

Introduction

The crystallization of struvite (magnesium ammonium phosphate, MAP, $NH_4MgPO_4 \cdot 6H_20$) represents an interesting technique to recover this element from wastewater. The product recovered, struvite, is easy to hand and free of sludge-handling problems. Struvite crystallization has been demonstrated to be an economical way to remove phosphorous of wastewater effluents, especially at high phosphorous concentrations. Traditional systems based on orthophosphate precipitation with metal salts (Al or Fe) are expensive and generates a large amount of sludge that must be treated. In contrast, struvite has a commercial value as a fertiliser, so is supposed to be really beneficial in all senses for nutrient recovery.

Struvite crystallization may be achieved with different plant configurations: Complete Stirred Tank Reactors (Regy et al. 2001) and either Two-phase or Three-phase Fluidized bed reactors (Battistoni et al. 1997). In this work the performance of a three-phase reactor was studied. An airlift reactor that was used to precipitate phosphate as struvite, from secondary treated industrial wastewater with a high phosphorus concentration. Wastewaters were generated in a fish-canning industry located near the shore of the sea. Magnesium should be added to the wastewater in order to precipitate struvite. For this reason seawater was used as magnesium source, during an experimental period, in order to treat the secondary treated wastewater with high phosphorus concentration.

Materials and methods.

A 2.7 L volume airlift reactor with a three-phase separator at the top of the system was using during this experiment. The operation of the system during 128 d could be divided in three periods of time:

*Period I: Days 0 to 34, Batch operation period: A*irlift was fed during some hours per day, feeding it with synthetic medium with similar P concentration to those found in the industrial wastewater, and two concentrated media were also fed with ammonia and magnesium salts.

Period II: Continuous operation with industrial wastewaters: Days 43 to 91, Secondary treated wastewater, was fed to the reactor and the two concentrated media with ammonia and magnesium salts.

Period III: Days 103 to 128, Synthetic media as phosphorous and ammonia sources were fed again. Nevertheless, in this case, seawater was the magnesium source.

The determination of orthophosphate and ammonium concentrations was carried out accordingly to the Standard Methods. Temperature, pH, conductivity, alkalinity, porosity, suspended solids, TOC, IC and solids hold-up were other measured parameters. X-ray fluorescence and ICP-OES was used for determine the elemental composition of the precipitates and the effluent. Scanning Electron SEM observation.

Results and discussion

Phosphorus recovery, through struvite precipitation, from either synthetic or real wastewater was achieved during the three experimental periods in which the reactor was fed with different sources of phosphate and magnesium. Microscopic observations of precipitates using SEM showed different kinds of precipitates depending on the period of operation (Figure 1). Obviously, the best results were obtained at first operation period, by using synthetic mediums. Nevertheless, a high phosphate separation was also achieved under different feeding conditions, by maintaining an adequate range of pH. Recovery efficiencies were above 80%. The higher phosphate removal was obtained when the system operated at a pH above 8,5. pH was maintained by adding NaOH to the reactor.

Amorphous calcium phosphate precipitates were also observed during the operation periods in which seawater was used as magnesium, and calcium, source. SEM observations demonstrated that this amorphous precipitate [$Ca_3(PO_4)_2$] also acts like a glue, leading to formation of rough agglomerates with struvite crystals. The mean inconvenient is the indicated increment of fines that was observed in the effluent. These fines caused a certain loss of the phosphate removal of the system as were principally composed by amorphous crystals of $Ca_3(PO_4)_2$. In accordance with that, during first operation period (feeding synthetic wastewater), a little amount of fines was detected in the effluent and needle-form struvite crystals grew as monocrystrals, not agglomerated. Kinetics studies demonstrated that magnesium excess is not a key factor in the formation of struvite. Therefore, molar ratios N/P and Mg/P of 1,2 were enough to achieve primary nucleation and struvite crystals. Apparent volume of crystals in the reactor reached 400 mL/L before observing operating problems in the system. Therefore, crystals concentration should be purged to guarantee systems stability.

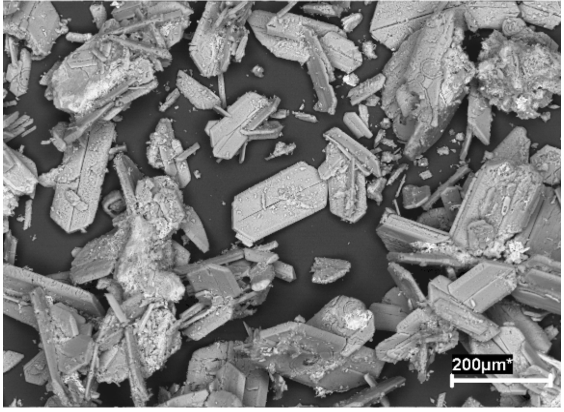

Figure 1. Microphotography of the struvite crystals**.**

Conclusions

Crystallization of struvite take place all over the experience, in the three periods studied. In both cases feeding with synthetics mediums and with real wastewater, the main problem is the formation of fines which were washed out of the airlift reactor. With respect to, an adequate reactor design is a basic factor. This design must provide settling of fines and eldest crystal purged. Therefore, controlling fines outflow, molar ratios and pH, magnesium ammonium phosphate precipitation using seawater like magnesium source might become a promising and a beneficial phosphorous recovery technology.

Acknowledgements: To the Ministry of Education and Science through the Novedar-Consolider project (CSD2007-00055) and to the Consellería de Innovación e Industria, Xunta de Galicia (PGIDIT06TAM008E) that funded this project.

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EcoDesign through a multi-criteria environmental decision tool based on fuzzy logic

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Keywords: Ecological Footprint, Life Cycle Assessment, Environmental Risk Assessment, Fuzzy Logic, Ecodesign Tool

Ecodesign may be defined as the systematic introduction of environmental concerns during product design and development (AENOR, 2003). The identification and valorization of the environmental burdens requires the application of evaluation tools.

Some traditional tools used in ecodesign are qualitative and subjective like the Life Cycle Design Strategies (LiDS) Wheel, or semi-quantitative like the Materials Energy Toxicity (MET) Matrix. On the other side, the more developed ones, like the Ecoindicators or the Life Cycle Assessment (LCA), include objective criteria and quantitative evaluation of the environmental impact.

The different kinds of tools can highlight different potential environmental problems (Byggeth and Hochschorner, 2006). Consequently, none of them can be believed to offer a totally comprehensive evaluation. In some cases the limitations stem from the application of a single approach can be assumed, but in other situations an integrative proposal needs to be considered. Ecological Footprint (EF), Life Cycle Assessment (LCA) and Environmental Risk Assessment (ERA) are considered as examples of objective and quantitative ecodesign tools. Besides, they provide complementary information about environmental performance of processes and products. The EF gives valuable information about the degree of sustainability of a particular process; however, some limitations were acknowledged for this methodology (Herva et al, 2008), and as a consequence it resulted interesting to complement it with LCA. The existence of a relation between EF and LCA has been identified by Huijbregts et al (2007).

Although LCA is claimed to offer an integrative assessment of a process, the information provided regarding human and ecosystem toxicity, for example, is more incomplete than desirable. This means that it has a limited capacity to predict toxicity effects. However, ERA provides an established methodology based on the assessment of different scenarios, exposure pathways, duration and frequency that allows for a more rigorous and exhaustive evaluation. Therefore, LCA and ERA are complementary tools (Leet and Geibig, 2006) that can be integrated (Huijbregts et al, 2000). As a result, ERA is used to estimate a risk index associated to certain hazardous substances that raw materials may contain (organic compounds, heavy metals…) and that would affect the final consumers or factory employees (Franco et al, 2007).

In this work, the integrative approach of the above mentioned environmental tools was built on the basis of fuzzy logic reasoning and features. The usefulness of fuzzy logicbased methods to address uncertainty and subjectivity in environmental problems has already been stated (Carrasco et al, 2002). This idea enabled the decision making at process level taking into account the values of the different indicators at a time. Thus, membership function were defined for the Cancer Risk (CR), Hazard Quotient (HQ) and Ecological Footprint (EF) (Figure 1), the latter including the Global Warming Potential (GWP) and Acidification Potential (AP) LCA impact categories transformed into required space of land. The established objective was to obtain a final Fuzzy

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EcoDesign Index (FEcoDI) that could help to distinguish the best option from an environmental point of view. To test the tool, different packaging materials for a beverage bottle were considered to identify the most environmentally friendly option. A FEcoDI of 30.0 for the PVC bottle was obtained, while a value of 66.6 was estimated for the PET one. The negative evaluation obtained for PVC was consequence of the carcinogenic effects of vinyl monomer. Consequently, this would lead to select the PET bottle as the best option from an environmental point of view.

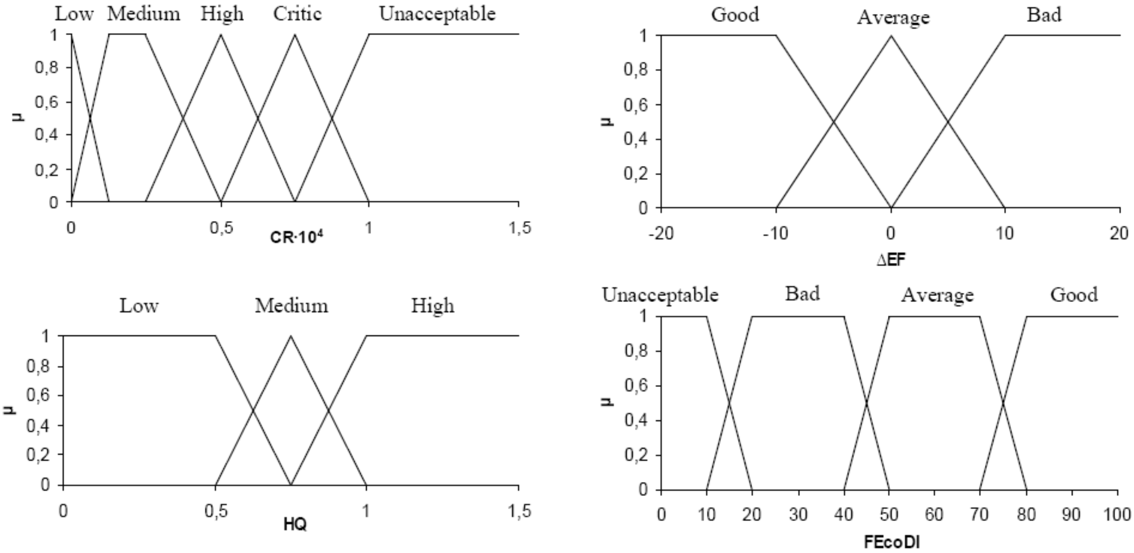

Figure 1. Membership functions for input variables (CR, HQ, EF) and for the output FEcoDI.

Acknowledgements

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Design of sustainable processes: Systematic generation and evaluation of alternatives

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Keywords: Process design, Sustainability metrics, Mass and Energy Indicators, Safety indices

Motivation:

Nowadays the concerns about the human future are growing everyday and consequently the need for improving the industrial plant operation and design is also increasing. The use of green compounds and the operability at sustainable conditions are two very important factors among many others that should be taken into account in the retrofitting of the already existing processes. Important questions to ask in this respect are - How should we improve an existing process (that is generate retrofit alternatives) without too much effort? What should be done in order to make the process more sustainable? Which are the process points that are going to significantly improve the plant performance? The same questions may also be asked as one is generating design alternatives for a new process by comparing a "reference" design. In order to address all these issues a new methodology, which allows the generation of more sustainable alternatives for any process (new or existing) has been developed. This methodology is able to generate, screen and then identify sustainable alternatives in any chemical process (new or old) by locating the operational, environmental, economical and safety related bottlenecks inherent in the process.

Methodology:

The methodology has been developed for the continuous processes (Carvalho *et al*., 2007) and now it has been extended to batch processes. A brief description about both approaches is given in the following below.

Step 1: Collect the steady state data

Process data needed to apply the methodology is collected, such as the mass and the energy balances as well as the purchase and sale prices for each compound. This data can be provided from the real plant data or through model-based simulations.

Step 2: Flowsheet decomposition

The flowsheet decomposition for continuous processes is performed to identify all the open- and closed-paths for each compound in the process flowsheet. In the batch processes, although the same decomposition takes place, in this case the accumulation paths are also determined.

Step 3: Calculate the indicators, the sustainability and the safety parameters

The mass and energy indicators are calculated for all the paths determined in step 2. Through the values obtained for these indicators it is possible to identify the locations within the process where the mass/energy "paths" face "barriers" with respect to costs, benefits, or accumulation problems. These critical points "indicate" high potential for process improvements. The set of indicators used in the continuous processes is the same as the one used in the batch, although some indicators were added to the batch analysis in order to take into account parameters such as equipment volume, operation time, etc. The analysis of the process impact is determined through the calculation of a set of sustainability metrics and/or the LCA analysis. The safety of the process is measured by the use of a set of safety indices.

Step 4: Indicator Sensitivity Analysis (ISA) algorithm
In this step the target indicators are determined using the ISA algorithm. To apply this algorithm the indicators having the highest potential for process improvement with respect to sustainability and other criteria are first identified. Then an objective function such as gross-profit or process total cost is specified. Then a sensitive analysis is performed to determine the indicators that allow the best improvements in the objective function and selected indicators.

Step 5: Process sensitivity Analysis
A process sensitivity analysis with respect to the operational (parameters) variables, which influence the target indicators, is performed. The analysis identifies the operational variables that need to be changed to improve the process in the desired direction.

Step 6: Generation of new design alternatives
New alternatives are generated using a systematic analysis where a collection of synthesis algorithm can be used.

Sustain-Pro:
Based on the above methodology, a software, Sustain-Pro, has been developed to allow an easy application of the methodology for generation of more sustainable design alternatives. The inputs for Sustain-Pro are the mass and the energy balance data as well as the prices of the compounds present in the process. Sustain-Pro follows all the steps of the methodology, allowing thereby, the application of the methodology to any chemical process.

Case Study:
The application of the indicator based methodology as well as the software will be highlighted through a detailed case study concerning the production of an important chemical product in batch processes.

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Modeling of Orange II advanced biodecolourisation in upflow stirred packed-bed reactor

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Keywords: Azo dye, biological activated carbon, packed-bed reactor, reduction

A typical drawback of azo dye colouration – mainly occured in textile industry – is that large amounts of the dyestuff are directly spilt to wastewater. These chemicals and their degradation products may cause serious problems of environmental pollution. Relevant factories have deficiencies of treating efficiently these effluents on industrial scale, particularly at higher dye concentrations and at lower energy consumptions.

Among diverse colour removal techniques, biological methods seem to be the most economic and environmentally friendly for azo dye wastewater treatment. Complete and efficient biomineralisation for these effluents can be obtained by a sequential anaerobic-aerobic process (Kalyuzhnyi and Sklyar, 2000). However, anaerobic reduction of many azo dyes can be considered as a relatively slow process. To overcome this problem, by using redox mediators, such as quinoid compounds during the reduction, anaerobic biodegradation can be enhanced resulting much higher removal rates. By immobilizing the electron mediator in the bioreactor, the problem of continuous dosing of the redox compound can be avoided resulting lower process costs. Activated carbon as a possible solid redox mediator containing surface quinonic structures, was reported to be enable to accelerate azo dye reduction (Van der Zee et al., 2003; Mezohegyi et al., 2007a).

To our knowledge, packed bed-type reactors using biological activated carbon (BAC) system have never been applied for anaerobic azo dye decolourisation by other authors. In our previous study (Mezohegyi et al., 2007a) the results cleared that comparing to other continuous and biological processes treating azo dyes, continuous upflow packed-bed reactor (UPBR) with BAC seemed to be one of the most effective systems for anaerobic azo dye biodegradation. It was also found (Mezohegyi et al., 2007b) that the application of special stirring in the carbon bed resulted in an increase of azo dye bioconversion compared to unstirred reactor system with ensuring high dye degradation rates at very short space times/hydraulic residence times.

In recent study the anaerobic decolourisation of an acid azo dye in continuous upflow stirred packed-bed reactor (USPBR) was investigated. Different decolourisation models were compared and the effects of initial dye concentration on biodegradation were discussed.

The azo dye Orange II (O-II) was selected as the model colourant. The electron donation for azo reduction was ensured by sodium acetate. The reactor and the biological activated carbon system used in this study were established according to the procedure given by Mezohegyi et al. (2007b). Quantitative determination of Orange II and acetate in the outlets was done by HPLC on a C_{18} Hypersil ODS column.

The upflow stirred packed-bed reactor provided reproducible data to make kinetic modeling of O-II biodegradation possible. A kinetic model with a specified reaction rate was developed involving both heterogeneous catalysis and biological decolourisation in the reactor. First-order, autocatalytic and Michaelis-Menten kinetics were all found to give good fittings to experimental points of dye conversion at lower inlet dye concentration. Michaelis-Menten (MM) kinetics was especially expected to describe dye biodecolourisation since it is a biological process and, also, the amount of consumed acetate by bacteria – providing the electrons to azo reduction – is directly proportional to dye conversion. Indeed, MM kinetics seems to be applicable for modeling Orange II degradation in our reactor system (Figure 1).

On the other hand, the MM model set before showed significant deviation from experimental points at initial concentration of 300 mg L^{-1} (Figure 2), thus suggesting that Orange II posesses concentration-dependent inhibition effects to the biomass at higher initial concentration. In addition, processing at very low hydraulic residence times together with high initial dye concentration resulted in toxicity to bacteria.

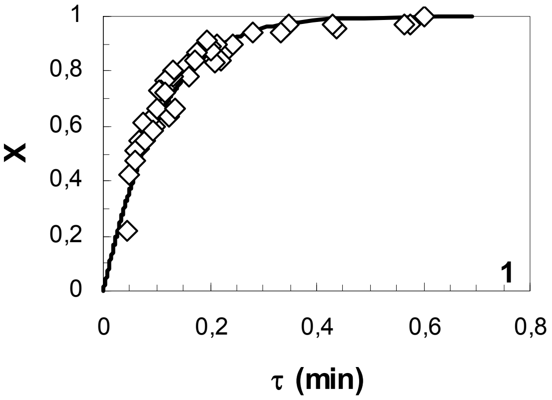

Figure 1. Kinetic modeling of Orange II anaerobic biodegradation in upflow stirred packed-bed reactor: Orange II conversion (X) in the function of space time (τ, min); line shows the fitting to Michaelis-Menten model.

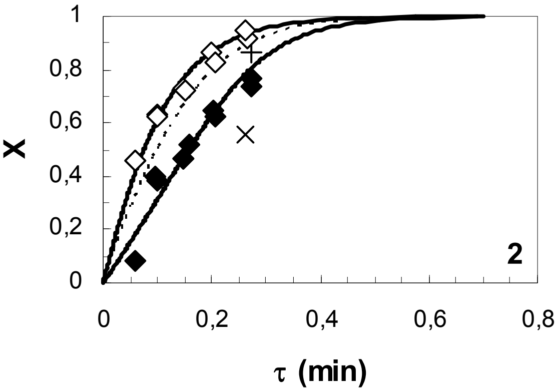

Figure 2. Substrate inhibition and toxicity effects during Orange II decolourisation in upflow stirred packed-bed reactor: (◇) shows repeated experimental points with initial O-II concentration of 100 mg L^{-1}; (◆) shows experimental points with initial O-II concentration of 300 mg L^{-1}; (×) shows O-II conversion, 1 day after biomass toxicity; (+) shows O-II conversion, 6 days after biomass toxicity; dotted line represents the Michaelis-Menten model at initial Orange II concentration of 300 mg L^{-1} supposing no substrate inhibition; continuous lines show the fitting to Michaelis-Menten model with substrate inhibition at initial O-II concentrations of 100 and 300 mg L^{-1}.

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Photocatalytic paint production for the abatement of nitrogen oxides

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Keywords: NOx, photocatalysis, $TiO_{2,}$ photocatalytic paint

Among the years people has recognized the importance of living in equilibrium with the environment. The sustainable development and the environment are nowadays hot topics all over the world and one of the major subjects with which people are concerned about. However, an unbalanced attention is being given to the causes and consequences of pollution. Carbon dioxide has a high impact on the greenhouse effect and has been chosen to be the indicator of it, somehow neglecting other equally important indicators such as nitrogen oxides (NOx) emissions. Moreover, in several EU countries the average urban NO_2 concentration is still above 0.02 ppm, the maximum allowable level in Europe after 2010 (EUROPA - Environment, 2007). In this way, new technologies must be developed in order to reduce the emissions and perform the abatement of the atmospheric NOx.

Photocatalysis appears as one of the alternatives for NOx abatement using free and clean solar energy. Recently, Maggos et al. (2008) reported 37 to 82 % NOx decrease in urban streets by using mortars incorporating titanium dioxide. On the other hand, our research group has recently suggested the use of decorative paint coatings for the photo abatement of atmospheric NOx (Mendes et al., 2007).

In the present communication we compare the performance of most of the commercially available photocatalysts (based on titanium dioxide), as well as synthesized new ones, on photooxidizing NO, when incorporated in a water-based paint coating. The studies were performed in a experimental set-up suitable to operate according to the relevant standard ISO 22197-1:2007(E) – figure 1. For the best performing photocatalysts, it is discussed the role of the temperature, relative humidity, light intensity, NO inlet concentration, photocatalyst concentration in the paint, pigment volume concentration (PVC) of the hosting paint and activation procedure, on the conversion of NO and selectivity during oxidation.

It is concluded that using a paint with a PVC concentration above the critical value, hosting 10 wt.% of photocatalyst, it is possible to photo oxidize more than 50 % of NO in the conditions described in standard ISO 22197-1:2007(E).

The synergy observed between photocatalysts and the coating paints can contribute to the effective abatement of the atmospheric NOx to values below the allowable limit and the involvement of the paint industry in this problematic.

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Figure 1 – Experimental set-up for studying the NOx photocatalysis. This unit is suitable for reproducing the operating conditions described in the relevant standard ISO 22197-1:2007(E).

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Starch filter-cake enzymatic degradation

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Keywords: starch filter-cake removal, openhole conditions, well completion

Horizontal wells are increasingly used to maximize well productivity since they are more efficient and cost effective to drain hydrocarbons from the underground. Successful well drilling systems usually comprise natural polymers like starch as the main component of drill-in fluids. Drilling fluid contact with the productive zone causes formation damage and reduces the productivity by fluid invasion in the borehole wall. During the drilling operation, the polymer deposit as a filter-cake on the wellbore wall in permeable formations as a result of filtration into the rock pores. The mainly function of such filter-cake is limit the leak-off of drilling fluid and filtrate into the formation. In many well completions, before prodution or injetion could start, filter-cake removal is a demand and it can be done by mechanical or chemical procedures (Hanssen *et al.*, 1999).

The filter-cake removal process reduces significantly the formation damage and improves the oil production (Queiroz, 2006). A traditional approach to well clean-up is the application of acids or strong oxidative solutions. However, these compounds are non-specific and can react with most species that are acid soluble or oxidizable, including formation walls, lines, tubulars, hydrocarbons, etc. Other undesirable side effect is the permeability damage by re-precipitation of dissolved components (Hanssen *et al.*, 1999).

Thus, enzymatic preparations are an alternative to filter-cake removal. First applications in oil industry were as blends of few generic hydrolytic enzymes. Enzymes are very specific catalysts, inherent environmental friendly and can be used to hydrolyse filter-cake main polymer, as starch (Batttistel *et al.*, 2005).

In a previous paper, Kameda *et al.* (2007) demonstrated that a commercial α-amylase solution were thermostable under determinated completion conditions (65^oC in brine solution - NaCl 3.5M or 203.57g/L), but at 80^oC some reduction in amylolytic activity occurred and at 95^oC no activity remained.

In this work, two enzymatic formulations, a filter-cake enzymatic breaker were also investigated and its performance was compared to the previously reported commercial α-amylase face to their starch hydrolysis capability under Brazilian wells conditions (65 or 80^oC in brine solution).

A kinetic study of both products was carried at 65ºC and 80ºC and the results demonstrated that they were able to hydrolize starch in the assay conditions. The enzyme preparations seem to behave according to Michaelis-Menten kinetics and the kinetic parameters (Km and Vmax) for both products were obtained under well operation conditions (65ºC and 80ºC in brine solution). Results showed a mixed-type inhibition in brine solution affecting both Km and Vmax values for the enzymatic formulations (Segel, 1993). High salt concentration in brine solution probably is the main factor to reaction velocity decline.

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Synthetic unconsolidated sandstone samples (Figure 1) were prepared appropriately accordding the method described by Queiroz (2006) to simulate a natural formation reservoir from typical deep oil field at Campos Basin (Rio de Janeiro-RJ, Brazil). Filtercakes were made in the top these unconsolidated sandstone plugs and after mechanical removal of the external filter-cake, the plug was inserted into a HPHT (high pressure high temperature) cell (Figure 2). A 5% (w/v) enzymatic solution at 65°C passed through the plug and internal filter-cake degradation was evaluated. Both enzymatic products were able to hydrolyse starch in the internal filter-cake, leading glucose and maltose as reaction products. Filtration rates were measured for each solution assayed and the filter-cake enzymatic breaker presented a higher filtration rate than the commercial α-amylase.

Enzyme products should contact the polymer into the filtercake to clean-up the rock pore. This contact could be established by enzyme flowing into the cake with the help of additives that could act as a surfactant. Filter-cake enzymatic breaker showed emulsification activity (28.57%) while the commercial α-amylase did not present any emulsification activity. Such result indicates that probably the enzymatic breaker attained a better self-diffusion into the filtercake, leading to such higher filtration rate.

Figure 1.Synthetic unconsolidated sandstone plug

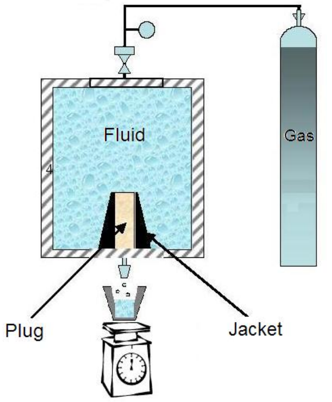

Figure 2. HPHT filtration system

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Robust multi-objective cascade controller for an anaerobic digester

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Keywords: Anaerobic digestion, ADM1, control, modeling, wastewater treatment.

Anaerobic digestion processes consist of a set of conversions of organic matter into methane and carbon dioxide, occurring in absence of oxygen (Lettinga, 1995). The use of methane as an energy source, the low growth of sludge and its ability to treat wastewater with medium to high organic loads are some of the reasons why the anaerobic wastewater treatment (AWT) has gained importance in the last few decades (van Lier et al., 2001). However, the AWT has classically been regarded as a difficult process to be controlled and managed. In this sense, the ideal control system must fulfill different characteristics for assuring altogether: (a) high quality of effluent; (b) maximum methane production; (c) general stability criterion; and (d) applicability to different types of wastewaters.

In the literature different process variables and promising controllers have been proposed using different control schemes and techniques. Most of the work has been done through two EU funded projects (AMOCO – Dochain et al., 2000, and Telemac – Bernard, 2005). However, this controllers lack a general stability criterion and portability for application to large scale and different types of wastewater. Moreover, they normally only consider effluent quality as an operational criterion.

In this work, a new multi-objective control strategy based on volatile fatty acids (VFA) and methane flow rate (Q_{CH4}) is derived and validated for an upflow sludge bed-filter reactor, which used for the anaerobic treatment of industrial wine distillery wastewater.

This approach is a novel one in the sense that: (i) it considers two operational objectives in the same simple control structure, the control on the effluent quality and the control on the maximum production of the methane gas; (ii) it takes advantage of the difference between the dynamics of the liquid and gas phase, using variables of both phases; and (iii) the selection of variables takes into account its applicability to different types of wastewaters.

The control system is a new multi-objective controller with cascade structure and a reference signal for methane flowrate. Other few authors have used cascade configurations successfully for controlling anaerobic digesters (Alvarez-Ramirez et al., 2002; Liu et al., 2004). Furthermore, in both cases the selection of variables doesn't take advantage of the different dynamic of the different phases (in this case gas/liquid phases) and only considers a single operational objective.

The controller designed is a MISO system. It acts increasing or decreasing the feed flow rate (Q) by an amount equivalent to ΔQ (delta feed flowrate). It requires two inputs or measures, the methane flowrate (Q_{CH4}) and the concentration of volatile fatty acids (VFA) expressed as acetic. The combination of VFA and Q_{CH4} gives us information about the occurrence of an imbalance situation in the AWT process. The variable of the liquid phase (VFA), which has a slower response, is used as a control variable in the external control loop, and against the variable in the gas phase (Q_{CH4}), due to its rapid response, is used as a control variable in the inner loop.

The feed flowrate rate (Q) is constrained by a saturation function (according to physical restrictions in the AWT plant). The minimum value for feed flow rate is zero, while the maximum was established using the criterion of minimum hydraulic retention time (HRT) of 9 hours for preventing wash-out of biomass. Furthermore, the feed flowrate speed of change (ΔQ) was limited for avoiding sudden changes to initiate the controller or whether the process reaches far away from the validity range considered for the design of the controller. Additionally, a dead band was implemented for avoiding oscillations due to the noise produced by the nature of this bioprocess.

The developed cascade controller was tested using data generated by simulation of different operational situations with a modified ADM1: (1) automatic restart-up, after short or medium stops, (2) organic overload, and (3) organic underload. The control system was able to restart-up and, handle the organic overload and underload. Restart-up was reached in 2 - 3 days. Effluent maintained good characteristics during the restart-ups, organic underloads and overloads experiments. The pH was kept stable near a neutral value, while the IA/TA ratio was lower than 0.25, an adequate value lower than the 0.3 limit stability value.

In this contribution, a robust multi-objective cascade control for regulating anaerobic wastewater treatment was developed, optimized and validated by simulations carry out with an ADM1 virtual plant. The proposed scheme could be improved with a supervision system and alkalinity control system.

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Fenton's treatment of aqueous phenol effluents in presence of anionic surfactants

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Keywords: Fenton process, phenol, anionic surfactants, micelles, wastewater treatment

Chemical process industry produces large amounts of polluted waters which need to be treated before being released to the nature or re-used. Typically, biological treatments are preferred as they achieve adequate abatement of organic compounds at low cost. Nevertheless, many organic contaminants, such as phenol, are biorefractory to biological treatments. Thus, phenolic effluents cannot be directly treated in a conventional biological wastewater treatment plant. In these cases, an effective pre-treatment step has to be applied prior to the biological treatment. However, it is not needed to totally destroy the biorefractory organic contaminants because the cost of the overall treatment would unacceptably increase. Hence, an effluent has to be treated just for providing enough biodegradability to allow the further biological treatment to successfully perform.

Physical methods, such as adsorption (Mukherjee et al., 2007), nanofiltration (Van der Bruggen et al., 1999), emulsion liquid membranes (Park et al., 2006) among many others can be employed as effective pre-treatments for removing phenol from wastewaters. However, physical methods only transfer the organic contaminant from one effluent to a secondary liquid or solid waste. In addition, physical methods often need regeneration or cleaning cycles. Thus, the environmental problem still remains after the physical methods.

Chemical pre-treatments, such as oxidation processes, are being designed for achieving a partial mineralisation of phenolic compounds. The biodegradability of the partially-treated effluent can be controlled as the formed oxidation intermediates and their concentrations depend on the operating conditions. Thus, the pair oxidation-biological treatment is an attractive and feasible option for dealing with phenolic effluents (Suárez-Ojeda et al., 2007).

Advanced Oxidation Processes (AOP's) are a group of chemical pre-treatments whose common characteristic is that a very powerful and non-selective radical, the hydroxyl radical ($OH\cdot$), is formed. Among them, it has been previously demonstrated that Fenton process is able to effectively degrade phenolic compounds at room temperature and atmospheric pressure (Chamarro et al., 2001) using the well-known Fenton liquor, i.e. ferrous ions (soluble salts) and hydrogen peroxide. The fast interaction between these two species leads to the formation of hydroxyl radicals, which then attack organic contaminants at different extent.

The Fenton process has been also used for the partial treatment of other organic pollutants such as dyes (Lodha and Chaudhari, 2007), chelating agents (Ghiselli et al., 2004), pharmaceuticals (Esplugas et al., 2007) and so on. However, no attention has been paid in the effect of the presence of a secondary organic product on the degradation efficiency of an easy-to-degrade organic contaminant, such as phenol.

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The aim of this work is to study the effect of an anionic surfactant (sodium dodecylsulfate, SDS) on the degradation of phenol by the Fenton process. In all the tests, carried out in a batch reactor, the initial concentration of phenol was 2500 mg/L and the solution pH was not adjusted. The bulk concentrations of ferrous ion (iron sulfate) and hydrogen peroxide ranged from 7 to 56 mg/L and from 2500 to 17500 mg/L, respectively. Therefore, the effect of both the catalyst and the oxidant concentration on the degradation of phenol was explored in both absence and presence of SDS. As surfactants are molecules having a hydrophobic head and a hydrophilic tail, they group forming micelles above its critical micelar concentration (cmc), which is 2300 mg/L for the SDS. All the experiments showing the effect of the catalyst and the oxidant concentration in presence of SDS were performed at a SDS concentration equal to its cmc. The conversion of phenol always decreased when SDS was present, regardless the iron and hydrogen peroxide concentrations. For instance, phenol conversion decreases about 20% when SDS was present at its cmc and the iron and hydrogen peroxide concentrations were 7 and 12500 mg/L, respectively. The phenol conversion decreases in presence of SDS could be related to the competence of SDS for the hydroxyl radicals or to the fact that SDS anionic micelles could attract the iron ions, lowering their availability to interact with the hydrogen peroxide molecules.

On the other hand, to test the effect of the morphology of the SDS (micelar or monomeric form), experiments at several SDS concentrations (from 577 to 3170 mg/L) were also conducted. The concentrations of iron and hydrogen peroxide were fixed at 7 and 12500 mg/L, respectively. The results show that the higher the SDS concentration, the lower the phenol conversion up to its cmc. At SDS concentrations higher than the cmc, the phenol conversion remained invariable. Thus, not only a possible degradation of the SDS molecules can occur, as previously stated, but also the morphology of the surfactant could affect both the phenol degradation and also the SDS degradation. In fact, the hydrophobic core of the micelles could host the phenol molecules, thus preventing its degradation.

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Electrochemical ozone production for *in situ* atrazine degradation in aquifer

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Keywords: underground water, remediation, electrooxidation, ozone, atrazine

Introduction

The impact of pesticides on the quality of underground waters has been subject of scientists and public health concerns in the entire planet, especially in the areas where the underground waters is mainly used for human consumption. The intensive pesticide use in the agriculture and the high persistence of many of them has required a rigorous control of possible environmental contaminations, especially of underground waters and other drinking waters sources.

Atrazine (2-chlorine-4-etil-6-isopropil-1,3,5-triazin), is a herbicide that continues to be used extensively in Brazilian agriculture, specifically for corn, cotton, sorghum and sugar-cane. It is one of the largest pollutants of groundwater in many countries (Dörfler et al., 1997) and was selected for study because of its widespread use.

Atrazine is frequently detected in aquatic waters, and has been known to affect reproduction of aquatic flora and fauna, which in turn impacts on the community structure as a whole (Graymore et al., 2001).

The *in situ* chemical oxidation technology uses chemical oxidants that directly destroy dissolved contaminants in a subsurface medium. Because the process is *in situ*, no surface treatment system for the contaminants is needed and there is no treated groundwater to be discharged.

Ozone is a powerful oxidant (e^0 = 1,51 V *vs* Standard Hydrogen Electrode Potential) that does not produce toxic byproducts when is decomposed and has been used in degradation of organic compounds present in soils and aquifers.

The technology of reactive permeable barriers (RPBs) is one of the remediation alternatives of greater acceptance. This technique has some advantages, such as: is an *in situ* method; it is adequate to treat persistent pollutants in aquifers; it does not produce important physical and chemical alterations to the ground.

A new technology uses groups of electrodes as active reactive medium of the RPB to treat contaminated groundwater. Electrolytic reactive barriers (e-barriers) consist of closely spaced permeable electrodes installed across a groundwater contaminant plume in a permeable reactive barrier format (Wani et al., 2006).

The electrolytic reactive barriers methodology can be used in combination with the advanced chemical oxidation technology *in situ* for the treatment of pollutants, that is, an oxidant (ozone) could be electrochemically produced in one of the electrodes that is part of the electrochemical barrier. Pollutants of interest are transformed by the generated ozone instead of being transformed directly on the surface of the electrode. Therefore an *in situ* degradation system constituted by an electrochemical cell that generates an oxidant substance which transforms the groundwater pollutants is created.

A study on the evaluation of the effectiveness, in a laboratory scale, of this in situ new treatment technique for atrazine is being conducted. The work is divided in three main stages: 1) electrochemical ozone production in an electrochemical cell at different values of applied current as well as the determination of the ozone dissolved concentration; 2) degradation of the atrazine in aqueous solution in order to obtain depletion curves of contaminants at different electrolysis current; 3) degradation of the atrazine in horizontally mounted column packed with quartz–feldspar sand used to simulate the aquifer material. The results of the study will be presented in this conference.

Materials and methods

The electrochemical cell used for the determination of electrochemical ozone production, is constituted by an anodic and a cathodic compartment. The electrolytic solution is a phosphate buffer 0.1 M (pH = 7). The cell contains one β-PbO_2 coated titanium anode (3 cm^2 of area), one stainless steel cathode (10 cm^2 of area). The coating anode process is accomplished by electrodeposition. A stream of nitrogen gas is bubbled into the reservoir removing the ozone to two bottles gas washing containing a potassium iodine solution. Ozone production is measured iodometrically.

Determination of ozone dissolved is made in a one-compartment cell. The electrolysis cell is cooled by water circulating around it. The electrodes and electrolytic solution are the same. The volume of solution is 150 cm^3. The ozone dissolved concentration is measured iodometrically and by Indigo Method.

The degradation experiments in batch conditions are made in the same one-compartment cell, and keeping the electrolyte and electrodes, too. The atrazine concentration analysis was made using HPLC technique.

The column of Plexiglas (25 cm x 5 cm ID) contains a single set of electrodes. Two expanded titanium mesh coated with β-PbO_2 and RuO_2 served as anode and cathode, respectively. The electrodes are separate 2 cm. A reference electrode of $Ag/AgCl/Cl^-$ (saturated) was installed in the vicinity of the electrodes to measure electrodes potential.

Three glass sample ports allow for collection of aqueous samples for bed profile analysis. One sampling port is positioned in the middle of electrodes and the others upstream and downstream of the electrodes.

Results

The results obtained up to now show that the ozone production gets higher with the increment of the current density. The density current range is between 1×10^{3} - 9×10^{3} A x m^{-2}. The rate of ozone production is around 10^{2} g x h^{-1} for square meter for current density of 9×10^{3} A x m^{-2}.

In addition, for all density current values considered in the dissolved ozone experiments (0.5×10^{3}, 1×10^{3}, 2×10^{3} A x m^2), the dissolved ozone concentration grows with time, remaining constant beyond a certain time (around 40 minutes). The constant value of dissolved ozone concentration was greater for current higher. A molar concentration of 3.5×10^{-5} mol x L^{-1} of dissolved ozone is attained after 40 minutes when the density current is 2×10^{3} A x m^2.

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Catalytic ozonation applied to the treatment of coloured effluents

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Keywords: ozonation, catalysis, activated carbon, cerium oxide, textile dyes

Introduction

Wastewater from spent dyebaths and dye rinsing operations in the textile industry contain unfixed dyes and are frequently strongly coloured. Besides the undesirable aesthetic impact caused in receiving waters, the persisting colour and the non-biodegradable nature of most of the dyes represent serious problems to the environment. The conventional association of biological and physical-chemical processes results in the removal of part of the organic content of textile wastewaters. However, the resulting effluent is still fairly coloured. Ideally, a final refining treatment, such as oxidation with ozone, is required for total decolourisation and extensive elimination of organic content, which is imperative for the eventual reutilization of water. In order to enhance the efficiency of the ozonation processes, new methods combining ozone with hydrogen peroxide, UV radiation or solid catalysts, such as activated carbon (Faria et al. 2005; Faria et al. 2008) or metal oxides have been investigated. The development of highly effective catalysts for the application in such processes and the understanding of the inherent mechanisms are of great importance. In the present work, the results of the catalytic ozonation of three commercial textile dyes are presented. An industrial effluent representative of a global effluent from a textile plant, collected after a conventional activated sludge biological treatment, was used as a case study.

Material and Methods

Three commercial textile dyes from different classes (CI Reactive Blue 5, CI Reactive Yellow 3 and CI Acid Blue 113), with known molecular structure, were selected for this study. The experiments were carried out at the natural pH of the solutions. The concentration of each dye in solution was followed by UV/Vis spectrophotometry. The mineralization of the solutions was followed by measuring the total organic carbon (TOC).

Cerium oxide (Ce-O) was prepared by precipitation from the corresponding nitrate aqueous solution ($Ce(NO_3)_3.6H_2O$). A composite of activated carbon/cerium oxide (sample AC_0-Ce-O) was prepared by a similar procedure used for the preparation of sample Ce-O, where a given amount of activated carbon (AC_0) was dispersed in the cerium nitrate solution before precipitation. The textural characterization of the materials was based on the corresponding N_2 equilibrium adsorption isotherms. Cerium containing samples were characterized by XRD, XPS and SEM.

Results and Discussion

The kinetic experiments of catalytic ozonation of the selected dye solutions at the initial concentration of 50 mg/L were performed in a laboratory scale reactor. Both single ozonation and adsorption on activated carbon were also carried out for all the compounds. Under the experimental conditions used in this work, activated carbon was not capable of removing the colour of the solutions efficiently. In fact, some dyes, such

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as the CI RY3, have a very low affinity towards activated carbon. On the other hand, total decolourisation is easily achieved by ozonation. The oxidation of dye molecules leads to the formation of oxidation by-products, which are resistant to direct ozone attack preventing complete mineralization. This accounts for the high efficiency of ozone for colour elimination but considerable less efficiency in the removal of TOC. Generally, catalytic ozonation notably enhances the extent of mineralization. However, no major improvements are attained in terms of colour removal, as this is achievable by direct ozone attack to the chromophore groups of the dye molecules.

Figures 1 and 2 show the kinetic data corresponding to both colour and TOC removal for a solution of 50 mg/L of CI RB5. As depicted in Figure 1, the disappearance of the visible band of the spectrum is achieved in ca. 5 minutes. The simultaneous use of ozone and activated carbon allows a significant improvement in the mineralization of the solutions and best results are achieved with cerium containing catalysts (Figure 2).

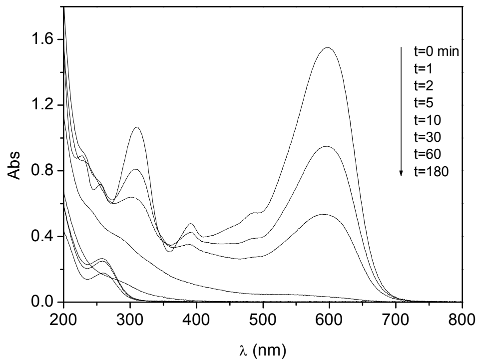

Fig.1 – Evolution of the UV/Vis spectra of CI RB5 during ozonation (C_0 = 50 mg/L, $CO_{3\,in}$ = 50 g/Nm^3).

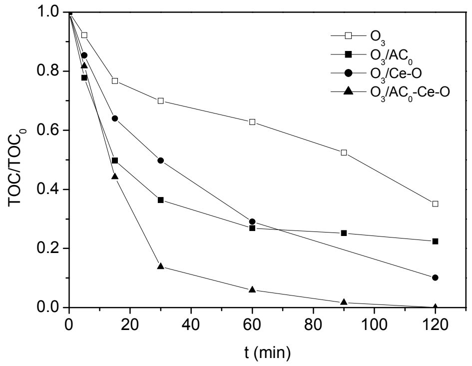

Fig. 2 – Evolution of dimensionless TOC of CI RB5 during non-catalytic and catalytic ozonation (C_0 = 50 mg/L, catalyst = 0.5 g/L).

After 30 minutes of ozonation of the CI RB5 solution, a TOC removal of 30 % is accomplished. The addition of activated carbon increases the removal of organic matter to ca. 64 %, while the composite containing cerium oxide allows a TOC removal of 86 %. The latter catalyst allows the nearly total mineralization of the solution for longer reaction times. Similar results were obtained with CI RY3 and CI AB113.

The results obtained in this study show that cerium oxide catalysts are effective ozonation catalysts for the mineralization of textile dyes. Comparatively to the performance of the commercial activated carbon, an enhanced mineralization extent was achieved. A strong synergic effect was observed between activated carbon and cerium oxide in the prepared composite, leading to enhanced mineralization degrees for all the studied dyes. The mechanism of the ozonation catalyzed by the ceria-activated carbon composite is believed to comprise both surface reactions, similar to what occurs with activated carbon promoted ozonation, and also liquid bulk reactions involving $HO^\bullet$ radicals, resultant from the catalytic decomposition of ozone on the surface of the activated carbon and mainly in the presence of cerium oxide.

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Ion exchange membrane bioreactor modelling for removal of anionic micropollutants from drinking water supplies

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Keywords: Drinking Water treatment, Ion Exchange Membrane Bioreactor, hybrid modelling, nitrate and perchlorate removal, design of experiments

The Ion Exchange Membrane Bioreactor (IEMB) is a patented process that combines a membrane process and biological reduction (Crespo and Reis, 2003). This process arose from the necessity of finding a technology able to remove anionic pollutants from water streams, avoiding both the formation of a concentrated brine and secondary contamination of the treated water. The IEMB combines the transport of the charged pollutants through a dense ion exchange membrane with the simultaneous biodegration by an anoxic microbial culture in a separated compartment (biological compartment). The membrane is positively charged, allowing the transport of anions like nitrate and perchlorate. Once in the biological compartment these anions are reduced to innocuous species, respectively nitrogen and chloride. Using this technology, no brine is produced and, due to a physical separation of the water and biological compartments, no secondary contamination of the treated water occurs. The transport across the membrane is governed by Donnan dialysis principle, using chloride as the driving counter-ion. Therefore, the target ions transport can be improved using an excess of chloride in the biological compartment.

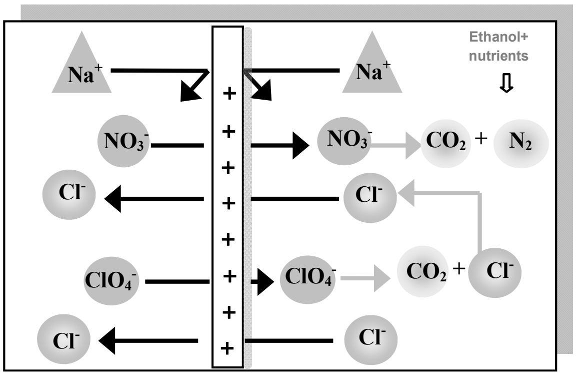

Figure 1: Shematic diagram of ion transport and bioreduction in the IEMB

The flux across the membrane can be described by a mechanistic model combining the driving force and the overall resistances to mass transfer (Velizarov *et al*, 2003). This model gives accurate predicted fluxes based on the physicochemical and hydrodynamic conditions. This model is based on the assumption that the overall process is controlled by transport step(s), meaning that biological conditions should be ideal for the effective bioreduction.

This study is focused on optimizing the IEMB process regarding the biomedium composition and biocompartment operating conditions. This optimization aims at increasing the economical viability of the process and minimizing the biomass produced. To fulfil this aim, the main variables affecting the biological compartment were identified. Seven factors were selected and, in order to design a proper number of experiments, a statistical design of experiments was used. The Plackett-Burman design is used for screening proposes since it used an orthogonal matrix in order to decrease the number of experiments to be performed. The results obtained cover a wide range of conditions under which the mechanistic model was not able to correctly predict the transport fluxes of the target pollutants. Therefore, the data obtained in these experiments were further analyzed by chemometric techniques, which use mathematical and statistical methods to obtain relations between data sets.

Due to intrinsic metabolic complexity and to non-linear kinetics of the biological activity and transport phenomena, the modelling of the IEMB can be extremely difficult. Therefore, in this study, a hybrid semi-parametric model (Azevedo *et al*, 1997) was adopted that combines mechanistic and black-box models according to the structure shown in Figure 1. In this strategy, two phases of the process will be modelled, the transport across the membrane and the consumption in the biocompartment. For the membrane transport, a black-box model (BB1) compensates for the mechanistic model inaccuracies. A second black-box model (BB2) is used to identify the biological reaction kinetics.

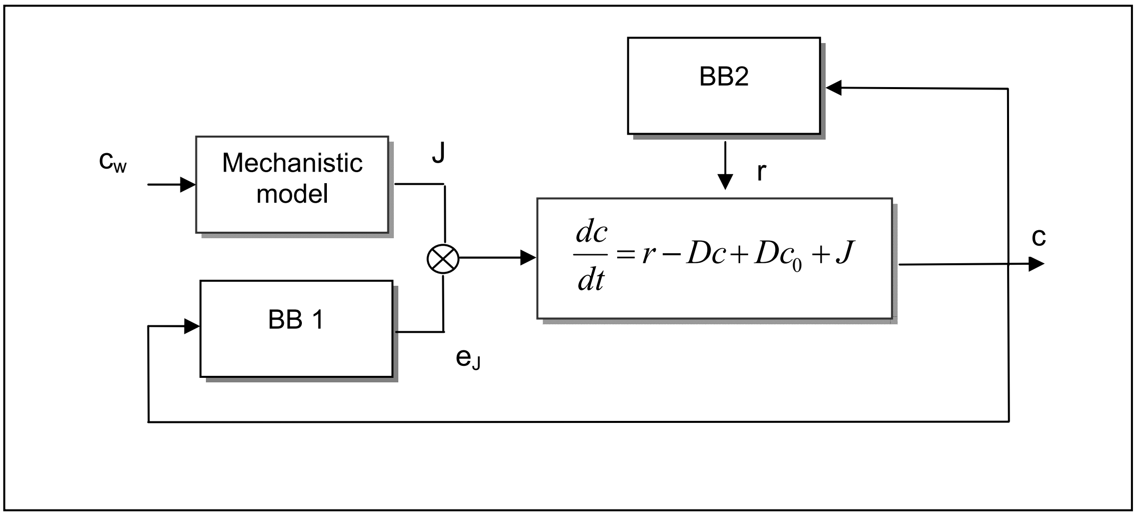

Figure 1: Hybrid model structure (c_w: ion concentration in water, e_j: transport kinetics residual)

This approach, based on hybrid structure, has the advantage of requiring less data than purely black-box models (Teixeira *et al*, 2006). The back-bone of the hybrid model consists of the dynamic material balance equations of the biological compartment, which account for the biological reaction kinetics (r), transport kinetics (J) and dilution terms (Dc_0-Dc). This global model can be used to predict the system performance, and furthermore, to be used in operational parameters optimization of the IEMB in the two compartments

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Performance comparison of biological and Fenton processes for treatment of urban landfill leachates

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Keywords: Biological treatment, Fenton process, landfill leachate, COD degradation

The water resources are becoming a serious concern nowadays and have contributed to motivate the development of processes and specific technology related to the treatment of effluents for allowing subsequent reuse of water.

The urban landfill leachate is usually considered as a refractory effluent, mainly due to the presence of different types of specific substances (e.g, xenobiotic organics compounds). Moreover, the composition of this effluent may be highly variable and depends on variables, such as the phase of degradation of the solid waste and the amount of the percolation water in landfill (Slack et al., 2005). The non-steady state conditions in the landfill, and in particular the flow rate variations of the leachate, as well as its composition leads to additional difficulties in the treatment systems.

Currently, the landfill leachate is usually treated by biological aerobic process mainly using activated-sludges systems (Renou et al.2008). The efficiency obtained with this type of treatment may be very good, unless when toxics or refractory components, are present in the effluent (Loukidou and Zouboulis, 2001). The Fenton process is an Advanced Oxidation Process (AOP) where the decomposition of hydrogen peroxide catalysed by an iron salt leads to the formation of hydroxyl radicals, which ones are very active species and allows the degradation of organic matter. Fenton reagent has the advantage of promoting the oxidation of toxic compounds. Nevertheless, small organic species, such as small chlorinate alkanes, parafines and small carboxylic acids may be very stable even in the presence of the oxidising agent.

This work compares the biological and Fenton treatment of the landfill leachate in which the effluent degradation by means of COD reduction is examined. Leachate samples collected in an urban sanitary landfill from the centre region of Portugal were used. The laboratorial biological experiments were performed in a batch reactor of 1L and the oxygen concentration was kept nearly constant in order to assure aerobic microbial growth. Six samples with different compositions were used, being the most important parameters monitored for evaluating the microorganisms activity, temperature and pH. The organic matter degradation of leachate was evaluated every 12 hours through the Mixed-Liquor Suspend Solids (MLTSS), the Mixed-Liquor Volatile Suspend Solids (MLVSS) and Chemical Oxygen Demand (COD). The experiments with Fenton process were also carried out in a batch operation mode using a Jar-test system providing a 200 rpm stirring speed with baffles paddles. A design of experiments was used in order to optimize the process conditions regarding the pH, concentration of hydrogen peroxide and concentration of iron. During the reaction, the COD was measured for evaluating the organic matter degradation and at the end also TSS and TDS were determined.

For comparing both treatments, the initial and final COD at the end of each treatment were represented in Figure 1 and 2, where the percentage values indicate the reduction in these parameters. From these Figures, it is noticeable that the largest removal occurred for the biological treatment (70% reduction maximum) against 27% for Fenton process. However, that the reaction time is much longer in biological treatment (order of days) while for Fenton process only a few minutes are necessary for partial chemical oxidation. These results were expected taking into account the characterization of landfill leachate tested. In fact, the effluent is characterized by high biodegradation level (high BOD_5/COD), thus one can conclude that the biological process is suitable for the treatment of this effluent. In this case, the Fenton process may be used in an integrated approach. At industrial level, the efficiency of the system of activated sludges is not enough for discharging the effluent into the environment. From our study, it seems that the Fenton process may be used after the leachate to be pre-treated by the biological process.

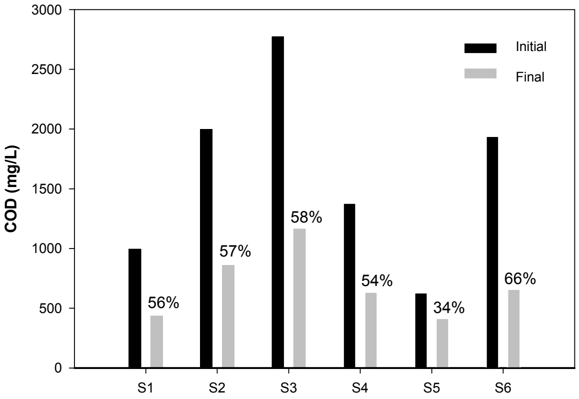

Figure 1 – COD removal for samples S1 to S6.

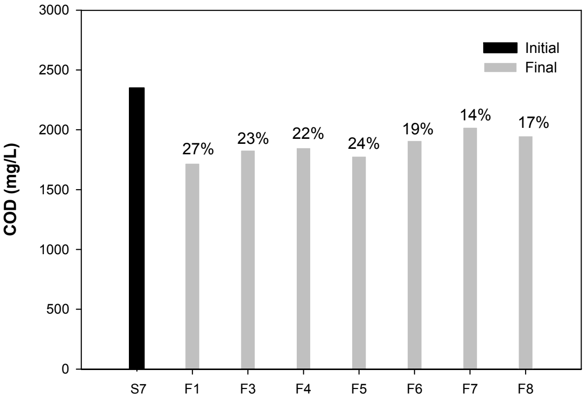

Figure 2 – COD removal for sample S7 and for experiments F1 to F8.

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Design and Construction of a Lab-Scale Simulated Moving Bed Unit. The FlexSMB-LSRE – from Theory to Practice

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Keywords: Simulated Moving Bed, Chromatography, Adsorption, Sustainable technology, Separation, Equipment development and Process intensification.

It is widely accepted nowadays that process innovation should be based not only on the challenges, problems, and needs but also on the social, political, and cultural conditions prevailing in the prospective market. Concepts as Sustainability or Sustainable Development (Brundtland report [1]), the problematic of resources depletion and eco-efficient techniques have to be taken into account. The reduction or renouncement of organic solvents represents not only the trend of the so-called green chemistry but also a sustainable, environmentally friendly production of chemical products that accounts for the economical as security (social) aspects.

The use of continuous chromatographic counter current operation modes maximizes the mass transfer driving force, providing a better utilization of the adsorbent as solvent consumption than the traditional batch mode. Nevertheless, in this counter current operation (so-called TMB-True Moving Bed), it is necessary to circulate not only the fluid phase but also the solid. The solid motion has some disadvantages (attrition, lack of efficiency, fluid velocity limited by fluidization phenomena, etc.) and from a technical point of view, is not easy to implement.

The solution to this major problem was first proposed through an inventive way by Broughton and Gerhold (1961) [2]; using simple fixed bed columns the solid movement is simulated by a synchronous shift of all inlet and outlet ports in the direction of the fluid flow. This simulated solid motion lead to the Simulated Moving Bed (SMB) concept firstly implemented by the UOP Inc.

Until the 90s the major implementation of the SMB technology continued to be in the petrochemical separation field with more than 100 industrial plants. Nevertheless, the application of the SMB concept to HPLC chromatographic separations in the earlier 90s, led to the second “boom” on the SMB technology applied now to the fine chemistry separations [3].

This late demand on the SMB technique has been the cause and consequence of an also emergent interest in the study and research on this particular matter, leading to the formulation of quite singular/different operation modes since the original patent. Non conventional strategies as the introduction of non synchronous inlet/outlet shifts (the Varicol process [4,5]) or the variable flux with/or variable composition in the inlet/outlet streams (the PowerFeed process [6,7]) and Modicon ([8,9]), as well as the utilization of multiple feed or distributed feed (the Two Feeds SMB in [10,11]) and the Outlet Streams Swing (OSS) [11], have increased the potential of this technique for a vast range on binary the separation field.

The practical application of the so-called “non conventional modes of operation” in Simulated Moving Bed (SMB) units is per itself a challenge. Generally an SMB unit, industrial, pilot of laboratory-scale, is limited to one or two modes of operation and to implement a new operating mode is necessary to contact the supplier, do adjustments, even to reformulate the entire unit if not to acquire a new one. Therefore, flexibility (at least at laboratory-scale) is seen as one of the more relevant qualities for this kind of

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equipment [12]. This aspect was the main reason that led to the design and construction of a 6 columns unit at LSRE, the FlexSMB-LSRE, here by presented.

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Inhibitory effect of phenolic compounds on the activity of Angiotensin I converting enzyme

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Keywords: angiotensin I converting enzyme, hypertension, phenolic compounds, grape seed

Several epidemiological studies have shown associations between the regular consumption of flavonoid-rich foods and a decreased risk for cardiovascular disease (Middleton et al., 2000; Nijveldt et al., 2001; Hung et al. 2004). Angiotensin II is a very potent chemical that causes the muscles surrounding blood vessels to contract and thereby narrows the blood vessels. The narrowing of the vessels increases the pressure within the vessels and can cause high blood pressure (hypertension). Angiotensin II is formed from angiotensin I in the blood by an enzyme, the Angiotensin I Converting Enzyme (ACE).

ACE is a glycoprotein peptidyldipeptide hydrolase, that cleaves angiotensin-I to produce angiotensin-II and also hydrolyzes and inactivates the vasodilator peptide, bradykinin to inactive peptides (Actis-Goretta, et al., 2003, 2006).

ACE inhibition is considered to be an important therapeutic approach in the treatment of high blood pressure, and the intake of certain synthetic inhibitors provides definitive positive health effects ((Middleton et al., 2000; Nijveldt et al., 2001; Hung et al. 2004). However, as ACE inhibitors are pharmacological drugs their use in healthy or low-risk populations is not advisable. The finding that certain flavonoid-rich foods can induce reductions in blood pressure and inhibit ACE activity, opens up the possibility that consumption of select flavonoid-rich foods may mimic synthetic ACE inhibitors and provide health benefits but without adverse side effects (Actis-Goretta, et al., 2003, 2006).

Grape seed (*Vitis vinifera* L.) is a well known oilseed crop containing typically between 5-10% of phenolic compounds, which may be obtained by solid-liquid extraction with methanol and acetone/water. Subsequent fractionation may be carried out by graded methanol/chloroform precipitation, producing a broad range of extracts with decreasing average degree of polymerization (DPn).

Our research team accomplished previous tasks and characterized such fractions by ESI-MS/MS (Passos et al., 2007) and thiolysis (pre-treatment) followed by HPLC-UV detection, from which the corresponding DPn values were determined. The same extracts have been also characterized by FT-IR spectroscopy within the region of 1300-800 cm^{-1} (Passos et al., 2008).

The main objective of this work is the evaluation of the impact such natural phenolic extracts have upon ACE activity. This study started with monomers catechin, epicatechin, catechin-*O*-gallate and epigallocatechin. Afterwards, fractions of natural extracts of grape seed have been also evaluated. A comparison is accomplished with captopril, a synthetic compound commonly used for the treatment of hypertension and some types of congestive heart failure.

In Figure 1, results for catechin-*O*-gallate and captopril are shown. In the case of natural extracts total inhibition of ECA activity has been surprisingly observed, for

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concentrations in the order of tens of $mg_{extract}/dm^3$. A comprehensive study for the remaining monomers and additional polymerised fractions will be presented.

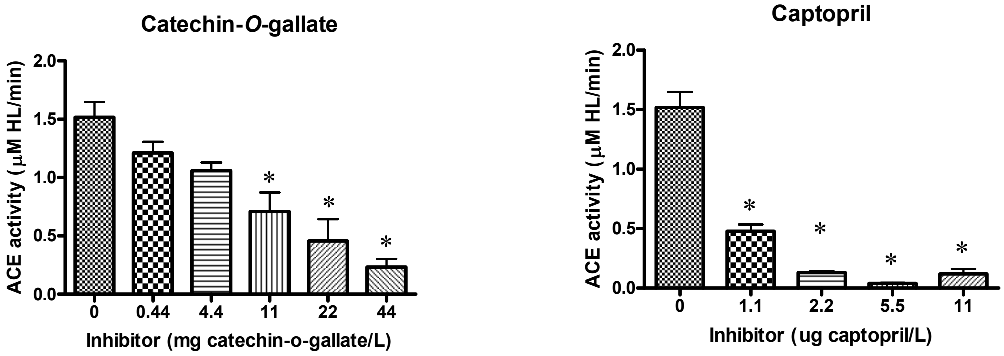

Figure 1 – Inibition of ACE activity by catechin-*O*-gallate and comparison with captopril.

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A novel view of the manufacture of polyurethane-polyurea aqueous dispersions

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Keywords: Polyurethanes, Aqueous dispersions, Solvent-free

Over the past few decades, polyurethane-polyurea aqueous dispersions (PUDs) have developed a solid reputation for high performance applications, particularly in the field of adhesives and coatings. PUDs are mostly environmentally compatible products; they are totally devoid or contain only low amounts of volatile organic compounds (VOC). This is an important feature in view of the present environmental policies where governments and internal agencies are placing emphasis on developing sustainable processes, improving work conditions and reducing emissions of toxic and polluting substances into the atmosphere. Moreover, polyurethanes are known as “tailor-made” products with properties resulting from the wide diversity of raw-materials which can be combined in different ways during the synthesis.

The industrial production of PUDs is nowadays a well established technology. A schematic representation of the process is given in Figure 1. They are produced by several companies across the world and for various applications. There are two main synthetic routes to produce PUDs: the acetone process (a former process developed by Bayer AG) and the pre-polymer process (developed as an alternative response to the acetone process). The pre-polymer process comprises several stages briefly described as follows: a macrodiol (usually a polyester or polyether) and a hydrophilising diol (the internal emulsifier) react with an excess of diisocyanate to form an isocyanate terminated pre-polymer. After neutralizing the acid groups of the internal emulsifier, the pre-polymer is dispersed in water until phase-inversion occurs. Finally, the dispersed pre-polymer is chain extended using a short diamine. If any solvent had been added during the process, for example to suppress process viscosity restrictions, the last stage will correspond to its removal (Dieterich, 1981).

The pre-polymer process, at present, is being forced to readapt due to ongoing developments, partly motivated by process constraints, raw-materials restrictions and the need to obtain a true solvent-free product. Allied to this fact it is worthy to mention the upcoming of European REACH legislation, which is expected to have a considerable influence in the PUD industry. Most of the industrially produced PUDs use dimethylol propionic acid (DMPA) as the internal emulsifier. DMPA is sparingly soluble in the reactive mixture and needs to be previously dissolved in an organic solvent. The chosen solvent must fulfil a series of criterions: dissolve DMPA, be inert towards isocyanates and be miscible in water. Additionally it must present low odour and low cost. Alternatives to the traditionally used option (methyl-2-pyrrolidone NMP) include ketones, cyclic ethers, amines and amides.

NMP has one major problem; it has a high boiling point, near 200 ºC, and thus remains in the final product. The upcoming product restrictions refer that all products containing more than 5% (w/w) of NMP will be considered toxic from June 2009 onwards (Mestach and Goossen, 2007) and at present almost all commercially available PUDs have NMP contents from 5-15 %. In this context, the NMP-free concept is gaining a

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growing importance, particularly for PUD industry which uses the pre-polymer process. Beyond this issue, the appearance of new markets for PUD, where restrictions are even more specific, such as adhesives for food contact, are justifying additional studies. In this case, besides the absence of solvents, the raw materials must be carefully chosen to avoid toxicity and noticeable smell.

Presently, there are some alternatives to achieve the NMP-free concept. Among them we may refer the direct NMP replacement by an equivalent solvent, DMPA replacement by an equivalent hydrophilising diol but with better solubility in the reactive mixture, and replacement of the macrodiol by one of the commercially available new macrodiols with incorporated ionic groups in the molecular backbone (Gertzman et al., 2007).

In recent years, our research group has been involved in the development of polyurethane-polyurea water dispersions for various applications. With this work we intend to review this theme and describe the most recent developments. Characterization of industrial dispersions (such as that shown in Table 1.) will be presented and examples of synthesis will be described. We must highlight that our experience has always been aimed at industrial applications.

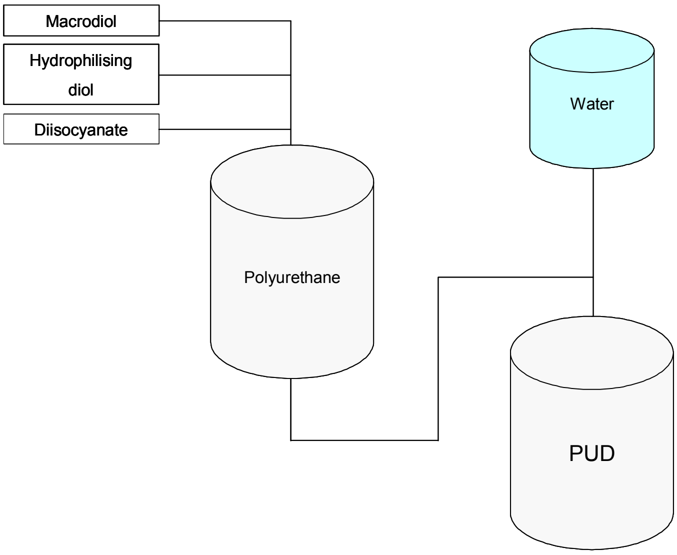

Figure 1. A process for polyurethane dispersion production (adapted from Mestach and Goossen, 2007)

Table 1. Characterization of a commercial PUD

Solid Content (%, w/w)	37.0
Viscosity (mPa.s)	40.1
pH	8.05
Mean Particle Size (nm)	
Volume distribution	114
Number distribution	109

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Constructing chemical reaction networks through decoupling, regression and rationalisation

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Keywords: Chemical Reaction Network, Regression, Stoichiometry.

One of the key issues for fine chemical and pharmaceutical companies is to reach the market with many new products as quickly as possible. Within these industries multi-purpose plants which are suitable for a variety of customer specifications are often used. This leads to fast changing discontinuous processes incorporating batch or semi-batch reactors. A major problem facing these companies is the scale up of a process from laboratory to full-scale production in the shortest possible time, preferably avoiding pilot-plant testing.

Modelling and simulation studies are often used to assist scale-up, for conventional equation-based modelling software it is necessary to formulate mathematical equations in order to describe the process dynamics. In general, the behaviour of process design variables such as flow-rates, reactor volumes and inlet concentrations of species are well understood. However, obtaining knowledge of the chemistry, in particular the chemical reaction network remains a limiting step.

Traditional methods for determining reaction networks involve postulating a number of different structures which are then fit to experimental data. The reaction network whose ordinary differential equation (ODE) model provides the highest prediction accuracy with respect to the experimental data is taken to be the correct structure. This is a time consuming procedure requiring both chemistry and modelling expertise.

From a system identification perspective, the task of identifying a useful network is one of determining the structure as well as the parameters of a set of non-linear ODEs. This is a non-trivial system identification task; however, recent work known as an incremental identification approach has been presented by Brendel (2007) and Brendel et al. (2006). This aims to facilitate the network identification problem using a stage-wise identification process that a) estimates the reaction rate terms individually using species concentration data, b) if the reaction stoichiometry is not known uses Target Factor Analysis (TFA) to test target stoichiometry and Singular Value Decomposition (SVD) to determine the number of reactions occurring (see Bonvin and Rippin, 1990) and c) given the stoichiometry calculates the reaction rates from the reaction fluxes. Brendel et al. (2006) argue that the decomposition of the identification process into stages reduces complexity and results in more robust and efficient identification strategy. Each of the identification steps provides additional information regarding the reaction network, which facilitates the selection of model candidates. Furthermore, due to this decomposition, the number of possible candidates at each stage is drastically reduced. However, as with all approaches reliant on TFA there is an assumption that the stoichiometries (or suitable target stoichiometries) are known in advance.

The primary objective of this paper is to present an alternative incremental approach to network identification. The method is based upon the use of an auxiliary data measurement, the rate of heat evolution (Q_R) which may readily be obtained from a

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reaction calorimeter. During early process development in the fine chemical industry reaction calorimeters are used for systematic and quick characterization of the relevant kinetic and thermodynamic reaction parameters. Q_R represents the sum of individual heats of reaction multiplied by the rates of reaction and may therefore be used to obtain kinetic data (see Wright et al, 1993 and Zogg et al, 2004).

The identification method proposed in this paper is split into five stages a) SVD is used to estimate the number of reactions occurring, the observed stoichiometric space and the conservation relationships[Θ] b) given the estimated number of reactions (N_R) and the Q_R data, mathematical and statistical tests are applied to reduce the number of concentration terms to a subset of N_R terms, c) multiple linear regression (MLR) is then used to determine the concentration terms that are influential on the individual ODEs, d) as each ODE is considered separately a rationalisation procedure is applied to ensure consistent chemical reaction network equations are obtained that preserve system stoichiometry and e) the isothermal rate constants are estimated and the network is constructed.

As opposed to the rationalisation strategy described in Burnham et al. (2008), the strategy here is to use topological features of the reaction network that may be obtained from the elemental matrix (particularly the atomic matrix) or process data. These topological features provide mathematical constraints that may be used to determine whether the estimated model parameters (and stoichiometries) are consistent with the atomic matrix and, given the event that they are not, constrain them to be. Finally it is checked that the (constrained) estimated model parameters (and stoichiometries) are consistent with the process data.

The method is developed using a simulated case study and, finally, a real experimental case study is used to demonstrate the technique.

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[Θ] One of the characteristics of chemical reaction networks is the conservation of molecular subgroups, termed moieties or conservation relationships.

A multi-objective optimization for the design and periodic scheduling of multipurpose facilities

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Keywords: Design, scheduling, batch, multi-objective, RTN

In multipurpose batch facilities, a wide variety of products can be produced through different processing recipes by sharing all available resources, such as equipment, raw-materials, intermediates and utilities. In order to ensure that any resource in the design can be utilized as efficiently as possible, an adequate representation is necessary in order to avoid ambiguities in the process/plant representation. The resource-task network (rtn) is one such representation, suitable for the design of multipurpose batch plants, as suggested by pinto et al (2003), which was later adapted to the design of multipurpose batch facilities (pinto 2005).

Sometimes this type of facilities is operated under conditions of relatively stable production demands, over extended periods of time. In such situations it is profitable to establish a regular periodic operating schedule, in which the same sequence of operations is carried out repeatedly, thus simplifying the operation and control of such facilities. Pinto (2005) presented a generic and detailed formulation for the optimal design and retrofit of multipurpose batch facilities operating in a periodic mode. Later on, castro (2003) presented a discrete and continuous time formulation for the optimal periodic scheduling of batch plants using rtn.

Like most real-world problems, the design of multipurpose batch facilities involves multiple objectives, while the existing literature on design has been mainly centred on mono-criterion objectives (Barbosa-Povoa, 2007). Therefore, the multi-objective optimisation is a modelling approach that requires further study when applied to such problems, in order to enable the resulting models to act as potentially powerful decision making tools handling different decision trade-offs. Decision makers can arrive at an optimal solution by weighing up the values of different objectives at the Pareto-optimum surface, i.e., by pondering how much one objective is worth in relation to the other.

In this work, the detailed design of multipurpose batch facilities with periodic mode of operation, proposed by Pinto et al. (2005), is extended with the incorporation of economic aspects, which are targetted as the multi-objectives within an optimization approach.

This allows the identification of a range of plant topologies, design facilities and storage policies that minimise the total cost of the system, while maximizing production subject to total product demands and operational restrictions. The ε-constraint method is employed to handle the multi-objective optimization, which has as its major advantage the fact that it can be used for any arbitrary problem with either convex or non convex objective space.

Several examples, where different situations are evaluated, are solved to test the model applicability.

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The influence of products' portfolio demand on the optimal supply chain planning

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Keywords: Supply chain management, optimal planning, uncertainty, partnership structure

Nowadays enterprises face important economical challenges. The trade-off between quality, price and service levels is definitively a key to success in a worldwide scale. Thus, enterprises need to provide high quality products around the world at the right time, in the right quantity and at the lowest prices. Supply chain structures have, in this area, an important role in the fulfillment of such goals in global markets (Varma et al, 2007). The supply chain planning is one of the areas that needs to be optimized in order to enhance the desired operational efficiency. Planning problems involve usually medium to large time horizons and accordingly, decisions on future events are frequently taken based on more or less accurate forecasts. Therefore, uncertainties regarding product demands and prices, amongst other supply chain conditions, need to be accounted for in planning decisions (Guillen *et al*, 2007).

In this paper, we look into the supply chain planning decisions that consider explicitly uncertainty on product portfolios demand and prices. A supply chain centralized managing strategy is adopted for the multi-period planning problem, where the supply chain operational decisions, at the actual period take into consideration both, contracted and uncertain products' demand and/or prices. The operational decisions on, supply, production, transportation, and distribution are evaluated in face of a defined supply chain economical performance objective (e.g. global operating costs/ profit realized), for different demand and price behaviors.

The economical flows (*cash-flows*) observed at each planning period are actualized based on a defined average market rate (Gupta et al, 2004). Regular *cash-flows* (i.e. equally spaced periods) are assumed, which are actualized based on a constant market rate. The actualized period profits (APP) are then considered as the problem economical goal.

On the other hand, the demand behavior is characterized by a set of polynomial functions that translate demand to price dependencies, according to the products considered. The demand uncertainty induced by price changes (i.e. different probabilistic price forecasts) is modeled through a set of planning scenarios with a probability occurrence corresponding to the price forecasts. In each of these scenarios the demand uncertainty induced by each price probabilistic change is considered through the demand to price elasticity coefficient.

A general model, based on the work of Amaro and Barbosa-Póvoa (2008), is proposed that considers the supply chain topology, operability and market demand/price uncertainty while accounting for product recovery through the integration of reverse material flows and remanufacturing operations. A Mixed Integer Linear Programming formulation (MILP) is developed for each planning scenario and the optimal solution is reached using a standard Branch and Bound (B&B) procedure. The final results provide details on the supply chain partners production, transportation and inventory, at each planning period, while accounting for demand/price uncertainties.

The model applicability was tested to a real industrial case involving a pharmaceutical supply chain. The results achieved are promising but improvements should be further

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explored. Namely, the demand to price elasticity coefficients can be extended and the price uncertainties should be studied to larger planning horizons with different periods of occurrence in order to capture the cumulative effect of demand uncertainties induced by price changes.

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Nonlinear dynamic modeling of a real pilot scale continuous distillation column for fault tolerant control purposes

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Keywords: continuous distillation column, nonlinear dynamics, dynamic modeling, fundamental models, fault tolerant control

Distillation columns are probably the most common process units within the chemical industry (Kister, 1990). Industrial distillation columns are complex systems which exhibit nonlinear dynamic behavior, so the development of models for control strategies is usually based on nonlinear models (Abdullah et al., 2007). In recent years, one of the major challenges in developing an effective model based control strategy is to construct a model which is used to describe the process under consideration.

Many sophisticated model based control algorithms have been developed under the assumption that all system components will not fail. Once a failure occurs, the system performance will degrade and may lead to divergence, generating dangerous situations, particularly when the failure occurs in the control loops.

The demand for increased efficiency, in various branches of industry, such as chemical industry leads to an increased degree of automation of the production process. In order to meet this demand while at the same time maintaining or improving the quality and safety, it is necessary that the (complex) control system is fault tolerant. This means that the effects of failures, such as component failures, actuator or sensor failures, on the quality and safety of the production process are minimized.

An important class of approaches to design fault tolerant control systems, consists of a so-called Model Based Fault Detection and Isolation part (Calado et al., 2001) and a Controller Reconfiguration part. The two key elements in designing these two parts are the development of a mathematical model and a suitable decision mechanism to localize the failure and to select a new controller configuration.

As a first step in this work, nonlinear dynamic models of a pilot-scale continuous distillation process were developed for the simulation of the dynamic behaviour of the real continuous distillation plant at the Chemical Engineering Department of ISEL, in the absence and in the presence of faults. The modeling of the overall pilot scale distillation plant includes mass and energy balances for the individual systems such as the distillation column sections, the condenser, the heated reboiler, the feed pre-heater, the reflux valve, the bottom product control valve, the PID regulatory controllers, the pumps, and other sensors. The complete dynamic models were validated against experimental data gathered from the plant under normal operating conditions in the absence and in the presence of faults, for the separation of a binary liquid mixture of ethanol/water. The models were developed within MATLAB®/SIMULINK environment.

The continuous distillation column system at the Chemical Engineering Department of ISEL, includes: a 2 m structured packing column (4.3 cm inside diameter) equivalent to 62 theoretical plates; a glass reboiler with two 2000 W electrically heated elements equipped with an internal overflow; a water cooled reflux top condenser; a reflux return

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valve solenoid operated, to provide for 0%-100% reflux, adjustable by electrical signal; a differential manometer connected to the top and bottom of the column, to monitor column pressure drop; sampling points throughout the system for composition analyses; peristaltic feed pump, range 0-15 dm$_3$/h adjustable by voltage input variation to the pump motor controller; a bottoms product water cooled heat exchanger; a 1200 W electrically heated feed pre-heater; a double temperature sensor for monitoring and control of the reboiler's temperature; a double temperature sensor for monitoring and control of feed temperature; temperature sensors in the flow stream entering the top condenser, in the flow stream entering the reboiler and at the middle of the column between the two elements of structured packing.

Since the ultimate use of the model will be for Fault Tolerant Control purposes, the process and instruments faults selected and tested in this distillation plant were taken into account and included in the complete model for the subsequent study of the controller reconfiguration steps. The benchmark consists of the MATLAB®/SIMULINK files with the nonlinear simulation dynamic model of the plant. It covers the whole operating region and allows the simulation of the following 3 different types of selected fault scenarios: (F1) change in reflux valve position (change in reflux flow); (F2) decrease in the heat delivered by the two reboiler electrically heated elements (change in reboiler liquid temperature); and (F3) leak in the peristaltic feed pump (decrease in feed flow rate).

Figure 1 shows some experimental results and the correspondent simulated dynamic model response obtained for the top distillate product outlet ethanol composition (xD) and for the bottom product outlet ethanol composition (xR), in the case of implementing reflux valve fault scenario (F1) at time 74.4 min, as a fault with the magnitude of 57% decrease in the reflux flow rate.

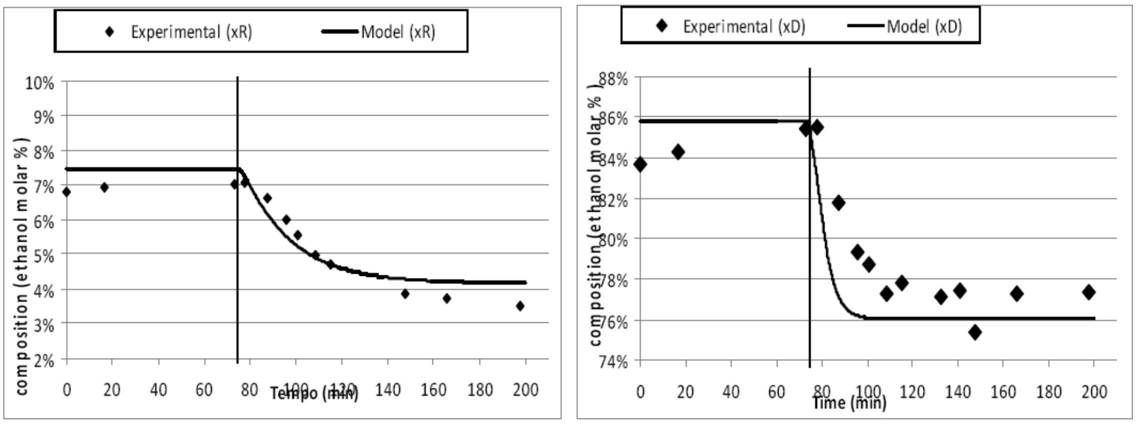

Fig.1 - Experimental top distillate product ethanol composition (xD) and bottom product ethanol composition (xR) and simulated dynamic model response for the testing of reflux valve fault scenario (F1) at time 74.4 min, with a magnitude of 57% decrease in reflux flow rate.

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Applications of model based design of polymer products

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Keywords: Dynamic optimization, Product Design, Polymerization.

The world market for polymeric materials is vast. It comprises high volume commodity products, such as plastics, and high quality polymers with very specific molecular properties. PSE tools can be applied in both cases in order to achieve innovative products. The properties of existent products can be improved accordingly to application demands and also new products can be designed thus broadening the applicability of polymeric materials. This is possible due to the high correlation between the molecular/morphologic structures and the physical properties of the product, and the impact that these have on its feasible end-uses.

The development of systematic optimization strategies, to be applied in the manufacture of innovative products, is extremely appealing given the increasing availability of very detailed and precise mechanistic kinetic models of polymerization systems. The information provided by the models can be combined with physical experiments specifically designed to better understand and thus predict the influence of the processing conditions on the physical structure of the final product.

Previous work addressed the search for operating conditions that improve productivity, resulting from minimum time formulations (Silva and Oliveira, 2002) and the effects of constraining the variance of the chain length distribution of the final product (Silva, 2006). In order to develop a systematic approach to polymer design, other aspects should be considered. Namely, it must be possible to control rigorously the size distributions (moments and shape) and to have as problem solution the complete choice of operating conditions (*e.g.* temperature, concentrations), processing agents, such as suitable initiators (which ones, how many, in which concentrations, when to add) and other additives like suspension or chain transfer agents.

The resulting design problem is characterized by considerable complexity, high dimensionality, highly nonlinear behaviour of the physical models, multi-objective nature and the need to perform discrete design decisions. To tackle most of these limitations, a two-step methodology was recently introduced (Silva, 2006; Silva and Oliveira, 2007). Initially the problem is reformulated in terms of the decision variables used, namely the reaction temperature and the initiation reaction rate. Given that the initiation reaction rate profile is independent of the set of initiators used, the choice of initiators may be considered in a second step, and formulated as a MILP problem, significantly decreasing the overall problem complexity.

Initial experience with the application of this methodology showed that, although it could be used to reduce the cost of manufacture of existing products and as a basis to manufacture innovative products with improved properties, in certain cases its unguided application could lead to undesired solution characteristics, such as high residual concentrations and rapid changes in the reaction temperature. Modifications in the original formulation are therefore considered in the present work, in order to originate solutions more suitable for practical application.

The application is demonstrated with the batch suspension polymerization of vinyl chloride producing polyvinyl chloride (PVC), one of the most used thermoplastics. These results are based on the kinetic information of Xie et al. (1991a,b).

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Global dynamic optimization of chemical and bio-processes using the Scatter Search metaheuristic

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Keywords: dynamic optimization, chemical engineering, global optimization, scatter search, metaheuristics.

Dynamic optimization of chemical and biotechnological processes has received major attention in recent years. A relevant example is the dynamic optimization of fed-batch bioreactors (Banga et al., 2003a).

Dynamic optimization allows the computation of the optimal operating policies to maximize a prede- fined performance index such as productivity or other economical indexes. Most bioprocesses present a nonlinear dynamic nature and constraints in both the state and the control variables, which calls for the use of robust dynamic optimization techniques in order to successfully obtain their optimal operating policies. Numerical methods for the solution of dynamic optimization problems are usually classified under three categories: dynamic programming, indirect and direct approaches (Banga et al., 2005). Direct approaches transform the original dynamic optimization problem into a non-linear programming (NLP) problem using either control vector parameterization or complete parameterization. The control vector parameterization (CVP) approach (Vassiliadis et al., 1994) seems to be the most convenient method for dealing with large scale ODE systems (Balsa-Canto et al., 2004), such as those resulting from distributed systems.

NLPs arising from the application of direct approaches (such as CVP) are frequently multimodal. Therefore, gradient based local optimization techniques (such as SQP methods) may converge to local optima. Global optimization methods are robust alternatives to local methods. Recent advances in global deterministic methods for dynamic optimization have been achieved in recent years (Chachuat et al., 2006) but they still need some requirements regarding the functions differentiability and the path constraints type to be handled. Besides, the computational effort is still a barrier for the application of these methods. Stochastic and hybrid global optimization methods have been successfully applied to dynamic optimization problems (Banga et al., 2003b).

In recent years, a special class of stochastic global optimization methods called metaheuristics (Glover and Kochenberger, 2003) has appeared as efficient optimization techniques. These methods were originally created to solve combinatorial optimization problems although many adaptations to continuous problems have been carried out lately (Michalewicz and Siarry, 2008). Some examples of the use of metaheuristics for dynamic optimization problems can be found in the recent literature (e.g., see Banga et al. 2003a and references therein).

Scatter Search (SS) is a population-based metaheuristic based on the formulations by Glover (Glover, 1977). Unlike genetic algorithms and/or evolutionary methods, SS does not use randomization for choosing population members to be combined. Instead, it creates sets of population solutions and combines them systematically. Another difference is that the number of population members is relatively small (e.g., between 10 and 20 elements depending on the problem size). SS incorporates a memory term to avoid doing combinations among population members previously combined. This

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strategy prevents the method from visiting already explored areas thus making the search more efficient. Like other meta-heuristics, SS was originally designed for combinatorial problems but it has also been adapted to continuous problems in bioprocess optimization (Egea et al., 2007).

Here we present a SS-based algorithm for the global dynamic optimization of chemical engineering processes using the control vector parameterization (CVP) approach. It is designed to overcome typical difficulties of non-linear dynamic systems optimization such as noise, flat areas, non-smoothness and/or discontinuities. It balances intensification and diversification by coupling a local search procedure with a global search and makes use of memory to avoid simulations in previously explored areas. It has been applied to four multimodal dynamic optimization problems from the bioprocess engineering area: A fedbatch reactor for ethanol production, a fed-batch fermentor for penicillin production, a drying operation of bio-products and the microwave heating of foods. Its results, compared with those obtained by other state-of-the-art global optimization algorithms, prove its efficiency and robustness, showing also a very good scalability.

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Integrating real-time optimization and control for optimal operation: Application to the bio-ethanol process

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Keywords: Bio-ethanol process, direct optimizing control, NMPC.

Despite recent advances in purification technologies as well as in the modification of microbial strains for making them more resistant to the different stress and inhibition factors, the economical feasibility of the bio-ethanol industry is still questioned, and therefore, much effort should be oriented to the optimization and control of the process. In the same way, as recently argued by Engell (2007) for chemical processes, the purpose of control should be to achieve optimal process operation despite the presence of disturbances. Therefore, the optimization and control tasks should be combined in order to operate the chemical or biochemical process at maximal productivity, which is the real purpose of any production plant. In the last years, many works coupling the real-time dynamic optimization and the Model Predictive Control frameworks, have been reported in chemical process applications (Manenti and Rovaglio, 2007); however, to the authors knowledge no applications on the bio-ethanol process taking into account more than one equipment has been presented. In this work, the problem of controlling the bio-ethanol process (including saccharification, fermentation and a first separation stage) for optimal operation is addressed from two different real-time frameworks: the two-layer integrated dynamic optimization and control (two-layer) and the direct finite horizon dynamic optimization (one-layer).

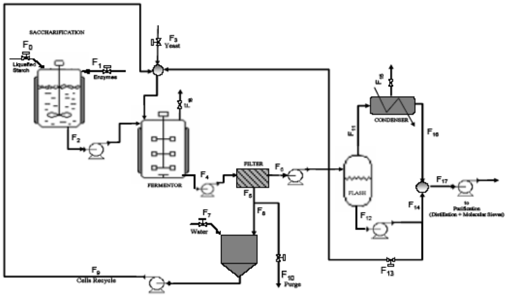

Figure 1. Bio-ethanol Process: Saccharification- Fermentation- Cells Recycle- First Purification step.

The process investigated (Figure 1) corresponds to the so-called extractive alcoholic fermentation studied by Costa et al (2001) and has been modelled assuming that pH, temperature and liquid volumes are regulated by internal control loops. The whole process including distillation, rectification and adsorption will be addressed in a future work. Figure 2 shows the structure of the one-layer and two-layer approaches. The main difference between them is that in the one-layer, the input variables applied to the plant are given by the optimization layer; whereas for the two-layer, the inputs applied are calculated by a control layer (NMPC) that uses as set points, the optimal values given by the optimization layer. The trigger blocks act like switches for re-calling the optimization and control layers when necessary (working based on a time criteria or on the disturbance dynamics). Although the bio-ethanol process (until the flash) has six available manipulated variables (F_0, F_1, F_3, F_7, F_{10}, F_{13}), only F_0, F_1 and F_{13} are taken as decision variables during the optimization, because the remaining are used in an internal control loop proposed for biomass control. This internal loop comprises a

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splitrange controller for keeping the biomass concentration in the fermentor at its optimal value and a ratio controller for achieving a suitable viscosity in the biomass recycle slurry. The biomass control loop proposed in this work is treated as independent because i) an optimal biomass concentration should be guaranteed, in order to avoid a misuse of the substrate (which can be quickly consumed for cells maintenance and growth instead of metabolite production) if a higher concentration than the optimal is available; also, if the biomass concentration is below the optimal, a slower metabolite production rate will occur, affecting the productivity of the process; and ii) the yeast is only involved in the fermentation, filter and cells recycle loop.

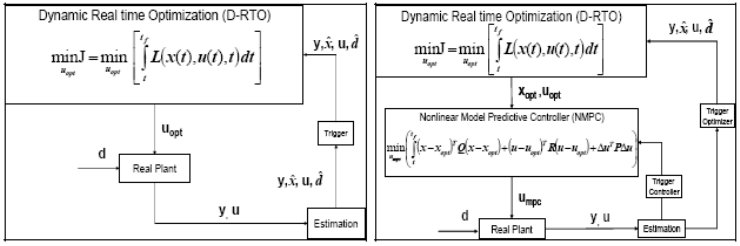

Figure 2. Dynamic Optimization based Control: One-layer (left) vs. Two-layer (right) approach.

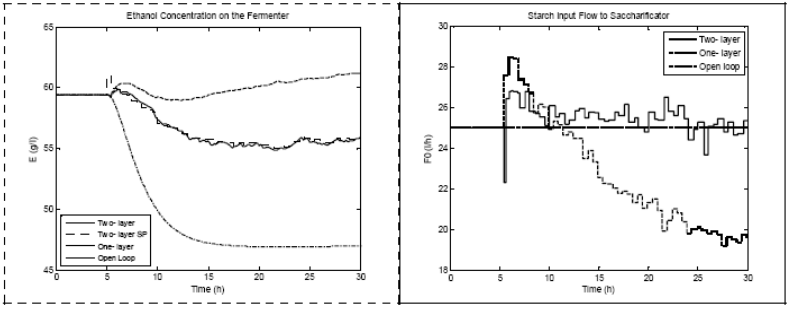

Figure 3. Results One-layer v.s. two-layer: Ethanol (left) Starch Input Flow (right)

The dynamic optimization problem for both approaches was solved by the direct sequential approach using the Metropolis Monte Carlo-simulated annealing optimization method as presented in Ochoa et al (2007). Figure 3 compares the results of the one and two-layer approaches to the open loop policy (which runs also with the biomass internal control loop). The process starts at steady state and at time t=5h, a step disturbance on the starch feed concentration occurs (a reduction of 20%). As shown, the one and two-layer approaches have good response to the disturbance, maintaining the ethanol concentration in a higher value when compared to the open loop policy, in which a fast decrease in the ethanol concentration takes place. Although the one-layer keeps the ethanol concentration higher than the two-layer, reaching values even higher than the starting steady state, application of the two-layer could be preferred because it is argued that the NMPC controller layer may take care of additional implementation issues such as the stability of the process, or avoiding large changes on the implemented manipulated variables between each sample time to the next. Finally, the results presented in this work, demonstrate that the integration of optimization and control in a real-time framework is a very promising approach for achieving optimal operation (higher productivity, good disturbance rejection, increased profit, etc.) in bioprocesses.

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On-Line adaptive metabolic flux analysis: Application to PHB production by mixed microbial cultures

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Keywords: Metabolic flux analysis, on-line, adaptive, polyhydroxybutyrate, mixed microbial cultures

In this work, on-line metabolic flux analysis (MFA) is proposed and applied to polyhydroxybutyrate (PHB) production by mixed microbial culture (MMC). MFA is a methodology that allows to determine the flux distribution of a given metabolic network from the knowledge of a smaller subset of measured fluxes, normally substrates uptake and endproducts formation rates (Stephanopoulos et al., 1998).

In MMC process, the population dynamics constitutes an important source of perturbation to MFA calculations since some stoichiometric and energetic parameters of the underlying metabolic network are continuously changing in time. In such problems the use of adaptive algorithms to adjust network parameters that are poorly known becomes a necessity. Therefore, in this study an adaptive MFA algorithm is proposed for the on-line monitoring of biological systems with dynamic metabolic networks. The algorithm is based on the application of the observer-based estimator (OBE) (Bastin and Dochain, 1990; Oliveira et al., 2002) to the central MFA equation, whereby the role of the OBE is to force the concentrations of intracellular metabolites to converge to null by adjusting the values of unknown network parameters. The algorithm was implemented in a reactor equipped with on-line measurements of dissolved oxygen and carbon dioxide, respirometry and titrimetric measurements. The oxygen and carbon dioxide fluxes were measured directly while acetate, PHB and sludge production fluxes were estimated indirectly using a PLS calibration model validated a priori with off-line measurements of 4 batches (Dias et al., 2008).

The metabolic network proposed in a previous work (Dias et al., 2005) was adopted in this study. The network is composed by six metabolic reactions: acetate uptake, biosynthesis for biomass growth and extracellular polymeric substances (EPS production, catabolism, maintenance, oxidative phosphorylation and PHB production. The metabolic parameters involved in EPS and active biomass synthesis, was estimated by the on-line adaptive MFA algorithm. The obtained results showed a considerable short and long term variability. The variability within each experiment was caused by the metabolic shift between cell growth and EPS synthesis, while the long term variability may be explained by the population dynamics.

Along with the parameters estimation, the unknown metabolic fluxes, acetate uptake, CO_2 production and $NADH_2$ oxidation, were calculated and compared with off-line concentration measurements. A good agreement between the estimate and the off-line data was observed for all experiments. The obtained results were very consistent with the observed sludge production. The results demonstrate that the algorithm converged exponentially with the steady state error always below 1 mmol/l.

The estimated respiration quotient (*RQ)* using metabolic network parameters followed closely the measured *RQ*. This result supported the parameter estimation results in the sense that the lumped metabolic reaction proposed for sludge production has biological significance in term of yields and *RQ*.

Finally, the Chi-square (X^2) test was used to check the consistency of calculated metabolic flux distribution (Wang and Stephanopoulos, 1983). The consistency index, h, was calculated using the known fluxes for a hypothetical very low measurement variance of 1 %. In practically all sampling instants, h passed the X^2 distribution test for a 95% confidence level. The higher values for h were observed mainly in the transition between EPS and cell growth metabolism, which causes a perturbation in the estimation of parameters and higher deviations to null intracellular concentrations.

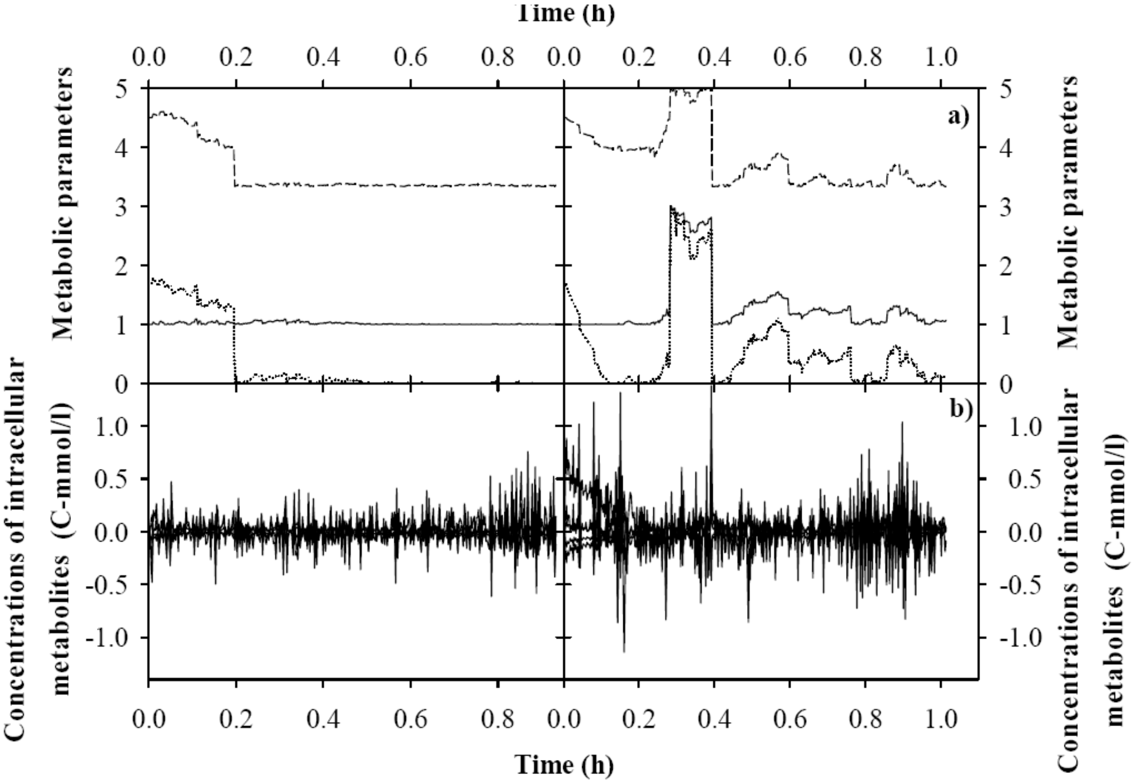

Figure 1- a) On-line estimation of the metabolic parameters involved in EPS and active biomass synthesis for two experiments using the adaptive MFA algorithm; b) Convergence of the concentration of the intracellular metabolites.

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Modelling of membrane bioreactors for wastewater treatment incorporating 2D-fluorescence monitoring data

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Keywords: Membrane Bioreactor (MBR), Modelling, PLS, 2D-fluorescence

Membrane bioreactors are widely applied for wastewater treatment. However, the progressive membrane fouling due to deposition of cells and cell products on the membranes reduces the permeability of the system, resulting in substantial maintenance costs and shortening membrane life span. The major biofouling agents in MBRs are Extracellular Polymeric Substances (EPS), and thus EPS monitoring and control in MBR operation is essential. EPS contain large amounts of proteins and aromatic organic substances that possess natural fluorescence properties, making fluorescence a powerful technique to monitor their production in MBRs.

2D-fluorescence spectroscopy is a highly sensitive, selective and non-invasive technique that can provide rapid information about the composition of complex media such as biological systems. Due to the fact that all organisms contain natural intracellular fluorophores, such as aminoacids (e.g. tyrosine and tryptophan), vitamins, coenzymes and aromatic organic matter in general, fluorescence is a technique suitable to be used in bioprocess monitoring. Furthermore, since this technique is non-invasive, it can be used in situ and on-line without disturbing the biological system. The excitation-emission matrices (EEMs) obtained by scanning the spectra wavelengths can cover a wide diversity of natural fluorophores. The maps obtained from the EEMs can thus correspond to a fingerprint of the physiological activity of a biological system.

The richness and complexity of information contained in EEMs should be taken into account when interpreting fluorescence results. Fluorescence fingerprints not only combine physiological patterns of different microorganisms, but also reflect the variation in concentration of some substrate components and microbial by-products which possess fluorescence properties. In addition, some molecules can act as quenchers to fluorescence, by interacting with the excited fluorophores, resulting in alterations of fluorescence signal. Therefore, it is necessary to deconvolute the contextual information contained in the fluorescence maps. Using chemometrics, EEMs can be correlated with key operational and performance parameters of the studied system. When using chemometric modelling techniques, fluorescence spectra are viewed as multivariate descriptors of performance parameters and treated with statistical approaches such as Principal Component Analysis (PCA) and Projection to Latent Structures (PLS). The statistical models are obtained by this approach using on-line fluorescence maps and their corresponding process performance parameters measured for different operating conditions. Through these calibrated models, the EEMs obtained on-line can be used to monitor the status of the process, as well as for prediction, allowing rapid correction of operational parameters for process optimisation.

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In the present study, 2D-fluorescence was applied to monitor a pilot scale MBR for domestic wastewater treatment (Lavis, Italy), by measuring EEMs of the influent wastewater, the bioreactor mixed liquor and the permeate.

PLS models were developed for this system in search for a correlation between inputs and outputs that would enable us to predict the systems behaviour. Operating conditions, wastewater characteristics and EEM were used as inputs of the models. Permeate characteristics, sludge characteristics and membrane performance (measured as transmembrane pressure, TMP) are the parameters predicted by these models (outputs). Using the PLS models it is also possible to infer about the contribution of each input parameter to determine the outputs, leading to a possible process optimization.

PLS models were used to maximize the co-variance between 2D-fluorescence maps, selected performance variables (e.g. trans-membrane pressure and Chemical Oxygen Demand removal) and operation conditions (e.g. aeration and influent Chemical Oxygen Demand). The model description of the output parameters with and without the use of the EEMs was compared in order to assess the significance of using 2D-fluorescence for monitoring of such a complex system.

It was found that the combination of the EEM additional information about the biological system state with the selected performance variables enhanced MBR modelling and performance prediction. In Figure 1 it can be seen that for the TMP prediction, models using EMM as input fit better the training points. Also the validation points (data not used to the model developing) fit better in PLS models with fluorescence.

The importance of fluorescence in PLS models is also being studied using sludge EEM and using the 2D-fluorescence of wastewater, sludge and permeate all together in the same model.

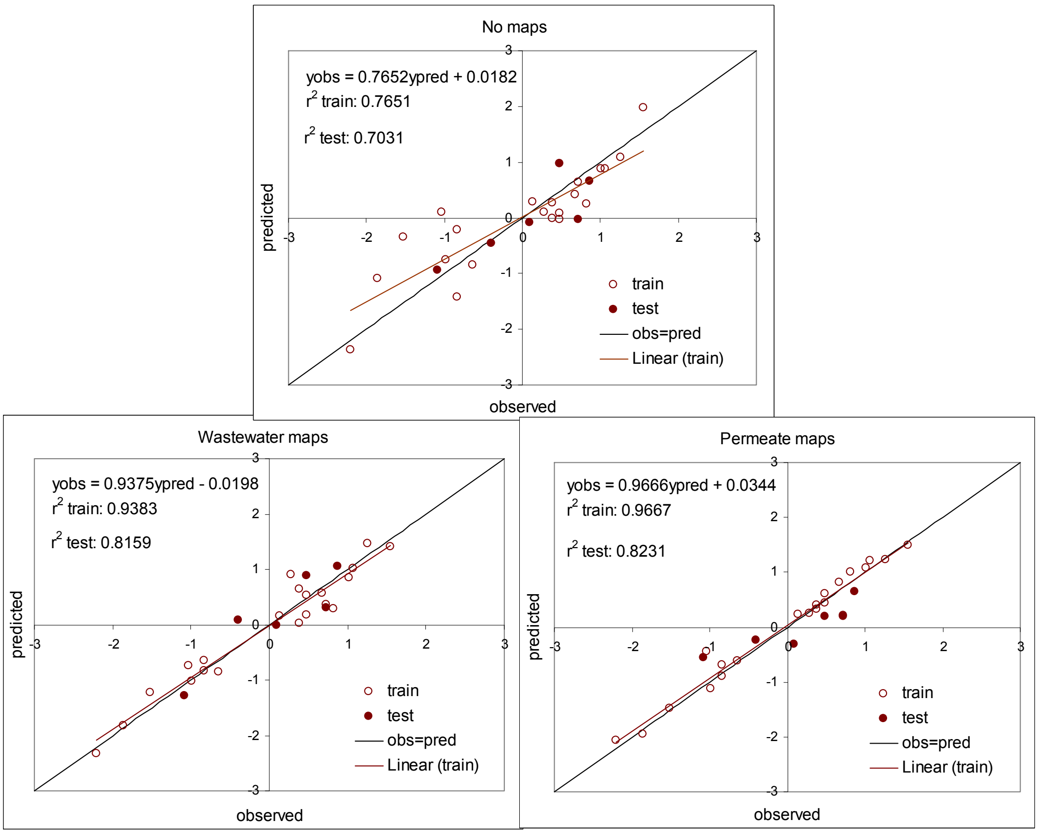

Figure1 – Normalized values of TMP predicted by PLS models against the experimental normalized values of TMP. The first plot shows predicted values by a PLS model done with no fluorescence information, the other two plots with a PLS model done with wastewater EEM and permeate EEM, respectively.

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Modeling, identification and, control of complex systems

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Abstract

Understanding the physical phenomena and 'engineering' the processes involve methods to correlate process parameters that determine the progression and state of the process. These correlations form the basis for engineering analysis, design, optimization and control of the process of interest. However, in practice there is no perfect mathematical model that can accurately represent a physical process. This is because of various reasons namely; uncertainty in the process, nonlinearity associated with cause and effect relationships, and time varying external disturbances. Mathematically such processes can be described by $f(u, y, x, \theta, w, v) = 0$, where *f* is a nonlinear function of $u \in R^m$, the input vector, $y \in R^n$, the output vector, $x \in R^l$, the state vector, $w \in R^l$, the state noise vector and, $v \in R^n$, the output noise vector. The uncertainty in the process can be described by (i) uncertainty in the parameter vector $\theta \in P$, where *P* is a family of probable process model parameters, (ii) uncertainty in the model structure $f \in F$, where *F* is a family of nonlinear models that can describe the process behavior in different states, (iii) uncertainty in the combined state and output noise, $z \in Z$, where *Z* is a family of noise vectors pertaining to a set of probability distributions. In addition, process may contain sub-processes with varied scales and rates. In this research, an attempt has been made to resolve some of the issues posed by above mentioned complexities in modeling, identification, and control of the dynamic processes. In order make sure that the outcome of the research to be applicable, most of the work has been carried out in linear systems framework, but easily can be adapted nonlinear systems theory.

In this talk, two of those techniques will be presented; identification linear time invariant models subjected to non-stationary disturbances, and online change detection and model validation under time varying disturbances. These techniques have applications in various fields even though the illustrations are made on model based control applications.

The task of identifying accurate process models in presence of stationary unmeasured disturbances is well addressed in literature for both open-loop and closed-loop cases. Bias in process model parameter estimates in case of inputs and/or outputs corrupted with colored noise can be eliminated using specialized identification algorithms such as errors-in variables, bias eliminated least squares etc. For the case of time varying systems (non-stationary processes) various variable forgetting factor recursive identification algorithms, variable structure nonlinear model based identification algorithms were proposed in the past.

However, the identification of unbiased process models in case of non-stationary external disturbances corrupting the process output measurements is yet to be resolved. Accurate identification of linear process models subjected to non-stationary disturbances in open loop is motivated by the improvement in the performance of model based control algorithms, whereas in closed loop it is motivated by the fact that along with the accurate model predictions, one needs accurate estimate of non-stationary disturbance progression in the immediate future for corrective action to be implemented to reject the disturbance efficiently. A novel recursive extended equation error algorithm for the identification of linear time invariant processes subjected to

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stationary and non-stationary disturbances. Figure 1 shows the comparative study of Auto-Regressive Moving Average eXogenous (ARMAX) model with the proposed Extended ARMAX (EARMAX) model identification. In presence of non-stationary disturbance shown in Figure 1, both in closed loop and open loop the EARMAX yielded accurate model parameters, whereas with ARMAX the model parameters are subjected to same oscillatory trend as of the disturbances and parameter estimates are heavily biased.

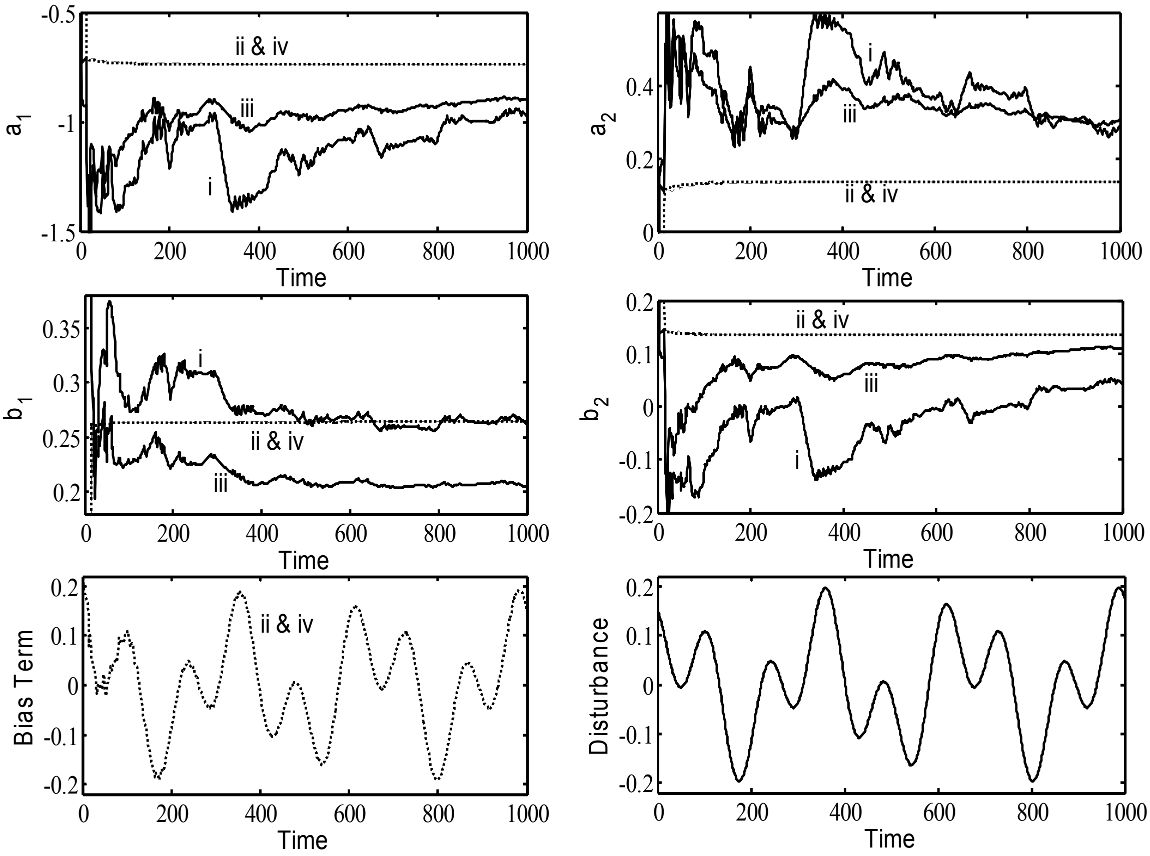

Figure 1: Variation in the identified parameters along the sampling time for deterministic case. Also present in the Figure 1 is external sinusoidal disturbance introduced (Disturbance) and identified disturbance (Bias Term). i) Open-loop identification with ARMAX, ii) Open-loop identification with EARMAX, iii) Closed-loop identification with ARMAX, and iv) Closed-loop identification with EARMAX.

Significant improvement in product quality, energy consumption and operating costs in current day process industries can largely be attributed to the model-based control. But, if the process changes significantly, the dynamic model used in control applications may no longer be adequate and thus the model-based control scheme may result in poor performance. However, poor agreement between model predictions and output data does not necessarily imply that model re-identification is required, because the unmodeled component may not be necessarily from change in process dynamics. External disturbances that are entering the process may also lead to plant-model mismatch. Hence, there is an explicit need to identify whether the plant-model mismatch is caused by change in process dynamics or purely because of external disturbances. The logical step to be followed this is to re-identify the new process model. Figure 2 shows the propagation of process model mismatch (PMM) for a step change in input for various kinds of changes in dynamic behavior of process. A novel online change detection and model validation algorithm for dynamical systems is presented. A univariate residual cross-correlation approach to test the independence of two time series is used a tool for model validation.

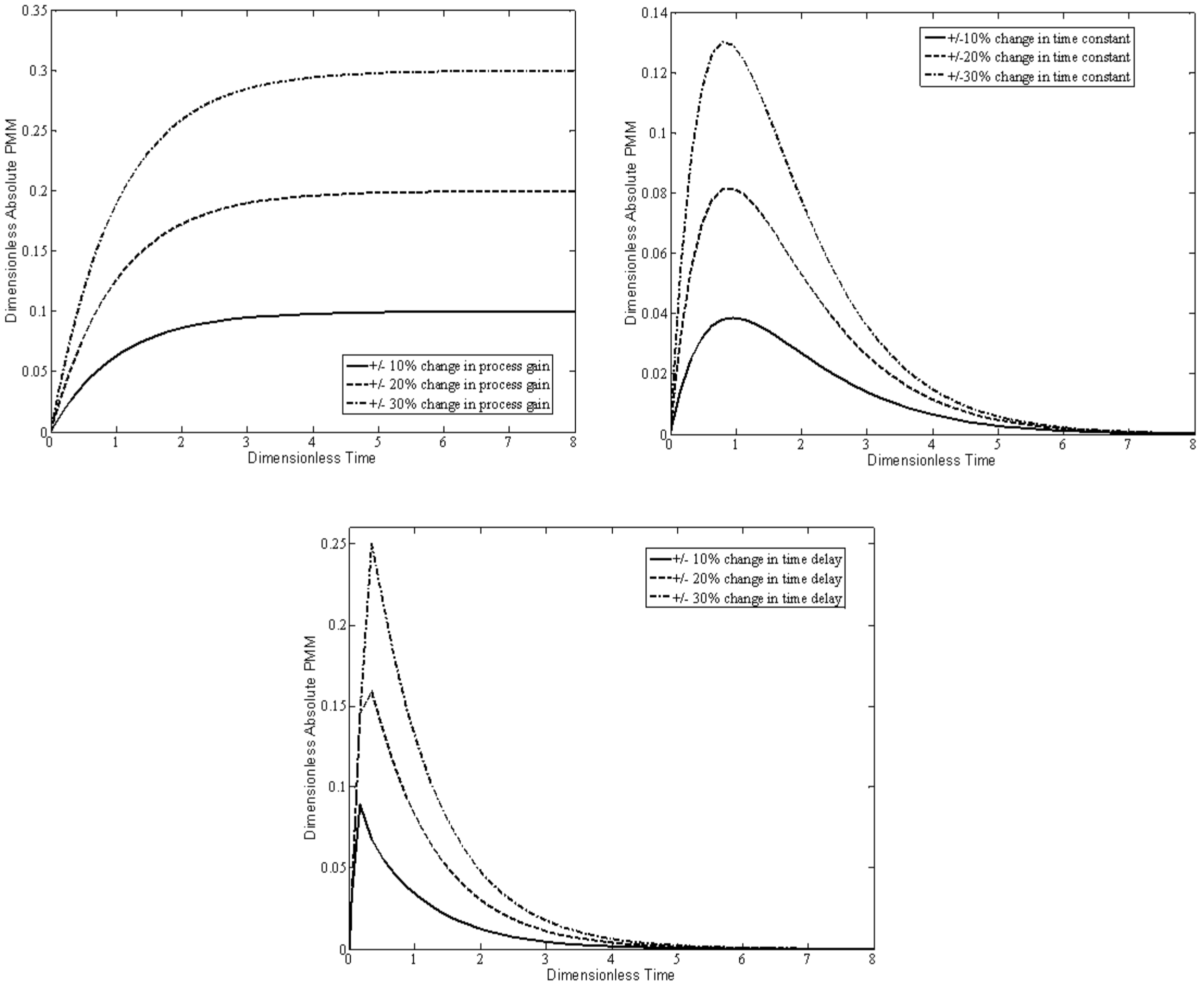

Figure 2: Propagation of PMM for various kinds of changes in process dynamics for a step change in input.

Performance indicators for reactive distillation design

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Keywords: Reactive distillation, multi-objective optimization, feed quality

Reactive distillation (RD) combines reaction and separation into the same physical shell. This combination can lead to both economic and environmental gains resulting from the process intensification (Malone and Doherty 2000; Taylor and Krishna 2000) and therefore to systems with significantly greener engineering attributes (Malone *et al.* 2003).

However the design of these systems can prove very complex and costly. Design should therefore be preceded by preliminary studies to assess first the potential adequacy of the technology. With this aim some work was developed to create a framework combining feasible regions and optimization techniques to the design and multi-objective optimization of complex reactive distillation columns, for reacting systems with variable degrees of relative volatilities (Filipe *et al.* 2006; 2007; 2008).

This led to the consideration of RD columns with distributed feeds, with many of them involving the combination of superheated and subcooled feeds. This combination provides a source or a sink of heat at specified trays of the columns, which favors reaction while reducing the total reactive holdup requirements.

To assess the impact of employing such combined feeds, a cost indicator, designated *capacity*, was introduced as a new optimization objective criterion (Filipe *et al.* 2006).

This cost indicator, based on the capacity variables (Jobson *et al.* 1996), was defined as the weighted sum of liquid and vapor internal flows divided by the total feed to the column.

Together with the total number of stages and reactive holdup this indicator was used to build the Pareto front that contains the efficient set of solutions. Considering the very large number of designs involved in the evaluation, an indicator was preferred to a detailed cost calculation.

Following a strategy of refining the Pareto optimal designs, some details related with the practical implementation of a few promising designs were investigated (Filipe *et al.* 2008) by means of a number of sensitivity tests on catalyst usage and feed quality. The advantage of using combined feeds was confirmed, as well as the need for a careful selection of the feed qualities.

The *capacity* indicator proved valuable as an optimization criterion, not only because it is efficient in comparing thousands of different designs, but also because it effectively limits the range of the feed qualities used. However, some drawbacks have been identified in its formulation, namely there is no distinction between reactive and non reactive trays and the RD effective capital and operational costs are not explicitly shown.

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This works presents a study where the effectiveness of the proposed cost indicator is assessed and, in addition, the optimal solutions identified in previous stages of the design procedure are tested at an advanced level. The size (height and diameter) of the column, energy and catalyst demands are quantified in the *ASPEN Plus* detailed model of some designs and the total cost of the column estimated using common cost correlations. These values are correlated with the correspondent *capacity* values giving a new weight for the factors of the initial *capacity* definition.

As an illustrative example, a system with ideal vapor liquid behavior is employed, i.e., the olefin metathesis system (wherein 2-pentene reacts to form 2-butene and 3-hexene). This particular reactive system has the reactant boiling point between those of the two products, allowing for a wide range of feasible column designs.

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the importance of models and practical integration in process development. Cases from the simulated moving bed technology

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Keywords: Simulated Moving Bed, Chromatography, Adsorption, Modeling, Simulation, Optimization, Separation, Process development and Process intensification.

This work is based on the presentation recently awarded with the prize jEPSI at the 6th Workshop Anual de Engenharia de Processos e Sistemas, 12th March 2008 at Coimbra-Portugal, and intends to illustrate why a solid method for process development should be the result of the integration of “theory” (the models) and “practice” (experimental applications). Therefore, are presented some cases from the Simulated Moving Bed technology where developments based on model analysis led to the conception of practical applications, as well as some cases where the restructuration or operation of experimental/industrial units guided improvements in the previously used models.

Simulated Moving Bed technology

It is known that industrial-scale continuous adsorption processes are preferable to batch ones. The use of continuous chromatographic counter current operation in particular (here by called TMB, True Moving Bed), maximizes the mass transfer driving force, providing a better utilization of the adsorbent, which may have a rather low selectivity. In addition, with TMB it is possible to reduce the solvent consumption, increase of productivity and purity and therefore less diluted product streams. Contrary to the TMB units, where the solid phase moves counter currently to the fluid phase, in the SMB (Simulated Moving Bed) [1] units the solid motion is simulated by a synchronous shift of all inlet and outlet ports, while the solid phase is held immobile. By this way several problems usually associated with solid motion, namely, particle and equipment attrition, high pressure drops, particle and flow redistribution are avoid. The SMB technology has been mainly applied to the so-called “difficult separations”, in fields such as the petrochemical area with the separation of p-xylene from C8 isomers mixtures. The expansion to new SMB applications in fields such as the biotechnological, fine chemistry and pharmaceutical industries is remarkable in the last decade [2].

From models to practice…

By means of mechanistic models, or more simplistic approaches, it is possible to describe an SMB unit accounting to all its particularities, even the ports discontinuities. The deep study of these models provided the development of the “non conventional modes of operation”, where, and by means of the introduction of asynchronous ports movements, variation of concentration and/or flow rates, it was possible to extend the versatility of this kind of units and by consequence its performance. The development of the OSS (Outlet Streams Swing) [3] mode of operation is one of these cases, and one of the objects of this presentation.

However, the practical application of these “non conventional modes of operation” is per itself a challenge. Generally an SMB unit, industrial, pilot of laboratory-scale, is

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limited to one or two modes of operation and to implement a new operating mode is necessary to contact the supplier, do adjustments, even to reformulate the entire unit if not to acquire a new one. Therefore, flexibility (at least at laboratory-scale) is seen as one of the more relevant qualities for this kind of equipment. This aspect was the main reason that led to the design and construction of a 6 columns unit, the FlexSMB-LSRE. The main advantage of this unit remains in its automation routines, and a particular attention was driven on that aspect.

…from practice to models.

In the published or patented innovative work related with SMB industrial separations, just a few references refer to the adsorbent ageing problems. The "time life" of the adsorbent, a predominant economical factor in the SMB units design as adsorbent cost, has considerable influence on the complete unit design, if not in the main project viability. Nevertheless, the information considering this issue remains restricted to the plant operators and technology owners. Usually an industrial SMB unit operates at the limit (the optimum conditions), a small deviation on its operating parameters will pollute the outlet streams. Initially, the unit is designed accounting to the industrial/economical requirements and to equipment/adsorbent restrictions. If the adsorption equilibrium data changes over the time, ageing phenomena, and the unit is kept on the initial operating parameters, the unit will pollute its outlet products. The observation of these aspects during an industrial unit operation led to the extension of the common models to account with ageing problems, from practice to theory…. The analysis of this "extended models" provided some diagnostic methods for the ageing mechanism as well as short cut methods for the compensation of this kind of problems [4,5].
resented.

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A chemometric tool to monitor high-rate anaerobic granular sludge reactors during load and toxic disturbances

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Keywords: Control, Organic Loading Disturbance, Principal Components Analysis, Quantitative Image Analysis, Toxic Shock Load

High-rate anaerobic digestion reactors gained significance during the last decades in the field of wastewater treatment processes. However, these systems are designed with reference to a nominal operating condition, in which the loading rate is assumed to be constant in time. Also, some compounds can have inhibitory or toxic effects to the microbial populations. These facts, coupled with the long start-up periods imply the need to monitor the anaerobic granular sludge stability in order to achieve an appropriate control and sustainability of the process.

Chemometrics based techniques such as Principal Components Analysis (PCA) can be useful to detect groups, trends, correlations, and outliers in datasets. This method allows identifying patterns in data, and expressing them in order to highlight their similarities and differences. PCA is a projection method for analyze data and reduce it from an n-dimensional space to few latent/hidden variables, while keeping information on its variability.

Three programmes previously developed (Amaral, 2003) were used as the final step of a procedure (Costa et al., 2007) to obtain quantitative morphological information from anaerobic granular sludge. The PCA method is described in Costa et al. (2008).

Four organic loading disturbances (OLD) (Table 1) were applied to an Expanded Granular Sludge Bed (EGSB) reactor fed with 1.5$g_{COD\text{-}ethanol}$/L and hydraulic retention time (HRT) of 8 hours, in steady-state conditions. Also, three toxic shock loads (TSL) were applied in EGSB reactors operating in similar conditions (Table 1).

Table 1. Organic Loading Disturbances and Toxic Shock Loads conditions.

Disturbance	OLD1	OLD2	OLD3	OLD4	TSL1	TSL2	TSL3
Ethanol (g_{COD}/L)	5	1.5	15	15	1.5	1.5	1.5
HRT (h)	8	2.5	8	8	8	8	8
[Toxic] (mg/L)	-	-	-	-	1.6	3.1	40
Exposure phase (h)	72	72	72	384	56	222	222
Recovery phase (d)	7	7	7	7	14	12	7

Three datasets were created gathering morphological, physiological, and reactors performance information. Datasets 1 and 2 included observations of OLD and TSL, respectively. The objective consisted in examine the sensitivity of the latent variables to recognize the disturbances. Dataset 3 encompassed all observations and was created to study the discrimination of the OLD from the TSL, and respective effects.

The PCA expressed the importance of the proposed morphological parameters to recognize, possible problematic, disturbances to high-rate anaerobic granular sludge reactors. The latent variables t[1] and t[2] showed high percentages of variation in the first hours of exposure in every disturbances applied. Besides the imposed variables, organic loading rate (OLR) and toxic concentration (Csd), the morphological variables, $D_{eq}\geq1$mm, $0.1\leq D_{eq}$(mm)<1, LfA, and TL/VSS, had the higher loadings/weights associated with the latent variables.

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The two firsts Principal Components gathered 51.2% of the total variability in dataset 3. However, analysing the score and loading plots of PC1-PC2 plane (Fig. 1) is possible to visualize the variables with higher influence to distinguish load disturbances from shock load observations and respective effects, i.e. the variables more affected by the disturbances. The organic loading disturbances displaced the observations in direction of negative scores in PC1 and PC2 (line 1 in Fig. 1a). Concerning to the toxic shock load, it was observed that observations were dislocated in the direction of positive scores in PC1 and negative scores in PC2 (line 2 in Fig. 1a).

Watching at the direction lines of the exposure phase observations (Fig. 1a) and the loading plot (Fig. 1b), is visible that the TSL affects mostly the morphological variables LfA and TL/VSS. The OLD caused increases in the VSS, and, decreases in the pH and COD removal efficiency. A severe fragmentation phenomenon was also observed, as showed by the increase in the percentage of aggregates projected area with equivalent diameter (D_{eq}) between 0.1–1 mm (>0.1), and consequent decrease in the % of aggregates area with $D_{eq} > 1$ mm (>1).

The recovery phases observations tend to return to close the inocula observations (Fig. 1a).

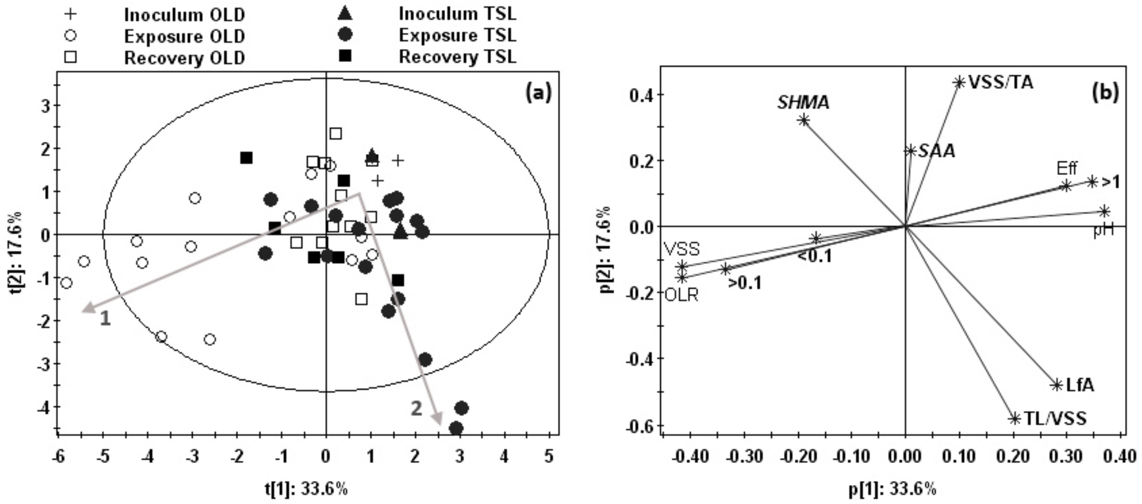

Figure 1. PCA of the first principal component versus the second principal component: (**a**) score plot t[1] *vs.* t[2], and, (**b**) loading plot p[1] *vs.* p[2].

The results enhance the important role that biomass morphology monitoring can have in the recognition of load and toxic disturbances in anaerobic granular sludge reactors. Indeed, the proposed morphological parameters were sensitive enough to detect the disturbances even before COD removal efficiency decreased.

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In-line monitoring of particle size during vinyl acetate and butyl acrylate emulsion copolymerization in a continuous pulsed sieve plate reactor using NIR spectroscopy

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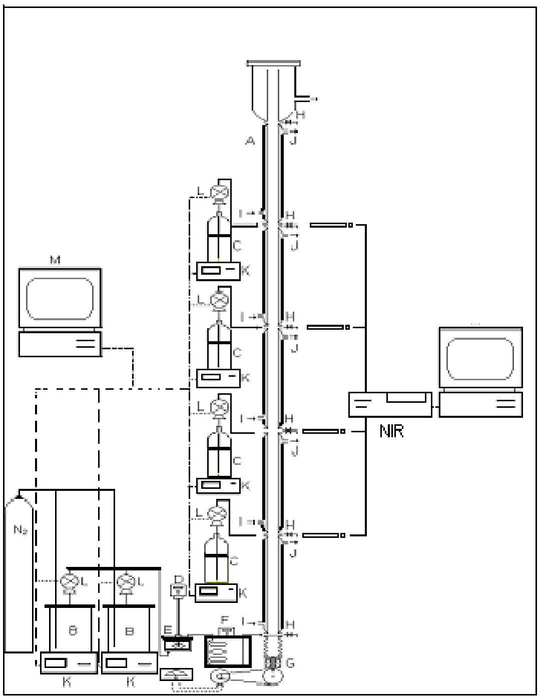
Keywords: NIR Monitoring, emulsion polymerization, particle size.

Emulsion polymerization processes are largely used for the industrial production of different commercial products. This polymerization process occurs in a heterogeneous medium composed by water as continuous phase, emulsifiers, monomers, water-soluble initiator and additives. Due to the multiphase and compartmented nature, high polymerization rates can be achieved thus favoring higher productivity and lower residual monomer content in the final latex. Polymer particle size strongly affects the physical and mechanical properties of the product (e.g., emulsion viscosity and latex stability). Paints, adhesives, varnishes are typical applications of filming-forming latexes.

Polymer particle nucleation and growing, changes in temperature and viscosity during the polymerization process, the myriad of mass transfer, equilibrium and kinetics events taking place make difficult the elaboration of comprehensive mathematical models for process simulation of emulsion polymerization processes. Similarly, the on-line monitoring of emulsion polymerization processes for tracking the polymer quality variables during the synthesis is very challenging. Actually, the lack of reliable sensors for on line monitoring is the main problem for the effective control of polymer quality properties, in special for heterogeneous polymerization processes. In the last decade the use of near-infrared spectroscopic (NIR) along with chemometric techniques becomes a powerful tool for noninvasive multipurpose monitoring of polymerization processes (Santos et al., 1998, Fontoura et al., 2003, Reis et al., 2003, Reis et al., 2004). Most works refers to batch and semibatch processes, and the use of such techniques in continuous reactors such as continuous stirred tank reactors (CSTR) and tubular reactors is still rare. Continuous reactors present the advantages of lower volumes and better control of polymer quality due the reduction of batch-to-batch variations. However, emulsion polymerization processes carried out in CSTRs may exhibit self-sustained periodical oscillations in monomer conversion and particle sizes. These oscillations can be minimizes or avoided in tubular reactors with pulsed flow, due to lower level of axial dispersion; moreover, tubular reactors present further advantages such as higher operational flexibility, less off-spec product due shorter transients during startup, shutdown and grade transitions, and better temperature control due to the higher heat transfer area-to-volume ratio (Sayer et al., 2002). In the present work the sensitivity of nir spectroscopy to the evolution of particle size was studied for emulsion copolymerization of vinyl acetate (VA) and butyl acrylate in a pulsed sieve plate column (PSPC, a tubular reactor with internals and pulsed flow). This reactor consists on five vertically mounted stainless steel sections, each one is 1000 mm long with 40 mm inner diameter. The plates, also made of stainless steel, have 39 holes placed in triangular arrangement, resulting in 23% of free area in each plate. Plate spacing between two consecutive plates is 50 mm, resulting in a reactor void fraction of 95.7%. Figure 1 presents the tubular reactor used in this work. In order to prevent possible polymer deposits on reactor walls or on its internals (“fouling”) and, as a consequence, a possible reactor clogging, a pulsation is applied to axial flow. The pulsator is located at the bottom of the column and the amplitude and frequency of pulses can be set to ranges varying from 5 to 25 mm and 0.2 to 3.5 Hz, respectively. The feeding flow rates of all reactants are controlled by a computer, using metering pumps and electronic balances. NIR spectra were collected with ifs 28/n Bruker spectrometer, equipped with a quartz bean splitter, using a probe (Hellma 661.622 NIR, with a transflection system with overall light path equal to 1mm) immersed into the reaction medium. Spectral range was from 9000 cm^{-1} to 14000 cm^{-1} in which the absorbance of chemical components is small so that the effect of light scatering in the polymer particles is significant. Photon correlation spectroscopy (coulter n4 plus) was used as reference technique for off-line measure the average particle diameters. Different techniques of spectral preprocessing were evaluated for developing, training and validation of calibration models. Experimental results indicate that in the spectral range selected the monitoring of particle size (figure 2) is possible and effective.



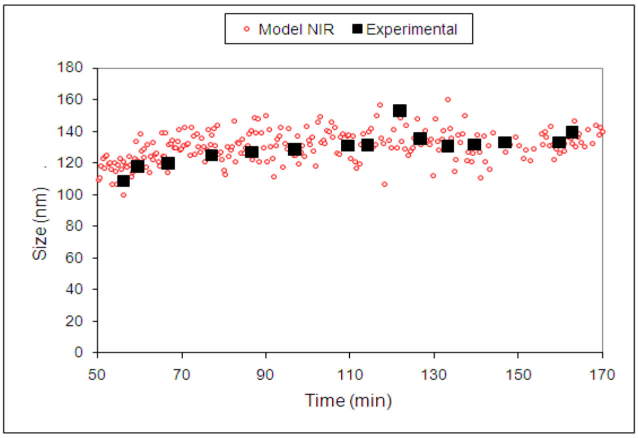


Figure 2. Evolution of the particle size during copolymerization reaction in a PSPC reactor.

Figure 1. PSPC: A - Reactor, B,C – feed tanks, D - agitator, E - pré-mixing tank, F - heater, G - pulsator, H - sampling points, I – jacket entrances, J - jacket outlet, K - balances, L - metering pumps, M - computer.

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Control of bromelain recovery process through conventional and fuzzy controllers

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Keywords: Fuzzy controller, PID controller, Bromelain, Fieldbus

Control systems have been developed with the goal to keep stable operational conditions with small values of overshoot, in a fast and safe manner. Although classic PID controllers present easy implementation and are plainly accepted in industry, they usually have low performance when applied to inherent nonlinear and transient systems, usual features of bioprocesses. Fuzzy logic can play an important role in process control due to its capability to incorporate the empirical knowledge about the process. Logical rules are used in the control algorithm, that can be easily implemented in systems with unknown structure. This makes fuzzy controllers an important alternative method to the conventional PID control for use in plants that are ill-defined or in nonlinear systems (Eker and Torun, 2006; Horiuchi and Kishimoto, 2002; Hiroyuki and Kobayashi, 2000; Martìnez, 1999). In this context, this paper presents an experimental implementation and posterior comparison between classic and fuzzy controllers applied to a bromelain precipitation process.

Bromelain presents high commercial value and an increasing demand in pharmaceutical and industrial applications (Kelly, 1996). In the bromelain recovery process from pineapple fruit, temperature control is crucial to avoid the irreversible protein denaturation, consequently improving the precipitation yield.

This paper focuses on the development of conventional and fuzzy controllers applied to a bromelain recovery process from pineapple juice through the precipitation process with alcohol at low temperature (5ºC) in a fed-batch sti rred tank (1000 mL). Samples containing 150 mL of pineapple juice were fed into the tank. A micropump was employed to continuously feed the alcohol (ethanol 99.5%) into the tank, at room temperature, until the liquid volume reached 750 mL, which corresponds to 1:4 v/v ratio between juice and ethanol (best protein precipitation condition). Inside the tank, the protein was exposed to unsteady operational conditions during the addition of the precipitating agent because the dilution ratio juice to ethanol and heat transfer area of the jacket changed. In order to avoid protein denaturation, the coolant flow rate was manipulated through a variable speed pump. A digital Fieldbus network architecture was employed, as well as the configuration system Syscon 7.0 and the supervisory software INDUSOFT web studio 6.1.

The Ziegler Nichols method was used to tune conventional controllers. The parameters Kc = 35 %/ºC, τi = 28 s e τd = 7 s were used in PID controller. The implementation of the fuzzy-PI controller (MISO strategy) was developed through software MATLAB 7.0.1.

In the early stages of the development of the fuzzy controller, some experiments were carried out to acquire expert knowledge. Positive and negative disturbances of same magnitude were applied in the manipulated variable at different moments of the batch run, with different volumes, and the controlled variable behavior was observed. With these tests, the process dynamic behavior was identified and precipitation process

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nonlinearities could be observed. In fuzzy controller tuning, different universe of discourse, base of rules and support sets of membership functions were tested.

In the present case-study, three linguistic variables were used: temperature error and change-of-error (input linguistic variable) and change in coolant flow rate (output linguistic variable). Triangular membership functions were obtained using cardinality 7.The linguistic expressions for the magnitude of the linguistic variables contain the following adjectives: negative large (NL), negative medium (NM), negative small (NS), zero (ZR), positive small (PS), positive medium (PM), positive large (PL). The rule base was implemented using the above adjectives and Mandani inference system. The center of area was used for the output variable calculation (defuzzification).

The determination of the enzyme activity was used as parameter for evaluation of the performance of both types of controllers. The analysis of the enzymatic activity is based on the biuret colorimetric method. Under alkaline conditions substances containing two or more peptide bonds form a purple complex with copper salts in the reagent. According to this method, a unit (U) of enzymatic activity corresponds to the amount of enzyme able to change in a unit the absorbance reading at 540 nm, during 10 min at 37ºC.

The experimental results showed the effectiveness of fuzzy controller in comparison with the conventional controllers. The fuzzy-PI controller presented reduced error parameter (ITAE), smaller electrical energy consumption (58 % smaller) and better recovery of the enzymatic activity (80 %).

The efficiency of the fuzzy intelligent controller is due to its adaptation to the nonlinearities of the system, presenting satisfactory behavior regarding the capability of maintaining the temperature of precipitation around set-point during the process.

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Optimal cleaning schedule for heat exchangers in a heat exchanger network based on continuous time approach

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Keywords: Cleaning schedule, Heat exchanger network, MINLP, Optimization, Continuous-time.

In all chemical industries, the energy consumed plays an important role in the operating costs. Heat recovery process is employed in order to intensify the energy consumption of the process. Therefore, heat exchanger networks are implemented in almost all chemical plants. However, fouling of heat exchanger is one major problem. Fouling in heat exchangers decrease heat transfer coefficients and heat transfer rate resulting in the increasing of external utility consumed and decreasing of heat exchanger e-ciency. Moreover, it also reduces production and increases operating, cleaning and maintenance cost. Thus, the effective methods to prevent or to remove fouling are necessary. There are many effective mitigation techniques (Smaili et al., 2001). One technique is to reduce the rate of fouling by interrupting the mechanisms causing fouling, which can be achieved by adding anti-fouling chemicals. This method results in increasing of the operating costs. Another technique is to improve the performance of heat exchanger by using more efficient heat transfer unit. However, this technique increases the fixed cost of the process. Another technique is to remove fouling in heat exchanger by regularly cleaning fouled units during operating to restore thermal performance of the network. Unfortunately, to clean heat exchangers aspects the operating costs. Generally, chemical industries use a combination of these techniques in order to minimize the overall operating costs.

Normally, cleaning improves the heat exchanger performance resulting in a reduction of energy costs. During cleaning period, the heat exchanger must be stopped, resulting in the requirement of external utility. Thus, cleaning too often may not be economically favorable. Generally, it is necessary to plan an optimal cleaning schedule to maintain the heat exchanger network in the optimal operating conditions. The optimal cleaning schedule involves consideration of which and when heat exchanger to be cleaned.

The optimal cleaning optimization problem is a mixed integer nonlinear programming involving binary variables and a number of constraints, where the objective function is postulated to minimize the operating cost. The optimization problem requires a reliable fouling model to predict performance of heat exchanger, and to determine the optimal solution. In previous research, Georgiadis and Papageorgiou (2001) developed a mixed integer linear programming (MILP) model by using arithmetic-mean temperature differences instead of log-mean temperature differences. This model could be solved to give a global optimum. Smaili et al. (2002) studied a mixed integer nonlinear programming (MINLP) model without approximate the nonlinear equations related to heat transfer or the fouling model. The formulated optimization problem was solved using outer approximation and extended relaxation algorithm. The approximation of the global optimal solution was provided by relaxed mixed integer nonlinear programming model. Thus, for small problems, the model is capable of rendering global optimality.

In this work, we presents a new technique based on continuous of time horizon approach to formulate the optimization problem as MINLP. In our proposed approach, the number of cleaning operation is fixed priorly, and the time period for each cleaning

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operation is then determined. In comparison, in the case of the discretization of time approach, the length of time horizon is divided to time period, where each period is subdivided into cleaning sub-period and operating sub-period. Further, the comparison between our technique and the discretization of time approach is discussed in terms of robustness and solution quality.

We illustrate the proposed technique in three case studies: (i) a single heat exchanger for a two year horizon, (ii) the optimal cleaning schedule of crude preheating heat exchanger network consisting 10 exchangers for 18 months horizon and (iii) the optimal cleaning schedule of Urea production unit in actual plant featuring 9 exchangers for two year horizon (Sanaye and Niroomand, 2007). The linear fouling model and asymptotic fouling model are considered. The performance of our technique compares favorably with traditional discretization of time approach approach. Our developed technique can overcome the difficulty in using logical variables encountered in the cleaning schedule optimization problem. The result of case study (i) does not show any different between discrete and continuity time approach. While in case (ii) and case (iii), the performance obtained in continuous time formulation are better than that obtained using discrete time formulation. This is due to the fact that in continuous time approach, the time period between each cleaning operation which is continuous in nature is not required to be defined priorly.

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Optimal design of water using networks

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Keywords: Water minimization, water reuse, mathematical programming, optimization

Freshwater supply around the world is under pressure due to a growing population and a significant boost in agriculture and utilization in an industrial wide range of products and applications. Water is, both as a raw-material and as an utility, probably the most broadly used resource in the process industries and it has also been intensively used in abundant quantities by other type of industries for multiple purposes. These usages lead to contaminated wastewater, which may pose an environmental pollution problem. Increasing water costs, restrictions on water use and increased environmental awareness have driven designers towards conceiving more efficient water systems. Several contributions have appeared in the literature that look into the targeting/design problem of the water-using system of a plant and/or its wastewater treatment system. This paper looks into the optimal design of the water-using network, WUN.

Mathematical programming approaches are an efficient way of addressing the WUN design problem and, when compared to pinch based approaches (Prakash and Shenoy, 2005), have the advantage of dealing with multicontaminant systems. All water reuse possibilities can be embedded into a superstructure and the problem formulated as a non-convex nonlinear program (NLP). However, unless global optimization solvers are employed (e.g. BARON), which can be computationally expensive, local optimal solutions will result.

One alternative is to use heuristic procedures to generate multiple, structurally different, starting points to the NLP. Recent examples of such methods can be found in Teles et al. (2008) for WUN and in Castro et al. (2007) for water treatment networks (WTN). In the former, the initialization procedure replaces the general superstructure by a particular substructure where the water streams go through the operation units in sequence. Each operation is then tackled one at a time from the first to the last in the sequence by solving a linear program (LP). While very effective in finding the global optimal solutions, the number of LPs and NLPs can become prohibitively high as the number of operations increases.

In this work, a new mixed integer linear programming (MILP) based heuristic method is proposed. Now, instead of selecting the operations (set O) for all positions in the sequence, the optimizer will select the most appropriate operation for a particular calculation stage. More specifically, the MILP corresponding to the first stage will consider all operations but will allow a positive inlet flowrate only to the one for which the allocation binary variable to stage 1 is equal to 1. The same for the second stage but now the solver cannot select the operation already allocated to the first stage and so on. To generate the single starting point, $|O|$ MILPs are required followed by 1 NLP to determine the optimal solution. The new procedure is much faster but it also less efficient in finding the global optimal solution due to the use of a single initial point. Nevertheless a compromise can be reached by specifying the first operation in the sequence and then solving all $|O|$ possibilities to generate multiple starting points. Overall, a new powerful procedure is proposed for the optimal design of water-using networks that can handle fixed contaminant load as well as fixed flowrate operations, and multiple water sources with multi-contaminants.

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A Shortcut Design Method for Hybrid Separation Processes

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In recent years, hybrid processes composed of a distillation column coupled with another separation process (membrane, pressure-swing adsorption unit, etc.) have received increased attention by both the industry and the scientific community. Given that combining such processes lead to a multiplicity of design arrangements, efficient design methods are required. In this paper we develop some general thermodynamic insights which may be used to integrate auxiliary separation processes in order to minimize the number of trays of a distillation column. Two new developments are discussed. The first one concerns the concept of power of separation (P_{sep}) previously introduced by Sorin and co-workers [1]. The second one is a method to best redistribute a given separation duty between unit operations within a complex separation system.

We begin by defining the power of separation of a steady-state isothermal separation process. By analogy with the mechanical power of heat engines, we call the output yield of the separation process (per unit of time) the power of separation of that process. Formally, it is defined by the following equation:

$$P_{sep} = \frac{T_0}{T} \Delta G$$

where T and T_0 are the temperatures of the process and the environment (respectively); ΔG is the increase in Gibbs free energy of the processes' products with respect to its feed. Using the relation linking the Gibbs free energy to the component fugacities and flow rates for the process streams, one obtains an equation that can be used to generalize the definition of the power of separation to processes which are not isothermal. It follows from this definition that one can decompose a system (S) into subsystems (S_l) in such a way that the power of separation of S is the sum of the power of separation of each S_l.

This property of additivity may be used to analyse hybrid processes. Indeed, the power of separation of a hybrid process is the sum of the power of separation of the distillation column and of an additional separator. As a result it is logical to assume that the number of trays within a distillation column may be reduced by increasing the power of separation of an additional separator. This hypothesis will be verified by using the example of the separation of the water-ethanol mixture in a hybrid process composed of a distillation column and a pervaporation membrane.

The following hybrid design problem is considered: for given flow rates and compositions of the hybrid's input and output streams, a given reflux ratio for the distillation column, a fixed (and constant) internal pressure for the column and fixed pressures at both sides of the membrane, determine the membrane surface area, the composition and flow rates of the permeate and distillate which minimize the number of equilibrium trays required in the column. It is assumed that the hybrid is such that the (saturated vapour) permeate is returned to the column with no mixing, i.e. the position at which the permeate enters the column is determined as the position where the vapour exiting the corresponding tray has the same composition as the permeate. By using the data for a distillation column given in [2] and the membrane model described in [3], the number of distillation trays was evaluated for different molar fractions of ethanol in the distillate. Through this numerical study it has been proven that the minimum number of trays corresponds to the maximum power of separation for the membrane. This observation offers the advantage of reducing the problem's complexity by considering only the design of the membrane unit in isolation of the rest of the system. It also forms the basis of a newly elaborated short-cut method to minimize the number of distillation trays for hybrid processes. The latter allows the rapid evaluation of hybrid design alternatives without the requirement for a full specification and provides understanding of the limits imposed to the hybrid processes by thermodynamics.

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Methods and tools for global sensitivity analysis of dynamic models of biological systems

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Keywords: Global sensitivity, identifiability, parameter estimation, systems biology.

Developing suitable dynamic models of biochemical pathways is a key step towards the development of predictive models for cells or whole organisms. Such models can ultimately provide explanations of the biological phenomena becoming a fundamental aspect in Systems Biology.

Model building can be regarded as a cycle: starting from a goal definition (purpose of the model), and some a priori knowledge (i.e. preliminary data, basic analysis and initial hypothesis), a model framework is chosen and a model structure is proposed. From the available data, parameter estimation is then performed, leading to a first working model. This initial model must be validated with new experiments, which in most cases will reveal a number of deficiencies. Thus, a new model structure and/or a new experimental design must be planned, and the process is repeated iteratively until the validation step is considered satisfactory. This is the typical model building cycle as considered in the area of systems identification.

Since the amount and quality of experimental data increases and the underlying mechanisms of biological systems are better understood, the complexity of the models necessarily increases. That usually involves an increase in the number of parameter often leading to over-parametrised models where the parameter identification problem turns very challenging. In that case, the sensitivity analysis (the study of the impact that changes in the value of the parameters have on the output of the model) can be used to decide which parameters should be the focus of the model calibration efforts, or even as an analysis tool to test model identifiability. Moreover, sensitivity methods should serve as diagnostic tools that help to improve mathematical models guiding model reformulations efforts when unidentifiable parameters are found.

Saltelli et al (2005) have reviewed and categorized the available sensitivity analysis methods. Roughly, they can be classified into local and global approaches. Local methods measure the variation of the output due to the change of model parameters around a given nominal value. They have the advantage of being straightforward to implement and computationally cheap. However, although these methods are valid for linear cases or when the value of the parameters is known, they can be misleading for the general nonlinear case. In contrast, global sensitivity analysis methods consider the effects of changing parameters simultaneously while spanning the entire admissible region of the parameter space.

In this work, global sensitivities are presented as a robust alternative for this type of analysis, detecting non influential parameters and their interactions in non linear dynamical models. The derivative based global sensitivity measures, presented in Kucherenko et al. (2007) for explicit functions, are here extended for models described by deterministic ordinary differential equations (ODEs). These values and the computational time required for their computation are contrasted with the well known Sobol' indices (Sobol', 2001).

A parameter ranking based on these measures is established and compared with the one based on local sensitivities. These rankings allow the prioritisation of parameters

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that can be subsequently estimated using robust global optimization methods (Egea et al, 2006). The dependence of the global sensitivities on the bounds considered for the parameters is also carefully studied. The performance of this methodology is illustrated with a benchmark problem regarding a model of a biochemical pathway with three enzymatic steps (Rodriguez-Fernandez et al, 2006).

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Stochastic modelling of the baculovirus/insect cells system: prediction of recombinant protein expression

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Keywords: Stochastic Modelling, Insect Cells, Baculovirus, Recombinant Protein Expression, Rotavirus-like Particles

Genetically engineered baculovirus are powerful vectors for expression of heterologous proteins in insect cells. This system is being applied to the production of rotavirus-like particles (RLPs) as a vaccine against rotavirus disease which currently has a higher mortality than AIDS/HIV in under-five children. RLPs based vaccines are non-infectious, highly immunogenic and safer to produce than existing live attenuated ones. Nevertheless, the optimisation of RLPs production is extremely complex as it involves the assembly of three viral proteins (VP_2, VP_6 and VP_7). To maximise RLP synthesis, the starting point consisted in evaluating the effect of MOI on final VP_2 titre (used as a model for the remaining VPs). For such purpose, a stochastic mathematical model was developed (Roldão et al., 2008) and used to find the optimal asynchronous infection regime. The model combines the stochastic nature of infection (randomness of virus binding to the host cells) with intracellular dynamics, resulting in a very large scale stochastic problem with high computation time associated. In order to reduce computation time, a finite number of subpopulations of "equal" cells (*npop*) were considered and two integration methods were evaluated: the direct Euler method and the 4th/5th order Runge-Kutta solver (MATLABs' *ode45* function). Both methods converge for low integration steps. Nevertheless, the Euler method for an integration step of t = 0.1 h and *npop* = 1000 was taken as a reasonable compromise between accuracy and computation time. Model calibration was performed using intra and extracellular data of vDNA, mRNA and VP collected over time in experiments with MOI= 5pfu.cell^{-1} and a starting cell density $n(0)$=10^{6} cells.ml^{-1} (Roldão et al., 2008a). All intracellular events were then simulated for MOIs of 0.01, 0.1, 1, 10 and 40 dna$_2$.cell^{-1} to extract process specificities and verify model robustness.

The population dynamics of healthy and infected cells showed that for high MOIs, 100% infection occurs within the first 3 hours post infection (hpi) whereas at low MOIs a high percentage of cells remain healthy and growing in the first 36 hpi, being only infected in a second round of infection due to virus budding (16-36 hpi). In terms of *vDNA* replication, maximum intracellular vDNA content is a trade-off between two different factors: high MOI represents more 'seeds' for vDNA replication but reduces the time window for vDNA replication. The other important factor is that for low MOIs, virus budding is extended over a long period of time, which will continuously infect and re-infect the existing host cells. Intracellular RNA_2 and VP_2 follows closely the *vDNA* replication pattern, thus a direct first order transcription law was applied.

The effect of MOI on final VP_2 (mg.L^{-1}) titre was assessed and compared with the values obtained using a deterministic model (Roldão et al. 2007)(Figure 1). A maximum productivity at MOI around 10^{-2} vDNA.cell^{-1} was observed using the stochastic model. Protein simulations show a local minimum at MOI = 10 which can be attributed to the no-saturation of the translation machinery although 100% infection was obtained in the first infection cycle. For very high MOIs, the infection is essentially synchronous and instantaneous; thus, both deterministic and stochastic models converge to the same solution due to the protein expression saturation. An optimal midterm MOI between 0.01 and 1 pfu.cell^{-1} maximised process productivity. Moreover, since the stochastic/structured model explores the hypothesis of gene size being the main factor affecting the rate of protein expression, the effect of both gene size and MOI on final protein productivities was addressed (Figure 2). Interestingly, the optimal MOI seems to be independent of the gene size. This result is rational because the recombinant gene size does not affect either the infection kinetics or the vDNA replication rate or the budding of progeny virus. It only affects the transcription and translation mechanisms, meaning that it only impacts on the intracellular accumulation of protein. Finally, as expected, the increase in gene size results in the decrease of the protein productivity (Palomares et al., 2002). The application of this modeling and optimisation methodology to the remaining VPs, will allow the establishment of optimal operational conditions for maximisation of RLPs titre.

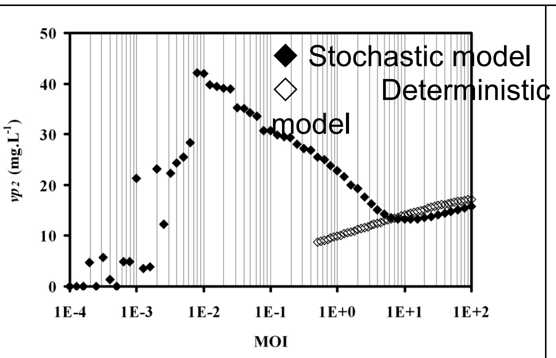

Figure 1. Effect of MOI on vp_2 production levels (mg.L^{-1})

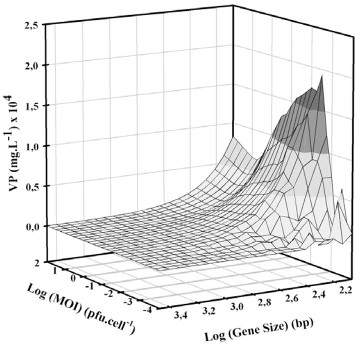

Figure 2. Gene size and MOI effect on final recombinant protein production (mg.L^{-1})

This study also highlights the relevance of having accurate analyticals for viral stock titers estimation as MOI variations may compromise process optimisation. Thus, several titration methods were compared. The techniques that better combined all important variables (accuracy, cost *per* titration, titration time and labor intensity) and therefore proved reliable for titer estimation were the $TCID_{50}$, MTT and flow cytometric assays with intra-variabilities and approximated costs *per* titration of 19% and 14€, respectively.

Mathematical/analytical tools were here demonstrated to play a determinant role in recombinant protein expression. The predictive capacity of the stochastic model will be particularly useful in designing co-infection experiments for RLPs production as infection statistics becomes a crucial factor and one must guarantee that the infected cells carry all genes required for RLP assembly.

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Scaling-up control of yeast fed-batch cultures

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Keywords: fermentation process, robust linear control, internal model principle, observer polynomial, biotechnology.

S. cerevisae is one of the most popular host microorganism for vaccine production. The possibility to easily express a variety of different recombinant proteins explains its important role in the pharmaceutical industry. In order to maximize productivity, a common strategy is to regulate the ethanol concentration at a low value, thus ensuring an operating point close to the edge between the respirative and respiro-fermentative regimes where the yeast respirative capacity is exactly filled (bottleneck assumption of Sonnleitner and Käppeli's model (1986)). Several applications of this principle can be found, for instance in Chen et al. (1995) and Valentinotti et al. (2003). However, these control schemes all require the on-line measurement of the ethanol concentration, implying the availability of an (unfortunately quite expensive) ethanol probe. This explains that alternative strategies based on more basic measurement signals, such as the dissolved oxygen concentration, have been proposed, e.g. in Akesson (1999), or that software sensors reconstructing ethanol from the measurements of basic signals have been designed (Hulhoven et al., 2006).

In a recent study of Renard et al. (2006), a RST controller with Youla parametrization is developed for the regulation of the ethanol concentration and tested successfully in real-life experiments. One of the main advantage of this approach is that it is based on a simple linear model linking the feed flow rate to the ethanol concentration, and a simple linear model of the disturbance, which represents the substrate demand for cell growth. The prior knowledge of only one stoichiometric coefficient is required, whereas the apparent growth rate can be easily estimated on-line (in order to ensure a good disturbance rejection).

The objective of the present study is to present an alternative and simpler formulation of the RST control scheme based on the observer polynomial (Dewasme et al. (2007)), and to demonstrate its large applicability range and robustness in the scaling-up of the ethanol regulation from laboratory bioreactors to industrial ones.

In all the experimental validations, the controller performs well (Figure 1) independently of the scale, demonstrating its reliability under various conditions. As compared to conventional open-loop operation, the application of the control scheme can lead to very significant productivity gain.

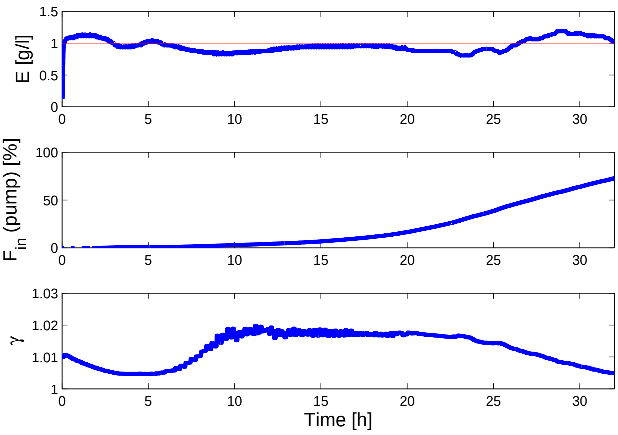

Figure 1: Experimental results of the ethanol regulation applied to laboratory-scale fed-batch cultures of S. Cerevisiae. Evolution of the ethanol concentration E around E_{ref}=1g/l, the feed flow rate F_{in} expressed in % of the maximal pump speed and the parameter γ, image of the cells growth rate.

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Adaptive extremum-seeking control of yeast fed-batch cultures

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Keywords: Extremum-seeking, fermentation process, nonlinear control, biotechnology.

Yeasts are one of the most important host microorganisms in manufacturing of biopharmaceuticals. Industrial vaccine production is usually achieved using fed-batch cultures of genetically modified yeast strains, which can express different kinds of recombinant proteins.

From an operational point of view, it is necessary to determine an optimal feeding strategy (i.e. the time evolution of the input flow rate to the fed-batch culture) in order to maximize productivity. The main problem that can be encountered at this stage is the presence of an overflow metabolism. The fermentation of an excess of substrate (glucose) can lead to the accumulation of ethanol in the culture medium, and in turn to the inhibition of the cell respiratory capacity.

To avoid this undesirable effect, a closed-loop optimizing strategy is required, which could take various forms (Chen et al. (1995), Renard et al. (2007)). In particular, the use of extremum seeking strategies for bioprocess optimization has received an increasing attention in recent years (Ariyur et al. (2003), Titica et al. (2003)).

In this study, we develop two adaptive extremum-seeking strategies based on Lyapunov stability arguments: (i) in order to determine on-line the critical substrate concentration and to avoid the production of ethanol. (ii) in order to keep the ethanol concentration constant.

The results show a satisfactory productivity as more than *150 g/l* of biomass are obtained within less than *40 hours* for both control strategies (Figures 1 and 2). Nevertheless, the substrate control presents more practical drawbacks than ethanol control once facing problems of probes sensitivity. On the other hand, substrate control leads to the optimal operating conditions in the sense of Sonnleitner and Käppeli's model (1986) while the ethanol control is a very good but sub-optimal solution.

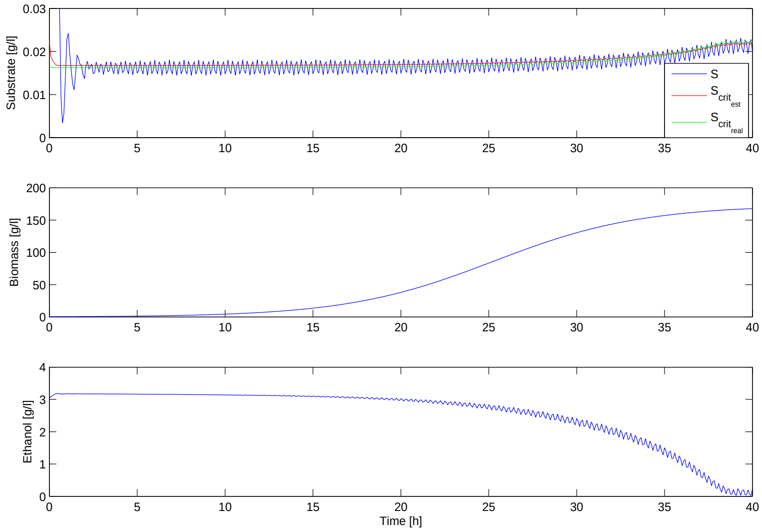

Figure 1: Substrate control. Substrate ($S, \hat{S}_{crit}$ and S_{crit}), biomass (X) and ethanol (E) concentration values.

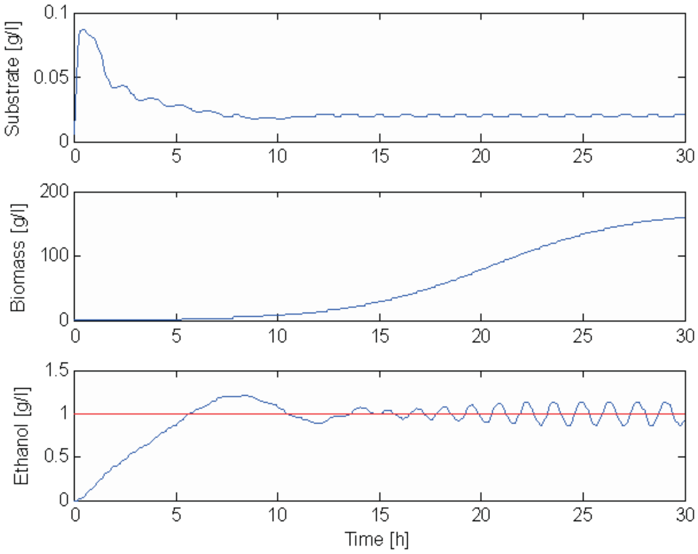

Figure 2: Ethanol control. Substrate ($S, \hat{S}_{crit}$ and S_{crit}), biomass (X) and ethanol (E) concentration values.

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Improvement of baculovirus vectors production based on the metabolic characterization and modelling of Sf-9 cultures

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Keywords: Sf-9 cells; baculovirus vectors; metabolic flux analysis; MOI; high cell density infection; nutrient supplementation

Baculovirus (BV)-infected insect cells have been used extensively as an expression system for the production of recombinant proteins (Summers, 2006). More recently, an increasing number of investigators have tackled the use of BVs to direct gene expression in mammalian cells (Kost and Condreay, 2002), for which efficient vector production at high cell density bioreaction is needed. The robustness of suspension cultures of *Spodoptera frugiperda* (Sf-9) cells and the high cell densities and productivities generally attained are advantageous for bioprocess development (Ikonomou et al., 2003). However, very few works have attempted to analyze the metabolism of insect cells, especially the effects exerted by BV infection. This knowledge can be important to unravel the limitations affecting productivity at high cell density infection and for the rational design of culture strategies to increase the efficiency of the bioprocess (Reuveny et al. 1993).

In the present work, Metabolic Flux Analysis (MFA) was applied for the metabolic characterization of *Spodoptera frugiperda* Sf-9 cells before and after infection at low ($1 \cdot 10^6$ cells/mL) and high cell densities ($3\text{-}5 \cdot 10^6$ cells/mL), and at different multiplicities of infection (MOI). The experiments were carried in batch mode using either spinner vessels or fully controlled bioreactors (1-5 L). Several metabolites in the supernatants (carbon sources, by-products and amino acids) and intracellular cofactor pools (NAD(H), NADP(H) and ATP) were followed. Additionally, in order to assess process performance, recombinant BV titration by end point dilution was performed based on the green fluorescent protein (GFP) signal.

Our results demonstrate that the major alterations of cellular metabolism occurring following infection are related to decreased fluxes through the tricarboxylic acids (TCA) cycle and growth inhibition, these effects being dependent on the MOI used (Fig. 1). Moreover, the increase in the intracellular level of ATP, together with the decrease in the NAD^+/NADH and $NADP^+$/NADPH ratios, clearly indicate that the events following BV incorporation have a profound impact on the energetic state of the cells. In terms of process productivity, the so-called “cell density effect” was less relevant when infecting the cells at high MOI (Fig. 2). Moreover, higher titers obtained after medium exchange at infection suggest that nutrient limitations may occur when working at higher cell densities (Fig. 2). By-product accumulation is not known to negatively affect growth and metabolic efficiency of Sf-9 cells (Ikonomou et al., 2003). Pursuing this, a detailed characterization of medium composition along culture time led to an increase in specific productivity by selected nutrient supplementation when infecting the cells at high cell densities. Altogether, the results obtained provide the basis for interconnecting cellular metabolism with infection process allowing culture optimization of BV vectors production.

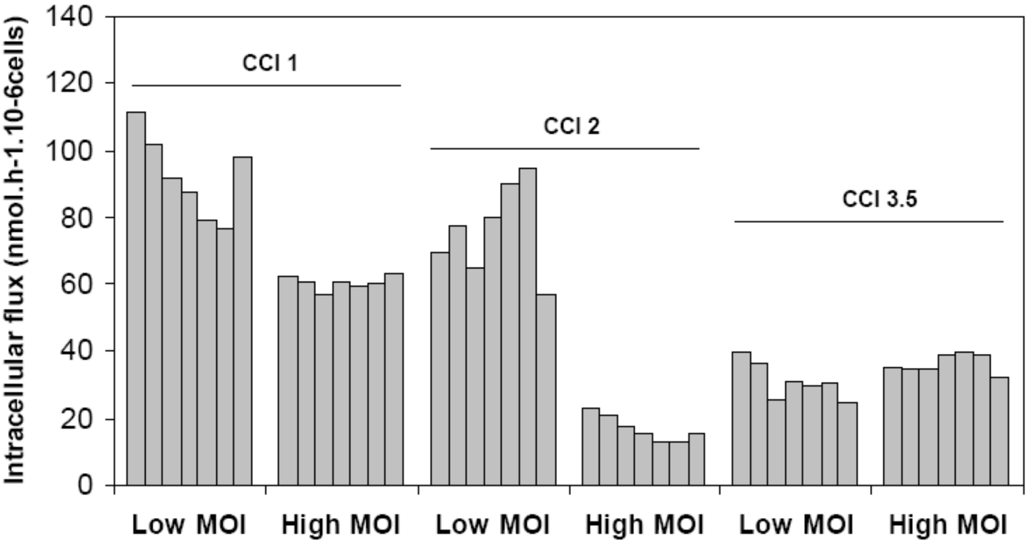

Figure 1 – Effect of different combinations of MOI and CCI on the TCA cycle fluxes.

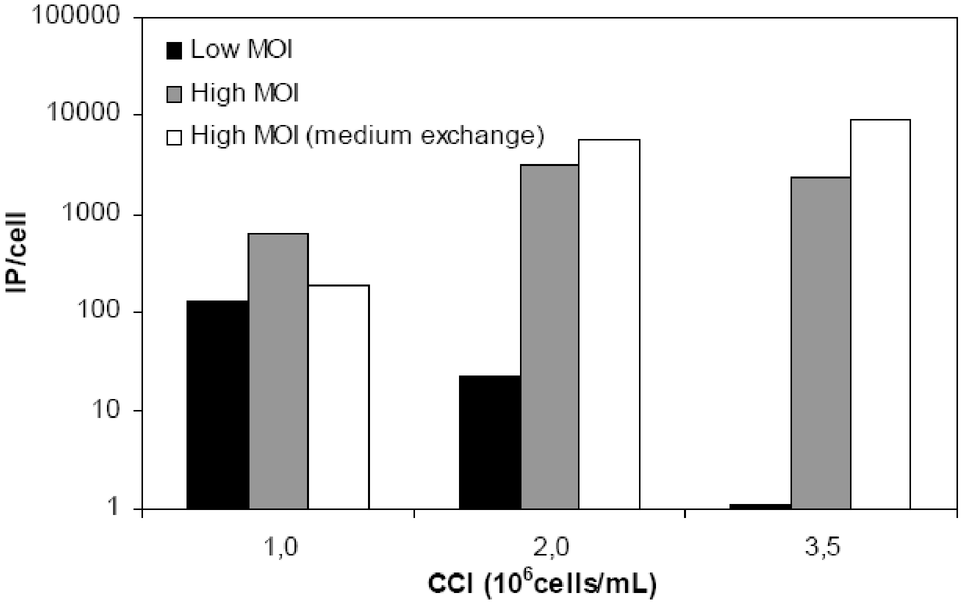

Figure 2 – Effect of different combinations of MOI, CCI and medium exchange at infection on specific productivity.

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Lactose fermentation by recombinant *Saccharomyces cerevisiae* strains

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Keywords: Fermentation, Lactose, Ethanol, Recombinant *Saccharomyces cerevisiae*

Cheese whey is a by-product of dairy industries, particularly the watery portion that is formed during the coagulation of milk casein in cheese making or in casein manufacture. Whey is produced in large amounts (about 9 litres of whey are generated per each Kg of cheese produced) and has a high polluting charge therefore creating a significant environmental problem (Siso, 1996). The world whey production is estimated to be around 82 million tons per year (Pesta *et al.*, 2007). In Portugal, the production of liquid whey is estimated to be 500 – 560 thousand tons per year (Frazão, 2001). Whey has a BOD (biochemical oxygen demand) of 30 – 50 g/L and a COD (chemical oxygen demand) of 60 – 80 g/L. Lactose is largely responsible for these high BOD and COD. On opposite hand, however, whey represents about 85 – 95% of the milk volume and retains 55% of milk nutrients. Among the most abundant of these nutrients are lactose (4.5 – 5% w/v), soluble proteins (0.6 – 0.8% w/v), lipids (0.4 – 0.5% w/v) and mineral salts (8 – 10% of dried extract). Whey also contains appreciable quantities of other components, such as lactic (0.05% w/v) and citric acids, non-protein nitrogen compounds (urea and uric acid) and B group vitamins (Siso, 1996). Therefore, whey has a vast potential as a source of added value compounds, challenging the dairy industry to face whey surplus as a resource and not solely as a waste problem.

The first step in most procedures for cheese whey valorisation consists in the recovery of the protein fraction, which has a high nutritional value. Separation of whey proteins is typically done by ultrafiltration, producing whey protein concentrates, as well as high volumes of a lactose-rich stream, the permeate, that remains a major pollutant since it retains the lactose. The lactose in the permeate (ca. 5%) may be used as a substrate for the production of valuable compounds by fermentation. The classical examples are ethanol and single cell protein production in yeast-based bioprocesses, although biotechnologists have proposed a multitude of alternative bio-products (Siso, 1996; Pesta *et al.*, 2007).

Thus, a bioprocess for the production of ethanol from whey lactose can perform two roles: on the one hand it can reduce the whey polluting charge, contributing to cheese whey bioremediation, and, on the other hand, an useful product (ethanol) is obtained that can be used as fuel or in food-related applications (e.g. in vinegar manufacturing or in the beverage industry). Being a waste product, whey has advantage over food-related fermentation feedstocks, such as corn, for bio-ethanol production.

Kluyveromyces fragilis has been the microorganism of choice for most industrial plants producing ethanol from whey (Siso, 1996; Pesta *et al.*, 2007), because *Saccharomyces cerevisiae*, the yeast usually chosen for industrial processes involving alcoholic fermentation, is unable to metabolise lactose.

We have developed a flocculent *Saccharomyces cerevisiae* strain that efficiently ferments lactose to ethanol, using a combination of genetic engineering and evolutionary engineering approaches (Guimarães *et al.*, 2008). This strain fermented efficiently and nearly completely (residual lactose < 3 $g \cdot L^{-1}$) lactose concentrations up to 150 $g \cdot L^{-1}$, including 3-fold concentrated cheese whey, producing ethanol titres up to 8% v/v. The ethanol productivity obtained with this strain (> 1.5 $g \cdot L^{-1} \cdot h^{-1}$) was higher

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than that reported for batch or fed-batch fermentations with other lactose-consuming recombinant *S. cerevisiae* strains. The strain is highly flocculent, a property that makes it interesting for the development of high cell density fermentation processes, which may attain much higher productivity (Domingues *et al.*, 2000).

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Studies on fed-batch operation mode on biosynthesis of short chain ethyl esters catalyzed by cutinase

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Keywords: Short chain alkyl esters, enzymatic processes, cutinase, fed-batch operation mode

Short chain ethyl esters belong to a huge group of flavor compounds that are very important components of natural aromas for the most part used in various food, beverage, cosmetic and pharmaceutical industries. Flavor esters are usually produced by chemical synthesis of an alcohol with an organic acid in the presence of an acid catalyst or by extraction from natural sources. The chemical synthesis of these fatty acid esters has low-priced but, they are characterized by low purity and not classified like as natural products. Natural aromas obtained by the extraction are very expensive owing to low concentration in the natural product and low extraction yield (Hari-Krishna et al., 2002, Berger R.G, 1995).

Biotechnology processes based on enzyme synthesis, as alternative catalysts to chemical route, produce also those fatty acid esters and other similar flavors. These enzymatic processes have technological and economical interesting characteristics as use mild reaction condition, synthesize flavors with high quality and purity due to the regio- and stereo-specificity of enzymes. The high purity of these flavors allows to be classified as *natural* by food regulatory agencies and better public acceptance as ingredients for food industry in relation to those synthesized by chemical processes (Welsh et al., 1990).

Cutinase are a group of enzymes that can be considered as a link between esterase and lipase. In recent years, the esterolytic activity of cutinase has been largely exploited and several applications in different industrial field have been presented. In the reaction of esterification cutinase showed selectivity for short-chain fatty acid esters (Carvalho et al.,1999).

The goals of these studies are to determine and optimize the conditions of the synthesis of fruit flavors by cutinase in organic media, in particular isooctane an organic solvent recognized as safety ingredient in food and beverage industrial processes.
The possibility and success to operate in fed-batch operation mode and to determine how much substrate and concentration would be possible to add in consecutive pulse of substrate supply and how these variables affect the enzyme activity and stability were evaluated.
In order to avoid substrate inhibition and to improve the esterification yield a discontinuous feed pulse of substrates strategy (pulse fed-batch mode of substrate supply) was tested which achieved concentration of ethyl caproate of 1.5M in isooctane media (Fig. 1). By monitoring the concentration of the substrates this type of cutinase from *S. cerevisiae* strain shows excellent properties for synthesis of short alkyl esters and special achieving high ester concentration.

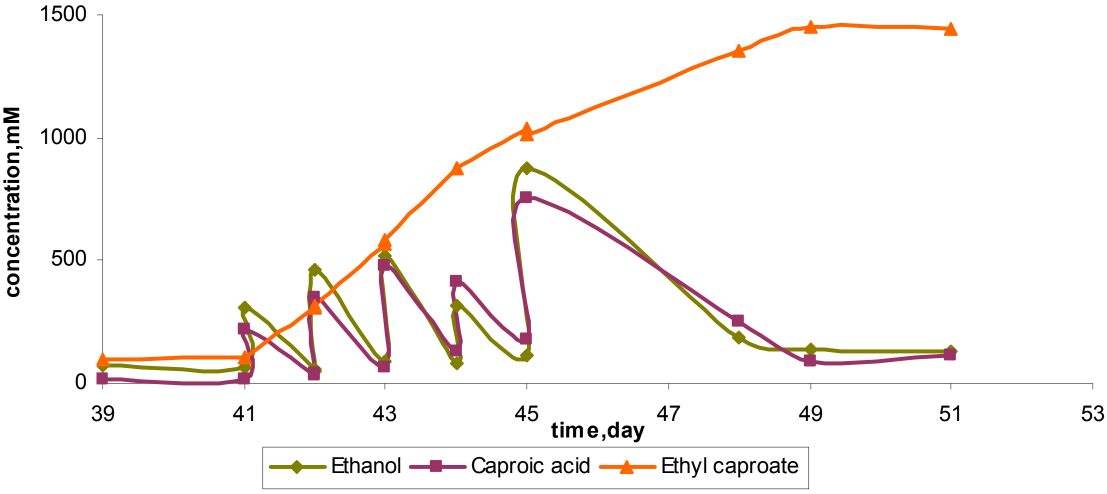

Fig. 1. Evolution of ester concentration in isooctane media obtained by discontinuous pulse of substrates (fed-batch mode).

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New enzymatical process for glycerol utilization

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Keywords: Glycerol; 1,3-propanediol; enzyme activities; glycerol dehydratase

For years we have been working on new enzymatic processes for glycerol utilization. The increasing abundance of glycerol, its renewability, and attractive pricing make it an appealing platform chemical. Glycerol is produced in two forms: natural glycerol, as a byproduct of the oleochemical and biodiesel industries, and synthetic glycerol, from propylene.

Biodiesel has become more attractive recently because of its environmental benefits and the fact that is made from renewable resources. The most commonly used method to make biodiesel is transesterification of plant oil with methanol. The main byproduct is glycerol arising in an amount of about 10% (Ma and Hanna, 1999). Once considered a valuable “co-product”, crude glycerol is rapidly becoming a “waste product” with an attached disposal cost (Soares and Dumesic, 2006).

Through the extension of the biodiesel production, probably huge amount of glycerol will be formed, which can not be fully used up by the classical glycerol utilizer cosmetic industry, so it is very important to work out alternative technologies converting this renewable byproduct into articles of high added value.

As glycerol is a nontoxic, edible, biodegradable compound, it will provide important environmental benefits to the new platform products. Glycerol can be utilized in various ways of biological transformation to industrially valuable products (Werpy and Petersen, 2004). Selective oxidation of glycerol leads to a very broad family of derivatives that would serve as new chemical intermediates, or as components of new branched polyesters or nylons. New bond breaking (hydrogenolysis) technology will lead to the formation of a number of valuable intermediates. In addition, there are some other important derivatives such as 3-hydroxypropionaldehyde ((3-HPA) commercial name: reuterin), dihydroxyacetone (DHA), and 1,3-propanediol (1,3-PD).

1,3-PD has the largest potential, which is proven by the fact that it is produced industrially by synthetic methods with more than 100.000 t/yr capacity mainly for the polimer industry (Chuah and Dalton, 1995). Dihydroxyacetone is needed by the cosmetic industry as self tanning agent and is produced by microbial fermentations. Several family of bacteria are able to ferment glycerol to 1,3-PD under anaerobic conditions, like *Citrobacter, Lactobacillus*, *Klebsiella, and Clostridium*. A new recombinant technology was worked out with the leadership of DuPont and Genecor in the last decade, in which a recombinant *Escherichia coli* converts glucose directly to 1,3-PD. A new 1,3-PD plant started with this biotechnological process in the last year (DuPont Tate & Lyle Bio Products, 2007).

Microbiological production of a molecule has always lower yield than an enzymatic method, because the cells also incorporate a given portion of the raw materials and they need another part of it for maintenace of their viability as well. On the other hand, they usually produce a lot of byproducts, which also can be avoided using enzymatic bioconversion.

We are working on this patented enzymatic way in order to produce 1,3-PD and DHA simultaneously from glycerol using a coenzyme regenerating membrane reactor system.

For the enzymatic production of 1,3-PD from glycerol, two key enzymes are needed (Papanikolaou et. al, 2000): Glycerol-dehydratase (GDHt, E.C.4.2.1.30) and a $NADH_2$-dependent 1,3-propanediol-oxydoreductase (PDOR, E.C.1.1.1.202). In our process the NAD^+ produced by PDOR can be regenerated by the third key enzyme called glycerol-dehydrogenase (GDH, E.C. 1.1.1.6) producing DHA.

These enzymes must be produced also by microbial fermentations. In our researches the enzyme source was *Clostridium butyricum VPI 1718* (Raynaud et. al, 2004).

We would like to present the results of developing the fermentation media, and the downstream processes for enzyme recovery. In addition, we would like to present the results of enzyme stability studies, and of improving the bioconversion (pH, buffer exchange, sterility, GDHt analytic, immobilization possibilities).

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Biosorption of hexavalent chromium by *Arthrobacter viscosus*

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Keywords: Chromium, Biosorption, *A. viscosus*, Detoxification.

Introduction

Among the different heavy metals, chromium is one of the most toxic and has become a serious health problem. Chromium is commonly encountered in wastewaters produced by industrial processes such as electroplating, metal finishing and those employed in tannery industries. The two typical oxidative states of chromium in the environment are hexavalent chromium, Cr(VI), and trivalent chromium, Cr(III). These two oxidation states have widely contrasting toxicity and transport characteristics. Hexavalent chromium is more toxic, with higher water solubility and mobility than trivalent chromium [1]. In recent years, the biosorption process has been studied extensively using microbial biomass as biosorbent for heavy metal removal. It is a promising method and the major advantage is evident particularly in the treatment of large volumes of effluents with low concentration of pollutants [2]. Numerous studies have demonstrated a reduction of toxic Cr(VI) to non-toxic Cr(III) by various types of biomaterials, such as bacteria The removal mechanism of Cr(VI) by biomaterials is partly through reduction, as well as through anionic adsorption [3]. The bacterium used in this work, *Arthrobacter viscosus*, is a good exopolysaccharide producer, which by itself allows foreseeing good qualities for metal ions entrapment through capture by the polysaccharide net developed by the bacteria [4].

The aim of this work is to investigate the ability of *A. viscosus* living cells for the removal and detoxification of Cr(VI) from aqueous solutions, for different biomass concentrations.

Materials and Methods

A medium with 10 g/L of glucose, 5 g/L of peptone, 3 g/L of malt extract and 3 g/L of yeast extract was used for the microorganism growth. The medium was sterilized at 121 ºC for 20 min, cooled to room temperature, inoculated with bacteria and kept at 28 ºC for 24 h with moderate stirring in a incubator. The cells were then harvested by centrifugation at 7000 rpm for 15 min and re-suspended in residual culture medium to obtain suspensions with different biomass concentrations to be used in the biosorption assays. Batch experiments were conducted in 250 mL Erlenmeyer flasks using 15 mL of each suspension of *A. viscosus* and 150 mL of a potassium dichromate solution (100 mg_{Cr}/L). Biomass concentrations of 1.2, 2.3, 4.3 and 5.3 g/L were used and determined by dry weight measurement. The initial pH value of the dichromate solution was adjusted to 3, using a 4 M H_2SO_4 solution. The Erlenmeyer flasks were kept at 28 ºC, with moderate stirring. Samples of 1 mL were taken, centrifuged and analyzed for chromium determination. Hexavalent chromium was analyzed by measuring absorbance at 540 nm of the purple complex of Cr(VI) with 1,5-diphenylcarbazide, in acidic solution [5]. For total Cr determination, the Cr(III) was first oxidized to Cr(VI) at high temperature by the addition of an excess of potassium permanganate previous to the reaction with 1,5-diphenylcarbazide. The Cr(III) concentration was calculated by the difference between the total Cr and Cr(VI) concentration.

Results

Cr(VI) removal and its reduction to Cr(III) was observed in the presence of *A. viscosus* cells. Fig. 1 presents the chromium concentration profiles and the removal efficiencies after 9 days of contact time, for the different bacterium concentrations in study.

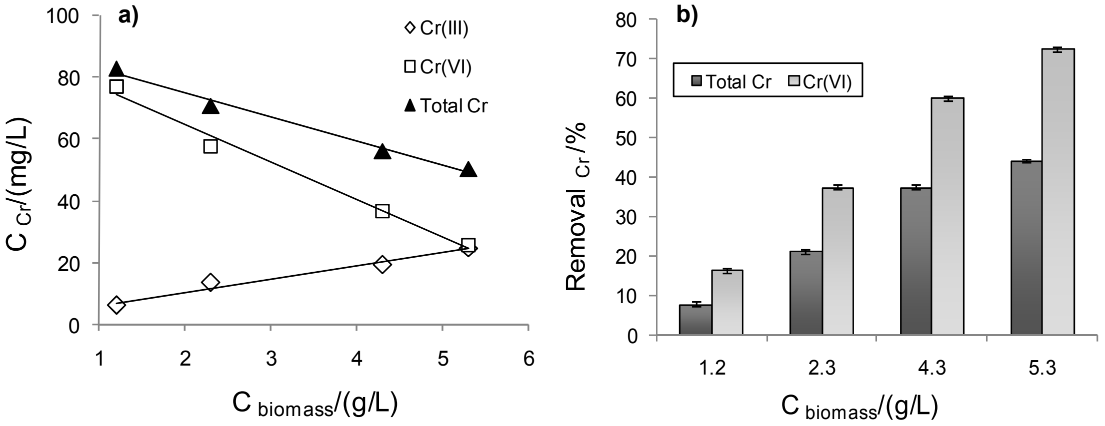

Fig. 1 Final chromium concentrations in solution **(a)** and removal efficiencies of Cr(VI) and total Cr **(b)** for different biomass concentrations.

It can be seen that the final chromium concentrations in solution showed a linear pattern with respect to biomass concentration, in the range of 1.2 to 5.3 g/L. Cr(VI) concentration decreased lienarly with the increase of biomass concentration, while Cr(III) concentration in solution increased proportionally with the increment of biomass. The lowest total chromium concentration was obtained for the highest biomass concentration, remaining in solution 25.4 mg/L of Cr(VI) and 24.6 mg/L of Cr(III).
As the biomass concentration increased the removal efficiency of Cr(VI) and total Cr increased linearly. The best removal efficiencies of Cr(VI) and total chromium were achieved for a biomass concentration of 5.3 g/L, 72.2 % and 44.0 %, respectively.

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Micro-scale and bioreactor systems for the expansion of mouse embryonic stem (mES) cells

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Keywords: mouse embryonic stem cells, expansion, microarray, spinner flask, microcarriers

Embryonic stem (ES) cells are continuous cell lines directly derived from the pre-implantation embryo. One of the main properties of these cells is their pluripotency, which allows them to be differentiated *in vitro* into a wide variety of cell types. Thus, ES cells have significant potential for tissue regeneration. However, culture and operational conditions for the *ex vivo* expansion and differentiation of ES cells still have to be optimized. The strategies for cell culture optimization include the use of both micro-scale cell platforms and bioreactor systems.

Cell-based microarrays can enable high-throughput parallel screening of a large number of small molecules. A particularly promising application of such cell-based microarrays is for investigating the influence of small molecules and cell culture conditions on cell proliferation and differentiation *in vitro*, as well as cell physiology and function. Thus, we have developed a microarray platform that enables the rapid and efficient tracking of stem cell fate and quantification of specific stem cell markers. This process integrates the use of 3D cellular microarrays that better mimic the cellular microenvironment, with an analytical method that provides quantitative information on cell function. Our results revealed that this platform is suitable for studying the expansion of mouse embryonic stem (mES) cells, as they retain their pluripotent and undifferentiated state. The 46C mES cell line was used as a model system. mES cells were encapsulated in 3D alginate spots at several initial cell densites (8.3×10^5, 1.7×10^6, 3.3×10^6 and 6.7×10^6 cells/ml) in volumes as low as 60 nL, which correspond respectively to 50, 100, 200 and 400 cells/spot. It was found that all growth curves comprise a lag phase of 24 hours. For the higher cell densities, a stationary phase was reached after 3 days, which is probably related to a lack of space inside the alginate matrix or to the accumulation of toxic metabolites (e.g. lactate, ammonia) inside the spot microenvironment. We have also developed a method based on an immunostaining technique scaled down to function on a cellular microarray. This on-chip, *in-cell* Western analysis, allows quantification of the levels of specific cell marker proteins on a microarray. As a proof-of concept, we quantified the levels of the target protein Oct-4 (a marker of pluripotency) after expansion of mES cells. Oct-4 levels were 4-fold higher when compared with a negative control, indicating that mES cells retained their pluripotency.

For the scaling-up of mES cell expansion while maintaining cell pluripotency, we implemented a microcarrier-based stirred culture system in spinner flasks (30-50 mL). The 46C mES cells were grown in serum-containing (DMEM/FBS) or serum-free (SF) medium, both supplemented with the cytokine leukaemia inhibitory factor (LIF) (Fernandes et al. 2007). The mES cells were imobilized onto porous gelatin microcarriers (*Cultispher* S). and inoculated at an initial cell density of 5×10^4 cells/ml on *Cultispher* S (1 mg/ml). The culture was performed during 8 days with half the medium being replaced everyday. The maximum cell densities achieved upon 7 days in culture were (2.6±0.7)x10^6 and (3.0±0.1)x10^6 cells/ml, which corresponds to fold increases of 50±15 and 68±1 for DMEM/FBS and SF, respectively. For both media, the maximum specific growth rate was 1 day^{-1} which corresponds to a doubling time of 0.7 day. The macroporous *Cultispher* S seemed to be advantageous in providing a protective environment against shear stress forces, which harmful effects are exacerbated in serum-free conditions. Concerning the metabolic characterization of mES cells expansion in spinner flasks, waste accumulation seemed to be the likely cause for the slowing down of the cell growth at the end of the cultures since in the cultures where lactate concentration was above 20 mM or where ammonia was above 2 mM, cell proliferation was reduced. Indeed, these concentrations were found to be inhibitory in other mammalian cell cultures. In addition, it can be considered that the feeding regimen performed in all cultures circumvented nutrients limitations since glucose and glutamine concentrations were never less than 5 and 0.6 mM, respectively. During these experiments, a key role upon culture starting was found for glutamine since its consumption was higher after 1-2 days in culture. Of notice, it was the observation that, after 8 days of culture under stirred conditions in SF medium, only 8% of the cells were apoptotic and less than 3% were necrotic. Therefore, approximately 90% of the expanded cells were viable. More importantly, the cells maintained the ability to differentiate into neural progenitors. Indeed, more than 90% of neural progenitors were obtained upon the neural commitment protocol. In addition, after expansion, cells stained positively for alkaline phosphatase, indicating that after 8 days in culture, a high percentage of cells remained pluripotent in their undifferentiated state.

Interestingly, when comparing both micro- and large-scale culture systems (microcarray spots and spinner flasks), it was concluded that mES cells proliferation is very similar in terms of growth rate values (0.7-1.3 day^{-1}).

We expect this work will have an important impact the design of highly controlled stem cell culture systems for tissue engineering and biological studies, as well as supply potential drug discovery assays in the biotechnology and pharmaceutical industries.

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Purification of human antibodies using affinity aqueous-two phase systems

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Keywords: Aqueous two-phase extraction, affinity, diglutaric acid functionalised triethyleneglycol, human antibodies.

Antibodies play an important role in the defence against invading pathogens. The invention of these “magic bullets” about 30 years ago provided the basis for new tools in biochemical research and a wide range of medical applications such as the treatment of several infectious diseases, cancers and autoimmune disorders (Farid, 2007; Gottschalk and Mundt, 2005). However, the downstream processing of antibodies is still considered the bottleneck in providing these therapeutics at reliable amounts and costs, representing, in fact, the major cost factor with 50–80% of total production costs. The critical steps have been identified as the selective purification steps such as the high-resolution techniques like affinity chromatography (Thiel, 2004). The use of inexpensive phase components and ligands in aqueous two-phase extraction (ATPE) makes this technique economically feasible and will yield a substantially enriched product, being in this way regarded as the non-expensive approach to affinity-based purification technologies (Labrou et al., 1994; Albertsson, 1986).

In this work, the purification of human Immunoglobulin G (IgG) from a Chinese hamster ovary (CHO) cells supernatant was studied using polyethylene glycol (PEG)/dextran/diglutaric acid functionalised TEG (TEG-COOH) aqueous two-phase systems. The results were in agreement with Albertsson’s theory. Higher partition coefficients, recovery yields and purities were obtained for higher TEG-COOH concentrations and lower PEG molecular weights. According to these results, the effect of the volume ratio was also studied. Higher purities and percentages of removed contaminants were obtained for lower volume ratios. However, higher recovery yields were obtained for higher volume ratios. In this way, it is possible to conclude that a compromise between volume ratio and TEG-COOH concentration should be obtained in order to achieve the best performance of affinity ATPE for the purification of human antibodies.

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Nano and micro-biocatalysts manufacture and their impact on the synthesis of β-lactamic antibiotics

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Keywords: Penicillin acylases, β-lactamic antibiotics, immobilization, sol-gel

Penicillins are the most widely used β-lactam antibiotics, but the excessive and too often inadequate use of these antibacterials has led to the development of resistant pathogens. Currently, the only method of overcoming the resistance problem is the use of newer Semi-synthetic antibiotics. These penicillins (e.g. amoxicillin, ampicillin, ticarcillin) and cephalosporins (e.g. cephalexin, cefaclor, cefadroxil) correspond to 65% of the ever rising worldwide production of antibiotics, exceeding 45000 tons in 2000 (Giordano et al., 2006).

The conventional processes of manufacturing these drugs demand harsh conditions, however, the industrial production of β-lactam antibiotics and their intermediates is undergoing a remarkable transformation where the traditional chemical conversions based on stoichiometry are being replaced by enzyme-catalyzed processes – the so-called "green chemistry" approach. In contrast to the complex solvent-based chemical approach, the one-step enzymatic conversion is regio-and stereo-specific and can be performed under mild conditions (Kim and Lee, 1996).

Penicillin acylases (PACs) (penicillin amidohydrolase, EC 3.5.1.11) are a group of enzymes which are mainly involved in the industrial synthesis of Semi-synthetic β-lactam antibiotics and production of 6-aminopenicillanic acid (6-APA), 7-aminodesacetoxycephalosporanic acid (7-ADCA) or 7-aminocephalosporanic acid (7-ACA). Availability of inexpensive enzyme catalysts with improved specificity, activity, purity and stability is a key issue in the development of successful methodologies for the enzymatic-based production of β-lactam antibiotics and their derivatives.

The advantages of using immobilized PGA over the soluble form, e.g., easier handling of the biocatalyst, facilitated separation of the biocatalyst from the product, lesser product contamination, possible continuous use or re-use of the biocatalyst and enhanced stability and productivity, allow for significant cost saving in the production However, immobilization brings along some drawbacks, such as loss of activity upon immobilization and diffusion limitations, which reduce the productivity of the immobilized enzyme system (Schroën et al., 2001).

A recent approach for enzyme immobilization is based in the sol-gel process, which allows the room temperature synthesis of silica glasses, although suitably modified to exclude the typical harsh conditions that would cause enzyme denaturation.The concomitant material offers the same beneficial properties as traditional silica based matrices and allows a high biocatalyst load, since the enzyme is entrapped within the polymeric matrix as it forms (O'Neil et al., 2002).

A well-established sol-gel processing technique consists in hydrolyzing the adequate precursors in aqueous solutions to produce soluble hydroxylated monomers, followed by polymerization and phase separation to produce a hydrated metal or semi-metal oxide hydrogel. Removal of water from the wet gel, which is usually accompanied by changes in the structure of the pores and of the gel network, results in a porous xerogel. The most widely used precursors are alkyl-alkoxysilanes (Reetz et al., 1998).

Entrapment in a silica matrix showed to be effective and efficient with lipases, but when applied to penicillin G acylase, the activity recovery was a poor 10%, which the authors ascribed to diffusion limitation in the silica matrix (Kallenberg et al., 2005).

In this work, particular emphasis is given to advances in penicillin G acylase immobilization by entrapment in a silica matrix with magnetic properties. Mechanically stable xerogel carriers containing magnetite were produced from tetramethoxysilane (TMOS). The presence of magnetite enhances the recovery of the biocatalyst, in the form of nano and micro-carriers (25 nm – 1 μm; see figure 1 and figure 2), from the reaction media. Diffusion limitations are minimized given the diameter of the carriers obtained. Immobilization of PGA in these carriers allowed for an immobilization yield of 95-100%, whereas the recovered activity was 60-70% at 37 ºC, as determined by the pH STAT method. Penicillin G hydrolysis was performed during 10 minutes at 37 ºC and pH 8.0, using the immobilized PGA in sol-gel in aqueous medium. Immobilized PGA retained 50% activity throughout 10 consecutive runs for penicillin G hydrolysis suggesting adequate biocatalyst stability.

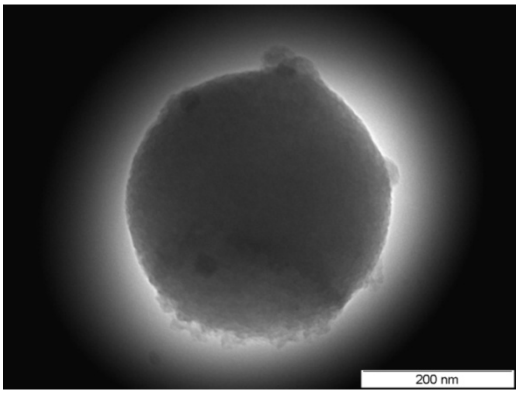

Figure 1: TEM micrographs of the sol-gel matrices with encapsulated magnetite and PGA.

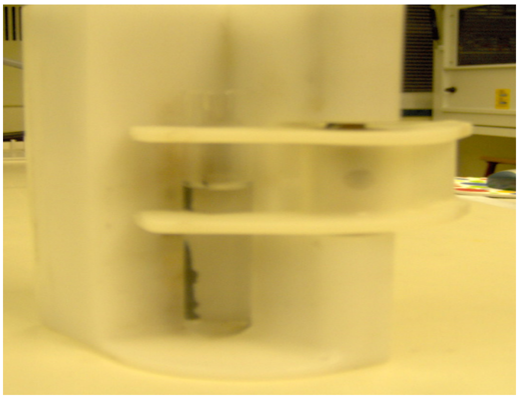

Figure 2: Magnetic particles recovery on a magnetic concentrer

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Biopolymers production by microbial fermentation on glycerol-rich product

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Keywords: Biopolymer, Fermentation, Exopolysaccharide, Polyhydroxyalkanoates, Glycerol-Rich Product

The market potential of biopolymers is restricted by the high production costs, mainly substrate cost and extraction and purification costs. Glycerol, a byproduct of many industrial processes, mainly the biodiesel industry, generated in quantities far beyond current consumption in traditional applications, is a residue for which interesting applications are lacking (Ashby et al., 2005). In this work, a process was developed for biopolymers production using glycerol-rich product (GRP) from the biodiesel industry as the sole carbon source for microbial fermentation. A *Pseudomonas* culture grown on GRP produced two distinct biopolymers, namely, an extracellular polysaccharide (EPS) and intracellular polyhydroxybutyrate (PHB) reserves.

Polysaccharides are biopolymers used in a variety of industrial applications, including food, painting, cosmetic, paper and pharmaceutical, as emulsifiers, stabilizers or thickening agents. Currently, polysaccharides obtained from plants (ex. Guar gum, Arabic gum), algae (ex. alginate, carrageenan) and crustacean (ex. chitin) still dominate the market, with microbial polysaccharides (ex. xanthan, gellan gum) representing only a small fraction of the biopolymers commercialized (Moreno et al., 1998). Poly(hydroxyalkanoates) (PHA) are natural polyesters synthesized as carbon and energy reserve materials. These biopolymers exhibit properties that range from semi-crystalline to elastomeric, making them biocompatible and biodegradable substitutes for petrochemical-based polymers (Ashby et al., 2005).

The microbial culture was grown aerobically, at 30ºC and pH controlled within 6.8-7.0, and glycerol was used as the sole carbon source. The culture produced the EPS, reaching a maximum concentration of 11.9 g/l after 6 days, and co-produced PHB (Figure 1). The process may be controlled to favor the production of either or both biopolymers. Concomitant with EPS production, there was a sharp raise of the culture broth viscosity, which induced mixing limitations, decreasing mass and heat transfer.

Thus, the fermentation run was terminated at 6 days, because it was no longer possible to maintain appropriate mixing, aeration or control of bioreactor parameters. Reactor operation strategies are being implemented to overcome mass transfer limitations.

The downstream process for polymer extraction and purification is relatively simple, involving cell separation through centrifugation of the culture broth, followed by its precipitation from the cell-free supernatant by addition of cold ethanol. The precipitated EPS is redissolved in deionised water and frieze dried.

The EPS produced from GRP has an average MW of 2×10^6 and is composed mainly by galactose (50-90%), with lower amounts of mannose (1-25%), glucose (1-25%) and rhamnose (0.5-20%). Although the presence of galactose residues is rather frequent among microbial polysaccharides, the chemical and physical properties of the EPS obtained from GRP distinguish it from other microbial EPS. The EPS forms highly viscous aqueous solutions with pseudoplastic fluid behavior (Figure 2). It is insoluble in

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organic solvents and has both flocculating and emulsifying activities, as well as film-forming capacity. These chemical and physical properties suggest a broad range of applications in the food, textile and paper industries, in the formulation of pharmaceutical and cosmetic products, for metal recovery in the mining industry, wastewater treatment and many others.

The use of GRP contributes for the sustainability of biodiesel industrial processes and to decrease microbial biopolymer production costs, thus making their market price competitive with those of synthetic and also plant or algal biopolymers.

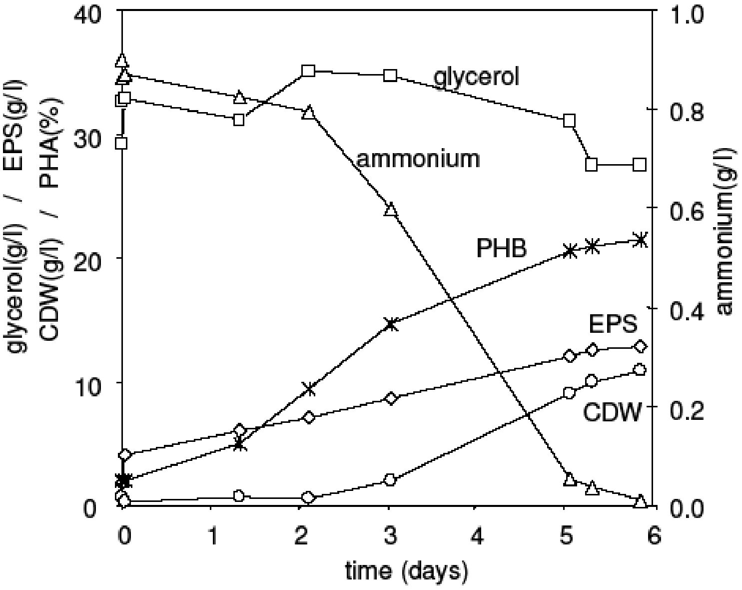

Figure 1. Bacterial growth on glycerol and production of PHB and EPS.

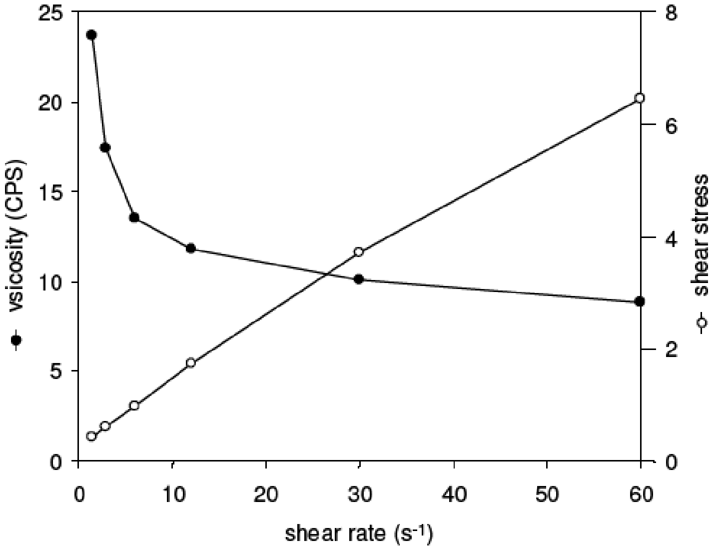

Figure 2. Rheological properties of a 0.1% w/v aqueous solution of the EPS.

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Antibody conjugated PLGA nanoparticles containing anti-cancer drugs for site-specific drug delivery

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Keywords: PLGA nanospheres, targeted drug delivery, anti-cancer drugs,polymeric drug delivery systems

Purpose: this work reports the development of plga polymeric systems attached to a unit able to recognize an antigen expressed on the surface of cancer cells in order to provide site-specific delivery of drugs and therefore contribute to the development of site-specific polymeric drug delivery systems (Liu et al., 2007). Docetaxel is the model drug chosed in this work. The preferential directioning of the drug containing micro/nanospheres marked with an antibody to the surface of epithelial cells and the internalization of the nanospheres by the cells was also evaluated (Farokhzad et al., 2006). The effect of the copolymer peg content on the drug release profile was studied. The obtained particles were characterised for size, morphology, polymer cristalinity properties and encapsulation efficiency.

Methods: Empty as well as rhodamine or drug containing nanospheres were prepared by the solvent evaporation method using PLGA and PLGA copolymers and PVA as emulsifying agent by sonication.The formed oil-in-water (o/w) emulsion was than gently stirred at room temperature until complete evaporation of the solvent. Nanoparticles were then collected by centrifugation, washed, filtered and liofilized.

The human antibody anti-CD8 marked with FITC was covalently immobilized on the nanospheres surface using NHS and EDC. The obtained nanospheres were characterized in terms of shape and external morphology by scanning electron microscopy (SEM) and cristalinity properties by differential scanning calorimetry (DSC). In vitro drug release studies were performed in (PBS; pH 7.4) at 37ºC (in sink conditions). Cell cultures were transfected with a plasmid encoding for the CD8 antigen and GFP. The preferential directioning of the drug containing micro/nanospheres functionalized or not with the antibody were tested on transfected and no transfected cell cultures. The cell lines used were kidney cells HEK293.

RESULTS:

Nanoparticles of plga and plga-co-peg containing docetaxel were obtained with high encapsulation efficiencies.

Morphology: the obtained nanoparticles present a spherical shape and a smooth surface with a mean diameter varying from 200 to 500 nm as can be seen in figure 1.
DSC analysis: the TG obtained for the copolymers PLGA-CO-PEG was around 30 ºc while the PLGA TG was 43 ºc. These results show that the presence of PEG increases the polymer chain flexibility.

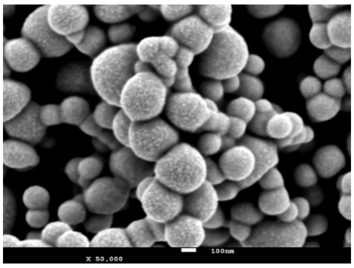

Figure 1: SEM of plga nanoparticle

In vitro drug release kinetics:

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The docetaxel release profile obtained for the nanospheres of PLGA and copolymers is presented in Figure 2.

As can be seen, the release profile show three regions: an initial burst effect due to the presence of drug in the spheres outer surface, a region with a diffusion release profile and after the 4th day a region with a constant release rate. This profile show that in the first 4 days the main release mechanism is the diffusion of drug througth the nanosphere polymer matrix and after 4 days the release of drug is mainly caused by the polymer degradation.

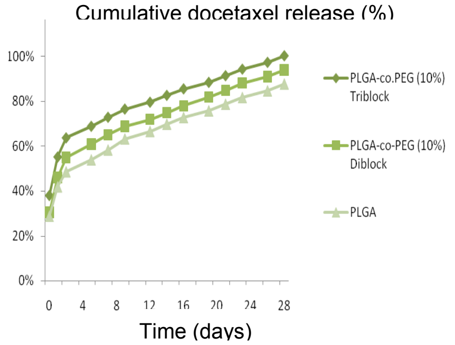


Figure 2: Cumulative docetaxel release rom PLGA and PLGA copolymers nanospheres

Also the presence of PEG increases the drug release rate. This fact can be due to an increase in the polymer hidrofilicity and to a higher flexibility of the polymer chains.

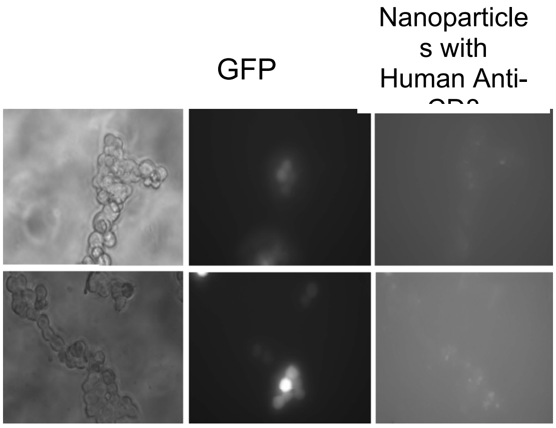
Conjugation of human anti-CD8 to the PLGA nanoparticle:

The chosen antibody was successfully immobilized on the nanospheres surface and the amount of immobilized antibody was assessed.

Cell experiments:

The results obtained after incubating the nanospheres - human anti-cd8 bioconjugates with hek29 cells are presented in figure 3. It can be observed that these conjugates bind preferentially to the cells presenting cd8 expressed in the cell membrane.

Conclusions: The drug release profile show that in the first 4 days the main release mechanism is the diffusion of drug througth the nanosphere polymer matrix and after 4 days the release of drug is mainly caused by the polymer degradation. Also the presence of PEG increases the docetaxel release rate. This fact can be due to an increase in the polymer hidrofilicity and to a higher flexibility of the polymer chains.



Micrographs of HEK293: after 1 h of incubation with Anti-CD8 conjugated nanoparticles by Fluorescence Microscopy using 400X magnification (A) visible (B) Green fluorescence (C) red fluorescence

Plga nanoparticles can be successfully attached to human anti-cd8. These bioconjugates bind preferentially to the cells presenting cd8 expressed in the cell membrane therefore providing a site-specific delivery of drugs.

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Bacterial community ctructure in a biotrickling filter treating H_2S by Terminal Restriction Fragment Length Polymorphism (T-RFLP)

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Although bioreactors for odours or, in general, for waste gas treatment have been frequently treated as "black boxes" due to the complexity of the underlying biochemical processes, a thorough study of the microbial community in these bioreactors has been shown to be critical to the understanding of how these bioreactors work (Borin et al., 2006). To our knowledge, very few studies have characterized the microbial community of such bioreactors using molecular techniques. Traditional cultivation-dependent methods of assessing the microbial community are often biased since it has been estimated that as many as 99% of all microorganisms are not cultivable by standard methods. However, molecular methods targeting the small subunit rRNA-encoding genes can be used to overcome this bias. Terminal restriction fragment-length polymorphism (T-RFLP) is a molecular approach for characterizing environmental microbial communities and for studying changes in their structure and diversity. T-RFLP is the most appropriate fingerprinting molecular method for frequent bioreactor monitoring.

In this study we spatially analyzed the microbial diversity in a biotrickling filter treating hydrogen sulphide (H_2S) using a combination of T-RFLP and clone libraries of the 16S rRNA gene. This combination allowed more accurate identification of individual T-RFLP peaks. The microbial community attached to the packing material presented a spatial distribution along the length of the reactor resulting from several factors, such as the sulphide to oxygen ratio. In this work, the spatial distribution of microbial communities throughout the reactor height was assessed by studying the changes in their structure and diversity. The most abundant clones from a clone library constructed for the reactor were used for T-RF identification.

In this work, an experimental reactor based on a conventional biotrickling filter with a separated oxygen supply system was used. Details of the reactor can be found elsewhere (Fortuny et al., 2008). The reactor was operated continuously for three months at an empty bed residence time (EBRT) of 180 s, an average liquid retention time (LRT) of 54 ± 7 h, an inlet H_2S concentration of 2,000 ppm_v (55.6 g H_2S m^{-3} h^{-1}) and a liquid recirculation velocity (LRV) of 3.6 m h^{-1} (241 ml min^{-1}). Biomass samples were withdrawn from two sampling ports situated at different heights on the reactor.

The DNA was extracted from the packing material using the MO-BIO soil DNA extraction kit according to the manufacturer's instructions (MO-BIO Laboratories, CA). DNA was visualized under ultraviolet (UV) light after electrophoresis in ethidium bromide-stained agarose gels and quantified (Nanodrop, Thermo Fisher Scientific, MA, USA). For T-RFLP analysis, the 16S rRNA gene was amplified with the labeled forward primer Fam-8F and the unlabeled reverse primer 926R (Molbiol, Germany). T-RFLP was performed as described elsewhere (Ho and Banks, 2006) with some modification as described by Egert and Friedrich (2005) to reduce partially single-stranded amplicon. Two 50-µl reactions were performed for each sample to ensure a sufficient amplicon concentration. The combined amplicon was checked for size and was treated with the Klenow enzyme to prevent partially single-stranded fragments. The amplicon was purified, and approximately 200 ng were digested for 3 h at 37◦C with HhaI, RsaI, or AluI restriction enzymes. The digests were analyzed on an ABI automated sequencer (Model 3100). The analysis of the collected profiles was performed using Genemarker 1.71 (Softgenetics, PA USA). A threshold fluorescence value of 40 was selected for the analysis. The profiles were visually inspected, and peaks were identified using T-RFs obtained from clones. To assess community similarity, terminal restriction patterns were standardized based on T-RF peak area (Kaplan et al., 2001). Hierarchical clustering was used to evaluate the statistical relationship of the T-RFLP profiles. T-RFLP fragments sizes were recorded and were compared with the data from the Ribosomal Database Project (http://rdp.cme.msu.edu/).

The results presented in the full manuscript will show that the bacterial community changes along the reactor due to the different environmental conditions. It was found that spatial distribution of putative sulphur-oxidizing microorganisms arises inside the reactor, providing a better knowledge of the performance of these types of reactors. *Thiothrix spp. and Thiomicrospira sp.* were more predominant in the upper part of the reactor, at low sulphide to oxygen ratios, whereas *Thiomonas intermedia* and *Thiobacillus plumbophilus* were more abundant at high ratios, in the lower part of the reactor, where elemental sulphur is produced in vast quantities. Nevertheless *Thiobacillus denitrificans* seemed to be equally distributed in the amplicon along the reactor showing its versatility to persist under different concentrations of oxygen and sulphide. In the paper a complete discussion of these results will be presented considering the different optimal growth conditions for the predominant species. This will highlight the microenviromental circumstances occurring in the system and will provide valuable information about the sulphide-oxidizing process. As an example, Figure 1 shows the type of information obtained from the T-RFLP techniques where the predominance of *Thiothrix spp*. and *Thiomicrospira sp.* can be observed.

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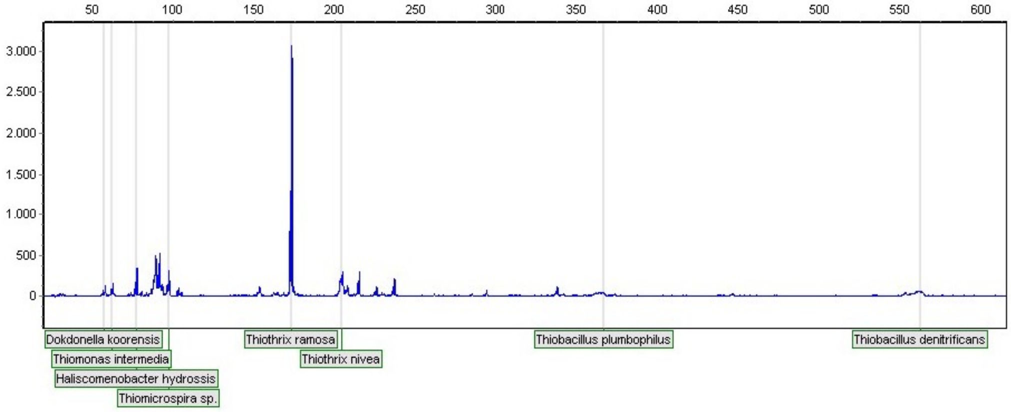

Figure 1.Electroferogram presenting the results for the upper part of the reactor. A tentative identification of the T-RFs is given based on Blast results obtained through a clone library.

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Biohydrogen production with an EGSB reactor using chloroform and 2-bromoethanesulfonate as inhibitors of hydrogen consuming bacteria

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Keywords: Anaerobic granular sludge; Bio-hydrogen; Chloroform; 2-bromoethanesulfonate; Homoacetogenesis, Lactate

Introduction

Hydrogen is a CO_2-neutral energy source with a very promising future as an alternative to fossil fuels for energy production. In a mixed culture system under anaerobic conditions the hydrogen produced by bacteria, such as *Clostridium* or *Enterobacter* is often readily consumed by hydrogen-consuming bacteria. In order to obtain hydrogen production from a mixed culture system, the seed sludge needs to be pretreated in order to suppress as much hydrogen-consuming bacterial activity as possible while still preserving the activity of hydrogen-producing bacteria. Effective pretreatment processes include heat, acidic or alkaline conditions, aeration, chemicals, and electric current. Heat treatment has been most commonly used for the screening of hydrogen producing bacteria (Lay et al., 1999). Although, some studies reported that heat treatment was unable not inhibit the activity for all hydrogen-consuming bacteria (Oh et al., 2003). Oh et al. (2003) found that some homoacetogenic bacteria may survive the heat treatment process and consume hydrogen to produce acetate. In the present study, two chemicals, 2-bromoethanesulfonate (BES) and chloroform were used to compare their efficacy as hydrogen-consuming bacteria inhibitors in a continuous system. Their effect was not only compared on the hydrogen production, but also on the presence of homoacetogenic and the distribution of fermentation intermediates.

Material and Methods

The experiments were carried out in plexiglas Expanded Granular Sludge Blanket (EGSB) reactors (1.3l). Reactors were operated continuously at a temperature of 37 ± 1 ºC. During the first 37 days, the reactors were operated with a HRT of 21 hours in order to acclimatize the biomass to the substrate. Reactor 1 was inoculated with 400ml of anaerobic granular sludge from brewery wastewater treatment plant subjected to a pretreatment with BES (15mM) for 72h at 37ºC. Reactor 2 was inoculated with 400ml of the same anaerobic granular sludge used in reactor 1 but subjected to a pretreatment with BES (15mM) and Chloroform (30µM) for 72h at 37ºC. The reactors were fed with glucose and L-arabinose (1/1) at a final concentration of 5gCOD l^{-1}. Sodium bicarbonate (1g/l) and macronutrients were also added to the reactor. The COD and VSS were determined according to Standard Methods (Standard Methods, 1998). Biogas flow rate was measured by a Ritter Milligascounter. Hydrogen and methane content of biogas was determined by gas chromatography. Volatile fatty acids (VFA), ethanol, L-arabinose, and glucose were determined by HPLC.

Results and Discussion

In both reactors acetate and n-butyrate were the most prevalent VFA's during the first period of operation, with an organic loading rate (OLR) of 5kgCOD/m^3/d (Fig.1). Hydrogen production was suppressed when acetate concentrations were ≥1200mg/l in both reactors, suggesting hydrogen consumption by apparent homoacetogenesis. When the OLR was increased to 10kgCOD/m^3/d, the acetate concentration in R1 increased to a maximum of 2100mg/l at day 60. At day 60, BES (15 mM) was injected into the reactor in order to inhibit hydrogen consumption. Subsequently, acetate concentrations decreased to approximately 800mg/l at day 75. In addition and at same time (day 75) hydrogen production resumed after an absence of approximately 40 days. In R1 after day 75, a pseudo-steady state of hydrogen production rate of approximately 200mlH_2/l/d was observed. Hydrogen production rates were observed to increase when the concentrations of acetate and lactic acid decreased and the concentrations of n-butyrate increased. Hydrogen production was lower and more unstable in R2 compared to R1.

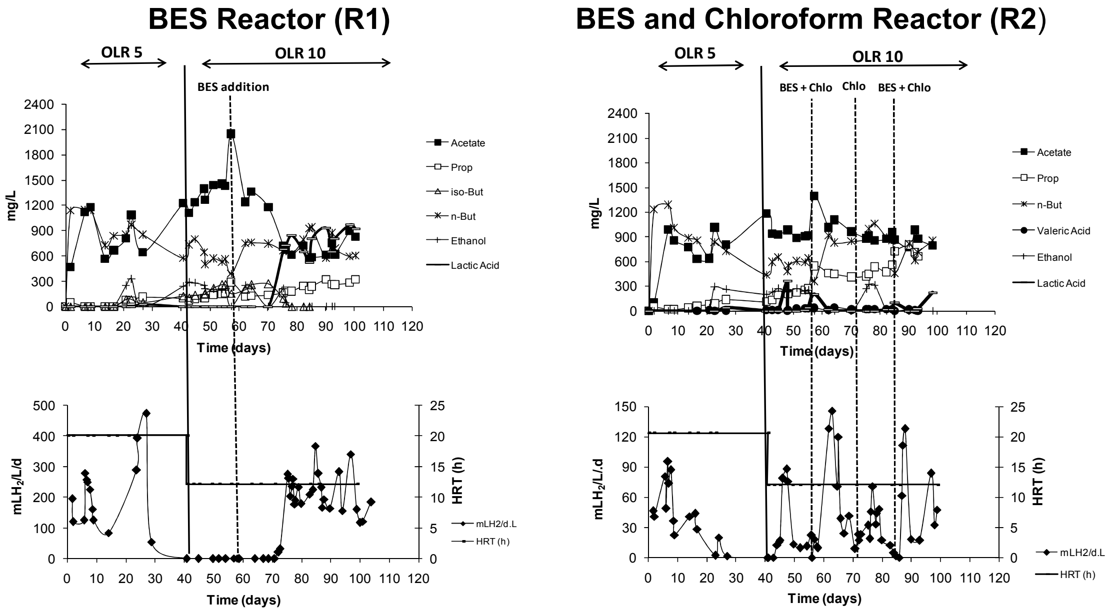

Figure 1 - Time course of reactor of R1 and R2 performance (a) profile of volatile fatty acids and ethanol; (b) hydrogen production rate and hydraulic retention time.

When an OLR was increased to 10kgCOD/m^3/d, acetate concentrations in R2 were stable at approximately 900 mg/l. However, acetate concentrations increased at day 60 to approximately 1400 mg/l. At this time, the first pulse of BES/Chloroform (BES (15 mM)/Chlo (30 μM)) was added to the reactor. This addition subsequently returned acetate concentrations to their previous level of 900 mg/l. Propionate was present in higher concentrations than in R1 suggesting that some hydrogen was consumed for propionate production. Hydrogen production rates increased for several days after the first BES/Chlo pulse addition. However, the rates were not sustained and they subsequently started decreasing. This same pattern was repeated with the addition of Chloroform only (day 71) and after the second addition of BES/Chlo (day 85). However, higher hydrogen production rates were observed after the addition of BES/Chlo rather than Chloroform only. Small amounts of methane was detected in both reactors (<5mlCH_4/l/d) during the entire operation. Pretreatment with BES and BES/Chlo and subsequent pulse additions during operation were effective at inhibiting hydrogen consumption by methanogenesis. BES/Chlo pretreatment was more efficient at inhibiting homoacetogenis as acetate concentrations were lower in R2 than BES pretreatment (R1). The lower hydrogen production rates obtained using pretreatment and pulses of BES/Chlo suggest that chloroform may not only inhibit hydrogen consumption but some hydrogen production as well.

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Biofilm technology: from support design to reactor operation

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Keywords: sequencing batch biofilm reactor (SBBR), support, nitrogen removal, poly-β- hydroxybutyrate(PHB)

The Sequential Batch Reactor (SBR) has been used to remove organic carbon and nutrients from wastewater in one single unit under properly controlled conditions (Brito et al., 1997; Rodrigues et al., 2001). The SBR can be combined with biofilm growth on the surface of a support material, originating the Sequencing Batch Biofilm Reactor (SBBR). In SBBR systems high concentrations of biomass can be maintained independently of the sedimentation characteristics of the biological aggregates and the hydraulic retention time of the reactor. SBBRs are particularly suitable when the required microbial population grow very slowly or when the biomass yield is low (Vieira et al., 2008).

Microorganisms in SBRs are exposed to continuous periodic environmental changes, namely varying liquid volumes and substrate concentrations (donors and receivers of electrons). When confronted with such interchanging periods of high ("feast period") and low ("famine period") substrate concentrations, bacterial populations adopt specific survival strategies. In particular, they often accumulate and set aside organic carbon as internal polymers such as polyhydroxybutyrate (PHB). While such storage phenomena are well studied for suspended biomass systems, as for example SBRs, they are still poorly documented in literature for systems using biofilms, such as the SBBRs (Alves et al., 2001). Nevertheless, they do play an important role in the optimization of operating strategies, which holds particularly true for multiple and interlinked degradation pathways, such as the nitrogen and carbon removal by biological nitrification and denitrification. Therefore, the aim of this work was to improve the know-how of a SBBR performing carbon and nitrogen removal: from support design to reactor operation.

The experimental part was conducted in two phases. In the first phase, biomass adhesion and biofilm formation was evaluated in a new developed thermoplastic support (*DupUM*) and in two supports currently available on the market (*Biolox10* and *Bioflow30*). The Extended Derjaguin-Landau-Verwey-Overbeek theory (XDLVO) was used to assess the affinity of the individual support materials to biomass adhesion. The thermodynamic characterization of the interaction support material-biomass was evaluated using contact angle measurements and the free energy of adhesion (ΔG) was calculated for the three support materials tested. According to the results obtained, the surface properties of *Biolox10* and *DupUM* (with values of ΔG of -34.60 $mJ.m^{-2}$ and -30.16 $mJ.m^{-2}$, respectively) are more favourable to biomass adhesion than the ones of *Bioflow30* (-4.04 $mJ.m^{-2}$) due to their lower free energy of adhesion (Salerno et al., 2004). Biofilm growth on the supports was directly evaluated in three 2.5 L SBBRs fed with synthetic water containing acetate and ammonium as carbon and nitrogen sources. *Bioflow30* and *DupUM* presented a higher biomass accumulation per unit of surface area (4454 $mg.m_{-2}$ and 5096 $mg.m_{-2}$, respectively) than *Biolox10* (2231 $mg.m^{-2}$).

The results from biomass adhesion and biofilm formation studies suggest that the hydrodynamic conditions established in the reactors and the geometry of the supports played a crucial role in biofilm formation. Biofilm growth was favoured in the supports

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that presented a higher internal surface area that protected biofilms from erosion and abrasion detachment mechanisms. Based on these experimental results *DupUM* was selected for the next phase of the experimental work.

In the second phase, the *DupUM* was used in a 28 L SBBR operating in cycles comprising three individual steps: mixed fill, aerated and draw. PHB was formed during the aerated phase immediately after acetate depletion, and was subsequently consumed for biomass growth, owing to high oxygen concentration in the reactor. Figure 1 depicts both PHB biomass ratios (f_{PHB}) of suspended biomass and biofilm (internal and external) and acetate concentration in the bulk liquid observed during a SBBR cycle.

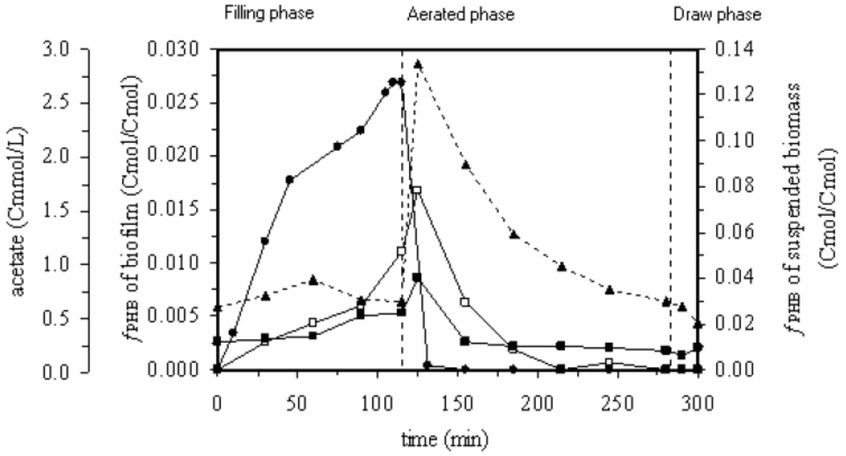

Figure 1: Profiles of acetate concentration and f_{PHB} during a SBBR cycle: acetate (●); f_{PHB} in suspended biomass (▲), in external (□) and in internal biofilm (■).

According to these results, suspended biomass was revealed to play a significant role in the carbon removal mechanism: about 79 % of the total PHB formed was stored in the suspended biomass, although this biomass represented only 20 % of the total biomass present in the reactor. This important result might be attributed to the higher accessibility of suspend biomass to acetate, due to existence of lower mass transfer limitation. The degradation of PHB by the suspended biomass and by the biofilm followed a first order kinetics with rate constants of 0.011 min^{-1} and 0.024 min^{-1}, respectively. PHB degradation rate of suspended biomass was considerably higher than corresponding literature values, which has been attributed to biomass acclimatization to operating conditions.

Time profiles of ammonium, nitrite and nitrate concentration in the aerated phase showed the typical behaviour of nitrification reactions, via nitrite formation and subsequent oxidation to nitrate. A nitrogen balance of this phase showed that 66 % of ammonium supplied was oxidized to nitrate with the remainder being used for biomass growth.

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Lignocellulosic materials as a feedstock for polymer synthesis

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Wood is one of the most abundant natural polymer, and despite its complex composition it can be used as a potential renewable feedstock for polymer production. The first step is the liquefaction of wood with multifunctional alcohols. The products are low molecular mass liquid oligomers. Liquefied wood can be prepared using all kind of lignocellulosic biomass, like wood meal and wood residues from wood furniture industry, wood waste from building industry, broken furniture, waste paper and other materials based on cellulose. Glycerine, the industrial byproduct from the production of biodiesel can be utilized in some experiments as the main reactant. Such a reaction mixture is made of 97% of waste materials.

An overview of the latest developments in wood and cellulose materials liquefaction will be given. Some suggested mechanisms of the cellulose degradation with glycols will also be presented.

In our research native hardwoods and softwoods were liquefied with different glycols and acidic catalyst. The liquefaction took place at 150 – 180 °C for 0.5 - 3 h with more than 95% yields.

We have been the first worldwide to utilize the liquefied wood as the feedstock for the synthesis of different polyesters where the liquefied wood is been incorporated into a large polymeric molecule. Such polymers have a potential use in adhesives and polyurethanes. Unsaturated polyesters were prepared by reacting liquid wood with maleic and phthalic acids. Polyester polyols were prepared by using adipic acid. These polymers were reacted with isocyanates to give a series of polyurethane foams that were comparable with commercial foams and could be used for isolation purposes.
Such utilization of lignocellulosic materials can reduce the crude oil consumption and might increase the use of the renewable resources in large extent.

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Bioethanol production from sugars by yeast having sugar uptake ability by expression of sugar transporter

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Keywords: *Saccharomyces cerevisiae*, bioethanol, transporter, xylose, xylose isomerase

Bioethanol production from lignocellulosic biomass, such like a wood, is one of ways to evade the competition with the food problem. Glucose and xylose are major component of the lignocellulosic biomass, and the efficient utilization of not only glucose but also xylose is advantage for large-scale ethanol production. Since the wild type strain of *Saccharomyces cerevisiae* could not metabolite xylose, engineered strains having xylose metabolic pathway had been well investigated previously by some research groups. In addition, *S. cerevisiae* has no xylose specific transporter, therefore the xylose uptake is very slow compared with that of glucose. The enhancement of the xylose uptake is extremely important to improve the productivity and economic efficiency of the bioethanol from lignocellulosic biomass. In this study, we carried out the ethanol production in a mixed culture medium consisting of mixture of glucose and xylose using xylose uptake ability enhanced yeast cells by the introduction of several transporters. Characterization of the xylose uptake into *S. cerevisiae* depending on hexose transporters have been reported, and the hexose transporters of *S. cerevisiae* indicated relatively low affinities for xylose (Hamacher et al., 2002, Sedlak and Ho, 2004, Saloheimo et al. 2007). Recently, a glucose/xylose facilitator *GXF1* and first yeast glucose/xylose-H^+ symporter *GXS1* from *Candida intermedia* have been reported and these transporters shows moderate and high affinity for xylose, respectively (Leandro et al., 2006). According to this previous report, we selected and constructed the recombinant yeast overexpressing following 4 transporters, hexose transporter *HXT1* and *HXT7* from *S. cerevisiae*, and *GXF1* and *GXS1* from *C. intermedia*, for enhancing the yeast xylose uptake ability, respectively. Furthermore, to introduce the xylose metabolite pathway, both of xylose isomerase *OXYLA1* from *Orpinomyces sp.* and xylulokinase *XKS1* from *S. cerevisiae* were additionally introduced into the engineered yeast cell. We evaluated the ethanol productivity of each recombinant yeast, overexpressing one transporter and both xylose metabolic enzymes in the mixed culture medium respectively.

Now, we are planing a co-fermentation system using these recombinant yeasts in the mixed culture medium for more improvement of ethanol productivity. Xylose concentration in the mixed culture medium decreases as the fermentation progress. Therefore, it is necessary that utilization of recombinant yeasts overexpressing transporter which show suitable affinity for xylose and xylose uptake against with changed xylose concentration to keep high xylose uptake thorough the fermentation. Figure 1 shows the prospective improvement of the xylose uptake profile by the utilization of recombinant yeasts overexpressing each transporter which show suitable affinity for xylose and xylose uptake in the several xylose concentration.

Furthermore, this co-fermentation system will be able to deal with several lignocellulosic biomass hydrolysates those composition of glucose and xylose differ according to the lignocellulosic biomass origin by changing of mixture ratio of recombinant yeasts. In the case of high xylose concentration (Figure 2a), raise of mixture ratio of the recombinant yeast overexpressing *HXT1* will be able to improve the consumption rate of xylose. Conversely, in the case of low xylose concentration (Figure

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2b), raise of mixture ratio of recombinant yeast overexpressing *GXS1* will be able to achieve complete consumption of xylose rapidly. Now we are verifying these our hypotheses.

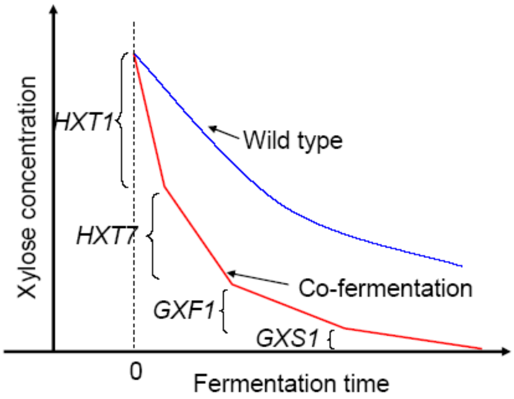
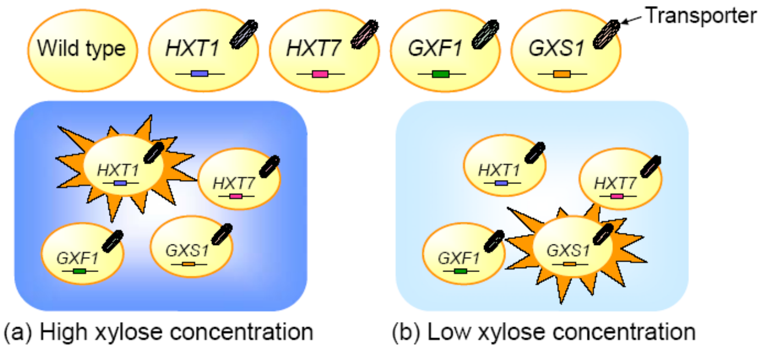


Figure 1. Prospective improvement of xylose uptake profire.



(a) High xylose concentration (b) Low xylose concentration

Figure 2. Co-fermentation system in several xylose concentration.

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Treatment of wood hydrolysates for bioethanol production by fermentation

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Keywords: bioethanol, wood hydrolysates, xylose, biorefinery*, Eucalyptus globulus*

Several well known factors lead to an increase demand of bio-based ethanol as an alternative fuel. Cane sugar in Brazil and corn in USA are the raw materials of the two major world scale bioethanol productions. However, in both cases, environmental impact due to intensive cultures and social problems related to the competition for basic nourishment, are showing that the sustainability of these production processes is far from desirable. The advantage of bioethanol as an ecological fuel, obtained from renewable resources, must be supported by other raw materials for its production. Cellulosic biomass is abundant, cheap and with a great potential to produce bioethanol, being composed of cellulose (40-50% w/w), hemicellulose (25-35% w/w) and lignin (15-20% w/w). To take greater advantage of the energetic value of lignocellulosic materials, significant research effort in the fields of non-food agro-engineering and chemical and enzymatic hydrolysis of cellulose and hemicelluloses, and the search of efficient microorganisms for hexoses and pentoses fermentation, are still required (Knauf and Moniruzzaman, 2004; Otero et al., 2007).

Biorefineries allow the use of renewable and sustainable raw materials in environmentally favorable and cost-effective industrial process. As an example, in the pulp and paper industry, hemicelluloses have been partly wasted during the cooking process being burned, simultaneously with lignin, in the recovery boiler to produce steam. These carbohydrates could be extracted before the cooking start up and could undergo an ethanolic fermentation process after hydrolysis into simple sugars. Xylans are the most abundant hemicelluloses associated to *Eucalyptus globulus* which is the main wood species used in Portugal to produce pulp and paper. After hydrolysis these biopolymers liberate xylose, but its fermentation is not easy, neither regarding the microorganism selection nor the productivity able to be achieved.

The valorisation of *E. globulus* wood hemicelluloses by using wood chips extracts prior to pulping to produce bioethanol was the aim of this work. Two liquid extracts obtained from i) an auto-hydrolysis process and ii) a hydrolysis catalysed with sulphuric acid were used. Xylose was the main fermentable monosaccharide available in the liquid extracts but its amount in the auto-hydrolysis extract was very low. Therefore, a secondary hydrolysis (catalysed by H_2SO_4, 4% w/w) was applied to the latter leading to an increase in the reducing sugars content of 50% (Carvalho et al., 2007). *Pichia stipitis* was the yeast selected due to its ability to ferment xylose into ethanol with a high productivity (Nigam, 2001).

The hydrolysates have a pH in the range of 1-3 and, besides carbohydrates, also contain a variety of other compounds such as lignin, acetic acid and furfural, which could have an inhibitory effect on the microorganism performance. In a previous study (Rocha et al., 2007) different synthetic growth media and environmental conditions, were checked. It was observed that furfural (0.1 and 0.8 g L^{-1}) did not inhibit the metabolic activity of *P. stipitis*, whilst lignin (1.3 and 7.0 g L^{-1}) induced a reduction of 20-30% on the yeast growth. Acetic acid (4 and 8 g L^{-1}), which lowered pH to 4, inhibited the yeast metabolism. The correction of pH with ammonia did not restore the

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yeast activity but better results were observed after correction with NaOH. Therefore, an adequate treatment to reduce or eliminate those substances and to achieve the desirable pH (5.5-6.5) had to be implemented so that the hydrolysates could be used as substrates for the fermentation process. Two different methods were tested using either NaOH or Ca(OH)2: one-stage method consisted in the continuous addition of NaOH or Ca(OH)2 until the pH 5.5-6.5 was reached, being the precipitates removed afterwards by centrifugation and vacuum filtration; the two-stage method consisted in increasing pH until 10, by adding NaOH or Ca(OH)2, and, after the precipitates removal, the pH was adjusted to 5.5-6.5 with H2SO4 and vacuum filtration. The hydrolysates treated by these two methods were supplemented with peptone (5 g L^{-1}), malt extract (3 g L^{-1}) and yeast extract (3 g L^{-1}) and used as culture media for *P. stipitis*, which had been previously adapted to the treated wood extracts. The use of an adapted strain provided the elimination of the initial lag phase during hydrolysates fermentation, as it is shown in Figure 1. In addition, the two-stage method, using Ca(OH)2, enabled a better yeast performance, probably due to a greater detoxification effect. The fermentation of the acid catalyzed wood extract, due to its higher content in xylose, led to higher ethanol concentration, yield and productivity, compared to those obtained with auto-hydrolysis and secondary acid hydrolysis extracts. It took 50h to achieve an ethanol concentration of 9 g L^{-1} in the fermentation of acid catalyzed wood extracts. Productivities in the range of 0.13 – 0.22 $g_{ethanol}$ (L h)-1 and yields based on xylose equivalents of 0.27 – 0.48 $g_{ethanol}\ g_{xylose}^{-1}$ were obtained, which are similar to the few data in the literature (Nigam, 2001), allowing to have high expectation on the energetic valorisation of *E. globulus* wood hemicelluloses.

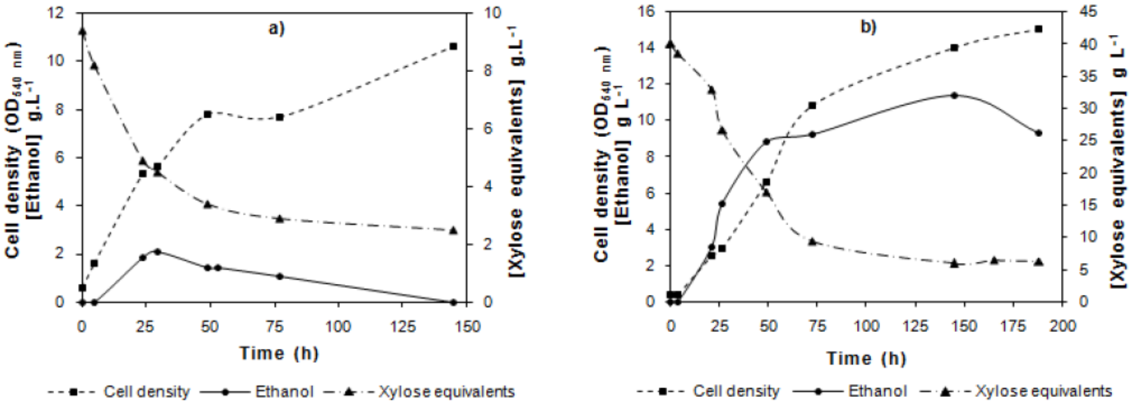

Figure 1 - Yeast growth, xylose consumption and ethanol production with a) secondary hydrolysate, b) acid catalyzed wood extract, both treated with $Ca(OH)_2$ by two-stage method.

Acknowledgments

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Integration of bioprocesses in polyesters microbial synthesis

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Keywords: polyhydroxyalkanoates, PHB, *Ralstonia eutropha*, bioplastics

The production of environmentally friend ecological plastics, since its manufacture from renewable raw materials till its degradation by biological processes, taking in mind that Nature always knows how to recycle what it is able to synthesize, it is a main contribution to mankind sustainability. The biocompatibility of natural materials enlarges their application to medicine, agriculture, food industry and other quotidian uses. The challenge is to explore Nature gifts without degrading its quality level, creating conditions to biological production in large scale, and economically competitive with traditional chemical synthesis. The polyesters from polyhydroxyalkanoates family are biopolymers that can be processed and used as a change of common petrochemical based plastics (Choi and Lee, 1997). They play the role of reserve compounds in many bacterial strains, such as *Ralstonia eutropha*, and their biosynthesis can be stimulated in fed-batch fermentation, under appropriate incentives and driven stress conditions, like the limitation of an essential growth nutrient and the excess of a carbon and energy source (Braunegg et al., 1998).

The large scale production of biopolymers still has several bottlenecks to be overtaken (Luengo et al., 2003). The large number of references found in literature, but the shortage of new experimental results, led to a set of experiments to test or confirm the effect of several factors in the production and yield of the most abundant homopolymer in many prokaryote's microbes, the poly-3-hydroxybutyrate (PHB). Simple reaction systems were carried out, always with the purpose of reducing overall costs of the production process. Three different carbon sources were used: glucose, sucrose and cane molasses. The temperature control and the culture medium homogenization were relatively easy to achieve, either in small and simple scale, like Erlenmeyer flasks in an orbital incubator, or in larger scale in fermentor. A limited oxygenation rate showed to favour the polymer production, which was confirmed in several fermentation conditions, in a qualitative way. A biopolymer content of *c.a.* 80% of cell dry weight was reached. The cell density achieved was not too high, showing that an undesirable nutrient limitation had occurred, probably a nitrogen limitation. The culture system by itself is not so decisive upon the concentration of polymer that it is possible to obtain (11.3 g/L in fermentor and 7.7 g/L in flasks), nor upon the yield related to the carbon source (0.2 – 0.3 $g_{PHB}/g_{glucose}$ in both culture systems), but it is upon the productivity, that is clearly favoured by the fermentor (0.13 *versus* 0.06 $g_{PHB}/(L\ h)$). The pH control close to neutral was shown to be an important parameter, either if done automatically in fermentor, or manually with periodic addition of alkali. The use of cane molasses, previously hydrolysed or not, led to some natural buffer effect, favourable when compared to added buffers to the culture medium. A microaerated culture in erlenmeyer flasks, with an initial concentration of 20-30 $g_{glucose}/L$, with a single supplemental addition of glucose, even without any pH adjustment, allowed to reach reasonable values of yield (0.22 $g_{PHB}/g_{glucose}$) as well as average molecular mass (1.8×10^6 Da), although still not so much of polymer concentration (7.7 g/L), for an eventual practical exploration; the smaller productivity can be compensated by the simplicity of the culture system and costs (operation and investment) associated. These last values compare well with the ones found in literature for equivalent fermentation systems (Khanna et al., 2005).

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The cane molasses showed to be a suitable carbon source to the production of PHB by *Ralstonia eutropha*. This carbon source is cheaper and compares well with the use of sucrose, mainly when molasses are previously submitted to a biocatalytic hydrolysis by the action of *Saccharomyces* yeasts (using the intracellular enzyme invertase). This previous treatment reduced the adaptation phase of the culture, making it able to reach the polymer accumulation phase earlier. Yeasts from a 2 days culture of 150 mL of a standard yeast growth medium at 30ºC and 125 rpm, were centrifuged and added to 200 mL of cane molasses solution (100 g L_{-1}, equivalent to 50 g L_{-1} of sucrose) and kept in an orbital incubator at 45ºC for 2 h. This temperature did not allow the yeast growth (so, no consumption of carbon source) and the biocatalytic hydrolysis of molasses was followed by DNS method, showing that a plateau was reached in 2 h. After centrifugation, the supernatant (hydrolysed molasses) was sterilized and diluted 1:5 with sterilized bacteria culture medium without carbon source. The resulted medium was inoculated with molasses adapted *Ralstonia eutropha.* The culture lag phase was reduced. Biopolymer productivity had a significant increase, even without pH control, when compared to the fermentation of non-hydrolysed molasses, under the same conditions. Cane molasses do not need to be sterilized before hydrolytic treatment with yeasts cells, and these ones can be re-used.

The product extraction strategy was also analysed, over the yield and the effect in polymer structure. The biopolymer extraction by ethanol precipitation gave larger polymeric chains (with higher molecular masses), acting as a separation process of the shortest chains, when compared to the simple chloroform evaporation. The total mass of polymer obtained is similar in both processes. Other polymer extraction and solvent recovery methods, such as the direct distillation of chloroform from the polymeric solution, or the solidliquid extraction in soxhlet, showed operational problems difficult to overreach.

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One-step cellulosic ethanol production

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In a traditional simultaneous saccharification and fermentation process, hydrolysis of cellulose to glucose by cellulase enzymes, and sequential fermentation of glucose to ethanol by microorganisms, such as *Saccharomyces* or *Zymomonas*, are accomplished in the same reactor. *Trichoderma* cellulases have been used widely to degrade cellulose. The optimal temperature for *T. reesei* cellulase activity is between 45 to 50 °C, where as ~ 30-37 °C is the optimum fermentation temperature using microorganisms. The main disadvantage of the *Trichoderma*-yeast SSF process is that the optimal temperatures for growth of these combined system are not compatible. Use of molecular biology techniques to express "cellulosome" on the cell surface is an alternative way to perform the SSF functions in a bioreactor, provided the host cell is robust, shear insensitive, tolerant of high concentrations of ethanol, and the expressed cellulases have high activity at normal fermentation temperature (~30-37 °C). This is the challenge, and it is the main goal of this work.

The key innovation in this research is the development of "mini-cellulosome" on the cell surface of a recombinant microorganism, so that the cells can uptake the cellulose and convert it to ethanol; for the proof of concept, we use the ethanologenic *Escherichia coli* LY01, a strain developed by Dr. Ingram of the University of Florida. This strain is robust, and can tolerate more than 6% ethanol. This is approximately the minimum acceptable concentration of ethanol, which can be economically produced in a commercial plant; lower ethanol concentrations result in higher distillation cost. This strain is also tolerant of acetic acid (2.5%), and hydroxymethylfurfural (HMF) and furfural, both up to 3.5%. This will reduce one unit operation, thus reducing the capital and operating cost of a cellulosic ethanol plant using cellulosic biomass. This method will also reduce the cost of enzymes, which is one of the major contributors to the operating cost of ethanol production. In a report by NREL (NREL/TP-510-32438), it was estimated that approximately 9%, and 7% of the ethanol selling price can be attributed to the enzyme, and the fermentation and saccharification costs, respectively. Since in the proposed methodology, the recombinant *E coli* will produce the necessary enzymes and express them on the cell surface, direct conversion of cellulose to ethanol is feasible without the need for externally added enzymes. It is estimated that at least 10% -12% cost reduction is feasible by this process.

To enhance cellulose-hydrolysis activity, three cellulases are simultaneously displayed on the surface of ethanologenic strain *E.coli* LY01. In order to display the designed mini-cellulosome which forms cohesin domain chain on the surface of cells, pHLA plasmid, from Dr. Kondo (Kobe University, Japan), is used for expressing the anchor protein PgsA in a membrane. For constructing the synergistic mini-cellulosome biocatalyst system, there are two main issues. One is to find cellobiohydrolase-cohesion and beta-glucosidase-cohesin interaction. Because only the cohesin 1 domain among cipC scaffoldin interacts with endoglucanase CelCCA of *C. cellulolyticum*, there is a need to screen other interactions between cohesin domain and catalytic proteins for synergistic effect of the biocatalyst. The other issue is to design the component such as a linker to construct the assembled mini-cellulosome model system. Various biological components such as peptides, DNA, and proteins are studied. There are several factors which need to be considered for manipulating the linker components: (1) the linker should be shear insensitive, (2) it should have well-defined accessibility and specificity and should not affect the adsorption rate of the enzyme and the substrate. Moreover, (3) the size of the assembled enzyme complex should not to reduce the hydrolysis rate. The cellulose binding domain (CBD) is able to anchor with PgsA on the surface of the cells. Different cohesion domains are connected to CBD via short linker. For LY01, pHLA basic plasmid is used to construct the bioconversion system. The overall concepts are shown in Figures 1 and 2

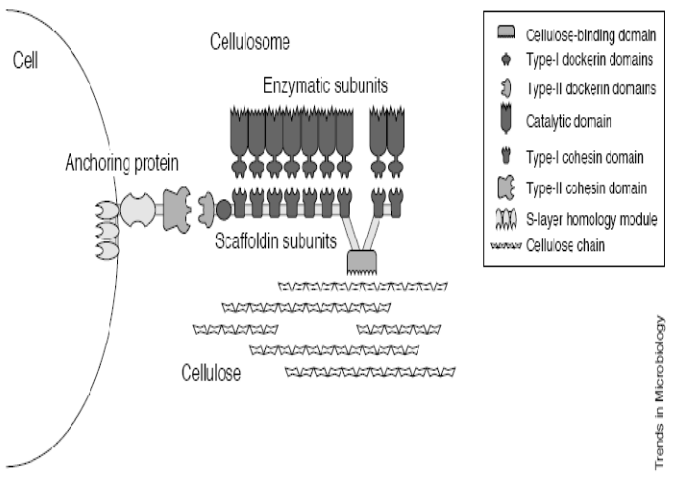

Fig. 1. Simplified schematic view of the interaction between the *Clostridium thermocellum* cellulosome and its substrate, and its connection to the cell surface via an associated anchoring protein.

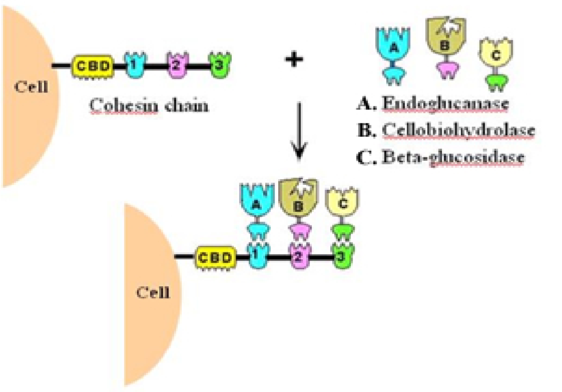

Figure 2. Design of mini-cellulosome.
Scaffoldin contains cellulose binding domain (CBD) and three different cohesin domains which can specifically bind with dockerin domain of catalytic enzymes. Each cohesion domain 1, 2, and 3 are interacted with endoglucanase (A), cellobiohydrolase (B), and beta-glucosidase (C), respectively (color-coded).

OptGene – a framework for *in silico* metabolic engineering

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Keywords: Metabolic Engineering, Evolutionary Algorithms, Flux Balance Analysis, Genome-scale stoichiometric models

Introduction

In metabolic engineering problems, due to the complexity of metabolic networks, it is often difficult to identify *a priori* which genetic manipulations will originate a given desired phenotype. Genome-scale metabolic models (Patil et al., 2004), available for several microorganisms, can be used to simulate the metabolic phenotype and therefore help the tasks of metabolic engineering. This simulation can be performed by calculating the fluxes through all metabolic reactions using techniques like the Flux Balance Analysis (FBA) (Edwards et al, 2002) or the MOMA (Segre et al., 2002 approaches, among others.

Several algorithms, like the framework OptKnock (Burgard et al., 2003) have been developed that use metabolic models to enable the identification of gene knockout strategies for obtaining improved phenotypes. However, the problem of finding optimal gene deletion strategy is combinatorial and consequently the computational time increases exponentially with the size of the problem.

OptGene Algorithms and Software

We have proposed the use of bio-inspired algorithms, namely Evolutionary Algorithms (EAs) and Simulated Annealing for the optimisation of the set of gene deletions to apply to a microorganism, in order to maximize a given objective function, associated with a given industrial objective (Patil et al., 2005) and (Rocha et al., 2007). The proposed algorithms enable solving large gene knockout problems in relatively short computational time and also allow the optimization of non-linear objective functions and additionally provide a family of close to optimal solutions.

Each mutant strain is evaluated by resorting to the simulation of its phenotype using the Flux-Balance Analysis approach, together with the premise that microorganisms have maximized their growth along natural evolution (Ibarra et al., 2002). The application of this methodology to several case-studies allowed to identify directed genetic modifications for the construction of improved bacterial and yeast strains for the production of valuable chemical compounds such as succinate. The results suggest that non-intuitive genetic modifications spanning several different pathways may be necessary for solving challenging metabolic engineering problems.

Based on these validated approaches, the user-friendly software framework *OptGene* was designed and developed that includes a number of tools to support *in silico* metabolic engineering. The application allows the user to load a genome-scale stoichiometric model of a given organism. This serves as the basis to simulate the phenotype of the wild type and of mutant strains. The simulation of these strains is conducted using a number of approaches (e.g. Flux-Balance Analysis or MOMA) that allow the set of fluxes in the organism's metabolism to be determined, given a set of environmental constraints. The core of the software tool is, however, the identification of metabolic engineering targets, performed by a number of optimization methods that allow to reach the best set of gene deletions given an objective function.

This application is complemented by an independent visualization tool, named *BioVisualizer*, that allows the visualization of graphs representing metabolic networks. These graphs have a number of distinct node types (e.g. metabolites, enzymes,

reactions) and connections. One of the major features of this tool is the ability to associate numerical values to the different types of nodes and edges in the graph.
The integration of the two applications allows the visualization of the metabolic network, superimposed by the values of the fluxes of a given simulation. The values of the fluxes for the wild type and different mutants can, therefore, be adequately visualized in this way, providing useful analysis tools for the researchers.
Additionally, this software is compatible with several SBML (Systems Biology Markup Language) standards (Hucka et al., 2003), allowing the use of models stored in public databases or built in other software tools both for simulation and visualization.
The *OptGene* application is being developed taking as a basis the *AIBench* framework. This is an environment for the development of Data Mining / Bioinformatics tools, using the Java programming language. The details of this project, a collaboration between the universities of Minho (Portugal) and Vigo (Spain), as well as updated documentation can be found at the web site www.aibench.org.

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The potential and the challenges in integrating thermodynamics into systems biology

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Keywords: Biochemical Thermodynamics, Systems Biology, Thermodynamic Feasibility Analysis

Systems biology is experiencing fast development, as a result of the development of high throughput experimental techniques as well as computational power. The goal is to integrate as much as possible biological data in the mathematical models that would eventually allow the creation of a completely quantitative in silico model of a live cell. However, in spite of the enormous amount of information that goes into a genome-wide quantitative analysis of a functional cell, systems biology results in mathematically underdetermined models, and instead of an exact solution one often has to deal with a whole solution space (Edwards and Palsson, 2000).

In an attempt to reduce this solution space, it was recently proposed to incorporate thermodynamics into system biology analyses (Beard et al, 2002 and 2004; Qian et al, 2004; Henry et al, 2006 and 2007; Kümmel et al, 2006). Indeed, thermodynamic feasibility analyses (TFA) can point out those solutions that satisfy all mass balances but are not allowed from a thermodynamic point of view. The authors were however confronted with such an overwhelming mass of unknown physical-chemical parameters that they had to introduce dramatic simplifying assumptions. Thus, the standard Gibbs energies of reaction were often estimated based on simple group contribution methods, the metabolite concentrations were either assumed to be 1 M throughout or they were adjusted within a very wide, conservative span of values, the solution were sometimes assumed to be ideal, the influence of ionic strength and of pH was either allowed for based on data estimated from group contribution methods or neglected altogether (Maskow and von Stockar, 2005).

In order to check to what extent these simplifications are justified, and if a TFA based on them is still able to provide meaningful and useful information, we have performed a thorough analysis of the thermodynamics of the well known pathway of glycolysis. The rationale behind this is that a) the result of TFA is known since glycolysis is undoubtedly a feasible pathway, b) most of the thermodynamic data as well as cytosolic concentrations for the glycolytic intermediates are known, c) glycolysis is an entirely cytosolic pathway, which simplifies the problem by avoiding the necessity to account for the compartmentalization of the cell.

Based on the mathematical procedure described by Alberty (2003) the corrections to the published standard Gibbs energies caused by changes in acid dissociation, magnesium complex formation, and ionic strength may be calculated for each reaction of the glycolytic pathway and for different pH, magnesium concentrations and ionic strengths. These transformed standard Gibbs energies were used in the subsequent TFA in order to check whether the pathway appears thermodynamically possible with and without different simplifying assumptions.

It turned out that the result of the TFA is extremely sensitive to i) the standard Gibbs energies of reaction used in the analysis, ii) the span of values within which the metabolite concentrations are assumed to vary, iii) the pH, ionic strength and pMg assumed to prevail in the cytosol, iv) the dissociation constants and the magnesium complexation constants of all the metabolites involved, and v) the assumptions concerning the precise nature of the reacting species. In view of the fact that no experimental data is available concerning these factors for most of the metabolites in a live cell, it can be concluded that systems biology in combination with TFA is still very far away from being able to predict the feasibility of a new, as yet unknown pathway. For a metabolic network known to function in nature, such as the metabolism of a well known bacterium, systems biology and TFA may be used to gain insight concerning unknown metabolite concentrations or pathway alternatives, but only if most of the information cited above is known with considerable reliability. An experimental program measuring this information at a genome scale is urgently needed.

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Macroscopic control of intracellular regulation: application to mammalian cell cultures

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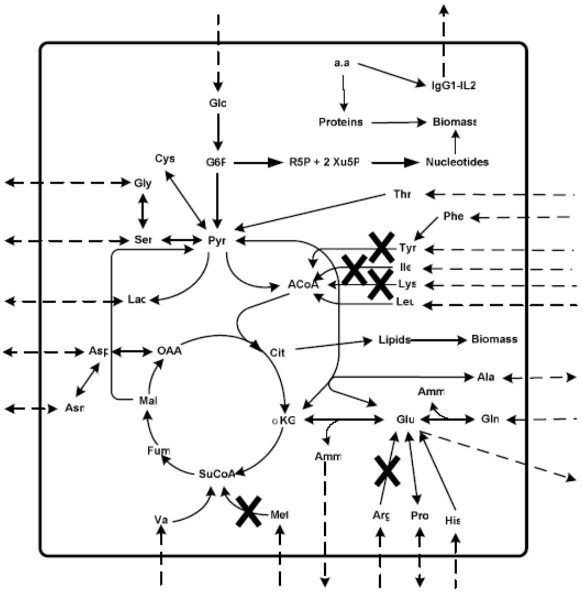
Keywords: elementary modes analysis, projection of latent structures, macroscopic control, mammalian cell cultures

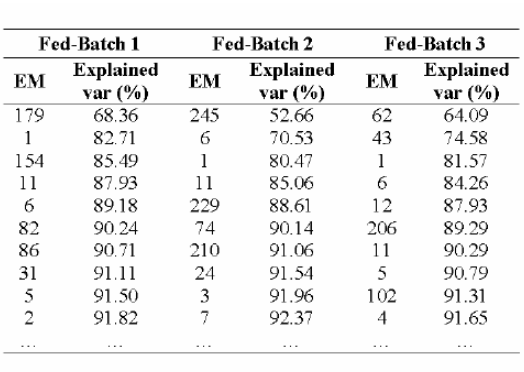
Cells in culture are exposed to manifold changes in their envirome, and normally they adapt to the new environmental conditions by fluxome rearrangement, without visible changes in their ability to support biomass synthesis and product formation. This metabolic redundancy is an obstacle for predictability and makes the inclusion of intracellular detail during bioreactor model development mandatory.

Some authors have been using the elementary modes (EMs) concept to represent metabolic networks by a set of macroscopic reactions connecting just extracellular compounds (Teixeira et al., 2007; Provost et al., 2006; Gao et al., 2007). The bioreactor model is then described by material balance equations over the compounds involved in the final reaction set. The main problem is that the number of EMs of a metabolic network increases exponentially with the number of reactions included, and the identification of the Ems reaction rates becomes impossible with the information of the envirome only.

To circumvent this problem, a method based on the projection of latent structures (PLS) was developed to rank the elementary modes that mostly explain the variance of the dynamics of the extracellular state variables. The main design principle is to identify the subset of Ems that allows the maximization of the covariance between the measured environmental factors and the observed extracellular rates. The algorithm was applied to select the "active" Ems among a set of 251 EMs, in different fed-batch cultures of Baby Hamster Kidney (BHK) cells expressing a fusion glycoprotein. The selected EMs were used to reconstruct the metabolic network, which normally corresponds to a simplified version of the initial one, since some intracellular reactions do not participate in any of the selected EMs (see Figure 1). To evaluate each rank, the well-known Metabolic Flux Analysis technique was used to estimate the intracellular fluxes in both simplified and original metabolic networks. The minimum number of EMs required to obtain consistency between experimental data and the stoichiometry of the resulting networks was the criterion used to decide when to stop the rank. It was possible to obtain consistent networks with less than 22 EMs (explaining over 90 % of the envirome data). Comparing the fluxes obtained in both networks, the powerfulness of the proposed algorithm in reducing large metabolic networks to a set of meaningful EMs is proved, since the excluded reactions have nearly zero fluxes and the remaining reactions have similar fluxes. Interestingly, the excluded reactions correspond to amino acids catabolism, which according to literature (Xie and Zhou 2006) only occur when the amino acids concentration inside cells exceeds the amount required for proteins synthesis. Thus, it can be argued that the algorithm was able to detect kinetic regulation at the enzymatic level. Besides the significance for bioprocess engineering, this approach offers advantages in relation to the well-known Metabolic Flux Analysis (MFA) technique, since it allows fluxome estimation in large scale underdetermined metabolic networks.

Moreover, the regression coefficients of the PLS can be used to infer about the effect that each envirome factor has in the flux of each EM, from which valuable clues about key variables promoting EMs for product formation and biomass growth can be obtained. The work here presented demonstrates the inherent potential for a macroscopic control of the intracellular reactions.





Fed-Batch 1		Fed-Batch 2		Fed-Batch 3	
EM	Explained var (%)	EM	Explained var (%)	EM	Explained var (%)
179	68.36	245	52.66	62	64.09
1	82.71	6	70.53	43	74.58
154	85.49	1	80.47	1	81.57
11	87.93	11	85.06	6	84.26
6	89.18	229	88.61	12	87.93
82	90.24	74	90.14	206	89.29
86	90.71	210	91.06	11	90.29
31	91.11	24	91.54	5	90.79
5	91.50	3	91.96	102	91.31
2	91.82	7	92.37	4	91.65
…	…	…	…	…	…

Figure 1 – (a) Simplified metabolic Network (marks identify reactions not included in the first20 selected EMs of fed-batch 1); (b) EMs ranks for the three fed-batch BHK cultures.

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Towards PAT bioprocess monitoring and control: near infrared and software sensor

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Keywords: PAT, software sensor, near infrared spectroscopy, feedback control

Introduction

Bioprocesses are characterised by natural variability in the raw materials, initial conditions, human intervention, and varying properties of the micro-organism. By setting fixed protocols the current GMP regulations do not encompass reduction of these deviations. This leads to products that have a variable quality, and are even out of specs. The FDA's guidance (2004) on Process Analytical Technology (PAT) finds more and more applications in the biopharmaceutical industry. It involves a shift from testing quality in finished drug products to building quality into products by introducing timely measurement and control of quality variables during the production process.

Wold et al. (2006) defined five levels of chemometrics analysis for PAT and states that the fifth level "PAT process control" in the sense of feedback control based on online monitored variables is still a challenge of the future. The use of spectroscopic instrumentation, although, is often seen as promising for PAT, however in the frame of feedback control it is less well developed. This work describes a step towards real-time feedback control for PAT by comparing two technologies for online monitoring of biomass growth. The ensuing controller design is based on the preferred technique. The dual substrate cultivation of *Bordetella pertussis* for the production of whole cell whooping cough vaccine was chosen as a test case.

Bioprocess monitoring

The specific growth rate and biomass formation are important variables that we would like to control during the cultivation of bacteria for vaccine production. This is particularly important when the produced bacteria form the basis of a whole cell vaccine like whooping cough. Biomass growth, however, can not be measured online, because there are no suitable sensors.

Software sensor. A software sensor based on an Extended Kalman Filter (EKF) was developed to reconstruct biomass and specific growth rate from easy to obtain measurements of the oxygen uptake rate (Soons et al., 2008). The software sensor accurately monitors biomass growth over the whole range from low to high biomass during different types of cultivation. Fig. 1 shows a typical batch cultivation.

Near infrared spectroscopy (nIR). It has also been investigated how nIR spectra can be used to yield an alternative measurement of biomass. The interpretation of the spectra is challenging due to the large number of wavelengths and due to the overlapping absorbance bands of specific components. An advanced method is proposed that allows automatic selection of wavelengths that contain relevant information on the biomass concentration. The automatic wavelengths selection outperforms the traditional procedures for nIR monitoring, thus enhancing the accuracy. A drawback of nIR monitoring is the sensitivity for temporary disturbances in the process conditions like pH and dissolved oxygen. Another drawback is that the estimations are relatively inaccurate and noisy estimation in the initial phase of the cultivation (Fig. 1C). The

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erratic biomass estimations from the near infrared sensor have important consequences for specific growth rate estimation and control. In the current state of development the software sensor is preferred when it comes to feed-back control.

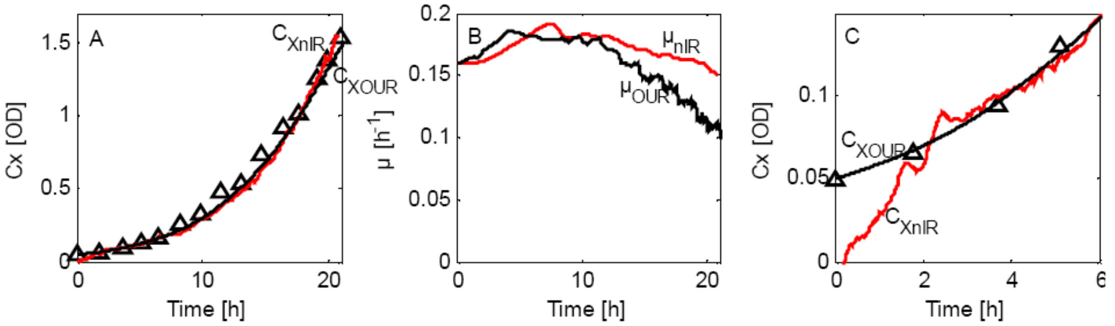

Fig. 1. Bioprocess estimations based on the near infrared and on the software sensor during batch cultivation. A. Biomass B. Specific growth rate. C. Detail of Fig. 1A: biomass estimations in the initial phase of the batch.

Bioprocess control

Control of bioprocesses is a difficult task due to the time-varying characteristics of cultivation processes. By combining a dual substrate model for biomass growth with a reference model, a controller is derived that adapts its settings in response to timevarying conditions. Consequently it is possible to extend the standard batch cultivation with a controlled fed-batch phase (Fig. 2), thus obtaining up to six times higher biomass concentrations compared to the standard batch production process, as is shown in experiments. The controller, therefore, contributes to enhanced batch-to-batch consistency and quality.

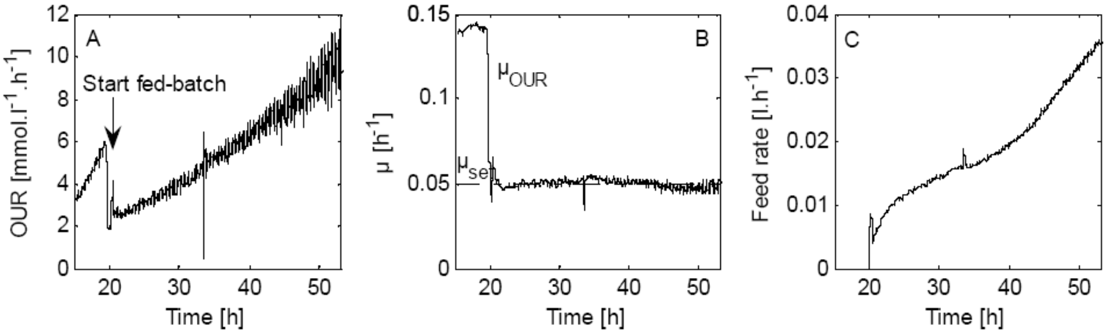

Fig. 2. Bioprocess control based on the software sensor during fed-batch cultivation. A. Oxygen uptake rate (*OUR*) B. Specific growth rate (*μ*). C. Feed rate.

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Near-infrared reflectance spectroscopy as a process analytical technology tool in pharmaceutical industry

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Keywords: Process analytical technology (PAT), Near-infrared spectroscopy, pharmaceutical industry, identification, qualification, quantification.

Abstract:

The FDA's Process Analytical Technology regulatory framework was born to support innovation and efficiency in pharmaceutical development, manufacturing, and quality assurance. PAT are a "system for designing, analyzing, and controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality".

Several PAT tools are described in the FDA guideline, but near-infrared (NIR) spectroscopy is those who developed a strait relation with the PAT concept, since it is nowadays, a very well known and advantageous analytical technique, to which doesn't remain much to prove.

Here we describe the use of near-infrared diffuse reflectance spectroscopy as a Process Analytical Tool (PAT) used in a Pharmaceutical industry. NIR has been used for identification and qualification of raw-materials and intermediate manufacturing products, and for quantification purposes, likewise the determination of moisture and active pharmaceutical ingredients (API's), in intermediates and final products.

NIR enhances understanding and control of the manufacturing process, at the same time it performs analytical results, according to the regulatory requirements.

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Enhancing engineering education in the area of chemical processes through a virtual platform (LABVIRTUAL)

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Keywords: Chemical Processes, E-learning, Virtual Laboratories, Web Platform

Recent advances on information and communication technologies (ICT) have facilitated the development of educational and laboratorial systems based on distributed multimedia technologies, namely virtual reality, under relatively low costs. The traditional methodology of education through the setting of classrooms does have a number of inherent limitations that include passive learning attitudes, difficulties in applying the concepts introduced to real life problems, and little responsibility on the side of students regarding self-learning. New educational tools, based on virtual learning environments, can contribute to stimulate each student, in order to develop competencies of autonomous and critical thinking, in accordance namely with the Bologna process. However, one needs to be aware of some potential dangers associated with the use of virtual laboratories as teaching aids. In fact, students have to be carefully monitored in their learning process, so that the use of a tool just to produce "numbers", without a proper analysis of the simulated situations, based on conceptual models, can be avoided. It is essential for students to go on receiving education based upon experimental work, though it may be directed towards simpler experimental set ups, aimed at getting acquainted with physical and chemical phenomena common to chemical processes.

The Chemical Engineering Departments of both the Universities of Coimbra and Porto have been experimenting, on their own, for quite sometime, new teaching methodologies [1,2], taking into consideration the concerns referred above, and also taking advantage of the information technology skills available in the new students' populations. Recently, these departments got engaged in a broader project, directed towards the development of a virtual platform with a wider scope, aimed at teaching/learning of Chemical Processes, at the graduation level in Chemical Engineering, for Portuguese-speaking students including those from African countries. A large group of academics (13 Chemical Engineering Faculty members and 5 research students), with complementary competencies and expertise were involved in this project.

This paper describes the different sections included in the web platform thus built, as well as the simulation strategies and web methodologies used for its construction. Moreover, some examples of application in the classroom are also discussed. The web platform structure adopted is illustrated in Figure 1. The functional areas of the platform are as follows: Separations and Unit Operations; Reaction Engineering; Process Systems Engineering; Biological Processes and Virtual Experiments. The first four sections deal with the chemical/biochemical processes and basically cover fundaments on the process and interactive simulators that enable users to perform different design tasks for a large range of operation conditions and case studies. The fifth section (Virtual Experiments) deals both with the laboratory visualization of the basic phenomena, related to the processes in the other four sections, and also with the remote monitoring of laboratory experiments. Additionally, our teaching platform also includes an area directed towards secondary school students interested in chemical engineering, where, besides general information on “What Chemical Engineering is all about”, fundamentals of physics and chemistry, in relationship with engineering applications, are covered.

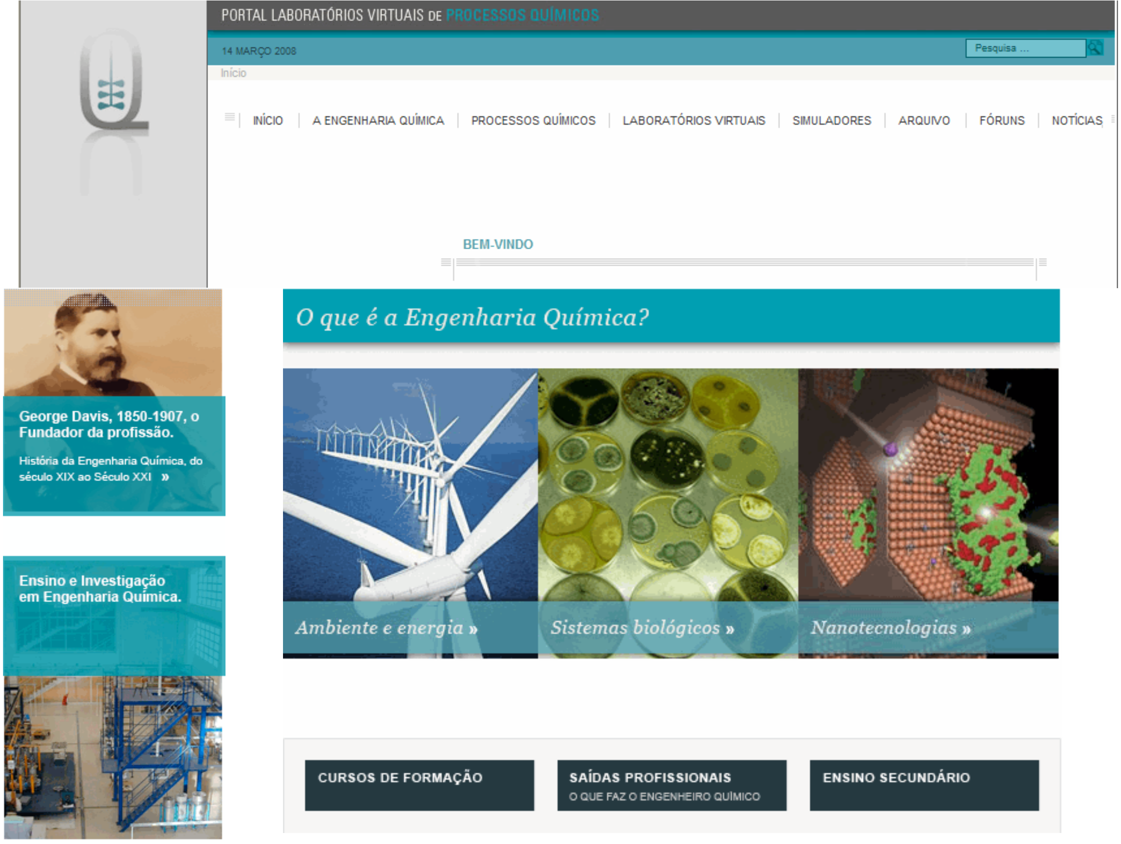

Figure 1 – Structure of our Teaching Web Platform.

The basic architecture of our simulation system comprehends two main blocks: the *Web interface*, which helps the user in entering data required for simulation purposes, and the *computational platform (MATLAB)*, where the mathematical models of the underlying chemical processes are solved. Exchanges of information between these two levels are mediated by a simulation gateway, designed in agreement with the CGI (*Common Gateway Interface*) protocol. The input data sets support numerical values as well as discrete options (chemicals to be processed, type of model to be used, different modes of simulation, etc.). On the other hand, simulation outputs may include graphics and tables. The Web platform is based on the *CMS Joomla*! - free software under the GPL license. This approach allows similar functionalities provided by other commercial software, with the flexibility to better adapt code to the specific needs of this Portal.

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Add-ins for shortcut design and sizing of distillation columns

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Keywords: Distillation, Shortcut Methods, Design, Column Sizing, Add-ins, Spreadsheet.

A computer program, in Visual Basic for Applications, was developed for design and sizing of binary and multicomponent distillation columns using shortcut methods. The program is in the form of two add-ins for Microsoft Excel, one for binary and the other for multicomponent distillation.

The add-in for binary distillation uses the McCabe-Thiele method for design, and allows the user to interact with the program by means of self-explanatory user-forms. The software is able to design and size binary or pseudo-binary distillation columns, with multiple feeds and sidestreams, for ideal and non-ideal liquid mixtures with different enthalpic conditions. The design of steam distillation processes is also considered. The non-ideality of the liquid phase can be described by the Margules, van Laar, Wilson, NRTL, UNIQUAC and UNIFAC models, or assuming constant volatility (Smith et al., 2001). The calculations are shown by means of a graphical interface, and the results summarized in a spreadsheet. The program gives the values of: minimum reflux ratio, operating reflux ratio, number of ideal trays, number of real trays, and estimates of the height and diameter of the column. The sizing of the column is based on the shortcut method purposed by Douglas (1988). The value of the actual reflux rate may be given as input or estimated by specifying the value of the ratio R/Rmin. This program does not only give to the process engineer a good first estimate of the design and size of binary distillation column, but is also an important teaching aid for students and operator training, showing graphically the influence of the different design variables in the final project. Figure 1 shows the McCabe-Thiele diagram for the binary system ethanol/water for a distillate with 89% ethanol and a liquid saturated feed with 35% ethanol (molar percentages). The ratio R/Rmin used was 1.3.

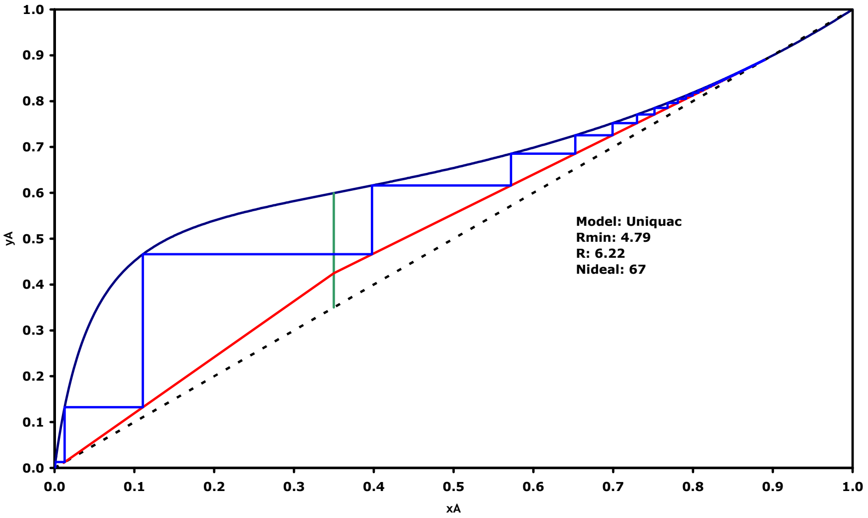

Figure 1 – McCabe-Thiele diagram for the system ethanol(A)/water(B).

The add-in for multicomponent distillation uses the shortcut method of Fenske-Underwood-Gilliland-Kirkbride. This method assumes constant internal flows and

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relative volatility. For systems of variable relative volatility, the equilibrium constants or the parameters for the Antoine equation must be given, to allow the program to calculate an average value for the relative volatility. Two equations are used to fit the Gilliland correlation: Martins (1999) and Molokanov et al (1972) equations. For application of the Gilliland correlation either the value of R/Rmin or N/Nmin must be specified. The program gives as output: minimum number of stages, minimum reflux ratio, composition and flows of both products, number of ideal stages, operating reflux ratio and feed tray. The program autonomously analyzes the possibility of the distribution of non-key components. The user can also choose to do the sizing of the column by the Douglas shortcut method (Douglas, 1988). Table 1 summarizes the results of the shortcut calculations for a multicomponent system. The feed composition is: 5% propane, 40% n-butane, 10% i-pentane and 45% n-pentane. The key components are n-butane and n-pentane, the operation pressure is 1000 kPa and the ratio R/Rmin is equal to 1.3

Table 1 – Results of Fenske-Underwood-Gilliland-Kirkbride shortcut method.

	Fenske			**Underwood**			**Gilliland (Martins, 1999)**	
Components	**xd**	**xb**	**Nmin**	**xd**	**xb**	**Rmin**	**N**	**R**
Propane	0.109	0.000	7	0.108	0.000	1.201	15	1.561
n-Butane	0.830	0.037	**D/F**	0.819	0.037	**D/F**	**Kirkbride**	
i-Pentane	0.032	0.158	0.458	0.044	0.149	0.464	**Nr**	**Ns**
n-Pentane	0.029	0.805		0.029	0.814		8	7

These two add-ins are easy to use and to incorporate in Microsoft Excel, a widespread working tool for engineers, and can be helpful as a teaching aid, a tool for operator training, and for preliminary design and sizing of distillation columns

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Continuous and batch distillation in an oldershaw tray column

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Keywords: distillation, rectification, McCabe-Thiele; Smoker and Rose, efficiency, Rayleigh

The distillation is by far the most frequently used separation process. Although not energy-efficient, it has a simple flowsheet and is a low-risk process. It is indeed the benchmark with which all newer processes must be compared. Following Null (1987), distillation should be selected unless $\alpha_{12} < 1.05$, whereas Nath and Motard (1981) and Douglas (1988) indicate $\alpha_{12} < 1.10$, a more conservative critical value. Generally, design heuristics point out that processes using energy separation agents should be favoured.

Undergraduate students of Chemical Engineering at Chemistry Department of the University of Aveiro receive lectures on distillation as part of their course of Separation Processes I (3rd year, 2nd semester), which is essentially devoted to equilibrium-staged unit operations. Rate-based separations are postponed to Separation Processes II.

Laboratory EQ is a weekly, six-hour lab course where students carry out experiments on separation, reaction and control. Due to its relevance, distillation is also included. Experiments are carried out in a laboratory scale Oldershaw column with 10 sieve trays. The system is run to separate cyclohexane/n-heptane under different modes of operation: total reflux, continuous rectification with partial reflux, and batch rectification with constant reflux. The effect of the reflux ratio and power supply to the reboiler flask upon the separation and column efficiency is studied.

The lab exercise along with subsequent modelling/simulation calculations allows students to train relevant concepts introduced earlier on their curriculum, namely: i) prediction of vapour-liquid equilibrium to generate $x-y$ diagram; ii) continuous and batch operation; iii) total reflux (minimum number of equilibrium stages) and partial reflux; iv) McCabe-Thiele method; v) Smoker and Rose method; vi) overall and tray efficiencies; vii) generalized Rayleigh equation with numerical integration; viii) modelling and simulation. Examining experimental results and theoretical predictions students gain insight in this unit operation.

Most important and typical results will be presented numerically and/or graphically to illustrate the methods and calculations mentioned above.

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Introduction of design tools in the experimental organic processes

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Keywords: Design of Experiments; Organic Processes; Factorial Design

The impact of globalization on the growth of developing countries such as Brazil requires some actions directed to both strengthening and promoting competitiveness.

The use of academic groups of high level in Research and Development (R&D) for acquisition of knowledge for technological development in areas like organic synthesis and processes optimization can join information on new products, with possible practical applications or create modifications of synthetic route for known products already. Since, with the exception of state-owned companies, very little research is carried out by industry communication between prospective partners at universities and firms is difficult. In the general form, the Brazilian universities staffs are not prepared to put together any type of proposal that is acceptable without some modifications to somebody from industry. In we can say them that they do not say the same language.

To minimize this gap is necessary to introducing management techniques into an academic. But this setting would certainly generate a lot resistance and might even jeopardize the teaching and research activities that are traditionally carried out by universities. Nevertheless, the term “Quality” has a strong appeal in the academic world and offering groups that were considering some form of interaction with industry techniques that could increase the quality of the services they intended to offer generated considerable interest.

For example, in the industry, the use of planning design is usual in fine chemical processes. But in the university, this use is limitated, principally for a few groups in a few uses. We proposed to expand this use of experimental design as tool in organic processes practices. This tool allows vary studies involved technology and teorical concepts and software to solve economic problems and optimization process in the chemistry industries.

Teaching with this focus we can describe a model of the processes in study, and decided the better conditions to work, with all details needed. We think that is also possible to learn organic chemistry involved in the processes, besides we want to shown that is not necessary a robust software to possible this work. Is possible to learn about the influence of each parameters involve in the process and create a new routes or conditions to obtain better results.

The students can also learn about statistical software and the calculations involving in the use of software less robust, to obtain the same results. After this training, is possible to engage students in research groups generating a program of quality in research and development designed for higher education and contribute to integration with research centers, government and companies.

This planning allows obtaining the threats and opportunities as such as to verify the power and deficiencies of the institution. After the attainment of the critical points, it is possible to obtain a model to attack them. The teams meet together to choose the better means to attack the problems suggesting actions of improvement, correctives and/or preventives, in form to permit the elimination of the gap, and make possible a gains in quality and productivity and implement the strategies for better organization performance. Periodical evaluations should be made for different clients to measure their degree of satisfaction and permit comparisons in order to reach better results.

The relevance of this model is that the universities are big organizations with different areas and emphases requiring a distinct approach to the implementation of TQM programs.

We suggest the initial presentation to relatively small groups that are open to change and commit them to this implementation. In the University Laboratories of R&D are necessary to search the Certification should focus in one test or process, and then try to introduce new tests or processes. The results of these groups can be adopted and propagated by the organization in order to "infect" new groups.

Such model could still be used by other universities in developing countries that they also desire to promote such integration, objectifying better adequacy of its scientific and technological level those practiced internationally.

Virtual laboratories in (bio)chemical engineering education

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Keywords: virtual labs, e-learning, (bio)chemical engineering education

Information and Communications Technology (ICT) has largely reconfigured the learning panorama over the last decade. The Internet paves for the creation and adoption of new learning and teaching styles. It facilitates the development of additional teaching strategies, including new ways to use them for illustration, simulation, demonstration, experimentation, operation, communication, and so on.

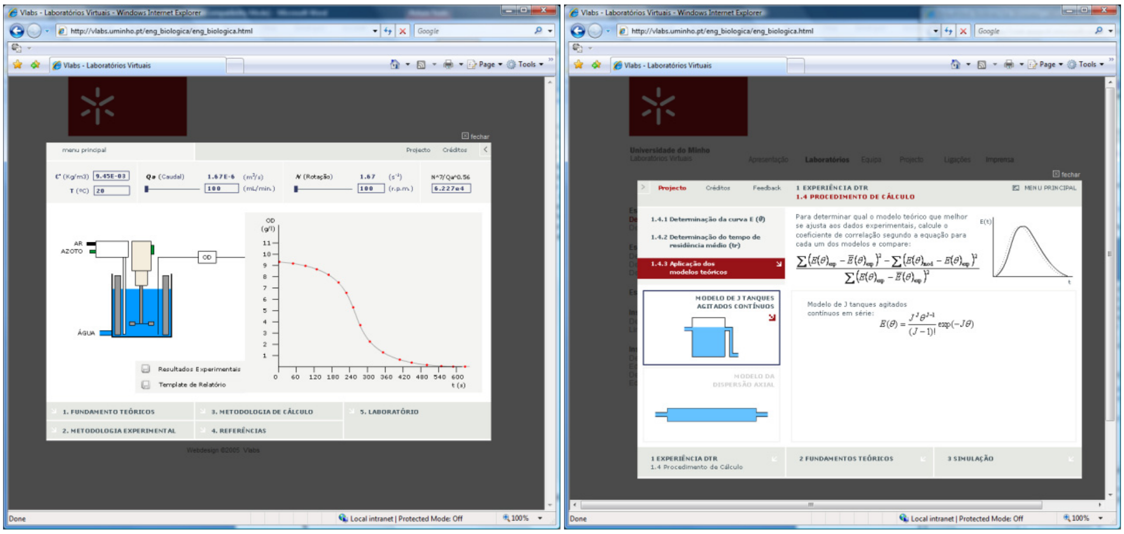
Hands-on laboratory experiments have enormous educational value, but traditional teaching laboratories are expensive and have complex logistics regarding space, staff, scheduling and safety (Selmer et al., 2007). Virtual laboratories (virtual labs) may overcome these limitations allowing the insertion of laboratory experiments in teaching at any time and the experiments can be performed from any place with internet access (Ferreira, 2004). Even though virtual labs cannot substitute hands-on laboratory experiments completely in the engineering curriculums, it has several advantages as a complementary educational tool.

The Universidade do Minho is being promoting a pilot project for development of components and software agents for assistance in laboratory lessons of curricular units from Science and Technology areas. These software contents will lead to the establishment of virtual laboratories and its offer via Internet will allow a better preparation of the students as well as e-Learning education activities. The portal http://vlabs.uminho.pt/ gives access to virtual laboratories where animations, videos and remote control of experiments can be handled, giving extra digital content for teaching in laboratory classes and for curricular units without laboratory schedule assigned. Contents and didactic material are being developed for virtual laboratories where the student can enter to know and explore objects like microscopes, reactors and "manipulate" intangibles realities like understanding how a molecule can catalyze a modification in another one.

Biological Engineering Department is engaged in this VLabs Project. The launching of virtual laboratories, with the insertion of these tools in teaching activities and his offer in internet, will permit the preparation of the students before having the laboratory classes or the examination, simulation or observation of certain key-experiments to understand the material for those curricular unities.

This paper describes the design and implementation of two virtual labs for biochemical engineering education. These virtual labs were designed for the course “Chemical Technology Laboratory” taught in the third year of Biological Engineering integrated Master studies. The first experiment was used by students in 2006. The effectiveness of the implemented system was evaluated through direct experimentation and survey (through questionnaires) with students taking the chemical technology lab course and these results will also be discussed.

One of the virtual labs is the determination of the correlation between oxygen transfer rate (kLa), aeration rate and agitation power. The designed web platform is presented in Figure 1. The main menu has five different areas: theory fundamentals, experimental methodology, calculation methodology, references and laboratory where the students can do the virtual experiment. This virtual lab replaces the hands-on experiment. The advantages over performing the experience are clear: reduced costs, reduced experience time, improved data with no loss of education efficiency. The other virtual lab is the determination of the residence time distribution (RTD) in continuous tank series and was implemented to support the experiment and not to replace it. In Figure 1 a screen of this virtual lab is also illustrated. It is divided in three main areas: theory fundamentals, experiment and simulation. With this virtual lab it is shown that the internet can also support the traditional hands-on laboratory experiments by providing the student with an experiment platform consisting of the theory underlying the experiment, pre-visualization of the experiment (videos, simulation) and generation of data. This web support will improve the student effectiveness and autonomy in the laboratory class, in result analysis and report writing.



C* (Kg/m3) 9.45E-03
T (ºC) 20
Qa (Caudal) 1.67E-6 (m³/s) 100 (mL/min.)
N (Rotação) 1.67 (s⁻¹) 100 (r.p.m.)
N^7/Qa^0.56 6.227e4

☐ Resultados Experimentais
☐ Template de Relatório

Para determinar qual o modelo teórico que melhor se ajusta aos dados experimentais, calcule o coeficiente de correlação segundo a equação para cada um dos modelos e compare:

$$\frac{\sum\left(E(\theta)_{exp}-\bar{E}(\theta)_{exp}\right)^2-\sum\left(E(\theta)_{mod}-E(\theta)_{exp}\right)^2}{\sum\left(E(\theta)_{exp}-\bar{E}(\theta)_{exp}\right)^2}$$

Modelo de J tanques agitados contínuos em série:

$$E(\theta)=\frac{J^J\theta^{J-1}}{(J-1)!}\exp(-J\theta)$$

Figure 1. RTD and Aeration/Agitation Virtual Labs.

The educational value offered by virtual labs can be gained in different ways and this is demonstrated by the two implemented virtual labs.

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Posters - Extended Abstracts

The research of the heterogeneous catalytic combustion of the combustible gases

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Keywords: The new synthesis, catalytic combustion, heterogeneous, gases.

The problem of catalytic combustion of the combustible gases has appeared in connection with necessity of elimination of explosive situations arising at their use. Most perspective of oxidic catalysts of free of platinum metals is cobaltite of cobalt. In the industry the cobaltite produce by decomposition of salts of a cobalt at the temperature 723-923 K. Catalytic activity of this catalyst was investigated in reaction of combustion of hydrogen by oxygen at T = 463-493 K and has constituted (according degree of transformation of hydrogen):

- with winterizing of water of vapour by liquid nitrogen - 50 %
- in the presence of saturated water steams - 7 %.

The complex of the executed researches has allowed to develop essentially new way of low temperature synthesis of active cobaltite cobalt and also way of precipitation of an active component of catalyst on a support - SiO2 - fibres having the high specific area of a surface.

The research of the kinetics of process of combustion of combustible gases have carried out on an example of oxidation of hydrogen by oxygen on catalyst Co3O4/SiO2 in gas dynamical installation of a flow type, in the reactor under ideal conditions of plug flow of gas (flowing plug reactor) with strict maintenance of the given parameters of process: temperatures of catalysis, volumetric speed of gas, mass of catalyst and pressure of water of vapour. The gas mixture before input in the reactor has been saturated of water of vapour. The pressure of steams water of vapour has constituted 48,1kPa. The rate of volume flow of gas mixture was varied in an interval 8,4 - 23,3 l/ hour. A necessary condition of reliability of the experiment was observance of the mass balance which was defined by a chromatographic method of the analysis of the composition of gas mixture before and after reaction.

To calculation of the experimental data was applied the equation of an efficient kinetic constant of the heterogeneous chemical process running in a stream when the product of the reaction is adsorbed strongly.

The kinetics of the catalytic combustion of hydrogen on catalyst Co_3O_4/SiO_2 submits to this equation. Therefore it can be considered as heterogeneous first-order chemical reaction for hydrogen.

The received experimental values of the effective constants of speed of reaction at the temperatures 453, 480, 513 K are submitted in this paper.

At the analysis of the experimental data is traced of the generality of the reduction of a degree of transformation of hydrogen, and catalytic activity with increase of speed of a gas stream at constant temperature. The catalytic activity (a degree of the transformation of hydrogen in the water) decreases with increase of the pressure of steams from 2,73 up to 48,93 kPa.

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Regeneration of sulfur poisoned nickel based catalysts for steam reforming catalysts in Razi petrochemical complex

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Keywords: methane steam reforming; nickel catalysts; sulfur poisoning; regeneration.

This paper reports the result of regeneration of Razi petrochemical catalyst used for steam methane reforming. Nickel catalyst was poisoned by sulfur component from abnormally available in natural gas. A remarkable loss of sulfur was observed and the catalyst gains its activity. No changes of crystal structure were detected by XRD analyses. The first part leads to the identification of the catalyst by X-ray diffraction (XRD), Scanning Electron Microscope (SEM), carbon and sulfur analyzer. It clearly shows that sulfur and carbon deposition was the main problem in deactivation of catalyst. The catalyst activity of the catalyst contaminated with sulfur before and after regeneration was measured on methane steam reforming at a CH_4:H_2O ratio of 1:3 at 850 ºC. The result shows that approximately, main quantity of sulfur is removed and the regenerated catalyst gained 80% of its catalyst activity.

One of the major problems in the application of nickel catalysts is sulfur poisoning. Even after a thorough desulfurization of the feedstock, sulfur-containing compounds in the feed may reach the nickel catalyst at a ppb level. Sulfur is a strong poison for nickel catalysts and blocks the active nickel sites. The sulfur adsorption capacity of steam-reforming catalysts is therefore an important catalyst parameter. Hydrogen sulphide inhibits the catalyst surface (M) following process (1):

$$H_2S + M \leftrightarrow M\text{-}S + H_2 \qquad (1)$$

Process (1) is a fast, reversible process in a large range of concentration. Feedstock with S contents lower than 10 ppb are recommended to avoid appreciable deactivation of the Ni catalytic surface.
Regeneration of nickel surfaces was clearly shown to be effective upon injection of water steam (oxidizing atmosphere) at 700 ºC.

$$Ni\text{-}S + H2O \leftrightarrow NiO + H2S \qquad (2)$$
$$H2S + 2H2O \leftrightarrow SO2 + 3H2\ (\Delta H^0_{298} = -207\mathrm{kJ/mol}) \qquad (3)$$

This sequence does not include the chemisorptions equilibrium and this may be reason for the improved regeneration.

The degree of regeneration is expressed by the ratio S/S0, the ratio of the sulfur content after and before the treatment. At PH_2O/PH_2 above 200-300 a significant change of the regeneration degree of the catalyst is obtain as shown in fig. 1 and also we have somehow the same situation for carbon content.

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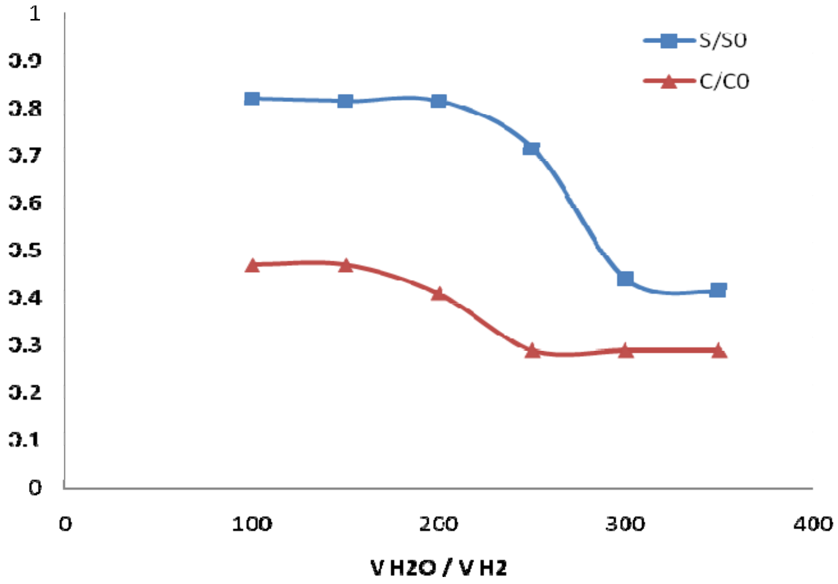

Figure1. Sulfur and carbon content before and after regeneration with steam stream.

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Total oxidation of ethyl acetate over TiO_2 supported noble metals catalysts

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Keywords: catalytic oxidation, VOC, noble metal, titania.

Introduction

Catalytic oxidation of volatile organic compounds (VOC) present at low concentrations in gaseous effluent streams is a promising treatment technology. The nature of the catalyst used plays a significant role in the performance of this process. In general, platinum and palladium supported catalysts are considered more active than metal oxides for the oxidation of several VOC. In this report, we study the oxidation of ethyl acetate over titania and noble metal catalysts supported on titania. The influence of several parameters was investigated, such as the structure of the support, the type and amount of noble metal (palladium or platinum), the metal dispersion and the activation conditions.

Experimental

TiO_2 with different amounts of anatase and rutile were used in this work. The first sample consists of 100% anatase and was synthesized by a sol-gel process, via hydrolysis of titanium alkoxides, in acidic medium. The second sample is a commercial product (Degussa P-25) containing 80% anatase. The third sample was obtained by submitting P-25 to a thermal treatment at 700 ºC under air, in order to reduce the content of anatase to 47%.

Platinum and palladium supported catalysts were prepared by incipient wetness impregnation on Degussa P-25 using H_2PtCl_6 and $PdCl_2$ as metal precursors, respectively (metal load: 1%). The resultant materials were dried at 110 ºC overnight and calcined at 500 ºC for 2 hours.

The activities and CO_2 selectivities of the catalysts towards total oxidation of ethyl acetate at low concentrations (2000 ppmv) were evaluated in a fixed bed reactor at atmospheric pressure and in the temperature range of 150-400 ºC. The space velocity in all experiments was fixed at 16,000 h^{-1}. The analytical system consisted of a gas chromatograph equipped with a flame ionization detector (FID) for the analysis of the organic compounds, and two non-dispersive infrared (NDIR) analyzers for CO and CO_2 quantification. Prior to the reaction, the platinum catalyst was activated according to three different protocols: a) air at 400 ºC for 1 hour; b) hydrogen at 350 ºC for 2 hours; c) mixture of steam and air at 500 ºC for 2 hours.

Results and discussion

Figure 2 shows the steady-state conversion of ethyl acetate and the conversion into CO_2 on the various catalysts, as a function of the reaction temperature. Over the TiO_2 samples, ethyl acetate is completely oxidized at about 340 ºC, but the CO_2 yields are low at this temperature (XCO_2 values between 47% and 67%, respectively for the 47% and 100% anatase). High amounts of by-products were formed, such as acetic acid, acetaldehyde and acetone. As expected, the presence of palladium or platinum, enhances the activity and selectivity into CO_2, decreasing the temperature at which ethyl acetate is completely converted into CO_2 to 330 and 270 ºC, respectively. It can be observed that the platinum catalyst is more active, and especially much more selective into CO_2 than the corresponding palladium catalyst. This behaviour is in

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agreement with that reported by other authors (Papaefthimiou et al. 1998). Figure 2 also shows that the structure of TiO_2 does not have a major influence on the catalytic behaviour of the catalyst, although the anatase structure is a little more active than the rutile phase.

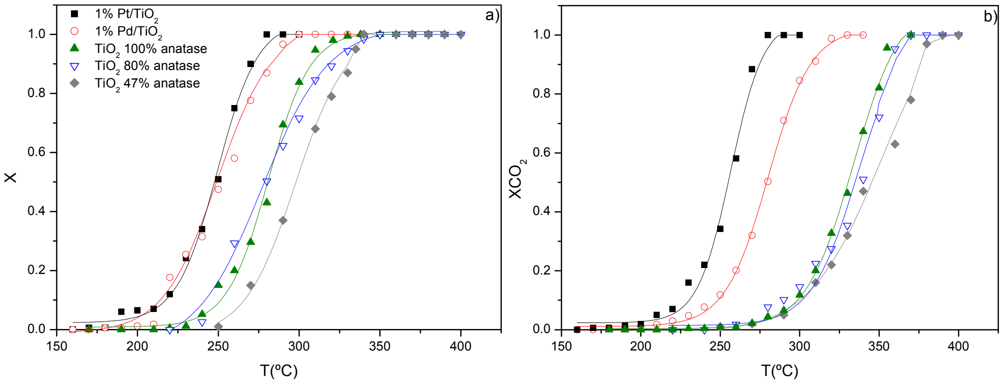

Figure 2- Catalytic activity of 1% Pt/TiO_2, 1% Pd/TiO_2 and TiO_2 (100%, 80% and 47% of anatase), on the oxidation of ethyl acetate: a) Ethyl acetate conversion (X) b) Conversion into CO_2 (XCO_2).

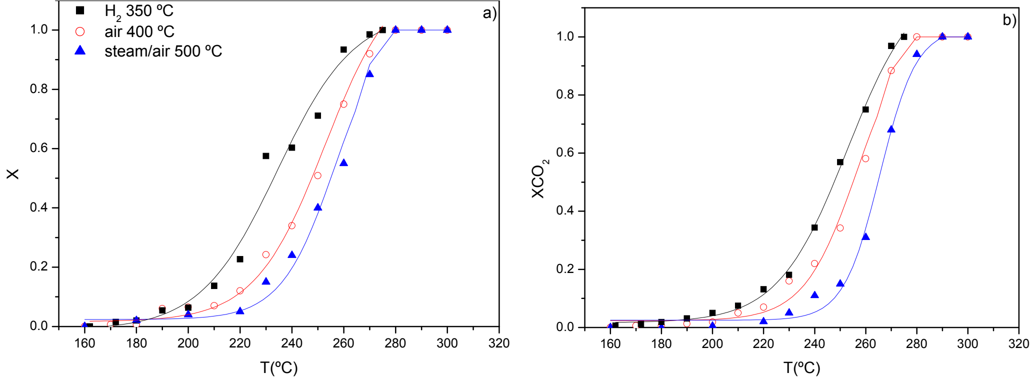

Figure 3- Effect of the activation conditions on the catalytic activity of Pt/TiO_2 catalyst: a) Ethyl acetate conversion (X); b) Conversion into CO_2 (XCO_2).

Figure 3 shows the effect of the different catalyst activation procedures on the oxidation of ethyl acetate. It seems that the hydrogen treatment slightly increases the catalytic performance, promoting the total oxidation of ethyl acetate into CO_2 at 275 ºC. This effect may be correlated with the electronic state of the platinum species. After pre-treatment with steam/air, the temperature needed for total oxidation into CO_2 increases to 290 ºC.

Conclusions

The following conclusions can be drawn from this study:

- The crystalline structure of TiO_2 does not have a major influence on the catalytic activity. However, better results were obtained with anatase.
- The platinum catalyst is more active and selective than the corresponding palladium catalyst.
- The catalyst that has been activated under hydrogen atmosphere shows the highest selectivity into CO_2, while the catalyst treated with steam/air has the lowest activity.

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Incidence of the preparation method of the support on the behavior in the n-butane dehydrogenation of Pt/$MgAl_2O_4$, PtSn/ $MgAl_2O_4$ y PtPb/ $MgAl_2O_4$ catalysts

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Keywords: synthesis of $MgAl_2O_4$, dehydrogenation, bimetallic catalysts

$MgAl_2O_4$ is a material with interesting properties to be used as a support of metallic catalysts for dehydrogenation. This material has a high thermal stability, low acidic character and good interaction with the metallic phase (Armendariz et al, 2001). The traditional method to obtain this material is the ceramic one (reaction in solid phase at high temperature). This preparation method has certain disadvantages such as the low specific surface area and chemical heterogeneity. In order to avoid these problems others preparation methods have been used: mechanochemical, sol gel and co-precipitation (Li et al., 2001). With respect to the nature of the metallic phase, it must be indicated that Pt has been intensively used as the active metal. Pt has a high activity of alcanes dehydrogenation and low hydrogenolytic capacity. In spite of these properties, the performance of the metallic phase can be enhanced by addition of metals of the 14 group as Sn, Pb and Ge (Pakhomov et al., 1980).

Two preparation methods of $MgAl_2O_4$ have been used: ceramic and co-precipitation. The supports were characterized by measurements of the textural properties (BET isotherms), X-Ray Diffraction and 2-propanol dehydration reaction (to determine the acid-base properties). Monometallic catalysts based on Pt and bimetallic catalysts (PtSn and PtPb) were prepared on the both supports. In the ceramic method, the support was prepared by a reaction in solid phase of MgO and γ- Al_2O_3 at 900 ºC for 24 h. In this case of co-precipitation method, $MgAl_2O_4$ was obtained by co-precipitation of solutions composed by $Mg(NO_3)_2.6\ H_2O$ and $Al(NO_3)_3.9\ H_2O$ (Mg/Al molar ratio = ½) with a NH_3 solution (28 wt%). After precipitation the solid was washed, dried in air at 120 ºC and finally calcined at 800 ºC for 4 h.

The different supports were characterized by X-Ray Diffraction. The specific surface area, pore volume and mean pore radio of both supports were determined through the BET isotherm in a Quantachrome Corporation NOVA-1000 equipment.

The monometallic catalysts were prepared by impregnation of both supports with a given volume of aqueous solution of H_2PtCl_6 such as to obtain a Pt loading of 0.3 wt%. The bimetallic catalysts (PtSn and PtPb) were obtained by impregnation of the corresponding monometallic ones with an aqueous solution of $SnCl_2$ (in HCl medium) or $Pb(NO_3)_2$. The Sn contents were 0.3 and 0.5 wt%. The Pb contents were 0.52 and 0.87 wt%. These catalysts were characterized by H_2 chemisorption, TEM, TPR, XPS, test reactions of the metallic phase (cyclohexane dehydrogenation and cyclopentane hydrogenolysis) and evaluated in the n-butane dehydrogenation reaction at 530 ºC in a continuous flow reactor fed with a mixture composed by n-C_4H_{10} and H_2 (H_2/n-C_4H_{10} molar ratio = 1.25) and in a pulse equipment at 530 ºC (pulse volume = 0.5 mL n-C_4H_{10} STP).

The textural properties were the following: i) $MgAl_2O_{4\ \mathrm{ceramic}}$: S_{BET}= 37 $m^2\ g^{-1}$ and pore volume = 0.11 mL g^{-1}; ii) $MgAl_2O_{4\ \mathrm{coprecipitation}}$: S_{BET} = 108.2 $m^2\ g^{-1}$ and pore volume = 0.33 mL g^{-1}.

For the $MgAl_2O_{4\ \mathrm{ceramic}}$, the XRD shows that the main product was $MgAl_2O_4$ with a spinel structure and small amount of γ-Al_2O_3 after purification. In the case of $MgAl_2O_{4\ \mathrm{co\text{-}precipitation}}$, XRD shows the presence $MgAl_2O_4$ spinel after the calcination of the coprecipitated gel at 800 ºC. 2-propanol dehydration reaction shows that γ-Al_2O_3 (reference material, which has acid Lewis sites) has an important activity. On the other hand, both $MgAl_2O_4$ display a very low dehydration activity, mainly $MgAl_2O_4$ $_{\text{co-precipitation}}$. This means that these materials are practically neutral from the acid-base point of view. The H_2 chemisorption results show that $Pt/MgAl_2O_4$ $_{\text{co-precipitation}}$ displays a lower metallic dispersion that the Pt supported on $MgAl_2O_4$ $_{\text{ceramic}}$, in spite of the $MgAl_2O_4$ $_{\text{co-precipitation}}$ has a higher S_{BET} that than of $MgAl_2O_4$ $_{\text{ceramic}}$. In the case of bimetallic catalysts it is observed that the chemisorptive capacity is clearly lower that than of the monometallic ones. This effect can be attributed to a blocking, dilution or Pt-Sn alloy formation by addition of Sn or Pb to Pt. The TEM measurements indicate the presence of Pt particles of higher size on $Pt/MgAl_2O_4$ $_{\text{co-precipitation}}$ that in $Pt/MgAl_2O_4$ $_{\text{ceramic}}$. The cyclohexane dehydrogenation results for mono and bimetallic catalysts supported on $MgAl_2O_4$ $_{\text{ceramic}}$ show that Pb had a certain electronic effect on Pt, but is very important to indicate the presence of blocking and dilution effects of Pb on Pt. Sn appears to produce a certain electronic effect in the $PtSn/MgAl_2O_4$ $_{\text{ceramic}}$. It can be observed from cyclopentane hydrogenolysis reaction that when Pb and Sn are added to Pt supported in the $MgAl_2O_4$ $_{\text{co-precipitation}}$ the effect on the CPH initial rate is lower than one order of magnitude, which means that the Sn or Pb are intercalated between Pt atoms, but in a low extension. In the case of Pb addition the blocking effect is clearly higher than that of Sn. The results obtained by TPR show that for $MgAl_2O_4$ $_{\text{ceramic}}$, after the Sn or Pb addition to Pt, the main peak is shifted to higher temperature and is broad with respect to the corresponding to monometallic one which can indicate a Pt-Sn (or Pt-Pb) co-reduction and a good interaction of both metals. For the catalysts supported on $MgAl_2O_4$ $_{\text{co-precipitation}}$ the reduction peak of Pt is shifted to higher temperatures when Sn is added to Pt. This behaviour indicates a good interaction between both metals. In the case of PtPb catalysts supported on $MgAl_2O_4$ $_{\text{co-precipitation}}$, TPR profiles would indicate a Pb segregation and a bad interaction between platinum and lead. XPS results of PtSn catalysts supported on both $MgAl_2O_4$ indicate that a small fraction of Sn is found as Sn(0), probably alloyed with Pt, thus indicating a good interaction between both metals in agreement with TPR results. Besides, it must be noted that the major fraction of Sn can be attributed to tin oxides and oxichlorinated species of Sn (accoding XPS results). The n-butane conversion as a function of the reaction time shows that the activity of $Pt/MgAl_2O_4$ $_{\text{ceramic}}$ is higher than the conversion of $Pt/MgAl_2O_4$ $_{\text{co-precipitation}}$. Besides, the addition of Sn to Pt supported on both $MgAl_2O_4$ increases the n-butane conversion in flow experiments with respect to $Pt/MgAl_2O_4$ ones. However, the Pb addition to Pt supported in both aluminates decreases the conversion with respect to the corresponding monometallic ones. In can be seen that the use of $MgAl_2O_4$ $_{\text{ceramic}}$ support and the lower Sn content in the prepared catalysts leads to a better catalysts (from the point of view of conversion), and the addition of 0.52 or 0.87 wt% Pb is negative for both preparation methods of the support. With respect to the selectivity to butenes, the Sn addition to Pt clearly increases the selectivity reaching values near to 98%. On the other hand, the Pb addition to Pt does not produce an increase of the selectivity to butenes, except for $PtPb(0.52\ wt\%)/MgAl_2O_4$ $_{\text{ceramic}}$ reaching an initial selectivity values of 90%.

With respect to pulse experiments, it can be said that the Sn addition to $Pt/MgAl_2O_4$ $_{\text{ceramic}}$ produces an important decrease of carbon retention, which is lower that for $PtSn/MgAl_2O_4$ $_{\text{co-precipitation}}$. The bimetallic catalysts doped with Pb show a low carbon retention which can be related with its low activity. The above mentioned results indicate that the $MgAl_2O_4$ $_{\text{ceramic}}$ has a better performance as a support of Pt, Sn and Pb with respect to the co-precipitated one.

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Hydrogen production from methane reforming: thermodynamic assessment

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Keywords: Methane reforming, hydrogen, chemical equilibrium, scilab software.

Hydrogen can be produced using several routes. At the present moment, the main route is the catalytic reforming of methane, which includes steam-methane reforming (SMR), partial oxidation (POX), autothermal reforming (ATR) and dry-methane reforming (DMR). Steam-methane reforming produces a H_2/CO ratio equals to three, which is high, when compared to others reforming processes (Seo et al., 2002). Nevertheless, it has the disadvantage of intensive energy requirements due to the overall endothermic nature. In order to reduce energy costs, partial or stoicheometric oxidation of methane has been investigated as an alternative (Corbo and Migliardini, 2006). Finally, dry-methane reforming produces a synthesis-gas with a H_2/CO ratio near one, wich can be used for adjusting H_2/CO ratio in steam reforming, suitable for Fischer–Tropsch reactions and methanol production (Schuurman et al., 2006).

Therefore, the main contributions of this study are to conduct a comparative thermodynamic analysis for these reactions and to asses the influence of key operational variables on chemical equilibrium. Equilibrium compositions are calculated by two distinct methods: (1) evaluation of equilibrium constants; (2) Lagrange multipliers. Both methods result in systems of non-linear algebraic equations, solved numerically using the open-source software Scilab (www.scilab.org). Comparison between experimental data and/or simulated equilibrium data, published in the literature, is the method of simulated results validation. Effects of temperature, pressure, initial H_2O/CH_4 ratio (steam reforming), initial CH_4:CO_2:N_2 ratio (dry reforming) and initial O_2/CH_4 (partial oxidation) ratio on the reaction products are studied.

Steam-methane reforming analysis is carried out considering the following species: CH_4, H_2O, CO, CO_2 and H_2. As the reactor temperature is raised from 600 to 800 °C, CH_4 conversion increases from 44 to 90 %. For temperatures around 800 °C, the raise of initial H_2O/CH_4 ratio from one to two increases H_2 yield in 4.67%. For temperatures around 430 °C, this yield is practically the same f or all conditions. Results are validated by comparison with CH_4 equilibrium conversion data of Lutz et al. (2003) and Seo et al. (2002).

Dry reforming analysis is carried out considering the same species as in the case of steam-methane reforming, with the addition of N_2. As the reactor temperature is raised from 600 to 800 °C, CH_4 conversion increases from 43 to 91%. H_2 yield and H_2/CO selectivity are proportional to temperature and to initial CH4:CO2:N2 ratio. Results are validated by comparison with CH_4 equilibrium conversion data of Akpan et al. (2007). For partial oxidation reforming, the following species are considered: CH_4, H_2O, CO, CO_2, H_2 and O_2. At 1000 °C, CH_4 is completely consumed when a initial O_2/CH_4 ratio of 0.5 is used. H_2 yield is proportional both to temperature and to initial O_2/CH_4 ratio. H_2/CO selectivity is proportional to initial O_2/CH_4 ratio and inversely proportional to temperature. Results are validated by comparison with equilibrium composition data of Zhu et al. (2001).

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Figure 1 shows equilibrium compositions at a wide range of temperature, for SMR, DMR and POX, at different pressures and/or reactor feed composition. Based on these results, optimal policy operation conditions for industrial reactors can be established, as well as, laboratory scale experimental studies involving new catalysts and new reactor configurations.

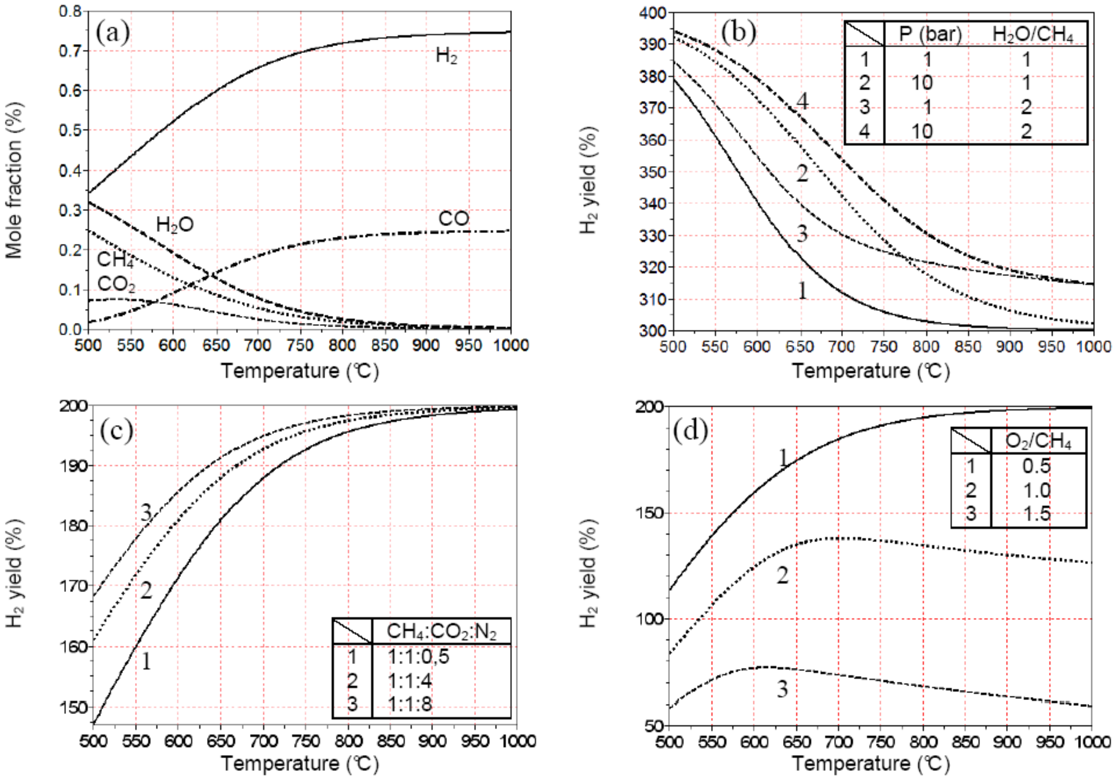

Figure 1. Equilibrium composition of methane reforming reactions: (a) SMR (P = 1 bar, H_2O/CH_4 = 1); (b) SMR, pressure and H_2O/CH_4 sensitivity; (c) DMR; (d) POX.

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Hydrogen production from methane autothermal reforming: mathematical modeling and parametric optimization

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Keywords: Modeling and simulation; process optimization; hydrogen production.

1. Introduction

In recent years hydrogen has attracted much attention as a clean energy source mainly as fuel for combustion engines and electricity production in fuel cells. As a result, hydrogen production from natural gas has been intensely researched. Steammethane reforming (SMR) is the main route for hydrogen production nowadays.

Nevertheless, the autothermal reforming of methane reaction (ATR) is an important alternative route that has been investigated since it is less energy consuming than SMR. This is because ATR couples steam reforming and partial oxidation methane reactions, yielding a system with high energy efficiency and a more satisfactory H_2/CO relationship for H_2 production (Ayabe et al., 2003; Dias and Assaf, 2004).

Although ATR has an interesting potential for industrial application, there are only a few studies that consider its reactor design and optimization in literature. Thus, the main objectives of this work are to present and to validate a mathematical model of a small scale reactor for the autothermal reforming of methane, and also to describe the influence of temperature on the methane conversion profiles, with the objective of maximizing H_2 production.

2. Modeling and validation

An one-dimensional model was developed for a differential packed bed reactor, filled with nickel catalyst, by means of mass balance equations (Barrio et al., 2007) in gas phase. Molar flow along axial direction, for each component, can be described by the following equation:

$$\frac{dF_i}{dz} = \rho_b * S * \sum_j r_{ij} \tag{1}$$

Kinetic rate equations of ATR throughout the reactor were calculated using composition dependent expressions (Halabi et al., 2008).

The model is constituted by a set of ordinary differential equations of initial value type and it was integrated using the function “ode” of the open source software Scilab™ (www.scilab.org), at different temperatures.

Experimental data, used for model validation, were obtained in the literature (Ayabe et al., 2003) for temperatures ranging from 573 to 1173 K (Figure 1).

3. Results and discussion

For each temperature, the molar flow was integrated along the reactor. At z = L, where L is the reactor’s length, methane conversion and dry mole fractions for other components, were calculated. Figure 2 shows the influence of temperature in the methane conversion and products composition profiles (light-off curve). These results show that high methane conversion is favored at high temperatures. At low temperatures, the methane total oxidation reaction is favored, generating CO_2.

Nevertheless, at higher temperatures, CO_2 and steam reforming also occurs, yielding synthesis gas (H_2 and CO). It should also be noticed that above 873 K all oxygen is consumed. Based on these results, optimal policy operational conditions, for laboratory

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scale ATR reactor, can be established. Additionally, this model can be a valuable tool in reactor scale-up studies.

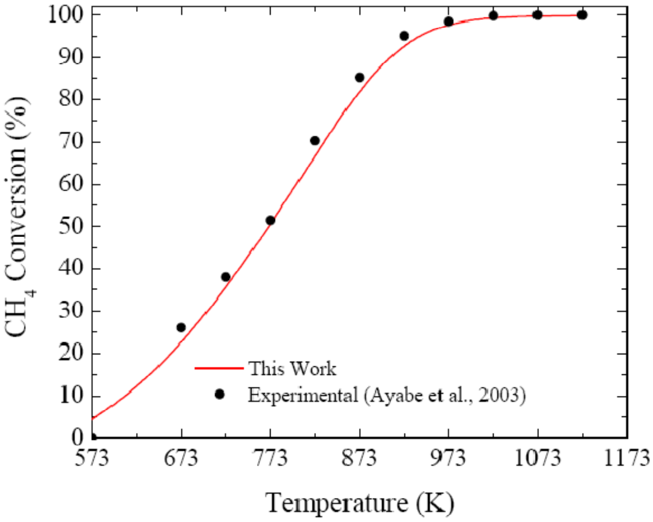

Figure 1 – Simulated and experimental (Ayabe et al., 2003) CH_4 conversion as function of temperature in the end of the reactor.

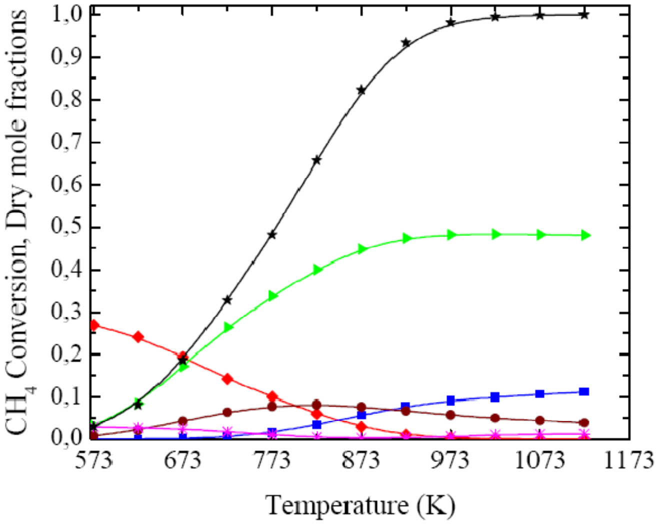

Figure 2 – Product gas composition in autothermal reforming of methane at different temperatures: (★) CH_4 conversion; (◆) CH_4 , (▶)H_2, (■) CO, (●) CO_2, (✳) O_2, all in dry mole fraction.

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Fluid-dynamics of fluidized beds operating with flat particles

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Keywords: fluidization, biomass particles; minimum fluidization velocity; vibro-fluidized beds

Many species of plants present therapeutic properties and constitute an important source of raw material for pharmaceutical and cosmetic industries. According to Rates (2001), about 25% of drugs prescribed worldwide come from plants, and of the 252 drugs considered essential by the World Health Organization, 11% are exclusively of plant origin. Because the harvesting is seasonal for most species, post-harvesting storage of dehydrated plants is an alternative to ensure good-quality products for processing industry along the whole year. The drying process aims the removal of water to a level to which deterioration and microbial activity are minimized. Due to the good mixing and high heat and mass transfer rates, fluidized beds have been widely applied in drying operations and many papers have been published on design and operation procedures for a wide range of particulate materials (Hajidavalloo, 1998; Colters and Rivas, 2004). Nevertheless, as pointed out by Cui and Grace (2007), limited research has been performed to investigate the fluid-dynamics of biomass particles in such systems. The authors observe that biomass particles (which include those originated from plants) have unique characteristics, such as extremely flat and irregular shapes. Thus, information based on conventional fluidization knowledge not always may be extended to such particles and further investigation is needed to optimize bed design and operation.

The purpose of present work is to investigate the fluid-dynamic behavior of fluidized beds operating with flat particles and to compare the results with those reported in literature for more conventional particles. As flat particles are difficult to be fluidized, vibrofluidization will be tested as an alternative to ensure the good mixing and homogeneity of fluidization for such materials.

The experimental apparatus is composed of a rectangular column, with a height of 0.30 m, and cross section of 0.20 x0.11 m^2, made of galvanized iron. The air is provided by a blower and the airflow rate is obtained using a previously calibrated orifice plate. Pressures at the orifice plate and at the bottom of the bed were measured by pressure transducers linked to a data acquisition system (acquisition board A/D PCI-6024E from National Instruments). For operation in vibrofluidized mode, an eccentric mechanism is used to adjust the amplitude of vibration and a mechanical controller located at the axle of the electric motor allows for the adjustment of frequency of vibration. Different amplitudes (A) and frequency (f) of vibration were tested, with vibration parameters Γ of 0.5; 1.0 and 1.5, where $\Gamma=A(2\pi f)^2/g$. In the fluidized mode, Γ was set equal to 0. Frequency of vibration is measured with an optical tachometer.

The final purpose of this work is to investigate fluidization of plant leaves, focusing initially on the flat shape of these particles. Details such as superficial roughness of leaves might difficult fluid-dynamic analysis at this stage. To avoid this and aiming to simulate bed operation with plant leaves, the particles used here were cut out from blank paper sheets 90 μm thick (ρ=0.883 g/cm^3). The use of these 'standard' particles has also the advantage of avoiding variations in shape and size inherent to biomass particles. Two shapes were investigated: the first one was leaf-shaped, with a projected area of 4.454 cm^2; and dimensions of 3.16 and 1.82 mm (measured at the longest and shortest axes passing through the centroid of the particle). The second one was square shaped, with length of 1.3cm and a projected area of 4.29 cm^2.

Pressure drops versus air velocity curves have been obtained for with a bed height of 3 cm, and Γ=0; 0.5; 1.0 and 1.5. From these curves were obtained the minimum fluidization velocities and bed pressure drops. The values obtained are being compared to those predicted by the usual correlations from literature for spherical particles. An important observation was that the imposition of a vibration to the bed causes the alignment of the particles in the packing, resulting in arrangements with all the particles having their shortest axis oriented in the flow direction. The time for full rearrangement is a function of the amplitude and frequency of vibration. At higher amplitudes and lower frequencies, the alignment occurs very fast, resulting in highly uniform beds. Such packing arrangements may provide an optimum configuration for drying, since they offer maximum heat and mass transfer areas.

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Heterogeneous model for mass transfer of dissolved lignin during kraft pulping of *Eucalyptus globulus*

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Keywords: Kraft pulping, mass transfer, lignin, kinetic model, *Eucalyptus globulus*

The kraft pulping of wood is a complex process that involves the chemical reactions of two distinct reagents - sodium hydroxide and sodium sulphide – with the several different components of wood, mainly lignin and carbohydrates. Moreover, the chemical reactions occur mostly inside the wood chip pores, meaning that the process involves two additional steps: the mass transfer of the reagents from the bulk liquor to the liquid inside the chips, and the mass transfer of the reaction products in the opposite direction.

In this paper a kinetic model is proposed for the mass transfer of the lignin dissolved in the interior of the chip to the bulk liquor, having the mass transfer of the reagents been addressed in a previous paper (Simão et al., 2007). The model presented here follows a lumped parameter estimation approach, where the spatial concentration profile of lignin inside the chip is represented by its spatial average.

The innovation in this model comes from being based on experimental data on lignin concentration, not only in the free liquor, but also in the liquor entrapped in the chip pores. The measured concentration of the dissolved lignin in the entrapped liquor corresponds to the spatial average concentration in the lumped parameter approach.

Furthermore, this approach allows estimating both the resistance to diffusion within the chips and the resistance to mass transfer in the film surrounding them. The extensive experimental data used to develop the kinetic model were obtained in laboratory batch cooks performed at the University of Coimbra. A pilot plant batch digester enabled to perform groups of six simultaneous cooks with the same operating conditions: temperature, pressure and concentrations and with varying pulping times. The cooking experiments were carried out with a target temperature ranging from 80 to 165ºC, the effective alkali charge varied from 15 to 45 g Na_2O/L and the sulphidity from 15 to 75%. At the end of each cook, a free liquor sample was collected, and the cooked chips were pressed in order to withdraw the entrapped liquor (Egas et al., 2002). The free and entrapped liquors were diluted with NaOH 0.01 N, and the dissolved lignin concentration was measured afterwards by reading the liquor's absorbance at 280 nm, following the method developed by Trinh (1988).

The experimental results revealed that the lignin concentration in the entrapped liquor is much higher than in the free liquor, and reaches a maximum value during the cook and then decreases, as shown on Figure 1. An increase in temperature, effective alkali charge or sulphidity increases the lignin concentration in both free and entrapped liquors, as expected.

For the model development it was assumed that the spatial concentration profile across the chip thickness can be represented by a second order polynomial, which enables to correlate the overall mass transfer coefficient (K_{OL}) with both the lignin effective diffusion coefficient (D_{eff}) and the mass transfer coefficient on the film surrounding the wood chips (k_L), allowing to estimate the corresponding resistance.

In order to estimate K_{OL}, the film mass transfer coefficient was calculated using correlations for liquid-solid systems, available in the literature (Dwidevi and Upadhyay, 1977). The effective diffusion coefficient was estimated by means of a modified Arrhenius equation. This equation includes the pulp yield raised to an exponent, which

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was introduced to account for a significant decrease in the lignin mass transfer rate, which was registered to occur along pulping time.

The model proved to enable a good prediction of the experimental data on the dissolved lignin concentration in the free liquor, as shown on Figure 2, with an R^2 of 0.986 for the 132 experimental data points used. The confidence intervals for the three parameters used to estimate the effective diffusion coefficient were very narrow. The estimated activation energy was 29 kJ/mol, which is higher than the activation energy obtained for the alkali mass transfer (Simão et al., 2007). The value of the yields´ exponent was 5.07, revealing a high contribution of this parameter to the effective diffusion coefficient, explaining an increasing resistance to diffusion along pulping time.

This may be justified by the fact that the lignin fragments with lower molecular weight diffuse faster to the bulk liquor, while the fragments with higher molecular weight, which are obtained later in the cook, have a slower diffusion rate.

The resistance to mass transfer in the film was found to have a significant contribution to the overall mass transfer resistance at the beginning of the cook. In the first ten minutes, the external resistance constitutes 20 to 30% of the overall mass transfer resistance, and therefore it should not be neglected, as it is in most pulping studies. However, since the internal resistance starts to increase after the heating period, the relative contribution of the external resistance becomes less significant from this point on.

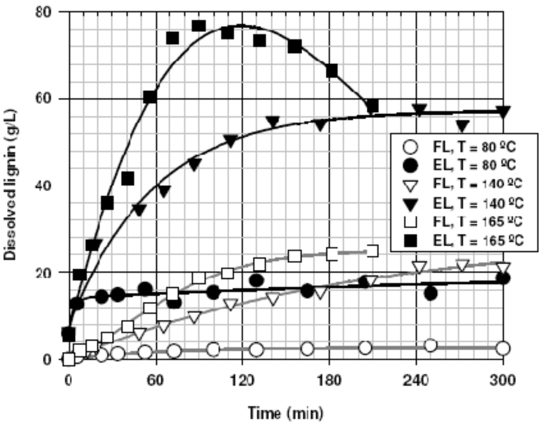

Figure 1. Effect of temperature (T) on the dissolved lignin concentration in the free (FL) and entrapped (EL) liquors (effective alkali charge = 15 g Na_2O / 100 g mad, sulphiidity = 30%)

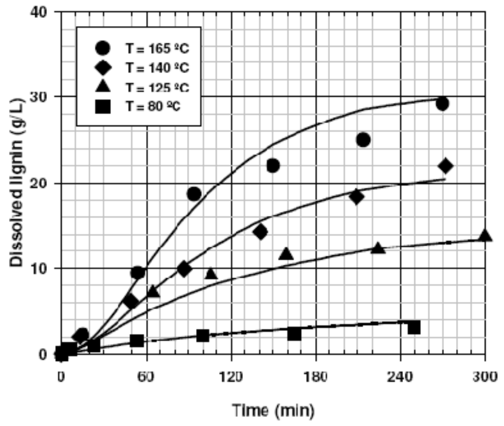

Figure 2. Experimental values and model predictions for the dissolved lignin in the free liquor (effective alkali charge = 15 g Na_2O / 100 g mad, sulphiidity = 30%)

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Longitudinal mixing in a peforated-plates extraction column

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Longitudinal mixing in the continuous phase of a liquid-liquid extraction column, without agitation mechanics and in scale of laboratory was studied in this work, using pulser tracer techniques. The column was 9,8 cm in diameter and contained 2 and 4 plates. The Levenspiel (1962) model is used for the determination of the values of the Axial Dispersion Coefficient through the behaviors of the Distribution Time Residence (DTR) obtained of the experiments. Analyses of effects of geometric and operational parameters are presented, using variations in the length of the column, in the free area of plates and in the water flow. Empirical equations for the Longitudinal Dispersion Coefficient are shown, as well as better ranges of use of column are presented.

Three-dimensional simulation of bubbly flow: influence of break up and coalescence models

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Keywords: Bubble Columns, Break up, Coalescence, CFD

Reactors based on bubbly flows such as bubble column and airlift reactors have been widely used in biochemical, petrochemical and metallurgical industries, due to their simple operation, high mass and heat transfer rates and no moving parts. In spite of the cited advantages the multiphase flow behavior that exists in such reactors is still a subject not fully understood due to its complexity.

However, the use of the adequate available tools can be very important in developing scale-up strategies and for the understanding of its flow behavior. Due to the arising of high speed computers and the advancement of numerical techniques, numerical studies of bubbly flows have increased. These numerical techniques are now capable to perform three-dimensional simulations of multiphase flows in complex geometries (Santos, et al. 2007).

Gas-liquid fluidization systems operate by the injection of the gas phase in the bottom of a column filled with liquid. This operation depends on several factors such as fluid physical properties, column dimensions and inlet gas velocity. There are basically two kinds of flow regime: the homogeneous and the heterogeneous. The homogeneous flow regime is characterized by low superficial gas velocity, and the bubbles are nearly uniform in size and shape, where bubble break up and coalescence phenomena is considered to be insignificant. On the other hand, in the heterogeneous flow regime, where the superficial gas velocity is high, it is observed higher turbulence, inducing bubble break up and coalescence; in this case, models that are able to capture these phenomena are necessary.

One important application of bubbly flows is the Fischer-Tropsch synthesis in slurry reactors. For applications in emerging technologies, such as natural gas conversion to liquid fuels through this reaction, it is necessary a deep knowledge on the hydrodynamics of this kind of reactor, increasing the industrial and scientific interest in this subject. Thus, for economic and logistic reasons, such conversions are best carried out in large-scale projects, so the capability of upscaling is an important consideration in the selection of reactors.

The present work shows three-dimensional gas-liquid simulations in a cylindrical bubble reactor with an external loop using the Eulerian-Eulerian approach. The commercial CFD package CFX 11 from ANSYS, which uses the finite volume method to solve the discretized system of equations that represents the flow, was used. The aim of this study is to present the effects of important parameters in CFD simulations, such as bubble size and geometrical modeling of gas sparger.

The effects of bubble average size, bubble size distribution and the geometry of the gas inlet plate were evaluated. The bubble break up model used was the Luo and Svendsen (1996), and for the coalescence it was used the Prince and Blanch model (1990). For the drag force the Ishii-Zuber model was considered. The k-epsilon turbulence model was applied only for the continuous phase and the dispersed one was considered laminar. The lift, Magnus and added mass forces were neglected. The fluids modeled were water for the continuous phase and for the dispersed phase was air at room temperature.

The tests were achieved with two kinds of inlets, one uniform and the other with a gas distributor in different superficial gas velocities. The studies referred to break up and

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coalescence models were developed only considering the uniform gas inlet, the particle size distribution was obtained by a population density function.

Results have shown that the approach used in this work provided physically consistent results, showing the transient effects in the column. Good agreement of time-averaged gas holdup with experimental data of gas holdup available in the literature was obtained. It was found that the simulation approach used in this work was able to capture the transient fluid dynamics.

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Comparison of hydrodynamic parameters between an internal and an external airlift column

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Keywords: airlift column/reactor, slug flow, gas-liquid systems

In many bubble columns the gas is injected at its base and rises through the liquid in the shape of bubbles that induce the circulation of the liquid. When the gas rises oriented by a riser tube the flow patterns become more ordered and the liquid flow rate increases. These are called airlift columns. In the internal loop airlift column, ILARL, the liquid rises along with the gas through an internal riser tube, separates total or partially at its top, and descends between the main column and the riser tube. In the external loop airlift column configuration, ELARL, the gas and liquid rise through a tube external to the main column and the liquid flows down through the main column.

Airlift columns are commonly used as gas-liquid reactors, in wastewater and sewage treatment processes, in nuclear, pharmaceutical and biochemical industries. The design of this type of columns involves the knowledge of the pressure drop, gas holdup and liquid recirculating velocity. This last parameter influences the mixture characteristics of these columns, the flow patterns and the heat and mass transfer coefficients, when mass transfer between two phases (gas and liquid) occurs. Each of these factors is conditioned by the physical and chemical properties of the gas and liquid, by the gas and liquid flow rates and geometry of the bubble column.

In the literature there are few studies about ILARL and ELARL especially for slug flow regime. In this regime, long bullet-shaped bubbles, usually called Taylor bubbles, occupy most of the tube cross section. Between the gas bubble and the tube wall flows a thin film of liquid and at the bubble rear there is a separated region, the wake, characterized by strong mixing. It is known that the flow pattern in the wake and in the liquid around and between gas slugs determine the mixing level in gas-liquid slugging columns. In the literature there is also a lack of information for laminar flow in the liquid, obtained when liquids of high viscosities are used.

To enlarge the knowledge about the performance of airlift columns/reactors, in the slug flow regime, an ILARL column was designed by Santos *et al* (2005) to ensure high precision and quality of the experimental results, with very simple experiments. This ILARL column can be operated for different submergence ratios of the riser tube and a wide range of operating conditions. In that study several hydrodynamic parameters were studied, namely:

- gas hold-up in the internal (riser) column;
- liquid superficial velocity in the internal column;
- bubbles rise velocity;
- length and average number of slugs in the internal column.

One the advantages of the ELARL column is that the volume of liquid used can be considerably reduced when compared to the ILARL column. So a ELARL was built, with a 2.80 m high riser tube, that could be operated under the same range of

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operating conditions used in the ILARL column i.e. different viscosities of the liquid solutions in internal columns with different internal diameters (22, 32 and 52 mm) of the riser tube and injection of different gas flow rates. The objective of the present study was to analise if similar results were obtained as in the ILARL column but spending less than 22% of liquid volume.

Several experiments were performed and analised for a 22 mm and a 32 mm internal diameter riser tube. An 78% aqueous glicerol solution was used (viscosity: 0.05 Pa.s), for different air flow rates (15×10^{-6} to 98×10^{-6} m^3/s), and a 0.94 submergence ratio was imposed.

The experimental data of the gas hold-up, the velocity of the Taylor bubbles and of the liquid circulation velocity were compared with the results obtained for the ILARL. In the conditions described above a decrease of 6% in the solution viscosity was observed due to the impossibility of working at the same temperature as in the ILARL. In general differences inferior to 3.3% were found in the liquid circulation velocity, 8.1% in the gas hold-up and 17% in the Taylor bubbles velocity.

The results described ensure that the two experimental designs lead to similar hydrodynamics parameters.

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Mass transfer coefficient in three airlift reactors with internal loop using Newtonian fluid

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Keywords: airlift reactor, holdup, scale-up, Newtonian fluid, volumetric oxygen transfer coefficient.

Introduction

Oxygen mass transfer is one of the most important design parameters of gas-liquid reactors employed for chemical and biochemical applications. Any shortage of oxygen significantly affects the process performance. An ideal reactor should have a maximal transfer rate, with efficient mixing at minimum energy input (Jurascik *et al.* 2006). In the present study the volumetric mass transfer coefficient (k_La) of oxygen was evaluated in three scales of internal loop airlift reactor (ALR) with different Newtonian fluids. The effects of reactor scale and viscosity on k_La were studied in three airlift reactors of different working volume (2, 5 e 10 dm^3) with similar geometric configuration. This study will be the basis for bioreactor design in the research on the optimization of clavulanic acid and cephamycin C production by *Streptomyces clavuligerus.*

Materials and methods

The three scales of ALR presented A_D/A_R ratio of 1.75 and a height-diameter ratio of 3.6. The holes of the cross-piece type sparger were 0.5mm of diameter and they were spaced 5mm along the length of the rods of the sparger (Badino *et al.* 2004). Distilled water and glycerol (99.5% purity) solutions were used as Newtonian fluids, the dynamic viscosity of the glycerol solutions ranged from 2.75 to 12.5 mPa.s. Gas holdup was determined by measuring the increase in height of the dispersion upon aeration (Chisti, 1989). The k_La was determined by the dynamic pressure-step method (DPM) (Jurascik *et al.* 2006).

Results and discussion

The oxygen mass transfer in the airlift bioreactor has been determined from measurements of the volumetric mass transfer coefficient (k_La), by the dynamic pressure-step method. The values of k_La as a function of the superficial gas velocity (U_{GR}) for the three reactors are shown in figure 1A. Some researchers have presented their k_La data by using equations of the type $k_La = \alpha \cdot U_{GR}^{\beta}$, with the two constants (α and β) depending of the two-phase mixture properties (Chisti, 1989). From the k_La values for water obtained from the three reactors, a model have been adjusted and the following values of $\alpha=0.535$ and $\beta=0.88$ were obtained. Both α and β are very similar those values presented by Shah *et al.* (1982) for tap water, $\alpha=0.467$ and $\beta=0.82$, respectively. The variation in the volume of the reactors had not caused a significant change in the k_La values as confirmed in figure 1A.

The influence of the kinematic viscosity (ν) on k_La can be observed in equation 1. The volumetric oxygen transfer coefficient (k_La) decreased with the increase of the kinematic viscosity, as observed by Shariati *et al.* (2007). The authors correlated values of k_La as a function of superficial gas velocity and kinematic viscosity and determined the following values: $\eta= 1.55$, $\gamma= 0.935$ and $\theta=-0.683$, respectively. In the presented work, similar values of constants η and γ were obtained ($\gamma= 0.875$ and $\theta=-0.780$).

$$k_La = \eta \cdot U_{GR}^{\gamma} \cdot \nu^{\theta} \tag{1}$$

Experimental results of k_La, ε_G and physical properties of the fluids (water and glycerol solution) were correlated according to the correlation originally proposed by Koide *et al.* (1983) utilizing dimensional analysis. The obtained correlation is presented by equations 2 and 3, which relate the Sherwood number to the dimensionless Schmidt (Sc), Froude (Fr), Bond (Bo), Galileo (Ga) numbers and holdup (ε). 212 k_La experimental values were determined from three scales of ALR with six different solutions of glycerol and water.

$$Sh = 0.00041 \cdot Fr^{0,31} \cdot Sc^{0,81} \cdot Ga^{0,68} \cdot Bo^{0,197} \cdot \varepsilon^{0,554} \qquad R^2=0.98 \qquad (2)$$

$$\left(\frac{k_L a \cdot D_{l2}^2}{D_L}\right) = 0.00041 \cdot \left(\frac{U_{GS}}{\sqrt{g \cdot D_{l2}}}\right)^{0.310} \cdot \left(\frac{\mu_L}{\rho_L \cdot D_L}\right)^{0.810} \cdot \left(\frac{g \cdot D_{l2}^2 \cdot \rho_L^2}{\mu_L^2}\right)^{0.680} \cdot \left(\frac{g.D_{l2}^2.\rho_L}{\sigma}\right)^{0.197} \cdot \varepsilon_G^{0.554} \qquad (3)$$

The result obtained by the fitting of correlation to the experimental data is illustrated in figure 1B, which confronts with experimental and calculated values of Sh. The correlation given by equation 2 has been very well adjusted to the experimental values, and the residues are ranged around ±15%.

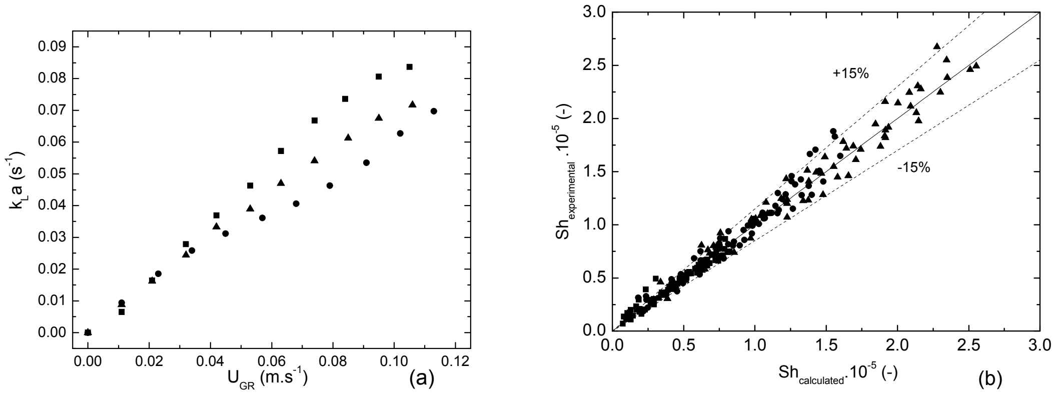

Figure 1. (a) Volumetric oxygen transfer coefficient (k_La) as a function of U_{GR} for water, (b) Comparison between calculated and experimental data of the Sherwood number for Newtonian fluid. (■) 2 Liters, (●) 5 Liters, (▲) 10 Liters.

The dimensional analysis is a very powerful technique whereby dimensionless groups of parameters are kept constant during the scaling. The application of this methodology in the present work provided the correlation given by equations 2 and 3, very useful for ALR scaling-up strategies aiming at the production of many important bioproducts, such as clavulanic acid and cephamycin C produced by *S. clavuligerus*.

Acknowledgements

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Prediction of mean bubble size in three scales of internal loop airlift reactor

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Keywords: airlift reactor, holdup, bubble size, Higbie´s penetration theory, volumetric oxygen transfer coefficient.

Introduction: The oxygen transfer is very important in bioprocess because aerobic microorganisms need oxygen to growth and produce bioproducts (Boltes *et al.* 2008). One of the most significant parameters in the design and scale-up of conventional or non-conventional bioreactors, such airlift reactors (ALR), is the overall volumetric mass transfer coefficient (k_La). Its value depends on the system, geometry and liquid proprieties which are related to several other parameters. The k_La is the product of the liquid-film convective mass transfer coefficient (k_L) and the interfacial area (a). Both k_L and a depend on the flow regime, hydrodynamics and bubble characteristics in the system (Ruen-ngam *et al.* 2008).

A new methodology is proposed to predict the mass transfer coefficient (k_L) and the mean bubble diameter (d_B) from experimental values volumetric mass transfer coefficient of oxygen (k_La) and gas holdup (ε) measured in three scales of ALR of 2, 5 and 10 dm^3 of working volume, with similar geometric configuration.

Materials and methods: Distilled water at 28 ºC were used as Newtonian fluid. The three scales of ALR presented A_D/A_R ratio of 1.75 and a height-diameter ratio of 3.6. The holes of the cross-piece type sparger were 0.5mm of diameter and they were spaced 5mm along the length of the rods of the sparger (Badino *et al.* 2004). Gas holdup was determined by measuring the increase in height of the dispersion upon aeration (Chisti, 1989). The k_La was determined by the dynamic pressure-step method (DPM) (Jurascik *et al.* 2006).

Theory: Prediction of mass transfer coefficient for airlift reactors was made according to a theoretical mass transfer model based on Higbie´s penetration theory.

$$k_L = \frac{2}{\sqrt{\pi}} \cdot \sqrt{\frac{D_L}{\theta}} \tag{1}$$

were D_L is the oxygen diffusivity and θ is the surface renewal time, which is defined in terms of the mean diameter of the bubbles (d_B) and the bubble liquid slip velocity (u_S) by Shamlou *et al.* (1995) as follows:

$$\theta = \frac{d_B}{u_S} \tag{2}$$

The bubble liquid slip velocity (u_S) is proposed by Ruen-ngam *et al.* (2008) as follows:

$$u_S = \frac{u_\infty}{(1-\varepsilon)} \tag{3}$$

Talaia (2008) proposed a new model to determine the terminal velocity of bubble (u_∞) given by equation 4:

$$u_\infty = 0.694 \cdot \left(\frac{g \cdot d_B \cdot \Delta\rho}{\rho_L} \right)^{1/2} \tag{4}$$

The relationship between mass transfer coefficient and bubble size was proposed by Chisti (1989), where ψ can be obtained experimentally using k_La and gas holdup (ε).

$$\frac{k_L}{d_B} = \frac{k_La \cdot (1-\varepsilon)}{6 \cdot \varepsilon} = \psi \tag{5}$$

Substituting equation 4, 3 and 2 into equation 1 and rearranging, it results in semi-empiric (or semi-theoretical) correlation to calculate the d_B from experimental values of k_La and ε. Values of k_L can be calculated from equation 5.

$$d_B = \left(\frac{4 \cdot D_L \cdot 0.694}{\pi \cdot (1-\varepsilon) \cdot \psi^2} \right)^{\frac{2}{5}} \cdot \left(\frac{g \cdot \Delta\rho}{\rho_L} \right)^{1/5} \tag{6}$$

Results and discussion: From equation 6, the values of d_B for the three scales of ALRs have been estimated and they are presented in Figure 1A as function of the superficial gas (air) velocity in the riser region (U_{GR}). The results obtained by the proposed model are in excellent agreement with the experimental values found in recent literature (Wongsuchoto *et al.*, 2003 and Ruen-ngam *et al.*, 2008). The mass transfer coefficient is presented in Figure 1B, where it can be observed that for a large range of superficial gas velocity in the riser (U_{GR}) from 0.01 to 0.11 m.s^{-1}, the value of k_L remained nearly constant for the three scale of ALRs studied. This demonstrates that the increase in the values of k_La with an increase of U_{GR} is due to the gas-liquid interfacial area (a) and not to the mass transfer coefficient (k_L).

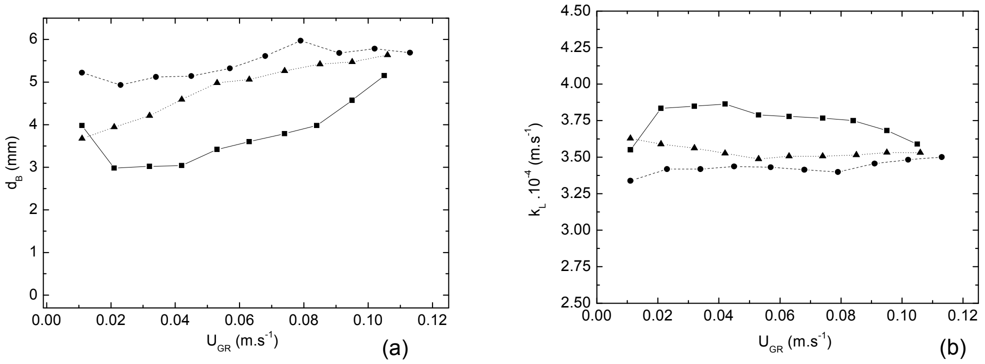

Figure 1. (a) Mean diameter of the bubbles as a function of U_{GR}, (b) Mass transfer coefficient as a function of U_{GR}. (■) 2 Liters, (●) 5 Liters, (▲) 10 Liters.

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Euler-Euler simulation of gas-liquid and gas-liquid-solid airlift reactors: case study

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Keywords: CFD, Euler-Euler, airlift, gas-liquid-solid

This contribution is aimed at CFD simulation of hydrodynamics of gas-liquid and gas-liquid-solid airlift reactors using Euler-Euler model. Commercial code Fluent 6.3 is used to solve model equations [1]. Studied cases include gas-liquid rectangular external loop airlift reactor and gas-liquid-solid cylindrical internal and external loop airlift reactor.

Goal

The goal of the work was to compare simulation results with experimental data available in literature.

Horizontal profiles of liquid and bubble velocity and gas holdup were compared with data of Becker et al. [4] in the case of rectangular gas-liquid airlift. Gas holdup and liquid velocity in the riser for different solid loadings were compared with data of Freitas et al. [5, 6] in the case of external and internal gas-liquid-solid airlift.

Model

In Euler-Euler model all involved phases (gas, liquid, (solid)) are treated as interpenetrating continua. There is continuity and momentum equation for each particular phase. Equations for phase q are (see e.g. [1], [2], [3]):

$$\partial(\alpha_q\rho_q)/\partial t + \ .\ (\alpha_q \rho_q \mathbf{u}_q) = 0$$
$$\partial(\alpha_q \rho_q \mathbf{u}_q)/\partial t + \ .\ (\alpha_q \rho_q \mathbf{u}_q \mathbf{u}_q) = -\alpha_q \ \ p - \ .(\alpha_q \tau_q) + \alpha_q \rho_q \mathbf{g} + \Sigma_p \mathbf{F}_{qp}$$

where α_q □is volume fraction of phase q, $\mathbf{u}_q$ is velocity, p pressure and $\Sigma_p \mathbf{F}_{qp}$ is sum of forces the other phases p act on phase q, □and $\tau_q = \tau_{l,q} + \tau_{t,q}$ is effective stress tensor which contains contributions not only from viscous effects ($\tau_{l,q} = \mu_q (\ \mathbf{u}_q + \ \mathbf{u}_q^{\mathbf{T}}$□□)) but also from velocity turbulent fluctuations.

These equations are coupled together through pressure, phase volume fractions (which sum to unity) and interphase force terms like drag, lift, added mass and turbulent dispersion force. Closure relations for the interphase forces are needed to solve the equations. These closures are difficult to obtain and their correct form may be unknown especially for higher phase holdup. Related problem is modelling of bubble size distribution since the interphase forces depend on bubble size. Also the turbulent contribution to the stress tensor needs to be modelled. This is usually done with single phase turbulence models such as k-ε extended for multiphase flows.

Simulations

Either steady state or transient simulations were done. Although the flow field is in general unsteady in airlift reactors, the steady state simulations may be useful to estimate total gas holdup and liquid circulation rate at least for lower gas flow rates. Simulations were done in 2D (2D Cartesian – rectangular airlift (Becker), 2D cylindrical (Freitas, internal loop airlift)) and 3D coordinates. The flow field in general is 3D so the 2D simulations may be used only as a first estimate.

Grid and time step independence was tested. Drag, lift, added mass and turbulent dispersion force have been accounted for in the model. Closures based on single bubble case were used. Simulations for different values of lift coefficient were done. Bubble coalescence or break-up were not accounted for in the model, dispersed particles were considered to be all of the same size. Several approaches to modelling of gas-solid interaction were tested. Interactions between gas and solid were either neglected or described in a similar way as gas-liquid interactions or an approach based on kinetic theory applied to granular flows was employed (see [1] for details).

Acknowledgements

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Interfacial instability of bubble during chemosorption

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Keywords: chemical reaction, interfacial instability, bubble, interference microscope.

Chemisorption is a complex and multistage process. It includes a diffusion-controlled chemical reaction near the interface accompanied with interfacial instability and the development of spontaneous small- and large-scale convection in the liquid phase. The problem of generation and time evolution of convective structures arisen due to chemical reaction is a subject of broad investigations aimed to the search for more intensive heat and mass transfer mechanisms than molecular diffusion. The phenomenon of chemo-gravitational and capillary instability manifests itself in the appearance of convective flows at the interface due to gravitation and surface forces.

The use of interference microscope enables real-time visualization of the evolution of interfacial phenomena for a spherical bubble of carbon dioxide formed at the end of microsyringe needle and placed in the water solution of a base. This gives the possibility to describe in detail the principle stages of instability, to estimate its duration and to suggest a physical interpretation of the experimental pattern. A polarizing interference microscope is schematically represented in Fig. 1.

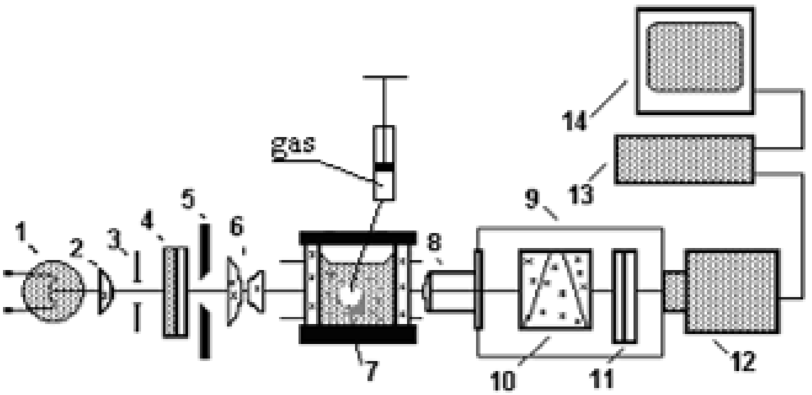

Figure 1. Polarizing interference microscope: (1) filament lamp; (2) lens; (3) aperture; (4) polarizer; (5) slit-shaped aperture; (6) condenser; (7) cell; (8) microlens; (9) polarizing interference unit; (10) prism; (11) analyzer; (12) TV camera; (13) video type recorder, and (14) TV monitor

The chemosorption cell (7) is made of transparent optic glass which enables observing the gas-liquid interface and phase regions near the interface. The design of the cell allows to wash it and to change chemisorbents without dismantling the experimental unit. The optical scheme of the polarization-interference microscope gives a 10 to 40 magnification of inhomogeneities near to the interface. The resolution of the unit evaluated from the number of lines on a monitor screen is no less than 50 μm.

The real-time observation of changes in the interference image close to the interface between carbon dioxide and the base aqueous solution allows us to track the dynamics of formation and evolution of the diffusion and hydrodynamic surface layers during chemisorption and the convection appearance on a scale of the whole experimental cell. The experimental patterns shown in Fig. 2 attest the complexity of time and spatial evolution of chemosorption.

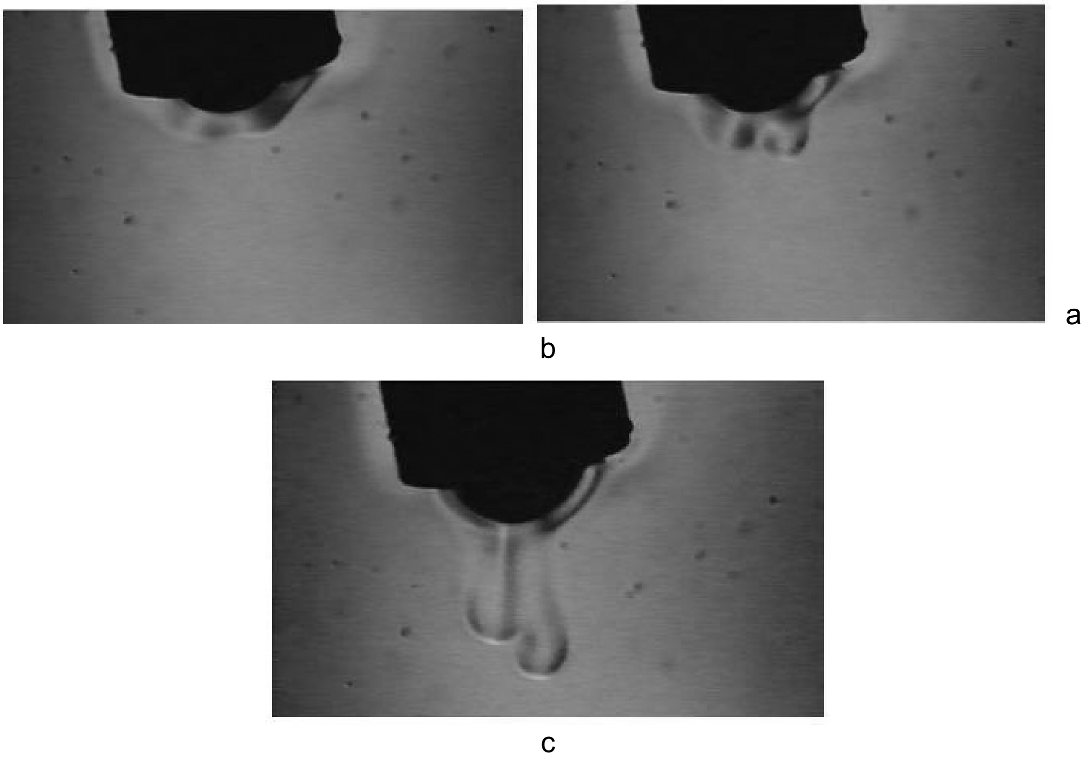

Figure 2. Interference patterns corresponding: (a) to the moment of instability generation at the interface; (b) to the appearance of droplets; (c) to the movement of product droplets from the interface

The appearance of the reaction product at the bubble surface is observed after some period which may last several seconds. One can see the flows of the liquid heated due to the reaction. At this stage, the reaction seems to proceed via the diffusion-kinetic mode. At the same time, the movement of the liquid along the interface is detected.

The film of reaction product becomes thicker and starts to precipitate along the surface to the bottom as if it is a separate quasi phase, possessing its own surface tension. In this case, the stability loss by the diffusion boundary layer can follow the Rayleigh-Taylor mechanism (chemogravitational instability). According to this mechanism, density stratification of liquids is unstable when a heavier liquid is present above and a lighter liquid below. Surface perturbations which develop with time acquire the form of separate drops, which grow gradually. As a result, the regime of convective transfer to the surface develops close to the interface which results in the intensification of the whole chemisorption process.

The delay in the appearance of drop chemogravitational convection after carbon dioxide is brought into contact with a chemisorbent is explained by the accumulation of the heavy reaction product close to the interface. This accumulation should precede convection. The appearance of macroscopic changes in the liquid phase due to instability, however, also takes some time even after a sufficient amount of the heavy product is accumulated and critical instability conditions are established.
At the last stage, droplets of the reaction product are formed at the bottom part of the bubble. The size of droplets is defined by the chemical composition of the water solution. Periodically droplets leave the surface and precipitate into the surrounding liquid without intermixing.

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Effect of kinds of gas on gas holdup in bubble columns

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Keywords: Gas holdup, Bubble column, Gas, Molecular weight, Bubble coalescence

Bubble columns are widely used as bioreactors and gas-liquid reactors. Gas holdup *EG* is a very important parameter for design and scale-up of bubble columns. Therefore, there have been many studies about gas holdup in bubble columns. It has been reported that gas holdup depends on many factors such as gas and liquid velocity, physical property of gas and liquid, type and arrangement of gas spargers, gas inlet height and inclination angle of bubble columns. However, the effect of kinds of gas on *EG* is not yet clear. In this work, the effect of kinds of gas on *EG* was experimentally studied.

A. Previous studies

1) Akita and Yoshida (1973) have reported that *EG* does not depend on kinds of gas in a 15cm square bubble column with 4.5 mm orifice, although the gas holdup with He appears slightly lower at higher gas velocities. They used Air, O_2, He, CO_2 / water system (see Fig.1).

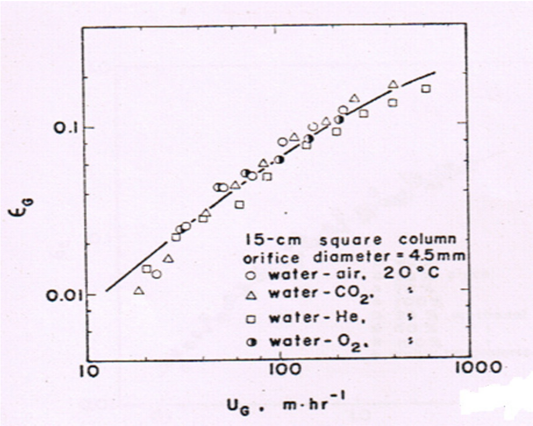

Fig.1 *EG* by Akita and Yoshida(1980)

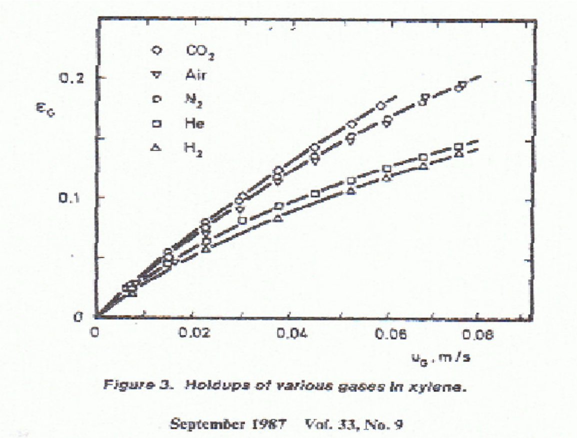

Fig.2 *EG* by Ozuturk et al.(1987) for Xylene system as liquid.

2) Hikita et al. (1980) studied the effect of gas and liquid properties on gas holdup in the bubble column of 10 cm inner diameter and 1.5 m height. They have reported that E_G depends on $\rho_G^{0.062}\rho_L^{0.069}\mu_G^{0.107}\mu_L^{-0.053}$ and presented the following correlation:

$$E_G = 0.672\,(U_G\mu_L/\gamma)^{0.578}[\mu_L^4 g/(\rho_L\gamma^3)]^{-0.131}\,(\rho_G/\rho_L)^{0.062}(\mu_G/\mu_L)^{0.107}$$

3) Ozturk, Schumpe and Deckwer (1987) have also studied the effect of kinds of gas on *EG* in the 9.5 cm I.D. and 85 cm tall bubble column with organic liquids and reported that *EG* depends on kinds of gas (see Fig.2). They have concluded that the gas-specific effects are probably related to the bubble formation at the sparger rather than the hydrodynamics in the bulk of the dispersion. Therefore, *EG* depends on kinds of gas in bubble columns with small height (*HT* < 1 m), however, *EG* does not depend on kinds of gas in tall bubble columns (*HT* > 2 m). HT means height of liquid layer.

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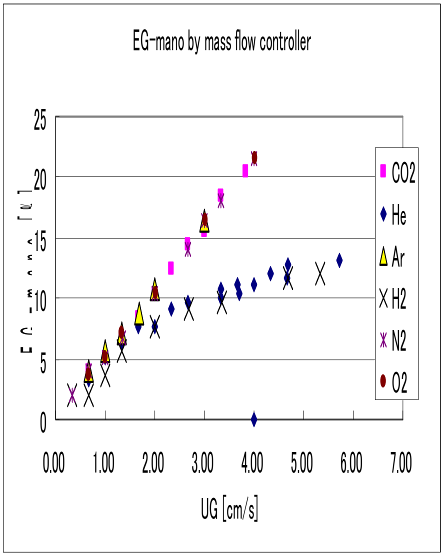
B. Experimental results

Fig.3 shows the effect of kinds of gas on *EG* in a 50 cm tall bubble column whose cross section is rectangular (5cm x 10cm). The gas sparger was a perforated plate.



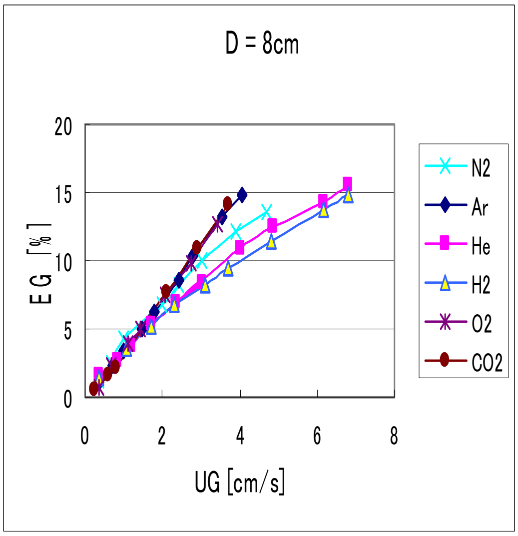


Fig.3 EG for a rectangular column. Fig.4 EG for a 8cm I.D. column

Fig.4 shows the effect of kinds of gas on *EG* in a 8cm I.D. and 140 cm tall bubble column. *EG* depended on kinds of gas. *EG* decreased in order of O_2, CO_2, Ar, N_2 >He > H_2. The reason why *EG* depended on kinds of gas is that bubbles of H_2 and He coalesce into large bubbles more easily than those of O_2, CO_2, Ar, N_2 at the same superficial gas velocity U_G. Moreover, the effect of kinds of gas on frequencies of bubbles which generated from the 7mm I. D. horizontal nozzle was experimentally studied. The results show that frequencies of bubbles which generated from the 7mm I. D. horizontal nozzle did not depend on kinds of gas.

Conclusion

1) *EG* increased with increasing molecular weight because bubbles of smaller molecular weight gas coalesce into larger bubbles more easily at the same superficial gas velocity.

2) Frequencies of bubbles which generated from the 7mm I. D. horizontal nozzle did not depend on kinds of gas at the same gas flow rate.

Simulation of dynamic performance of ozonation treatment of surfactants in a semi-batch bubble column reactor by CFX

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Keywords: Simulation, Ozonation**,** Surfactant, Bubble column, CFD

Much attention has been paid to clean environment. In order to keep clean environment, waste gas and liquid must be completely treated. Ozone is a strong oxidizer and has been widely used for treating water and wastewater. Surfactants are predominant pollutants of wastewater and must be decomposed. There have been many researches about decomposition of pollutants by ozone. Uchiyama et al. (2007) studied the dynamic performance of ozonation treatment for nonionic surfactants SS-90 (Polyoxyethylene Alkyl Ether) in a bubble column and simulated the experimental results by using the tank-in series model (see Fig.1).

Recently, CFD has developed remarkably because of development of cheap efficient PC and good software. Simulation by CFD is very useful for research, design and scale up of bubble columns. In this work, simulation and optimization of dynamic performance of the ozonation in a semi-batch bubble column reactor by CFX software were done. The simulation results were compared with results of Kawase et al. (2007) and this work. Fig.1 shows the change of TOC, Yout and ΔC with time by Uchiyama et al. Yout and ΔC mean ozone concentration in gas at the outlet of the column and concentration of SS-90 in the water of the bubble column, respectively. Solid lines mean simulation results of the tank-in series model by Uchiyama et al. Fig.2 shows the simulation results by CFX. Fig.2 well resembles Fig.1. However, O_3out (= Yout) by the simulation in Fig.2 is larger than Yout in Fig.1, because Yin in Fig.2 is 20 mg/L though Yin in Fig.1 is 15 mg/L. Yin means ozone concentration in gas at the inlet of the column.

Fig.3 shows the comparison between experimental results and simulation results for dynamic behavior of ozone concentration in a bubble column with ultra pure water and no reaction. In Fig.3, simu and exp mean simulation results and experimental results, respectively. C means ozone concentration in water. The reason why O_3out by simulation was much larger than experimental results may be because the bubble column used has the gas chamber in the bottom of the bubble column.

Moreover, in order to optimize the reactor, the simulation of effects of baffle plates and draft tubes on the dynamic performance of the ozonation in a semi-batch bubble column reactor were now under study by CFX software.

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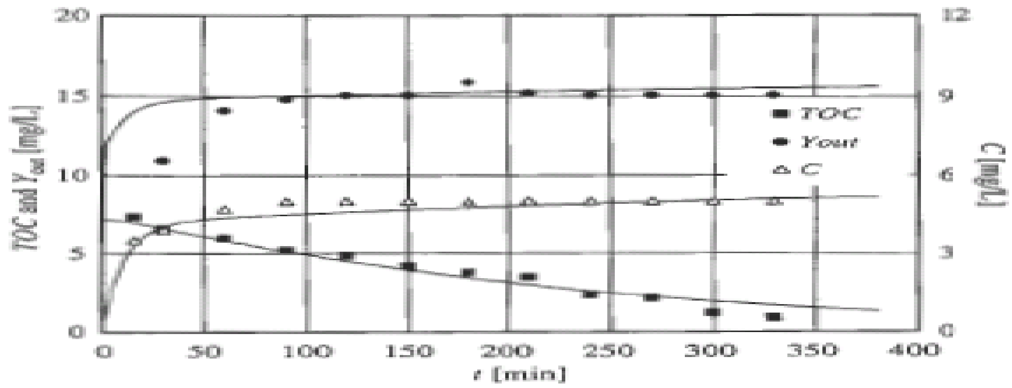

a) S_0=10 mg/L ($(TOC)_0$= 7.16 mg/L), Y_{in}=15 mg/L, Q_g=0.5 L/min, T=298 K

Fig.1 Dynamic simulations for ozonation of Sannonic SS-90 by Uchiyama et al.(2007)

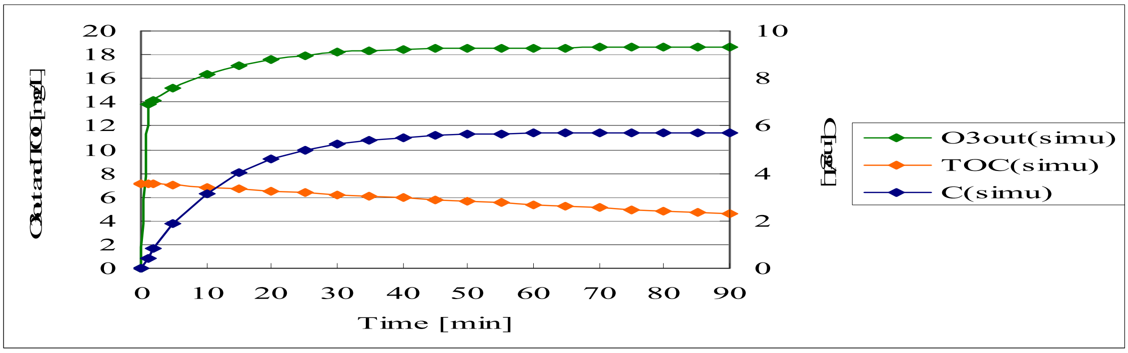

Fig.2 Simulation results by CFX. Yin = 20mg/L.

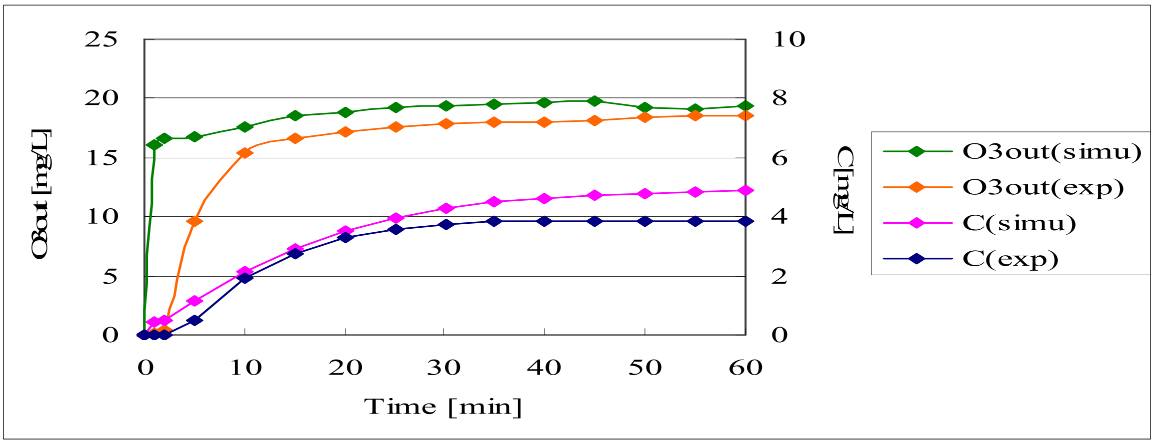

Fig.3 Comparison with experimental and simulation results for ozone solution into water. Yin =20 mg/L.

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Particle interaction with coherent structures in a confined two-phase jet

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Keywords: Gas-Solid Flows, Coherent Structures, Particle Interaction

The purpose of this research was the study of flow structures and the contact between phases in gas-solid two-phase flow. Flow structures are related to those coherent ones of large scale, quasideterministics structures - randomly distributed in the space and time, which are important in the turbulent transport and in the noise generation, and that the transport and the interlacement are in its majority of better directed induction than the provoked one by the diffusion gradient. The analysis of the contact between the phases abides the verification of the two-phase mixture gas-solid behavior, the gas-particle and particle-particle interactions. These interactions are two basic phenomena in dense particulated flows, which interact mutually to form several flow structures. Obviously to judge the precision of the available theories and finally to establish a complete fluidization theory, it has to get detailed information about the gas-solid interactions (hydrodynamics), particle-particle interactions and particularly its individual roles in the flow structures formation and evolution.

In an plexiglass chamber with 1m tall and pentagonal geometry, radial profiles, from the exit of the jet formation orifice, average velocity, velocity fluctuation (velocity RMS), turbulence intensity and interparticle arrival time (in terms of Chi^2 distribution and deviation number) were determined with the Phase Doppler Anemometry technique in five axial levels, with high solids rate and distinct gas phase superficial velocity. The reasons for the application of this technique are the non-intrusivity, directional sensitivity, high resolution in the space and time and high precision. The chamber had three entrances in the top for jet nipples, two of these for purely gaseous flow and one for the mixture gas-solid, this last one in the central position. The objective of this configuration was a bigger interativity between particles and the analysis of the influence on the flow structures formation. The two-phase mixture used was between 50μm and 90μm, in two different ratios.

The gas phase is analyzed of indirect form, only particles are analyzed. It is important to be clear that the gas phase flow have proper characteristics, as literature studies, thus the visualized structures are only effects of these related to the particulated phase. The variations of the initial velocity conditions, particle distribution and load ratio allow the attainment of important information related to the local flow structures and its effect on the macrocospic and turbulent transport of particles between the jet center and the jet contour regions.Thus it becomes possible to identify that in the jet center the large scale coherent structures formation does not exist, the flow is dominated by incoherent structures. Strong indications of these structures formation in a radial region between the center and the contour region also exist, and these dependents on the initial condition of the gas phase superficial velocity, which has more influence in the velocity radial profiles than the solid rate.

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Numerical investigation of unsteady flow and heat transfer from a porous square cylinder

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Keywords: Heat transfer, porous cylinder, gas - solid reactors

Newtonian fluid past a porous square cylinder in an unbounded domain. The flow past permeable bodies is a topic that manifests itself in several practical situations such as heat transfer enhancement of porous inserts in flow field (Martin et al, 1998), the instability of segmented solid propellant rocket motors (Couton et al., 1997), and the use of porous plates as motion damping devices (Downie et al., 2001).

A one-domain approach of (Basu and Khalili, 1999) has been adopted in the present study to numerically simulate the flow and heat transfer characteristics of a porous square cylinder in the Reynolds numbers (based on the height of the cylinder and the incident stream at the center-line of the cylinder) range $50 \leq Re \leq 250$. The Darcy numbers (*Da*) is varied from 10^{-6} to 10^{-2} while the porosity (ε) varies from 0.68 to 0.997. Numerical simulations are performed using a finite volume method SIMPLE to solve the governing equations of fluid flow and heat tranfer. With these flow parameters, vortex shedding and its subsequent effect on the heat transfer from a porous square cylinder is investigated. The change of unsteady vortex shedding flow to the steady flow regime at high *Da* can be viewed from the vorticity contours. The drag coefficient of the porous cylinder reduces considerably with increase permeability compared to an impermeable cylinder as more and more fluid oozes through it at high *Da*. The Nusselt number increases with increase in *Da* for a fixed *Re*.

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Heat transfer in the freeboard of a bubbling fluidized bed

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Keywords: bubbling fluidized bed, heat transfer coefficients, propane combustion

The main objective of this investigation was to experimentally study, in the free board of a bubbling fluidized bed, the heat transfer towards a water cooled confining membrane wall. Published data concerning the heat transfer in this region are scarce, and are essentially concerned with the heat transfer towards tube bundles placed in the free board region, while data on heat transfer towards membrane walls are almost non-existent. Wen–Ching (2003) refers numerous works on laboratory scale fluidized bed reactors and confirms that the available information in the transport region of bubbling beds is quite limited.

Several heat transfer studies were carried out in fluidized beds for operating conditions different than those considered in the present work. Al-Busoul (2002) used bed immersed surfaces while several other studies consider the membrane wall, but only in the bubbling bed region. Pagliuso and Lombardi (2000) presented results for an experimental set-up quite similar to that considered in this investigation and so their work was used for a preliminary analysis. However, these authors refer to a circulating fluidized bed operating at around 150 ºC, while the present study covers a higher range of temperatures.

The literature search carried out also reveals no information about studies of heat transfer in fluidized beds with the combustion of propane. Some authors (Gayan et al, 2004; Dounit et al, 2001 and Ribeiro and Pinho 2004) experimentally studied combustion reactions in fluidized beds, but only to characterize reaction kinetics and not the heat transfer phenomena involved.

The current investigation presents new results on the heat transfer mechanisms occurring in the membrane walls placed above a bubbling fluidized bed. To perform these experiments a laboratory scale fluidized bed reactor was built. A full description of the apparatus can be found in Neto (2008). The system was built in stainless steel and was thermally insulated with Kaowool ceramic fibre. The riser portion of the reactor was formed by two vertical sections with water jacket membrane walls, each with a length of approximately 1000 mm. The first part of the reactor, from the distributor to the flange separating it from the first membrane wall section, was 420 mm long, and the internal diameter of the reactor was of 54.5 mm. The fluidized bed reactor was heated by a 2 kW electrical resistance wrapped around the bubbling bed region of the reactor. Alternatively, propane could be injected and mixed with the fluidizing air, with combustion taking place in and above the surface of the bed, depending on temperature conditions and bed particle size.

Two types of experiments were performed: for the first type the bed was fluidized with air and the bed temperature reached values ranging from 400 – 700 ºC. During the second type of experiments propane was injected and mixed with the fluidizing air and combustion took place in and above the bubbling bed. In this case the temperature in the bed reached values above 700 ºC and the bed was always operated with excess of

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air. For all experiments the bed was formed of silica sand with the following average diameters: 107.5, 142.5, 180.0, 282.5 and 357.5 μm.

From the first set of experiments (bed fluidized by air) it was found that the thermal power received by the cooling water inside the membrane wall heat exchanger was dependent upon the three following mechanisms: gas convection towards the membrane wall, radiation heat transfer between the bed surface and the membrane wall surface, and projection of particles away from the bed surface towards the membrane walls. The relative importance of these three mechanisms was evaluated as a function of a global heat exchanger coefficient related to the membrane wall internal surface area.

For the propane combustion experiments, the thermal power received by the water was much higher than that received during experiments without combustion, particularly for beds formed from the three smaller particle sizes (d_p =107.5, 142.5 and 180.0 μm). For these sizes, the combustion reaction occurred both inside and at the surface of the bed. During the experiments it was observed that the mass of particles in the bed decreased with time, as particles were transported by the explosions occurring during the combustion process. For the experiments carried out with particle sizes of 282.5 and 357.5 μm, combustion initially occurred inside the bed, but rapidly moved to the transport zone. For the determination of heat transfer coefficients, only the data obtained for combustion inside the bed was considered.

Based on the experimental data a correlation for the overall heat transfer coefficient was developed, covering non-combustion and combustion experiments.

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Dispersion in packed beds

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Keywords: porous media, nuclear magnetic resonance, flow, packed beds

The experimental characterization of voidspaces and flow within natural rocks, packed bed reactors, chromatography columns, or in simple packs of mono-disperse solid spheres generally includes measurements of volume averaged properties such as permeability, porosity, dispersivity, and sometimes the hydrodynamic radius r_h=V/S, where V and S are the volume and surface area of the pore space respectively. Displacement encoding NMR experiments have made significant contributions to this area of research, with measurements of short time restricted diffusion coefficients yielding the hydrodynamic radius of a pore space (Mitra, Sen and Schwartz, 1993), and with alternating pulsed field gradient stimulated echo NMR (APGSTE-NMR) flow propagator (Kärger and Heink, 1983) and dispersion (Ding and Candela, 1996) experiments in packed beds determining pore space dispersivities or effective diffusion coefficients. It is clear, however, that NMR derived dispersivities in packed beds - the one random porous system for which there exist canonical but incompatible theoretical predictions with few or no adjustable parameters (Saffman, 1960; Koch and Brady, 1985) - can be affected by the same experimental complications which have substantially contributed to the puzzling scatter in published dispersion results based on elution experiments (Han, Bhakta and Carbonell, 1985). Notable among these are fast flow near walls, and inhomogeneous flow injection.

We will discuss how, with data analysis accounting for such macroscopic flow heterogeneities, displacement encoding APGSTE-NMR experiments were employed to obtain the intrinsic dispersivity of beds packed with solid monodisperse spheres (Scheven, Harris and Johns, 2007). The NMR dispersion measurement conducted on water flowing through a tube of Diameter D packed with monodisperse spheres of diameter d yields the dispersivities, for a range of Peclet numbers, of the infinite pack of spheres, provided that d<<D and provided that lateral mixing is poor over the time and length scales of the experiment. Longitudinal dispersivity data obtained in this manner are reproducible to within a 5%, in separate experiments using different packs of sheres having different porosities and hence packing arrangements, *when dispersivities are scaled by the hydrodynamic radius*. This universal behaviour opens the door for quantitative tests of dispersion models, and of optimized packing strategies.

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Operating the segregated flows of particulate materials as a principle of technological process organization

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Keywords: particulate solids, mixing, separation, segregated flows, operation, modeling.

The treatment of the nonuniform particulate solids mixtures with high inclination to segregation is a widespread technological problem. First of all, this problem is the consequence of the necessity to provide the distribution homogeneity of particles differing in size or density in the apparatus working volume. The ignorance of this condition leads to the different residence time of nonuniform particles in the apparatus working volume and reduce the product quality.

On the other hand, the high segregating components make difficult their mixture production and place exacting technological standards upon the mixing equipment. In consequence of high nonuniformity of the mixture particles their segregated flows arise. These flows are situated in dominate areas of the working volume and differ from each other by structural and kinematical characteristics. Traditionally these flows are destructed by means of several special methods.

In the present paper the principle of operating segregated flows is suggested in order to solve the technological problems during the treatment of nonuniform particulate solids. The basic advantage of this principle consists in its realization possibility for many technological applications. These applications may be as follows:

a) differentiation of technological conditions for different particles by means of operation of their residence time in the apparatus working volume;
b) increasing the mixture quality of particulate solids inclined to segregation due to the intensification of longitudinal and transversal mixing segregated flows;
c) separation process organization of traditionally hard separated mixtures by means of intensification of segregated flows of mixtures.

In the present paper a version of practical realization of the above mentioned principle is discussed. This version uses the traditional construction of apparatus for heat and mass transfer processes, which consists of horizontal rotating drum with peripheral lifting blades. Basing on the investigation results in terms of segregated flows in the apparatus of the traditional construction we suggested a device allowing the operation by named flows.

The device operating the segregated flows realize the principle of the multistage counter current flows of nonuniform particles [1] and additionally provides the possibility to change their value and direction along and across of the drum axis. The addition of the traditional apparatus by the suggested device allows to set the different treatment time for particles differing in size and density. If the main technological aim is the qualitative mixture production then the residence time of nonuniform components is equalized and longitudinal and transversal mixing segregated flows is organized in the working volume.

Moreover this equipment may be used for separation of particles differing in size and density. In this case the longitudinal countercurrent flows of segregated particles are intensified in the apparatus.

The paper presents the results of experimental and analytical investigations of technological efficiency and dynamic characteristics for multifunctional apparatus at various versions of its application.

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The basic application versions are the periodic and continuously working mixers also the separator for hard separated materials. Thereby it is noteworthy, that the apparatus construction is rather suitable simultaneously to organize heat-mass transfer processes, because of existence of the falling particles bed in the apparatus working volume.

The mixture nonhomogeneity was estimated by means of the variation coefficient determination. This coefficient is calculated as the ration of the standard concentration deviation of the test component to its mean concentration in the mixture expressed as the percentage. As an example the experimental dynamic characteristics of the periodic mixer are shown on Figs. 1 and 2.

The experiments on the mixing and separation carried out with the use of hard mixed and hard separated materials respectively. In order to prepare these materials we have used glass beads and polymeric particles differing in size and density. The particle sizes were choused to complicate the mixing and separation processes.

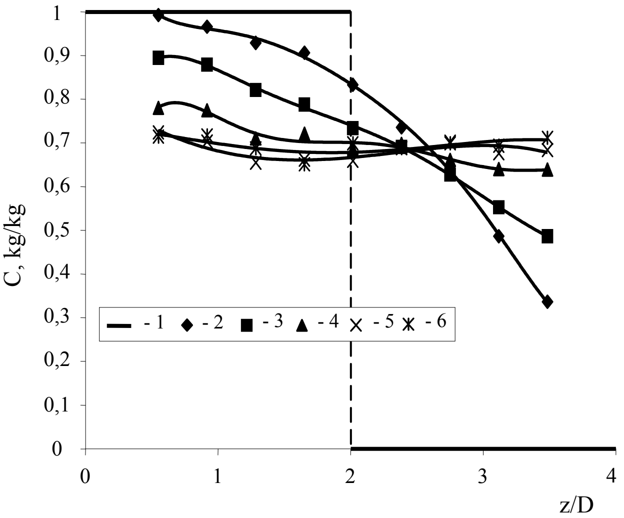

Fig. 1 Experimental dynamics of the test component distribution along the apparatus length z/D (z is the longitudinal coordinate; D is the apparatus diameter): 1 – 0 s., 2 – 24 s., 3 – 72 s., 4 – 168 s., 5 – 288 s., 6 – 360 s.

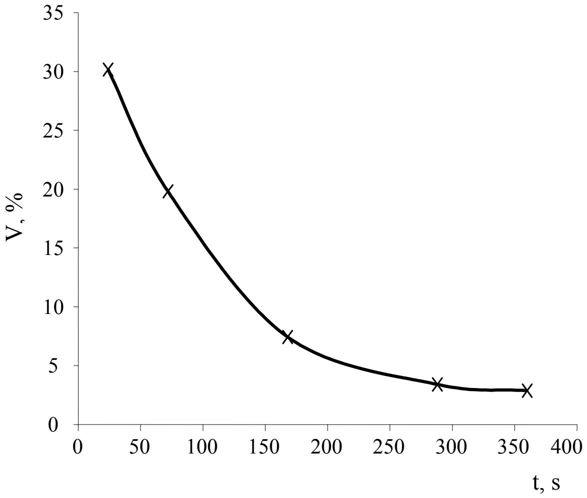

Fig. 2 Variation coefficient evaluation in the course of mixing high segregating particles.

The investigation results reveal that the suggested apparatus is rather effective especially when it is important to process the high segregating materials without sieves and separating gas-liquid flows.

In order to forecast the effect of the segregated flows operation an analytical approach is developed in the paper. This approach is based on an adaptation of the general mass transfer equation for conditions of segregating particulate media.

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Hindered motion in highly porous media: steric and fractal approaches

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Keywords: Hindered diffusion, Porous media, Fractal, Simulation

The growing need for higher resolution in the bio-separation of macromolecules, nano-particles, viruses and microorganisms, together with the need to keep their full bioactivity by preserving their shape and conformation is demanding new approaches in the field of bioseparation methodologies. A hydrodynamic theory developed for the diffusion and convection of micro-objects in porous media for the case of hindered motion was presented in a previous work (Mota et al., 2006) and can be summarised as follows.

The effective diffusion coefficient D_e of a solute in a channel is composed of two correction functions D_e and $F_2(\lambda)$: $D_e = D_0 F_1(\lambda) F_2(\lambda)$, where D_0 is the bulk diffusion coefficient and λ is the ratio of Stokes-Einstein diameter of the diffusing micro-object d_s to the equivalent pore diameter d_{por}, $\lambda = d_s / d_{por}$. The parameter $F_1(\lambda)$ is the steric partition coefficient, which is defined as the cross-sectional area of the pore available to the solute molecule divided by the total cross-sectional area of the pore

$$F_1(\lambda) = (1-\lambda)^2 \qquad (1)$$

The correction factor $F_2(\lambda)$ accounts for the effect of the pore wall on the solvent properties (an increase in the local solvent viscosity near the pore wall) and is often represented by a polynomial series, as displayed in equation (2), or by an exponential function, as displayed in (3). Other model functions are particular cases of (2) or (3)

$$D_e / D_0 = (1-\lambda)^2 \left[1 - 2.1044\lambda + 2.089\lambda^3 - 0.948\lambda^5\right],\ \lambda \le 0.5 \qquad (2)$$

$$D_e / D_0 = (1 - 1.83\lambda + 4.18\lambda^2)\exp(-6.52\lambda) \qquad (3)$$

Numerous experimental results show that hindered diffusion and convection depend not only on equivalent micro-object and pore sizes, but on the pore topology as well. To consolidate the experimental observations, a two-dimension porous media simulation was made with mono and binary packing of disks. Pore area fractal analysis was performed by a test-box method where the box simulates a compact micro-object inside the pore space. The measured parameter was the fractal dimension of pore area D_{pa} versus the aspect ratio r/d: the ratio between the test-box size and the equivalent pore size.

To compare the measured fractal dimension and the adimensional diffusivity D_e / D_0, a re-normalisation procedure was performed based on the assumption that the equivalent pore diameter is $d_{por} = (2/3)[\varepsilon/(1-\varepsilon)]d$, where ε is the sphere packing porosity, ε = 0.26 for hexagonal packing. Hence, the equivalent pore diameter is $d_{por} \approx 0.234\,d$. For other packing structures $d_{por} \approx \alpha \cdot d$. Normalised pore size was substituted into equation (1): $\lambda = d_s / d_{por} \sim r / d_{por} = r/(\alpha \cdot d)$. Results obtained for hexagonal packing are shown in Figure 1.

The most important simulation result is that the range of molecule's "sensitivity" to the pore network cross-section area coincides to the hindered diffusion range. The main measured values of D_{pa} occupy an area between curve 1, equation (1) for steric partition coefficient, (cylindrical pore and spherical molecule) and curve 3, equation (3)

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for hindered diffusion of a macromolecule, whereas Renkin equation (2) is close to the scattering zone of the determined fractal dimension.

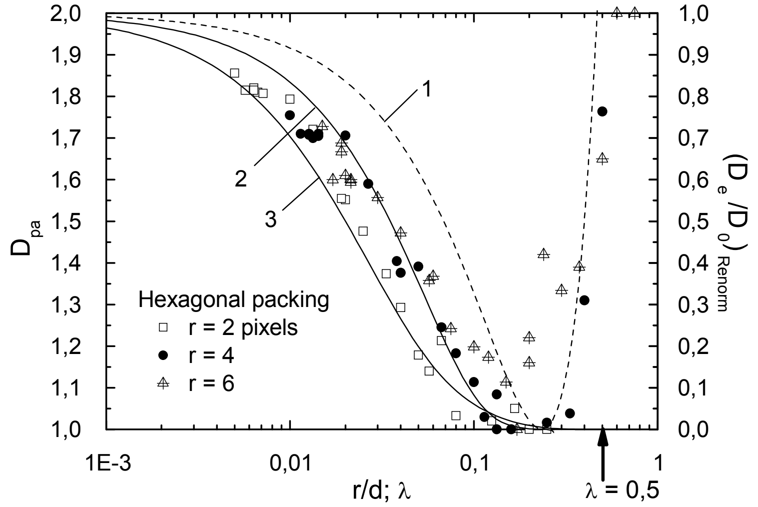

Figure 1. Dependence of D_{pa} and re-normalised diffusivity $(D_e/D_0)_{\mathrm{Renorm}}$ on r/d and λ for hexagonal packing of uniform discs. 1 – Equation (1); 2 – Equation (2); and 3 – Equation (3).

Concerning three dimension porous media, obtained results can be treated as follows: 1). Starting from large pores with λ > 0.001, molecules recognise the pore volume as a partially restricted space with reduced fractal dimension. 2). The restriction impact on a molecule depends on the pore topology (in present case on the type of packing). 3). Dramatic reduction of D_{pa} is observed when λ overcomes values around 0.01 and approaches λ ~ 0.1 in 2-D approach meaning that the test object recognises the pore as one dimensional rather than a 2-D space; in a 3-D system the test object will recognise the pore more with a behaviour closer to a 2-D system. 4). Concerning the hydrodynamic chromatography (Mota et al., 2006) the simulation explains why macro-objects become significantly retarded even at λ ~ 0.01, that is, even when the ratio between the Stokes-Einstein diameter of the diffusing micro-object d_s to the equivalent pore diameter is very low.

The developed approach confirms the fact that even for simple micro-object geometry (= test box) a molecule might be much more sensitive to the pore topology than the predicted by the steric effect. It is possible to expect an even more pronounced effect for asymmetric micro-objects in tortuous channels.

These results show that the problem of molecular sensitivity to pore topology may be satisfactorily solved using fractal features. Further work will apply this kind of approach to molecular diffusion inside gel-like, fibre-like or foam structures.

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Another basic segregation mechanism in aerated gravity flows of particulate solids

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Keywords: granular material, gravity flow, mixing, segregation, migration

Transversely aerated gravity flows of particulate solids are used widely in particle technology for the processes of heat-mass transfer and dust removal as well as for pneumatic transport in open channel. The results of experimental and analytical investigations presented earlier in [1] have shown that the aeration intensity and aeration direction considerably influence the segregation-mixing process in gravity flows of non uniform particles.

The prediction of segregation effects in aerated gravity flows of particles is of great significance for the industrial application because segregation may considerably upset the mixture uniformity which will influence the character of the subsequent technological processes and the quality of the finished products.

Earlier in the work [1] in order to forecast the segregation-mixing effects in aerated gravity flows of particulate solids differing in size and density the general segregation model [2] was adapted. In our further investigation [3] the general segregation model was developed by means of a closer definition of segregation kinetics and consideration of a quasi-diffusional separation mechanism.

The recently developed general model of segregation dynamics [3] is formulated for steady twodemensional shear flow as follows.

$$\frac{\partial c\cdot\rho_b}{\partial t}=-\frac{\partial\left(u\cdot c\cdot\rho_b\right)}{\partial x}+\frac{\partial}{\partial y}\left(\rho_{\cdot b}\left(D_{dif}\cdot\frac{\partial c}{\partial y}-D_m\cdot c\cdot\frac{\partial \ln s}{\partial y}-K\cdot\Delta M\cdot c\right)\right) \qquad (1)$$

It takes into account the fluxes of convection, quasi-diffusional mixing, migration and hydromechanical segregation. In case of rapid shear flows of cohesionless non-elastic spherical particles all the parameters in Eq.(1) excluding coefficient *K* are calculated analytically as functions of traditional physical and mechanical characteristics of particles and flow parameters [3]. Only the kinetic constant *K* is determined experimentally by the method described in Ref [3].

In this paper in order to reveal more accurately physical mechanisms of aeration influence on particle segregation in gravity flows the mathematical modeling of segregation dynamics is carried out on the basis of Eq (1) taking into consideration additionally the migration effects.

The segregation driving force ΔM in aerated flows is calculated as the excess momentum of gravity, friction, impact and hydrodynamic pressure forces acting on the test particle [1].

Equation (1) was integrated numerically for the initial condition

$$c\left(0,x,y\right)=c_0 \qquad (2)$$

where c_0 is the mean concentration of test particles.

Boundary conditions were formulated in the following way

$$D_{dif}\cdot\frac{\partial c}{\partial y}=D_m\cdot c\cdot\frac{\partial \ln s}{\partial y}=K\cdot\Delta M\cdot c=0, \qquad \text{if} \quad \mathrm{y=0,h} \qquad (3)$$

The profiles of velocity and fraction of void volume presented in the work [1] were used to obtain *u* and *s* values. The calculated results (as an example shown in Figs. 1 and 2) were obtained on the basis of the two different calculation versions. The first version

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takes into account all the particle fluxes in accordance with Eq.(1) and the second one ignores the migration flux, i.e. it assumes that D_m=0.

It should be pointed out that the adequate modeling results in case of non-aerated gravity flow of particles differing in size, may be obtained often without taking into consideration the migration flux as it shown in Fig.1. However, analyzing the concentration profiles calculated in case of aerated flows (Figs.1,2) we observed the considerable migration effects in the vicinity of the free surface of the bed and the segregation increase in the central part of the bed. These phenomena are explained by the shear rate increase in all the bed depth and expanding the loose zone in the upper part of the bed .

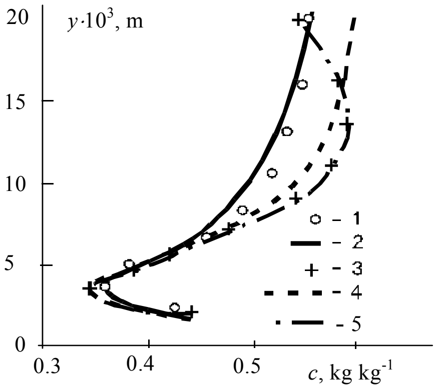

Fig.1. Concentration profiles in the gravity flow of silica gel granules (-3.75+3.5mm - 50%; -4.25+4.0mm -50%) for various aerating air velocity towards the bed bottom: 1 - w/w_m=0 (experimental [1]); 2 - w/w_m=0 calculated at D_m=0); 3 - w/w_m=-0.46 (experimental); 4 - w/w_m=-0.46 (calculated at D_m=0); 5 - w/w_m=-0.46 (calculated according to (1)).

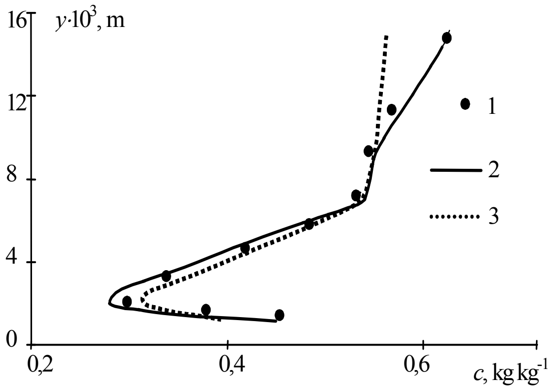

Fig. 2. Concentration profiles in the aerated gravity flow of superphosphate granules (-2.2+2.0mm -50%; -2.8+2.6mm -50%) for aerating air velocity w/w_m=0.405 towards the bed bottom: 1 – experimental [1]; 2 – calculated according to Eq.(1); 3 – calculated without migration flux (D_m=0).

Thereby it is important to note that the same value of segregation coefficient K is used for modeling in aerated and non-aerated flows. This fact can be considered as an indirect proof of the relatively high forecasting properties of the mathematical model.

Thus the investigation results allowed us to maintain that the quasidiffusional separation (migration) is one more basic segregation mechanism in aerated gravity flows of granular materials.

List of symbols

c - test component concentration ($kg \cdot kg^{-1}$); D_{dif} - quasi-diffusion coefficient ($m^2 \cdot s^{-1}$); D_m - migration coefficient ($m^2 \cdot s^{-1}$); K - segregation coefficient $(N\ s)^{-1}$; ΔM - segregation driving force ($N\ m$); s - mean distance between particles (m); u - particle velocity towards flow direction x ($m \cdot s^{-1}$); w - air velocity ($m \cdot s^{-1}$); w_m - minimum fluidization velocity ($m \cdot s^{-1}$); x, y - Cartesian coordinates; ρ_b - bulk mixture density ($kg \cdot m^{-3}$)

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Maxwell-Stefan based model for ion exchange in microporous materials

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Keywords: Ion Exchange, Titanosilicates, Maxwell-Stefan Equations, Nernst-Planck Equations

The use of microporous zeolites as ion exchangers has gaining considerably attention, mainly due to their interesting adsorption properties and ion exchange capability (Rocha and Anderson, 2000).

Beyond experimental measurements, modelling and simulation are unquestionably valuable tools to study and design a chemical plant. It is fundamental to be able to predict the dynamic behaviour of any unit, optimize their operating conditions, and scale up from laboratory to large scale. The objective of this work is to present a theoretical model comprising external and intraparticle mass transport resistances to describe and predict the concentration profiles of a batch ion exchange process in a microporous solid.

The kinetics of ion exchange is commonly described by models that are not able to evaluate multicomponent systems appropriately and do not take into account ionic interactions properly; they are proved to be valid only under dilute conditions (Graham and Dranoff, 1982; Pinto and Graham, 1987; Wesselingh et al., 1995). In the Maxwell-Stefan based model presented here, the exchanger solid pellets are considered to be a pseudo component in the ionic mixture, in a way similar to the Dusty Gas Model approach (Jackson, 1977), and both ion-ion and ion-solid interactions are considered and distinguished. In this treatment one diffusivity coefficient is defined for each pair of components, being dependent on their properties only. In addition, surface diffusion of adsorbed species is taken as the transport mechanism occurring in zeolite micropores, since ions never escape from the force field of the matrix co-ions, due to their small diameters.

In this work a batch ion exchange process is described using a model based on the generalized Maxwell-Stefan equations. Model capability to represent and predict experimental data is analyzed using data available in literature concerning batch experiments on mercury (II) removal from aqueous solution using ETS-4 microporous titanosilicate. Model results are compared with results obtained using the Nernst-Plank equations.

Results reveal both models provide good and similar representations as well as fine predictive capability. Therefore, under the particular conditions investigated here, both models can be successfully applied to describe intraparticle ionic transport.

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Competitive adsorption of heavy metals by two different types of soils

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Key words: Heavy metals, convection dispersion, retardation factor, soils.

Abstract

Soil is a key element in human survival and can be described by various definitions according to its main utility. Nowadays, it is accepted that soil is a very complex heterogeneous medium which consists of solid phases containing minerals and organic matter and soil solution, defined as the fluid phase where soil reactions, transport and adsorption occurs (Alloway, 1995).

Unfortunately, in the last decades, soils have been used as a deposit of residues. Therefore, the contamination and pollution of soils, sediments, surface and groundwater are of main concern.

There are several ions and compounds of organic or inorganic nature that can contaminate soil and water. The inorganic contaminants include heavy metals like cadmium, chromium, cooper, lead and zinc, which are focused in this study. These metals are commonly present in industrial wastes (electroplating, textile and dyes, etc.), in fertilizers or in sewage sludge, but they can also contaminate soils through atmospheric deposition or runoff water (Serrano et al., 2005).

Since adsorption is well recognized as the main process affecting the mobility of heavy metals through soils (Bradl, 2004), the aim of this work is the study the non competitive adsorption of these five metals, as well as the competitive adsorption in two different typical soils of the North of Portugal

The soil samples were collected in Póvoa de Varzim, from O-horizon and A-horizon (0 cm -30 cm), one from a culture land and another from a beach land.

To evaluate the adsorption equilibrium, batch tests were performed by adding to 2 g of air dried soil, 20 mL of 0.01 M $CaCl_2$ solution, containing different initial concentrations of Pb [$Pb(NO_3)_2$], Cd [$CdN_2O_6 \cdot 4H_2O$], Cu [$Cu(NO_3)_2 \cdot 3H_2O$], Zn [$Zn(NO_3)_2 \cdot 6H_2O$] and Cr [$K_2Cr_2O_7$], isolated (non-competitive system) and coupled (competitive system).To eliminate the mass effect, the multi-metal solutions were prepared in terms of molar concentrations (Echeverría et al., 1998). To avoid precipitation the pH of initial solution was adjusted to 2. Isotherm models like Freundlich and Langmuir equations were adjusted to experimental data.

Batch tests are useful for the understanding of the adsorption process. However, advection and dispersion may occur in dynamic systems justifying the need of flow experiments (Miretzky et al., 2006). For continuous experiments, soil samples were packed in a column (25 cm × 3.2 cm) and a single – or multi – element solution of 50 mg/L was pass upwards during seven days. Then, using the CXTFIT code, the convection-dispersion equation was adjusted to the breakthrough curves in order to determine the retardation factor (R) and to compare the behavior of the different ions and soils.

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A sequencing method applied to the solution of a linear model of an adsorption column

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Keywords: fixed bed adsorption column, numerical solution, sequencing method, finite difference method, breakthrough curve

Adsorption is an important step in industrial downstream processing in the pharmaceutical food industries (Dünnebier et al, 1998, Pais et al 1997 and Rodrigues et al 2005). However, the high costs of equipment, adsorbents and operations involved in this process motivate engineers to seek ways of developing more cost-effective separation systems. One way to reduce these costs is to stop the adsorption stage before the saturation of the adsorbent is completed, which requires a thorough understanding of adsorption characteristics.

Purification processes based on sorption technology use continuous flow columns. Starting at the inlet, the saturated solid sorbent zone gradually extends throughout the column, the sorbate eventually breaking through the column. This breakthrough is usually recorded in the form of a typical s-shaped curve whose shape and slope are the result of the equilibrium sorption isotherm relationship, the mass transfer to and through the sorbent in the column, and operational macroscopic fluid-flow parameters such as axial mixing, which affect the deviation from the ideal plug flow. Any optimized column system is based on the accurate prediction of breakthrough time under given specific operating conditions. When the sorbate concentration in the effluent stream reaches a predefined level, the column operation is terminated. At this point, the regeneration process may begin before the next operating cycle is activated.

Several common numerical methods have been used to approximate partial differential equations (PDEs) to ordinary differential equations (ODEs) for numerical simulation: finite difference methods (FDM), finite element methods (FEM) and orthogonal collocation methods (OCM) (Santacesaria et al 1982. Srivastava and Joseph, 1984 Gu et al, 1992, Altenhöner et al 1997). When treating a method without diffusion, most methods introduce some form of diffusion called numerical diffusion (Finlayson, 1992). However, many problems may arise in low dispersion systems, such as numerical diffusion or oscillation and even instability caused by inappropriate use of the Danckwerts boundary conditions (Renou et al, 2003). A numerical method called the Sequencing Method (SM) for solving convection-dispersion-reaction equations of homogeneous tubular reactor was recently proposed (Renou et al, 2003).The SM is based on the separation of the various phenomena. At each time step, convection, dispersion and reaction are applied successively on the reactor mesh. This method exhibits excellent shock capturing features for weakly dispersive reactors, and accurate simulations are also obtained for highly dispersive reactors. In the latter case, the only minor drawback is the fact that the SM has some problems to capture the inlet gradient correctly. However, this effect can be decreased by increasing the number of grid points.

Mathematical modeling - Due to their relevance and wide utilization in the downstream processes of fine chemicals, adsorption columns are the focus of attention of many research works. The effect of axial dispersion is neglected because in real chromatographic systems, which are dominated by convection rather than by diffusion, this simplification can be applied without loss of accuracy. The differential equations governing linear adsorption are given by two equations, the first a mass balance in the fluid phase and the second a mass balance in the stationary phase.The initial

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conditions give the concentration in the fluid phase and adsorbed phase at time zero, which, in this example, is no concentration at all.

Simulation results - The model proposed here consists of a system of partial differential equations, whose solution is required for the transient simulations. Two approximation methods were used: the sequencing method and the finite difference method.

A new algorithm was proposed for simulating adsorption columns, based on a time solution approach of the PDE model, and was validated by phenomenological arguments. This approach implies the successive solution of a specific sub-problem describing each phenomenon. The column simulations showed that the SM method yields adequate transient responses to step inputs. The FDM method also provided good results, but the concentration profile was steady and considerably less steep than the sequencing method solution. Because the SM method can account for sharp inputs, it can more accurately predict breakthrough time. Finally, a comparison was made of the computational efficiency of two numerical methods for solving the transient problem, i.e., the sequencing method and the finite difference method used in this work.

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Industrial air separation using pressure swing adsorption: A fluidization study

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Keywords: Pressure Swing Adsorption, minimum fluidization velocity, adsorbents

Pressure Swing Adsorption (PSA) is nowadays a very important technology, in different industrial contexts. Over the last decade, various companies start producing PSA units for different applications, resulting on a very competitive market. As a consequence, the PSA technology swiftly evolved over the past years, since it beginning in 1932. Also, the development of new adsorbents, exhibiting more capacity and selectivity, opened new trends to the development of this technology. PSA technology is currently used for air-drying, oxygen production, nitrogen production, hydrogen purification and hydrocarbon recovery and for many other applications.

In a PSA unit, gas flows through the adsorbent beds at a high surface velocity, however limited by the bed fluidization. Though very attractive in order to maximize the productivity of a PSA unit, fast cycles and upward high flowrates result in fluidization, which leads to abrasion, dusting and, often, destruction of the adsorbent beds (White and Barkley, 1989). In the case of *SYSADVANCE PSA GENERATORS*, the pressurization step is performed upward and high flow velocities of the inlet gas are observed, making possible the adsorbent fluidization. Similarly, during the top-to-top equalization step, the sudden decrease of pressure is responsible for high flowrates and, subsequently, fluidization is likely to occur. The knowledge of the minimum fluidization velocity allows to better design a PSA unit and to operate it most efficiently, preventing the deterioration of the adsorbent. It would therefore be useful to predict this velocity instead of having to measure. However, due to the very own morphology of each adsorbent and its adsorption capacity it is difficult to predict accurately the minimum fluidization velocity for a given bed (Coltters and Rivas, 2004).

A set of experiments was performed in a small pilot PSA unit for obtaining the minimum fluidizing velocity at different pressures and for various adsorbents. These experiments were performed for both critical PSA steps: pressurization and equalization. Ergun's and Coltters-Rivas models were then employed for obtaining the fluidization minimum velocity and results critically compared with the previous experimental results and with results obtained in industrial PSA units. Four adsorbents were studied: a carbon molecular sieve (CMS) for nitrogen production, an activated alumina for air drying, and two zeolites for oxygen production.

There has been observed that Ergun's model correctly fits the experimental results obtained in the small pilot PSA unit. These equations will be fed to a complete PSA model, which simulates the axial velocity. Combining the fluid velocity, minimum fluidization velocity and pressure over the bed slice under study, it will be possible to determine if there would be or not fluidization problems.

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Study of the adsorption process of methanol and water over a SAPO-18 catalyst by means of pulse techniques

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Keywords: adsorption, methanol, water, SAPO-18

This paper deals with the effect of the operating conditions on the adsorption process of methanol and water over a SAPO-18 catalyst. Water vapour and methanol compete with each other for the acid sites of the catalyst. Aguayo et al. (2005) have reported the low hydrothermal stability of catalysts such as γ-Al_2O_3 (an acidic function commonly used in the synthesis of dimethyl ether) in comparison with NaHZSM-5 zeolite, more suitable in mediums with high concentration of water. Nevertheless, water in the reaction medium may attenuate deactivation by coke deposition (Morán, 2002).

The catalyst used in this study is a SAPO-18 silicoaluminophosphate, prepared in the laboratory following the method proposed by Chen et al. (1994). Runs have been performed by using pulse techniques, in a mass spectrometer (Omnistar from Pfeiffer) coupled to a catalyst characterization equipment (Autochem II 2920). Mass transfer coefficients, diffusivities and kinetic constants of the adsorption process have been calculated by using the data from the mass spectrometer. The mass balance for the catalyst is:

$$\frac{\partial x}{\partial t} = \frac{k}{(1-\varepsilon)\rho_{solid}}\left[(K_{eq}y) - x\right] \tag{1}$$

where *x* is the adsorbate (water or methanol) concentration in the solid phase, *y* is the concentration of the adsorbate in the gas phase, ε the porosity, *k* the kinetic constant and K_{eq} the equilibrium constant of the adsorption process. On the other hand, the mass balance for the gas phase is:

$$\frac{\partial y}{\partial t} = -\frac{Q}{S\varepsilon}\frac{\partial y}{\partial l} - \frac{RT}{\varepsilon}k\left[(K_{eq}y) - x\right] \tag{2}$$

where *Q* is the gas flow and *S* the cross section of the catalyst bed. The system of partial differential equations has been solved by means of a program written in MATLAB, by using the Langmuir model to describe the adsorption equilibrium:

$$\frac{1}{K_{eq}} = \frac{1}{k_L x_m} + \frac{y}{x_m} \tag{3}$$

where k_L is the Langmuir equilibrium constant (ratio between the adsorption and desorption rates) and x_m the maximum amount of adsorbate in the solid phase.

First of all, the adsorption process of water vapour over SAPO-18 has been studied, using four different temperatures: 200, 250, 275 and 300 ºC (Figure 1 shows, as an example, the TCD signal obtained at 200 ºC). The data of the TCD signal show that, as temperature increases, the level of physical adsorption increases. Moreover, an increase in the partial pressure of water or in the adsorption temperature leads to a decrease in the equilibrium constant of adsorption. The experimental data have been fitted to the Langmuir isotherm. The following values of the parameters have been obtained: x_m= 1.66 mol (kg of catalyst)$^{-1}$, k_L= 17 atm^{-1}.

The adsorption process of methanol over SAPO-18 has also been studied. It is concluded that the adsorption is fundamentally physical at temperatures above 250 ºC. The equilibrium constant decreases as the partial pressure of methanol increases. The

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Langmuir isotherm is also suitable to describe the equilibrium of the methanol-SAPO system: x_m=0.12 mol (kg of catalyst)$^{-1}$; k_L=400 atm^{-1}.

Figure 2 shows the effect of temperature on the equilibrium for the methanol-SAPO system. The adsorption equilibrium is severely affected by temperature. Furthermore, the value of the equlibrium constant is higher at low temperature and for the first pulses, due to the higher level of chemical adsorption.

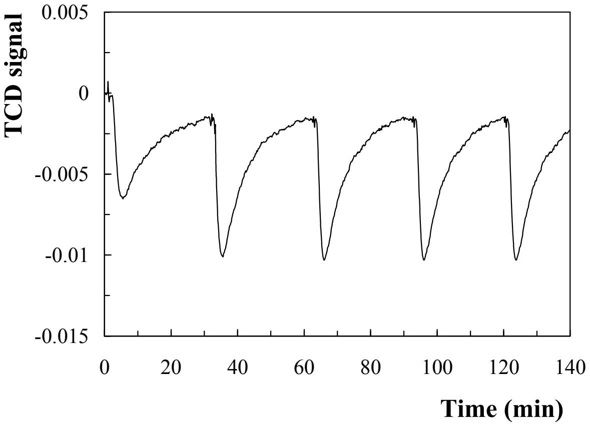

Figure 1. TCD signal of the adsorption of water over SAPO-18 at 200 °C.

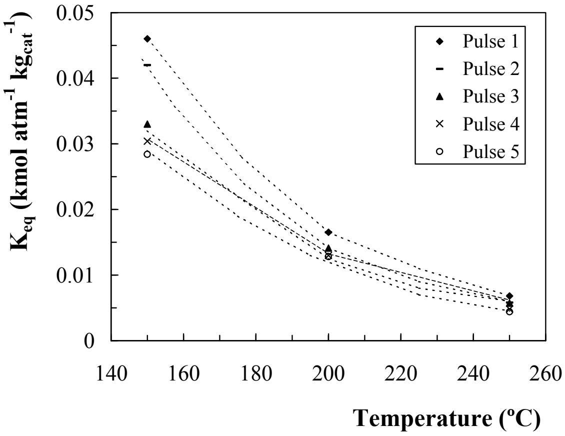

Figure 2. Influence of the adsorption temperature on the equilibrium for the methanol-SAPO system.

Table 1 shows the values of the kinetic and equilibrium constants of adsorption for both systems. The value of the equilibrium constant is similar in both cases. Nevertheless, the adsorption process of water vapour is much faster than that of methanol. Consequently, the low hydrothermal stability of SAPO-18 catalyst must be considered in reactions in which water concentration is high, such as the dehydration of methanol to dimethyl ether.

Table 1. Values of the kinetic and equilibrium constants for the adsorption of water and methanol over SAPO-18.

System	Kinetic constant (min^{-1} m^{-3} kg$_{cat}^{-1}$)	Equilibrium constant (mol atm^{-1} kg$_{cat}^{-1}$)
water-SAPO	$>$ 500	5-40
methanol-SAPO	50-75	5-45

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Optimization of mobile phase composition for preparative separation of profens enantiomers by chiral liquid chromatography

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Keywords: Mobile phase composition, Preparative liquid chromatography, Chiral separation, Non-Steroidal Anti-Inflammatory Drugs, Profens.

Ketoprofen (R,S)-2-(3-benzoylphenylpropionic acid) and Flurbiprofen (R,S)-2-(2-fluoro-4-biphenylpropionic acid) are both examples of profens, the actual most relevant subclass of the Non-Steroidal Anti-Inflammatory Drugs (NSAIDs). These drugs are frequently prescribed worldwide in relieve of pain and in the treatment of several forms of inflammation and in the treatment of main articular diseases such as rheumatoid arthritis, osteoarthritis, ankylosing spondylitis (Burian and Geisslinger, 2005).

In recent literature we can found several studies that indicate different pharmacological proprieties for both R and S enantiomers of profens. Using this point of view, chiral resolution of some profens enantiomers can contribute to the development of new drugs with distinct therapeutic applications and/or safer pharmacological actions (Panico *et al.*, 2006).

Preparative chiral liquid chromatography optimization using Simulated Moving Bed (SMB) technology is a complex task that requires a careful selection of mobile phase composition. In this selection, high resolution (or high selectivity) is not the only aspect since other parameters, such as high solubility or low retention times, are crucial aspects that must also be considered. (Ribeiro *et al.*, 2008).

In this work it will be presented and discussed the experimental and simulation results obtained for the preparative separation of ketoprofen and flurbiprofen enantiomers by chiral liquid chromatography. Experimental results include solubility studies, elution and frontal chromatographic experiments and the measurement of competitive adsorption isotherms, using different mobile compositions (see Figure 1, as examples). Modelling and simulation tools are used to predict the behaviour and the performance of fixed bed and simulated moving bed processes. These prediction tools are used to select the proper mobile phase composition for the enantiomers separation in a preparative and production-scale point of view.

For the separation of ketoprofen enantiomers, best situation is achieved using pure ethanol as mobile phase. This solvent allows high enantiomers solubility with higher selectivity and lower retention times, even at preparative conditions (i.e., high concentration values). Elution and frontal chromatographic experiments confirm a good agreement between model and experimental data over a large concentration range. Simulation predictions reinforce the previous conclusion that pure ethanol is the better choice for mobile composition since it allows bigger SMB separation regions, higher productivity and lower solvent consumption when compared with other studied mobile phase compositions.

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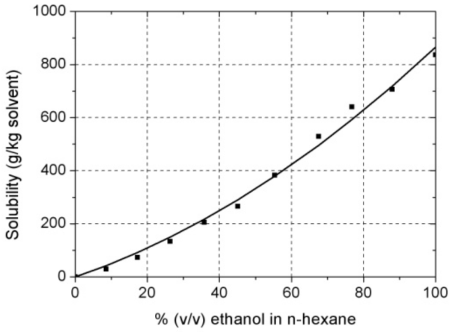
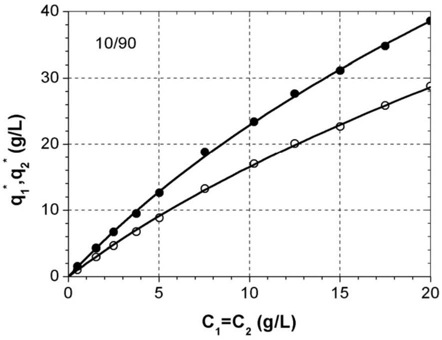
The experimental results obtained for flurbiprofen enantiomers lead to different conclusions. Adsorption isotherm measurements indicate that higher selectivity is obtained on mobile phase compositions with low alcoholic content (ethanol and methanol). However, low ethanol or methanol content means higher retention times and lower enantiomers solubility values. A 10%ethanol/90%n-hexane composition is the better choice since it means a good compromise between selectivity, retention time and solubility. Frontal chromatographic experiments and SMB predictions also clearly indicates that a 10%ethanol/90%n-hexane is the better choice for preparative separation of flurbiprofen enantiomers.

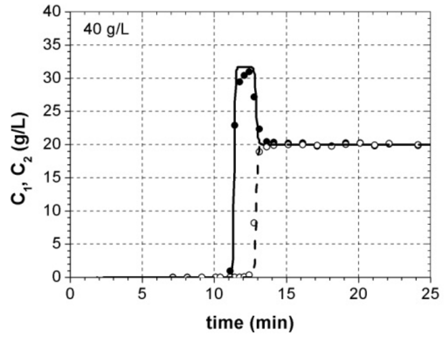
These results show that individual studies must be carried out for each enantioseparation system, since different NSAIDs drugs can show different behaviours.



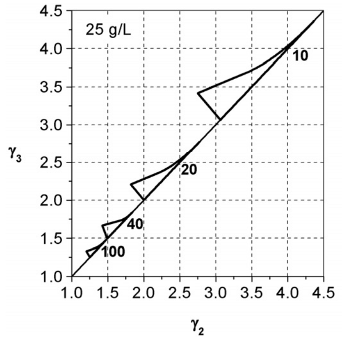
Solubility measurements: effect of the alcoholic content.



Measurement of adsorption isotherms: comparison between experimental results and model predictions.



Fixed bed frontal chromatography: comparison between experimental results and model predictions.



Prediction of the SMB separation regions: influence of the mobile phase composition (alcoholic content).

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Uncertainty assessment on chromatographic separation of lapachol

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Keywords: Uncertainty assessment, simulation of chromatographic units, lapachol separation

The development of efficient new methodologies to design large-scale manufacturing operations for the production of active pharmacological ingredient (API) is still a continuous challenge in the pharmaceutical industry. Computer optimisation and other process system engineering tools have been intensively used for this purpose. *A priori* knowledge of the most sensitive input parameters in the selected mathematical model is a measure of its prediction capacity, and it is essential when PSE tools are used (Johnson and Bogle, 2006).

The main objective of this work is to present an analysis of the parameter uncertainties impacts in one particular chromatographic process: lapachol separation from crude ethanolic extract. Based on this, a sequential design of experiments is proposed aiming the minimization of uncertainties of the most sensitive parameters of the developed mathematical model.

Lapachol is a naphthoquinone derived from the inner bark of the *Tabebuia Avellanedae* or *Tabebuia Impetiginosa* tree grown in South America. This substance is one of the major constituent presented in acid fraction of ethanolic extract of the *T. Avellanedae*. Medical practioners in South America have used lapachol as a primary treatment for cancer for many decades. Recently, lapachol and its derivates have already been tested in clinical studies. These substances are active against the intramuscular Walker carcinosarcoma and show antiviral, antischistosomal (Barreto Jr., 2005), antimalarial (Andrade-Neto *et al.*, 2004) and anti-inflammatory activities (Steinert *et al.*, 1995).

The lapachol chromatographic separation process treated in this work is an anion-exchange column, and the crude ethanolic extract of the *Tabebuia avellanedae* is composed by acid and neutral naphthoquinones. In the adsorption step of the process, crude extract feedstock is injected on a packed column with Lewatit MP 64 resin (Bayer). After this step, the column is sequentially washed with ethanol, eluted with formic acid, followed by the regeneration step.

The behaviour of the unit on each step was predicted through the numerical solution of the ion-exchange column microscopic mass balances. An algebraic-differential equations systems are generated when the mass balances are space discretized. Mass and thermodynamic parameters were estimated beforehand from independent experiments, the first ones through tracer experiments in the column and the adsorption isotherm parameters through finite bath experiments (Barreto Jr, 2005).

Sensitivity parameter analysis was conducted simulating the column response when positive and negatives deviations from the nominal parameter estimates are applied. Following this, a sensitivity analysis is used to estimate the ranking priority of the key parameters contributing to the uncertainty in the stochastic process output criteria. After all the model parameters were ranked, a sequential design of experiments was proposed seeking to reduce key parameters uncertainties. The volume of uncertainty for each estimated parameter (Atkinson and Bogacka, 2002) was calculated using the estimated posterior parameter covariance matrix (Bard, 1974).

Saturation capacity (q_m) and association constant (b) were identified as the key parameters of the lapachol separation process. Based on this fact, a sequential of bath finite experiments were designed in new operating conditions aiming the minimization of isotherm parameters uncertainties, according Atkinson and Bogacka (2002). This procedure generated seven experiments sequentially until variances of both parameters reached a plateau. After reestimate the values of the parameters, the impact of their uncertainties was evaluated from several simulations of the column considering random perturbation in both parameters confidence intervals. A statistical test, based on the t-student, between calculated and experimental outlet column concentration confidence intervals showed that after the fourth new experiment both confidence interval are equivalent and their variances are of the same order of magnitude.

The main conclusion of this work is that a significant improvement of the prediction capacity of the chromatographic column mathematical model capacity was verified with very few new experiments conducted in a simple apparatus, the finite bath adsorption system operating in a closed loop.

The main results of this contribution will be applied to design operating conditions of lapachol chromatographic separation, in batch and in semicontinuous mode.

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Gas filtration at high pressure

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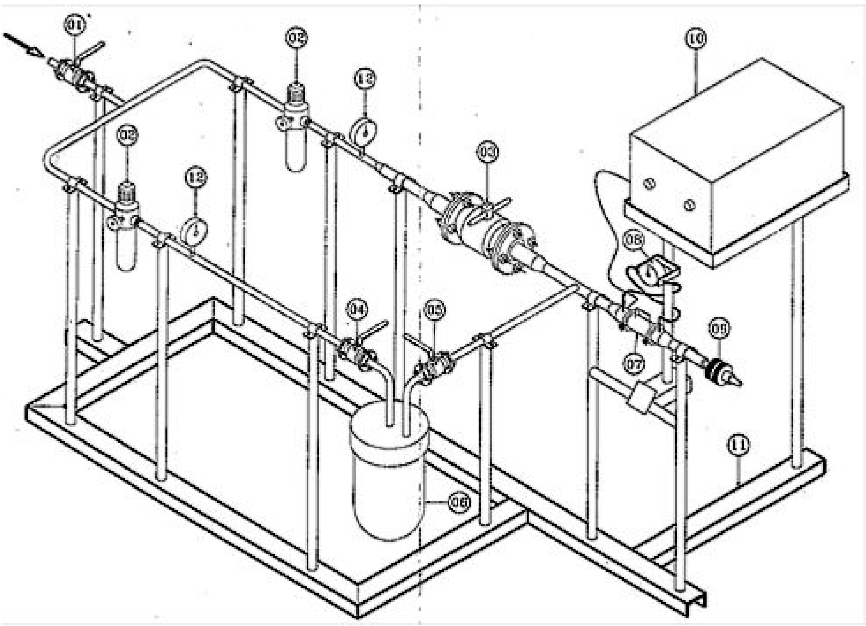
Keywords: Filtration, High pressure, air filtration, gas cleaning, filter media

Filtration can be defined as the process of separating phases, such that the desired phase is attained free of the unwanted phase (Matteson and Orr, 1987). Thus, for example, particles that are dispersed in a fluid can be separated using porous media. Filtration is used in various applications, such as: respiratory protection, cleaning the air of effluents from boilers, processing nuclear materials, cleaning rooms, recovery of particulate matter and filtration of natural gas.

Natural gas has been widely used as an energy source in the sectors of commerce and services. It is a much less polluting source than the fuel oil or other fossil fuel. But during their transport, occurs accumulate of waste inside the pipeline, which are usually removed using a high-pressure filtration.

In literature, rare studies have been conducted using high pressure, which is why this work has as objective the study of the filtration of air at high pressure for removal of particles changing the filter media and the particulate matter used.

The experimental unit, as illustrated in Figure 1, consists, basically of, a powder feeder, a high-pressure compressor, three filters to clean the air, a air drier, a filter absolute, a particle counter that operates in the range of 0,3 to 10 µm, rotary and safety valves and manometers.



1– Ball valve class 150
2 – High Pressure Regulator 3 – Ball valve class 150WCB
4 – Ball valve class 150
5 – Ball valve class 150
6 – Powder feeder
7 – Filter media
8 – Differential manometer
9 – Absolut Filter
10 –Particle Counter
11 – Support
12 – Safety manometer

Figure 1 – Experimental Unit

During the filtration experiment, previously-dried and filtered air was used, since it is cheaper and safer than natural gas for the performance of experiments in the laboratory, and powdery material having a particle size range smaller than 35 µm. Various filter media were also used. The scale of pressure used ranged from 5 to 9 bar.

Images of the clean filter media were obtained, before and after resistance tests were carried out, in order to verify the behavior of the fibers when submitted to higher than atmospheric pressures. The analysis of these images was performed in an analyzer of images called “ImagePro Plus”, in order to visually verify the arrangement and the possible wear and tear of the fibers.

Several filtrations were carried out with the different filter media in order to observe their behavior when submitted to similar filtration conditions. Different powdery materials were used and new filtrations were carried out with several filter media. The efficiency of the filters was determined through the counting of the particles that reach the surface of the filter media, and the particles that cross it, according to the equation (1).

$$Efficiency\ (x) = \left(1 - \frac{1}{\beta_x}\right) *100 \qquad (1)$$

where $\beta_x = \frac{\text{particles that reach filter media}}{\text{particles that cross filter media}}$

At the end of the study, more detailed information was obtained concerning the efficiency of various media filter found on the market when subjected to high pressure and different powdery materials. With these results, it was possible to select the best media filter to remove different particulate matter present in the gas, using high air pressure.

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Pervaporation separation characteristics for the quaternary mixture water + ethanol + ethyl lactate + lactic acid

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Keywords: Pervaporation, PERVAP®, esterification, lactic acid, ethyl lactate

Introduction

Pervaporation separation process may be used as part of a hybrid system in combination with a distillation column or with a chemical reactor. In combination with an esterification reactor, pervaporation is used to continuously remove one of the reaction products, in most cases water, to shift the reaction to higher yields.

For a correct design of a reaction-pervaporation hybrid process, reliable information on pervaporation characteristics for the mixtures involved in the process is necessary. In a previous study (Delgado et al., 2007), the pervaporation performance of PERVAP® 2201 membrane for different binary mixtures involved in the lactic acid esterification with ethanol were studied: water/ethanol, water/ethyl lactate and water/lactic acid. PERVAP® 2201 membrane was water selective for all the binary mixtures. It was found that for water/ethanol and water/ethyl lactate mixtures the permeation flux was of the same order. However, higher permeation fluxes were obtained for water/lactic acid mixtures probably due to a higher swelling of the membrane in contact with lactic acid. Rautenbach et al. (1991) explained that experiments with the real multicomponent mixture must be done since a representative system can only be found by comparison with the real system.

This work reports experimental results for the pervaporative separation through the commercial hydrophilic membrane PERVAP® 2201 of the quaternary mixture involve in the ethyl lactate synthesis: water/ethanol/ethyl lactate/lactic acid. The main aim of this work was to study the effect of feed temperature and feed composition in the permeation characteristics for the quaternary mixture. The results obtained for the quaternary mixtures can be compared with those obtained for different binary feed mixtures. These results will be useful for the design of a combined esterification-pervaporation process to improve ethyl lactate yield.

Results

The experiments were carried out with a laboratory pervaporation set-up with a test cell unit (170 cm^2 effective membrane area, Sulzer Chemtech) following the procedure described elsewhere (Delgado et al., 2007). Permeate and feed concentrations were measured off-line. Water, ethyl lactate and ethanol were analyzed using a Hewlett Packard (6890) gas chromatograph (GC). Lactic acid and its oligomers were analyzed by HPLC using a Hewlett Packard 1100 series liquid chromatograph.

In this work, two series of experiments were carried out: (1) pervaporation experiments of quaternary feed mixtures with variable feed water concentration (2) pervaporation experiments of quaternary feed mixtures with a fixed water concentration in the feed but variable feed lactic acid concentration. Two different feed temperatures were studied for each set of experiments, 54 ºC and 75ºC.

Effect of feed water composition. Feed water concentration was varied in the range between 12 and 63 wt%. Figure 1 shows the total permeation flux for this first set of experiments as a function of feed water concentration for the quaternary mixture at 54ºC and 75ºC. As the feed water concentration increases total permeation flux also increases for the two temperatures considered in this work. This is probably due to a

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better swelling of the membrane in contact with water which results in an easier passage of the molecules through the membrane, increasing the total permeation flux (Huang and Rhim, 1991).

Effect of feed lactic acid composition. Lactic acid concentration in the feed was varied in the range between 9 and 30 wt%. Feed water concentration was keeping in the range 13-17 wt%. Figure 2 shows the total permeation flux as a function of water and lactic acid feed concentration at 57 ºC and 75 ºC. As lactic acid concentration increases keeping constant feed water concentration, a more or less steady permeation flux was obtained. It can be concluded that total permeation flux is not much affected by feed lactic acid concentration, being nearly constant. In this figure two additional pervaporation experiments at 75 ºC are also plotted. In these experiments feed lactic acid concentration was keeping constant (around 27 wt%) while feed water concentration was increased (22 and 29 wt%). Total permeation fluxes sharply increases with feed water concentration. From this figure can be concluded that total permeation flux must be dependent on water driving force.

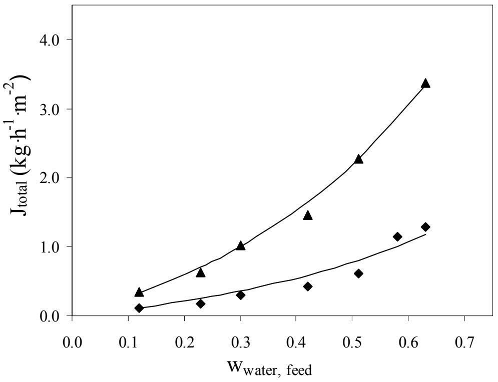

Figure 1. Influence of feed water concentration on total permeation flux for the quaternary feed mixture at different operating temperatures (◆ 54 ºC, ▲ 75 ºC).

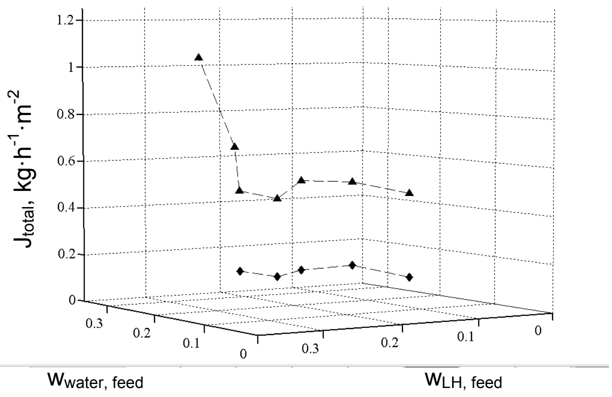

Figure 2. Influence of feed water and lactic acid feed concentration on total permeation flux for the quaternary feed mixture at different operating temperatures (◆ 54 ºC, ▲ 75 ºC).

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Kinetics of carbon dioxide absorption with chemical reaction in piperidine aqueous solutions

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Keywords: absorption, piperidine, kinetic

Solutions of several alkanolamines are an industrially important class of compounds used in the natural gas, petroleum chemical plants, and ammonia industries for the removal of CO_2 and H_2S from gas streams. A wide variety of alkanolamines such as monoethanolamine (MEA), diethanolamine (DEA), di-isopropanolamine (DIPA), N-methyldiethanolamine (MDEA) have been used industrially for a number of years. A recent advancement in gas treating technology is the application of sterically hindered amines which offer absorption capacity, absorption rate, selectivity, and degradation resistance advantages over conventional amines for CO_2 removal from gases.

Other possibility developed in the last years has been the use of blended amines to react with acid gases with specific advantages over the use of single amines related with the enhancement properties and stripping characteristic.

Present work develops the use of a different amine compound, piperidine (Hexahydropyridine) that reacts with carbon dioxide with an important reaction rate higher than other common amines employed in industry.

In this study the kinetics of the reaction between carbon dioxide and piperidine in aqueous media has been studied at different temperatures from 15 ºC to 55 ºC and compared with the experimental values obtained for other amines commonly employed in carbon dioxide removal processes.

The experiments were conducted in a stirred cell having a planar interfacial area working in batches as regards both phases. The gas to be absorbed, pure carbon dioxide, was passed through humidifiers to prepare the gas phase. A soap flow-meter was used to determine the absorption rate of carbon dioxide by the absorption accompanying the chemical reaction produced by the piperidine present in the liquid phase. The absorption rate was measured by analysing the movement (produced by the consumption of carbon dioxide) of the soap film along the calibrated glass tube.

An example of the experimental results obtained in present work is shown in figure 1 about the influence of piperidine concentration in the liquid phase upon the carbon dioxide absorption rate at 25 ºC. The experimental results show that an increase in amine concentration in the aqueous solution increases the carbon dioxide absorption rate. These experimental values allow determine the value of the reaction order and the kinetic constant for each operation conditions.

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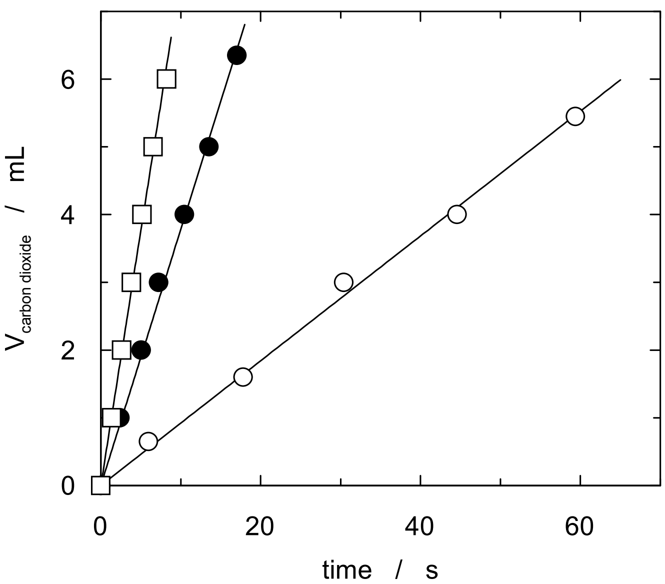

Figure 1. Influence of amine concentration on carbon dioxide absorption rate in gas-liquid kinetic studies. (○) [piperidine] = 0 M; (●) [piperidine] = 0.05 M; (□) [piperidine] = 0.1 M. T=25 ºC.

Absorption of carbon dioxide by MDEA aqueous solutions in a bubble column reactor

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Keywords: absorption, MDEA, bubble column, interfacial area.

Present work analyzes the behaviour and mass transfer of N-methyldiethanolamine (MDEA) in the removal process of carbon dioxide using a bubble column reactor (BCR) as gas-liquid contactor. The use of this type of equipment requires the interfacial area determination for the following mass transfer coefficient calculation based on absorption kinetics. In this work, a photographic method based on the bubble diameter determination, has been employed. The effect of contactor, operation conditions, liquid phase nature and chemical reaction upon the mass transfer coefficient and interfacial areas has been analysed.

Solutions of several alkanolamines are an industrially important class of compounds used in the natural gas, petroleum chemical plants, and ammonia industries for the removal of CO_2 and H_2S from gas streams. A wide variety of alkanolamines such as monoethanolamine (MEA), diethanolamine (DEA), di-isopropanolamine (DIPA), N-methyldiethanolamine (MDEA) have been used industrially for a number of years. Certain authors revealed that the addition of tertiary amines with primary or secondary amines present certain advantage to increase CO_2 absorption capacity (Versteeget al, 1996). In contrast to primary and secondary amines, the tertiary amines did not react directly with CO_2 to form stable carbamates. Specifically, MDEA (N-methyldiethanolamine) seems a very good amine to get the previously commented characteristics.

Absorption experiments were performed in a bubble column reactor, 1.03 m height, and has a square cross-section (side length 6 cm), operating in batches with respect to the liquid phase. The gas flow-rate fed to contactor was controlled with a mass flow controller (5850 Brooks Instruments) and measured with a soap flow-meter. The gas flow-rates employed have been included into 15-30 $L \cdot h^{-1}$. The outlet gas flow-rate has been measured using a soap gas flow-meter and gas absorption rate was calculated as the difference between inflow and outflow rates.

The bubble diameter was measured using a photographic method based on taking images of the bubbles along the height of the column, from bottom to top. A Sony (DCR-TRV9E) video camera was used to obtain the images.

The studies developed in present work allow to conclude that the bubble diameter and interfacial area in bubble contactor is important influenced by the gas flow-rate and amine concentration. Both parameters produce also an important effect on mass transfer coefficient and enhancement factor.

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Simulation of membrane separations using a modified Maxwell-Stefan model

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Keywords: Modeling, Ultra-filtration, Transport phenomena, Apparent rejection, Adaptive methods

The modeling of mass transfer phenomena in solute separations through inert membranes is essential for the efficient design and optimisation of these operations, namely ultra filtration processes. In this work, a model is applied to simulate the ultra-filtration of aqueous dextran solutions. This model considers both the concentration polarization and the transport through the membrane and incorporates a binary friction model based on the Maxwell-Stefan-Lightfoot equation (Kerkhof, 1996). Hence, for the polarization layer, we have:

$$\frac{\partial c}{\partial t} = -\frac{\partial N}{\partial z} \qquad (1)$$

with the flux, *N*, defined as,

$$N = -(D + D_t)\frac{\partial c}{\partial z} + u_v c \qquad (2)$$

where, $D = D_{12}\Gamma_c$, being *D*, the Fickian diffusion coefficient; D_t, the turbulent diffusivity; D_{12}, the Maxwell-Stefan diffusion coefficient; and Γ_c, the thermodynamic factor. The turbulent diffusivity is defined taking account the cinematic viscosity, computed by the Vieth correlation (Brito et al., 2004). For the membrane, on the other hand, the molar concentration temporal gradient is given by:

$$\varepsilon \frac{\partial c'}{\partial t} = -\frac{1}{\tau}\frac{\partial N_m}{\partial z} \qquad (3)$$

in which, τ is the tortuosity factor, and ε is the porosity. The intramembrane flux, N_m, is calculated by:

$$N_m = -\frac{\Gamma_c}{G}\frac{\partial c'}{\partial z} + u_v \frac{F}{G} c' \qquad (4)$$

where, *F* and *G* are the convective and friction factors respectively, defined as in Kerkhof (1996). The model is normalized and completed by the definition of: boundary conditions, fixed bulk concentration at the polarization layer extreme, and equilibrium and flux equalization conditions at the membrane/polarization and membrane/permeate interfaces; and initial conditions, zero concentration profile start-up.

The numerical solution of the partial differential-algebraic equation system, defined over the space-time coordinate system of variables, is accomplished with an adaptive resolution algorithm, based on the Adaptive Method of Lines, and presented by Brito and Portugal (1998). This method is used to solve simultaneously the two modules of the model (polarization layer and membrane) over a one-dimensional discretized space direction, in the time direction until a steady state solute concentration profile is reached. The procedure briefly described above provided promising results in the simulation of PEG-3400 aqueous solutions ultra-filtration (Brito et al., 2004, Ferreira et

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al., 2003). Now, the purpose is to test the ultra-filtration simulation performance for more massive solutes. Therefore, using the membrane conditions depicted in Brito et al. (2004), the dextran (*M*=98000) aqueous solutions ultra filtration is tested. Evolutionary profiles like the ones presented in Figures 1 and 2, for normalized concentration (*y* and *y´*) and space (*z**) variables, are obtained

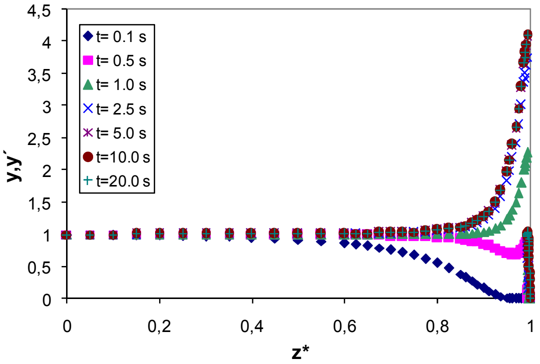

Figure 1. Normalized concentration profiles (polarization and membrane) in conditions: u_t= 1.57 m/s, u_v= 1.0×10^{-5} m/s and K_{eq}= 0.25.

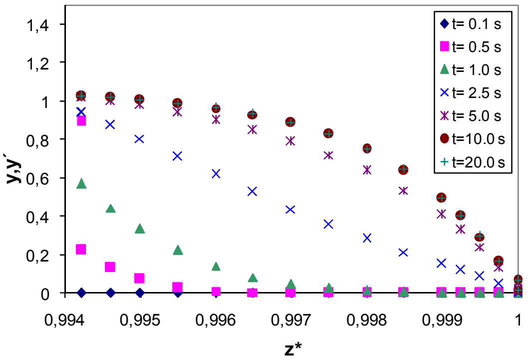

Figure 2. Normalized concentration profiles (membrane) in conditions: u_t= 1.57 m/s, u_v= 1.0×10^{-5} m/s and K_{eq}= 0.25.

It is verified the formation of significant solute accumulation at the membrane/polarization interface (in the presented case, the solute concentration is roughly four times higher than bulk concentration - c_b) and diminished permeate concentrations leading to high levels of apparent rejection of solute, which is defined by: $R_{ap} = 1 - {}^{c_p}\!/_{c_b}$. In these conditions, rejection values directly correlate with experimental results. The repeated simulation procedure for a range of filtrate average fluxes (u_v) allows the construction of simulated R_{ap} and pressure drop versus u_v profiles, that are readily comparable to available experimental data, obtained for equivalent operational and equipment conditions. These comparison leads to the model validation and consequent parameter tuning, namely of the interface equilibrium constant (K_{eq}). Furthermore, it allows the test of model sensitivity relating other operating parameters, like bulk concentration and circulating flux (u_t).

Finally, the study will be extended to ultra-filtration simulation of polidispersed solutes and multisolute transport.

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Influence of pyrolysis parameters on the performance of CMSM

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Keywords: CMSM, Pyrolysis, Gas Separation, Carbon Membranes

Carbon molecular sieve membranes (CMSM), pioneered by Koresh and Soffer in the 80's, are a very recent research topic in the area of gas separation. These inorganic membranes present great advantages over polymeric membranes, since they have comparatively high permeabilities and selectivities, together with high thermal and chemical stability (Saufi and Ismail, 2004; Ismail and David, 2001). The main applications for this type of membranes are, among others, air separation, landfill gas recovery, olefin/paraffin separation, hydrogen recovery and natural gas processing.

Carbon membranes are produced by pyrolysis of a polymeric precursor under an inert atmosphere (Saufi and Ismail, 2004). The preparation of CMSM should always be directed towards the tailoring of the final carbonaceous micropore network. The influence of the pyrolysis parameters and complementary treatments used for producing the final carbon membranes should be studied in order to better suit a certain application. Some previous studies indicate general requests to be fulfilled. The precursors should have high yield of carbon and should be thermosetting. The temperature programs followed in the pyrolysis, the cooling steps and the gas atmosphere employed, are all important aspects that have to be studied and optimized according to the precursor selected, having in mind the final application. Nevertheless, CMSM have two major disadvantages that still have to be overtaken: brittleness and aging effects (Ismail and David, 2001; Menendez and Fuertes, 2001). Nowadays, it is known that oxygen chemisorption on the surface of CMSM reduces the performance of these membranes (Menendez and Fuertes, 2001). This phenomenon may reduce the size of the pores affecting the separation of the gases. In cases that the feed mixture is humidified, the oxygen content on the carbon matrix may lead to the adsorption of water vapor which may block the passage of other species (Lagorsse et al, 2005).

Carbon hollow fiber membranes have been prepared by pyrolysis of a co-polyimide P84/S-PEEK blend. Permeation experiments and scanning electron microscopy (SEM) have been performed on the resulting carbon membranes (see figure 1). This study aimed at understanding the influence of pyrolysis parameters, end temperature, quenching effect and soaking time on the membrane properties.

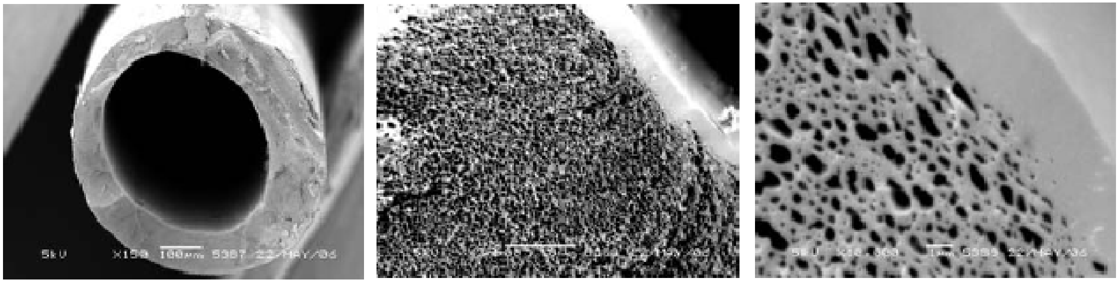

Figure 1. SEM pictures from carbon hollow fiber membranes.

The precursor was submitted to a proximate analysis performed in a thermogravimetric balance, revealing a yield of fixed carbon approaching 40 %. The pyrolysis occurred under a nitrogen atmosphere, with nitrogen flowing at 50 $\mathrm{ml_N \cdot min^{-1}}$. The temperature

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program employed is based on a temperature program described by Barsema (2004). The main differences from this protocol relate to the end temperature, quenching and soaking time. Permeability values towards CO_2 of 180, 467 and 104 Barrer were obtained, respectively, for 700, 750 and 800 ºC end temperatures. The ideal selectivities CO_2/N_2 were, respectively, 18.0, 14.2 and 10.4. All the membranes were tested for a possible decrease in performance caused by the exposure to CO_2, using N_2 as reference.

The pyrolysis parameters studied influence the characteristics of the resulting carbon membranes. The highest permeabilities and ideal selectivities were obtained for the membranes submitted to an end temperature of 750 ºC, previous to quenching. It was also concluded that the existence of a final soaking time at the end temperature, just before quenching, did not improve the performance of the carbon membranes. The permeabilities in this case were lower than the ones obtained without soaking time.

Furthermore, it was observed that the membranes revealed higher permeabilities when quenched at the end temperature than the ones naturally cooled down to room temperature. No decrease in the performance of the membrane due to CO2 exposure was recorded.

Acknowledgments

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Low-temperature water-gas shift reaction in a Pd-Ag "finger-like" configuration membrane reactor

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Keywords: Pd-Ag Membrane Reactor, Water-gas shift reaction, Hydrogen, Membrane Reactor Configuration, Permeation.

Since the economic boom of the 1990s and the inherently energy increase demand, associated with the growing concerns about environmental issues, the idea of a hydrogen economy has been revived and deeply developed [Blanchette, 2008]. Hydrogen is the most abundant element in the universe, however it does not exist naturally in large quantities or high concentrations in the Earth. Therefore it should be derived from all current forms of power generation including fossil fuels like natural gas, coal and petrol, nuclear energy and renewable energies such as biomass, solar and water [U.S. Department of Energy, 2004].

The water-gas shift (WGS) reaction is an important catalytic step in the hydrogen process production where carbon monoxide together with steam is converted into carbon dioxide and hydrogen. However this reaction is limited by equilibrium constraints; the WGS is an exothermic equilibrium-limited reaction, with conversion being therefore more thermodynamically favorable at lower temperatures. On the other hand, at these lower temperatures the reaction rate becomes considerably smaller [Ruettinger and Ilinich, 2005]. A promising approach to overcome this issue is the use of membrane reactors, combining the reaction and the H_2 separation through a selective membrane. In this way, the WGS reaction and the hydrogen separation steps required in the conventional power production processes can be replaced by a single WGS membrane reactor step, moving in the logic of the Process Intensification Strategy [Stankiewicz and Moulijn, 2002]. Additionally, the equilibrium can be shifted, achieving a higher CO conversion and improving the overall efficiency of the process [Tosti et al., 2003a; Tosti et al. 2003b; Basile at al., 2006].

Palladium-based membrane reactors have been identified as a promising option for hydrogen production in the future power systems. The most common types of Pd-based membranes are self-supporting metal foils with thickness of 25-100 μm due to its unique hydrogen permselectivity. The most commonly used alloys, for H_2 separation, are Pd-Ag membranes, where the H_2 permeability increases with silver content to a maximum of 23 wt.% Ag [Shu et al., 1991]. Nevertheless some issues related with membrane lifetime remain to be solved. With this concern in mind Tosti et al. [2006] developed a new Pd-based pemeator tube, in a "finger-like" configuration that reduces the constraints between the membrane tube and the module and avoids the presence of any mechanical (cyclic) stresses, assuring a long lifetime and no change in the H_2 separation ability. Also the sealing and joining of the Pd-Ag membrane tube to the metal module was improved.

In this work we study the use of a Pd-Ag "finger-like" configuration membrane reactor in the WGS reaction, carried out at temperatures in the range of 200–300 ºC and in a feed pressure range of 1 – 5 bar. A commercial Cu-based low-temperature shift catalyst was used. The conversion attained was compared with that achieved in a traditional packed-bed reactor and also with the thermodynamic one based on the inlet conditions (pressure, temperature and feed composition). The Pd-Ag membrane was

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also characterized in terms of permeance and selectivity by means of permeation tests carried out before and after reaction.

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Influence of pressure on the yield of supercritical CO_2 extraction of linseed (*Linum usitatissimum* l.) oil and investigation of its antioxidant potential

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Keywords: linseed oil, supercritical CO_2 extraction, lipid composition.

Introduction

The extraction technique using supercritical fluids to obtain natural products has promoted significant scientific and technological development in recent years, mainly in the food, pharmaceutical and chemical industries. In the food sector, in particular, there has been an explosion of consumer interest in the role of specific foods or physiologically active food components, the supposedly functional foods that improve health (Roberfroid, 1999). In this context, the linseed (*Linum usitatissimum*), recognized for its rich content of w-3 fatty acids, has received considerable attention. Although it is an oleaginous seed widely used in industrial oil production, literature data on linseed oil recovery using supercritical extraction is scarce. Accordingly, the present study proposes to investigate the effect of pressure on the yield of supercritical CO_2 extraction of linseed oil and its influence on the lipid profile of the oil obtained.

Materials and Methods

Raw Material Preparation**.** Brown linseeds (from a single lot collected in March 2007 in the city of Guarani das Missões, Brazil) were homogenized, strained and stored in glass vials until use. Sample moisture was determined by the AOCS 2-54 method. To prepare the particle bed, the seeds were ground in a domestic multiprocessor for 10 seconds and separated them in a sieve agitator containing Tyler sieves. Sample granulometry consisted of a mixture containing 30% of 24, 30% of 28, 20% of 32 and 20% of 48 mesh. A fixed mass of 140 g of linseed was introduced into the extraction cell forming the fixed particle bed for each experiment.

SC-CO_2 Extraction. The assays were performed isothermally (at 50°C) for two extraction pressures (P = 150 and 250 bar) in a fixed bed extractor operating at a constant solvent flow of 1.5 gCO_2/min. Experimental extraction time was set at 5h. The oil samples were collected at predetermined time intervals (so that the kinetic extraction curves could be obtained) and stored in freezer for subsequent analyses.

Analysis. The oil samples were esterified and submitted the chromatographic analysis. Hartman and Lago's (1973) method was used to prepare the fatty acid methyl esters (FAME).

Antioxidant Activity. The antioxidant potential of the linseed oil through thin-layer chromatography (TLC) and the evaluation of the co-oxidation of substrates of the system β-carotene/ linoleic acid were determined. For TLC, the detection reagents β-carotene/ linoleic acid and ferric chloride/ potassium ferricyanide were used. For the antioxidant activity measured spectrophotometrics of solutions contends the system oxidizer β-carotene/ linoleic acid were done, with readings at each 15 min/2h.

Results

Process yield**.** The yield values for each pressure condition tested in the supercritical CO_2 extraction assays varied from 3.8% (at 150 bar) to 8.3% (250 bar). The yield of each extraction was calculated from the ratio between the mass of oil obtained in each assay and the mass of linseed introduced into the extractor.

FA Composition**.** The main fatty acids (FA) found in the brown linseed oil were palmitic (6.51-6.52%), stearic (5.19-5.31%), oleic (21.96-22.09%), linoleic (13.85-13.86%) and α-linolenic (51.23-51.49%). The percentage of each fatty acid found in linseed oil did not vary significantly with extraction pressure used. Brown linseed oil had a mean value of 65.22% of polyunsaturated fatty acids (C18:2n-6 and C18:3n-3), 22.03% of unsaturated fatty acids (C18:1) and 11.77% of saturated fatty acids (C16:0 and C18:0).

Antioxidant Activity. The presence of antioxidant phenolics compounds was evidenced in all the samples, however, the best protection factor against the oxidation (21.33%) was obtained for the extracted oil samples at 250bar (volume of 200 uL).

Conclusions

The pressure range used in the supercritical extraction of linseed oil resulted in yield percentages of 3.8% to 8.3% (at 150 and 250 bar, respectively) for a total extraction time of 5h at a temperature of 50°C and mean solvent flow rate of 1.5 gCO_2/min. The obtained results suggested a proportionality relationship between the increase of the linseed oil recovery and the elevation of the operational pressure. As to oil composition, no significant differences were observed between FA concentration values as a function of the extraction pressure used in the experiments. With relationship to the potential antioxidant of the linseed oil, the presence of antioxidant phenolics compounds was evidenced in all the samples, however, the best protection factor against the oxidation (21.33%) was obtained for the extracted oil samples at 250bar (volume of 200 uL). The results showed that the linseed oil besides being an important source of essential fatty acids, also presents natural antioxidants, ratifying its role as an important food for the human nutrition.

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Supercritical fluid extraction of grape seed oil using an enzymatic pre-treatment

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Keywords: Supercritical Fluid Extraction, Enzymatic treatment, grape seed

Grape seed (Vitis vinifera L.) is a well known oilseed crop and a major by-product resultant from the wine industry, containing typically between 7-15% (w/w) of oil (Gomez et al. 1996). This research group is investigating ways of increasing the worth of such sub-products.

The extraction of vegetable oils using supercritical carbon dioxide is being studied as a potential alternative to conventional extraction methods. The most important reasons in this application come from the use of a harmless solvent, mild operating conditions, and the possibility to tailor inherent important properties such as solubility, selectivity, etc.

In this work, grape seed oil extraction is carried out using supercritical CO_2. To enhance the yield of the process, the extraction is combined with an enzymatic pre-treatment (cocktail of enzymes with pectinase, xylanase, protease and cellulase actions).

Figure 1 illustrates an extraction curve where experimental data for runs with and without enzymatic treatment are compared. Pre-treated samples (under optimized conditions: pH, temperature, concentration, and reaction time) exhibit an improvement higher than 30% relative to non-treated ones. In Figure 2 some eppendorfs with collected oil are shown.

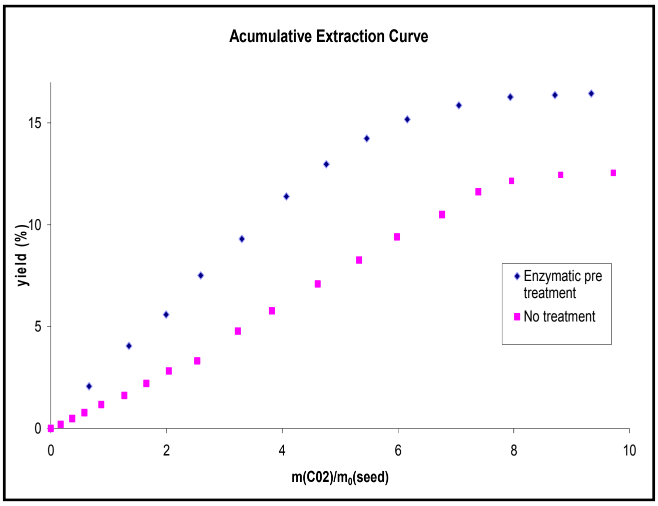

Figure 1 – Effect of the enzymatic pre-treatment upon extraction yield. Operation conditions: T = 40ºC and P = 160 bar.

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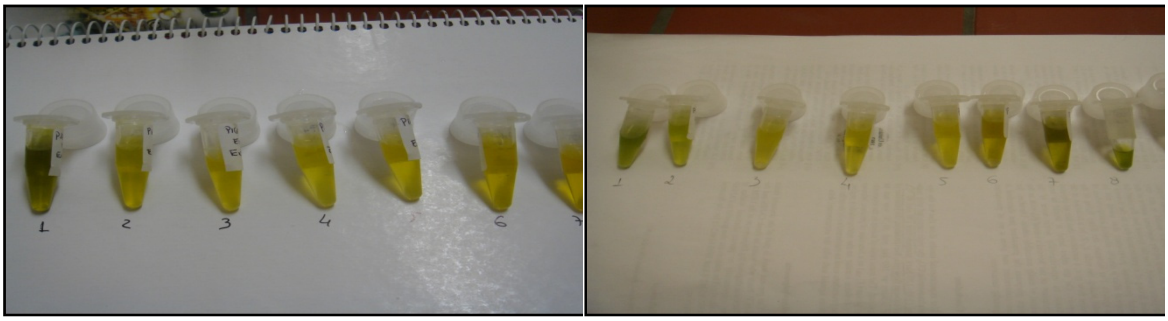

a **b**

Figure 2 – Eppendorfs containing grape seed oil collected along time under supercritical conditions with (a) and without (b) pre-enzymatic treatment.

Modelling has been also carried out in order to simulate the process and optimize the fundamental operating conditions. Following Sovová (2005), the model assumes that the oil is distributed between an easily accessible fraction of the seed and their intact core cells. In Figure 3 calculated results for T = 40ºC and P = 160 bar are shown.

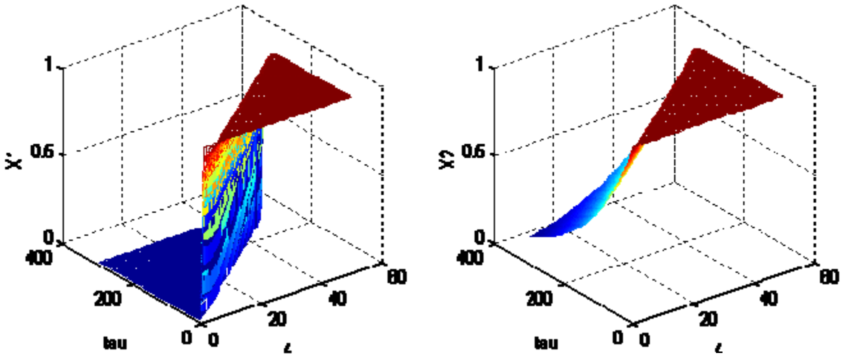

Figure 3 – Distribution of the oil concentrations in easily and non-accessible fractions of grape seed (X_1 and X_2) as function of both the axial coordinate of the bed (z) and dimensionless time (tau). Experimental conditions: T = 40ºC and P = 160 bar.

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Measurement and correlation of solubility of quinizarin and 1-(methylamine)anthraquinone in supercritical carbon dioxide

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Keywords: Dyes, Supercritical carbon dioxide, solubility, Quinizarin, Disperse red 9

Abstract

Solubility measurements of Quinizarin (1,4-Dihydroxyanthraquinone) and Disperse Red 9 (1-(Methylamine)anthraquinone) in supercritical carbon dioxide were carried out in a flow type apparatus, at the temperature range from 333.2 to 393.2 K and for pressures from 12.0 to 40.0 MPa. The supercritical fluid mixture including the dye was released at the expansion valve and the dye was trapped in a system of filters without organic solvents. The dye precipitated in the system during the depressurization was recovered by washing out with ethanol and analyzed in a UV-Vis spectrophotometer.

The values of solubility change from 1.43×10^{-6} to 3.16×10^{-4} to Disperse Red 9 and from 2.98×10^{-6} to 2.87×10^{-4} to Quinizarin. Semi empirical density-based models will be used to correlate the solubility of booth dyes in supercritical carbon dioxide. From the correlation results, the head of dye-CO_2 solvation and that of solute dye vaporization can be calculated and compared with the results in literature.

Introduction

Supercritical carbon dioxide is an alternative solvent that is considered for dry dyeing processes in the textile industry and has received attention due to increased environmental concerns (Montero et al., 2000; Guzel et al., 2000; Sicardi et al., 2000). The application of this process requires a study to determine the dye solubility in supercritical carbon dioxide (SC CO_2). The temperature and pressure of dyes solubilities in SC CO_2 must be know with precision, as well the mathematical modelling of the systems with the aim to promote the development of supercritical fluid process. When the critical properties are unknown semi empirical density-based models (Huang et al., 2005) can be applied with relative success.

Materials and Methods

The supercritical fluid extraction experiments were performed in a flow apparatus (Coelho et al., 2005). This equipment allows carrying out studies at a temperature up to 393.2 K and a pressure up to 60.0 MPa.

Conditions of solubility studies were: CO_2 flow rates of 4.90×10^{-3} g.s^{-1} (liner velocity of 6.20×10^{-3} cm.s-1), pressures up to 40.0 MPa and temperatures up to 393.2 K. The conditions were supervised during all experiments. The extracts were collected in a system of filters, at the atmospheric pressure and a temperature controlled with an ice bath.

The CO_2 (99.995% purity) was supplied by GasIn (Portugal) and the compounds was supplied by Sigma-Aldrich with a purity of 98%.

The solubility of Quinizarin and Disperse Red 9 was determined by a spectrophotometric method (Thermo Electron Corporation, model Nicolat evolution 300LC) on the basis of the absorbance of the composition (UV) at 481 nm and 503 respectively, being the filters washed and the solute collected in a solvent organic (ethanol). Calibration was made with twelve samples over a composition range of $1.0x10^{-4}$ – $1.0x10^{-6}$ mol/L. Three replicates were performance at each experimental conditions and the solubility was obtained by an average of these results. The uncertainty of the solubility measurements was 4 %.

Results and Discussion

Figure 1 and 2 shows the equilibrium solubility of the dyes in supercritical carbon dioxide as function of the pressure and to different temperatures. With the increase of pressure at constant temperature, the solid solubility increases due to an increase of the fluid density. However, solubility curves intersect each other around 22.5 MPa.

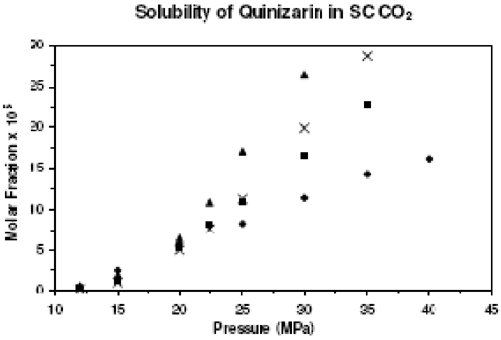

Figure 1 – Experimental solubility of quinizarin in SC CO_2 at temperatures of: ♦-333.2 K, ■-353.2 K, X-373.2 K, ▲-393.2 K.

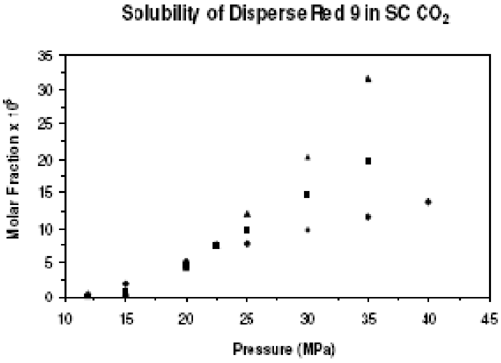

Figure 2 – Experimental solubility of disperse red 9 in SC CO_2 at temperatures of: ♦-333.2 K, ■-353.2 K, ▲-373.2 K.

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Supercritical antisolvent process applied to the pharmaceutical industry

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Keywords: supercritical antisolvent, nanoparticles, precipitation, carbon dioxide, antibiotics

Pharmaceutical preparations are the final product of a technological process that gives the drugs the characteristics appropriate for easy administration, proper dosage and enhancement of the therapeutic efficacy. The design of pharmaceutical preparations in nanoparticulate form has emerged as a new strategy for drug delivery [1]. Particle size (PS) and particle size distribution (PSD) are critical parameters that determine the rate of dissolution of the drug in the biological fluids and, hence, have a significant effect on the bioavailability of those drugs that have poor solubility in water, for which the dissolution is the rate-limiting step in the absorption process [2,3].

Supercritical AntiSolvent (SAS) processes have been widely used to precipitate Active Pharmaceutical Ingredients (APIs) [4, 5] with a high level of purity, suitabledimensional characteristics, narrow PSD and spherical morphologies. SAS process is based on the particular properties of the supercritical fluids (SCFs). These fluids have diffusivities of two orders of magnitude larger than those of liquid resulting in a faster mass transfer rate SCFs properties (solvent power and selectivity) can be also adjusted continuously by altering the experimental conditions (temperature and pressure). As consequence, the SCFs can be removed of the process by a simple change from the supercritical to room conditions which avoid difficult post – treatments of waste liquid streams. Carbon dioxide (CO_2) at supercritical conditions, among all possible SCFs, is largely used because of its relatively low critical temperature (31.1 ºC) and pressure (73.8 bar), low toxicity and low cost.

Supercritical antisolvent process uses both the high power of supercritical fluids to dissolve the organic solvents and the low solubility of the pharmaceutical compounds in supercritical fluids to cause the precipitation of such compounds when, firstly, they are dissolved in the organic phase and then they are put on contact with the supercritical fluid. Therefore, the supercritical fluid has an antisolvent effect regarding the solute which is tried to micronize. The dissolution of the supercritical fluid into the organic solvent is accompanied by a large volume expansion and, consequently, a reduction of the liquid density, and so, of its solvent power, causing a sharp rise in the supersaturation within the liquid mixture. Because of the high and uniform degree supersaturation, small particles with a narrow particle size distribution are expected.

In this paper, we show some results about processed antibiotics (ampicillin and amoxicillin), two of the worlds most widely prescribed antibiotics, when they are dissolved in 1-methyl-2-pyrrolidone (NMP) and carbon dioxide is used as antisolvent.

Samples of the powder precipitated were observed by a SIRION FEG scanning electron microscopy (SEM). SEM pictures of amoxicillin and ampicillin as received are showed in Figure 1 and processed amoxicillin and ampicillin in Figure 2 [6, 7]. The SEM images were processed using Scion image analysis software (Scion Corporation) to obtain the particle sizes. X-ray diffraction pattern analysis (Bruker D8 Advance) was performed in this work to determine the possible changes in the physical characteristics of the precipitate obtained by the SAS process.

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The SEM images of amoxicillin and ampicillin samples micronized show the formation of spherical nanoparticles with a mean particle size in the range of 99 – 395 nm, uniformly distributed.

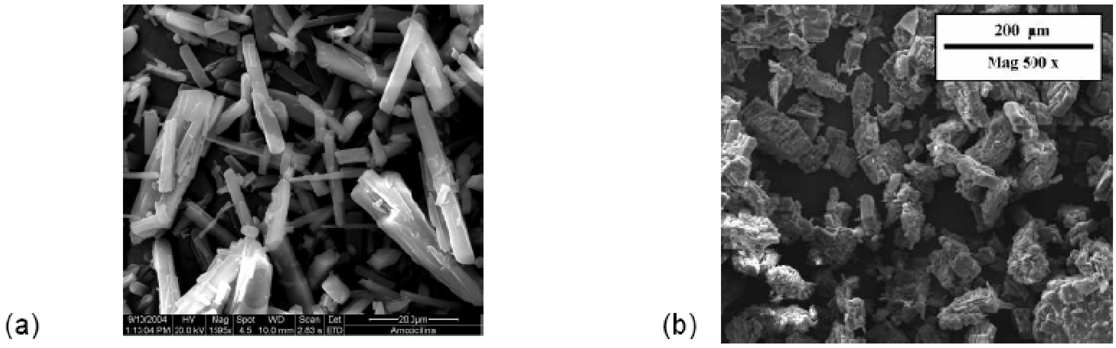

Figure 1. SEM images of unprocessed amoxicillin (a) and ampicillin (b)

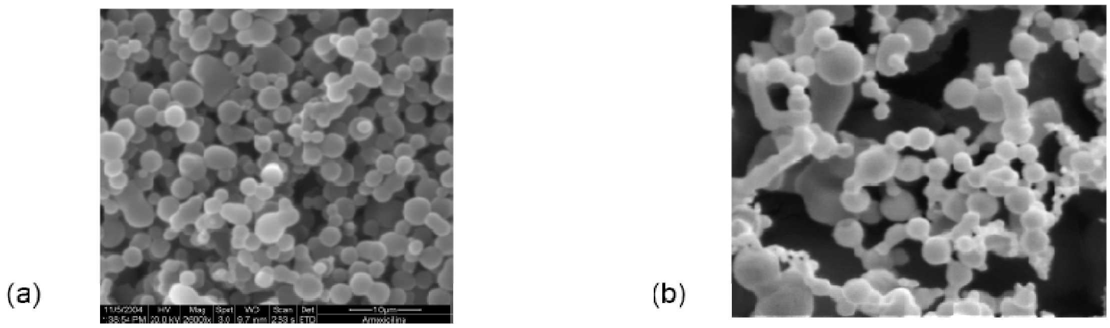

Figure 2. SEM image of ampicillin nanoparticles precipitated from 100 mg/mL, 308.15 K, 90 bar, 5 mL/min solution, 32 g/min CO_2, tW=180 min, noozle 100 μm

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Solvent effects on ionic association of 2,6-lutidinium chloride salt at 60.00 ºC: A QSPR study

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Keywords: Conductance, organic salts, association constant, QSPR, solvent effects.

Conductivity measurements of salts in a diversified set of solvents are of great importance due to their contribution for an in-depth understanding of solvent effects on ion association and mobility phenomena (Prini et al., 2000).

A considerable amount of work has been devoted to establish mathematical models to accurately reproduce molar conductivity (Λ_m) *vs.* concentration (*c*) curves. Expressions such as the Fuoss-Hsia equation (Hsia and Fuoss, 1968), later modified by Prini (Prini, 1969) or newer models which use a full radial distribution function as given by the Mean Spherical Approximation (Blum and Turq, 1992) encompass some possible examples.

The ionic association of salts, defined as an equilibrium between dissociated ions and associated salt molecules [ion$^-$] + [ion$^+$] $\leftrightharpoons$ [ion pair], is among the first recognized influences on the referred plots. An association constant (K_A) can thus be defined from the equilibrium equation which represents the concentration dependence of the process of accounting for strong Coulombic forces at short interionic distances (Cotê et al., 1998).This description of the association constant implies that log K_A can be considered an energetic related term dependent on both salt and solvent features.

Quite recently, the use of a suitable equation (eq. 1) allowed us to determine K_A values and respective errors at 60.00 ºC for 2,6-lutidinium chloride salt in ten different non-aqueous solvents (Moreira et al, 2006). In eq. 1 Λ^0_m represents the molar conductivity at infinite dilution, *S* a dielectric constant and viscosity dependent parameter, α the ionization degree and $f_\pm$ the mean ionic activity coefficient.

$$\Lambda_m = \Lambda_m^0 - S(c\alpha)^{1/2} - K_A \Lambda_m f_\pm^2 c\alpha \qquad \text{eq. 1}$$

The obtained results revealed strong deviations from the predictions of classical dielectric theories. It was therefore reasoned that specific ion-solvent-solvent interactions could play an important role in explaining the observed results. The aim of this work is the application of a suitable methodology to determine and quantify the relevant solvent properties which influence the ionic association of the above mentioned 2,6-lutidinium salt at 60.00 ºC.

Quantitative structure-property relationships (QSPR) are among the most updated methodologies used to study solvent effects on physicochemical processes and, in particular, the TAKA equation (Abraham et al., 1987) has been one of the most used equations built upon these concepts. We have enlarged its scope by including an extra solvent property related to the solvent's stiffness, thus deriving the denominated enlarged TAKA equation (eq. 2).

$$\text{Energetic term} = a_0 + a_1\pi^* + a_2\alpha + a_3\beta + a_4 C + a_5 \log\eta \qquad \text{eq. 2}$$

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According to the above equation, solvents are characterized by their dipolarity/polarizability (π^*), taken as a measure of non-specific solvent–solute interactions related to the capacity of the solvent to generate a spread of charges in the cybotactic region of the solute; their hydrogen bond donor (HBD) acidity (α) and hydrogen bond acceptor (HBA) basicity (β) abilities, regarded as measures of specific solvent–solute interactions of the Lewis type (see fig. 1); their structuredness, given by the cohesive energy density parameter, C, which accounts for solvent–solvent interactions determining the disruption and reorganization of the solvent structure; and the solvent's stiffness, given by the logarithm of viscosity ($\log \eta$). The a_i coefficients are the complementary solute-dependent coefficients of the solvent parameters.

The determination of K_A values (from eq. 1) in a more diversified set of solvents allowed a proper application of eq. 2 and the accomplishment of statistically meaningful results. The attained a_i values and respective errors are consistently analyzed and interpreted.

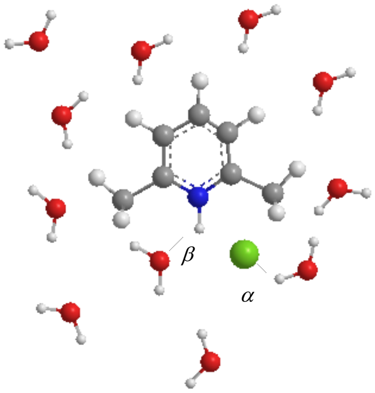

Fig. 1 - Scheme describing α (HBD) and β (HBA) solvation modes on a 2,6-lutidinium chloride – water cluster representation.

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Modelling of the solvent extraction equilibrium of cadmium (II) in neutral chloride medium using Cyanex 923

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Keywords: Cadmium (II), Cyanex 923, Solvent Extraction, Stability Constants, Extraction Equilibrium.

Speciation of aqueous cadmium (II) chloride complexes and the determination of the equilibrium extraction constant for cadmium (II) in neutral chloride medium by applying solvent extraction with organophosphorous solvating extractant Cyanex 923 have been investigated. The cumulative stability constants of Cd–Cl complexes in aqueous neutral chloride solution have been evaluated. The modelling of the extraction equilibrium constant for cadmium (II) by Cyanex 923/Exxol D-100 system has been described.
In neutral chloride medium in equilibrium are formed the following Cd–Cl complexes: $CdCl^+$, $CdCl_2$, $CdCl_3^-$, $CdCl_4^{2-}$. Its formation can be described by stepwise or cumulative stability constants. The overall equation expressing the cumulative stability formation constant of each of the cadmium (II) chloride complex can be proposed by the relation:

$$\beta_n = \frac{[CdCl_n^{2-n}]}{[Cd^{2+}]*[Cl^-]^n}$$

(1)
where n=1, 2, 3, 4 is the number of Cl^- ions complexed with metal ion.
The extraction mechanism of cadmium (II) from chloride medium using Cyanex 923 is related to the solvation of the neutral $CdCl_2$ species and formation of the $CdCl_2L_2$ complexes in the organic phase, where L is the extractant. The extraction of the neutral $CdCl_2$ species and the equilibrium extraction constant can be decribed by the equations:

$$CdCl_2 + 2\ L \xleftrightarrow{K_e} CdCl_2L_2 \quad (2)$$

$$K_e = \frac{[CdCl_2L_2]}{[CdCl_2]*[L]^2} \quad (3)$$

There are a few reviews in the literature reporting the determination of the speciation of aqueous cadmium (II) chloride species in equilibrium, all of them using different values of the stepwise or cumulative stability formation constants of Cd-Cl complexes. Unfortunately, none of them could have been fitted with success to the experimental data of the proposed investigation. Therefore, the numerical values of the studied cumulative stability constants of cadmium (II) chloride species have been determined through the mathematical modelling of the extraction equilibrium of cadmium (II) from neutral chloride medium using Cyanex 923 as extractant and consequently through comparison of the experimental data to the theoretical results by applying the curve-fitting simulation technique.
As an experimental part have been studied:

1. the effect of the concentration of extractant Cyanex 923 in the organic phase varying from 0 to 20% (v/v), with the initial metal concentration of 1g/L and two different total concentrations of chloride ions: 0.5 and 1M in the feed solutions;
2. the influence of the varying total concentration of chloride ions in the aqueous feed phases between 0 and 3M, with the initial metal content of 1g/L and for different concentrations of Cyanex 923: 5 and 10% (v/v);

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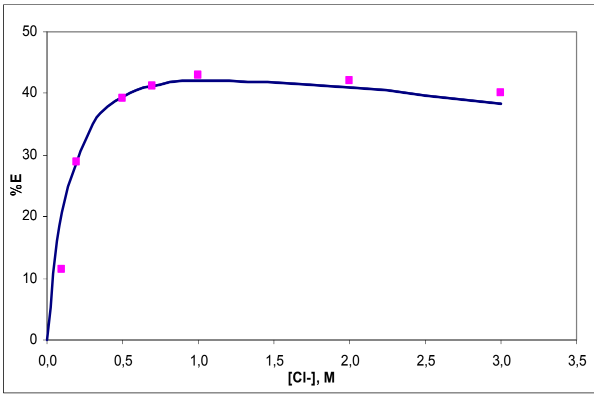
3. the extraction isotherm in the range of initial cadmium (II) content in the feed solutions of 10 – 0.3 g/L, with the total concentration of chloride ions established as 0.5M and for two different concentrations of extracting agent: 5 and 10%.

The back extraction investigations have been carried out using distillate water as a stripping agent.

Modelling of the extraction system has resulted in determination of the cumulative stability constants of aqueous neutral Cd–Cl species and, posteriorly, the equilibrium extraction constant for cadmium (II) in neutral chloride medium. The corresponding results are as follows: β_1=95.5, β_2=398, β_3=60, β_4=6 and K_e=19.8, respectively. The designed model fits well to the experimental data for all studied experiments.

The results for two experiments, where the experimetal data is fitted to the determined model of the extraction system, are given in Fig. 1 and Fig. 2.



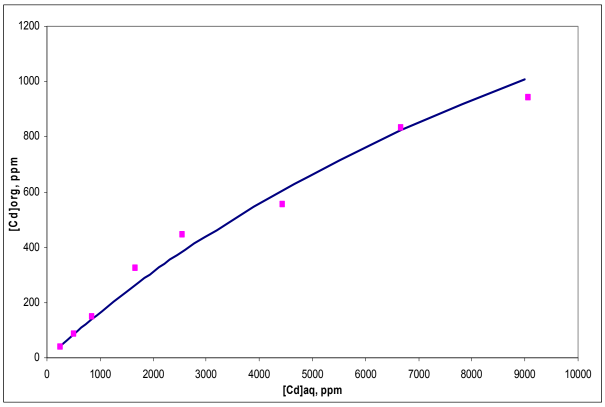


Fig. 1. Effect of the concentration of chloride ions
Fig. 2. Extraction isotherm: [Cl^-]=0.5M[Cy923]=5% (v/v)
[Cd^{2+}]=1 g/L, [Cy923]=10% (v/v)
■ experimental data, □ modelling results

experimental data, □ modelling results

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Models to correlate the surface tension of solvent mixtures

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Keywords: surface tension, chemical model

Surface tension has high industrial importance in chemical reactions as the reactions in the surface region are quite different from the bulk region. The surface tension also is an essential property in mass-transfer calculations, in various chemical engineering processes such as, distillation, extraction and absorption, because surface tension has a determining effect. In electrochemical reactions, biological membranes operation, environmental engineering and several other processes namely; corrosion, adherency, detergency, floating and lubricating the surface tension plays a significant role.
Nowadays*, ab initio* prediction of the surface tension of solvent systems is not possible, however, it is possible to calculate surface tension after collecting a number of experimental data and then prediction of surface tension using interpolation techniques. The aim of this study is to analyse the different models to correlate surface tension of mixed solvents.
Some of interesting models for surface tension prediction or correlation are:
The JAM model (equation 1), which is based on the Redlich-Kister extension

$$\ln\sigma_m = x_1\ln\sigma_1 + x_2\ln\sigma_2 + x_1x_2\sum_{i=0}^{2}K_i(x_1-x_2)^i \tag{1}$$

The chemical model of Connors and Wright (equation 2)

$$\frac{\sigma_1-\sigma_m}{\sigma_1-\sigma_2} = \left(1+\frac{bx_1}{1-ax_2}\right)x_2 \tag{2}$$

An empirical model (Asprion, 2005), which is based on a modified mixing rule (equation 3)

$$\sigma_m = x_1 + \sum_{i\geq 2}\frac{S_ix_i}{1+\sum_{j\geq 2}(S_j-1)x_j}(\sigma_i-\sigma_1) \tag{3}$$

Cheong and Carr model (equation 4)

$$\ln\sigma_m = A\sum_{i=1}^{m}B_ix_i^{\ i} \tag{4}$$

According several articles these equations contributed suitable results for different liquid phase systems.

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Thermodynamic analysis of surface tension in water / pyrrolidone system

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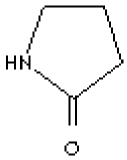
Keywords: 2-pyrrolidone, N-alkyl-2-pyrrolidone

2-Pyrrolidone is an organic compound consisting of a five-membered lactam. When the proton on the lactam is replaced for an alkyl group, this results in a great solvency and as N-alkyl chain increases the surface activity increases. This reaches a maximum at a chain with 12-14 atoms of carbon (1).



Nowadays, pyrrolidones have increased their industrial interest as solvent for a wide variety of applications such as hard-surface cleaners, extractor agents of antibiotics, formulation agents for shampoos, personal care products and agricultural products, etc. This is possible because pyrrolidones besides being surface-active compounds, they have low vapour pressure and low toxicity.

In the present study, the surface tensions of aqueous solutions of several pyrrolidones have been determined using a Krüss K-11 tensiometer using the Wilhelmy plate method to different concentrations and different temperatures from 20 to 50ºC and its behaviour has been analysed.

The reagents used in this paper, the pyrrolidones have been supplied by Fluka, with a purity of > 99%. Bidestilled water was used to prepare the mixtures of water and pyrrolidone. The surface tension of pure water was determined and compared with literature to confirm that this method contributes suitable results.

The addition of small quantities of pyrrolidone to water produces a drastically decrease in surface tension value. The bigger decrease in the surface tension was produced in low concentration of pyrrolidone. However, in high concentrations of pyrrolidone, the influence of the mixture composition can be considered despicable. This behaviour seems to indicate that an aggregation phenomenon exists between the two components pyrrolidone and water.

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volumetric properties for the ternary mixture methanol-formamide-acetonitrile

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Keywords: solvents, ternary mixture, molar volume, partial molar volume

Several chemical industrial processes use mixtures of organic solvents, and a significant number of these mixtures are non-ideal. The optimization and adequate design of equipment are conditioned by the correct assessment of the mixing properties of these mixtures. The mixing properties and their corresponding derived values are also of primary interest for theoretical purposes namely for model development, estimation of parameters, etc.

Following previous studies on characterization of solvent mixtures (Leitão, 2002), in this work we present molar volumes/densities´ values for the ternary mixture methanol-formamide-acetonitrile.

The densities were determined in an Anton-Paar density meter (cell unit DMA 512, processing unit DMA 60 and temperature unit DT 100-30), and obtained, at 298.15 ± 0.01 K, for a total of 61 molar fractions including ternary, binary and pure solvents.

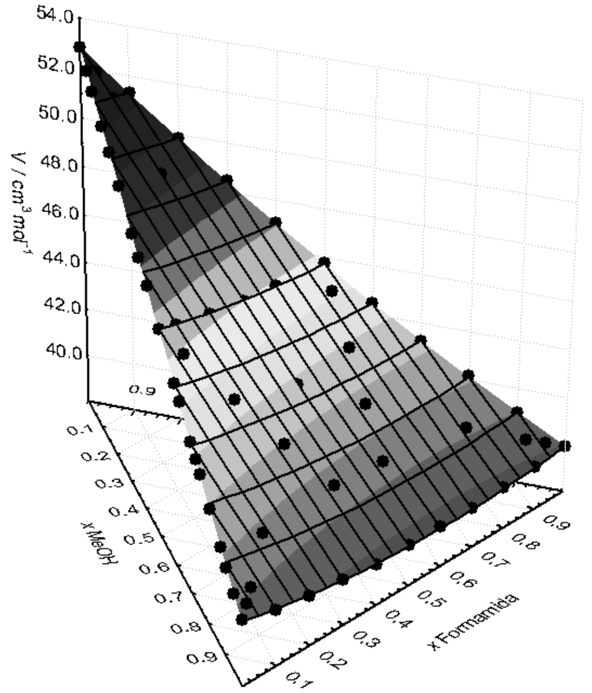

Figure 1 – Molar volume for the ternary mixture methanol-formamide-acetonitrile.

Derived properties such as the excess molar volume and the partial molar volume (Lepori, 1998) were also calculated allowing the identification of the various molecular interactions among the different solvents.

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The experimental values of the volumetric properties were adjusted by the Redlich-Kister (Redlich, 1948) and Cibulka (Cibulka, 1982) equations leading to a complete description of the ternary surface.

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An effective method for calculation of homogeneous azeotropes in reactive and nonreactive mixtures

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Keywords: azeotropy, global optimization, chemical equilibrium, phase equilibrium

Azeotropy is a phase equilibrium phenomenon that occurs in many industrial applications and its presence restricts the separation grade of a multicomponent mixture that can be achieved by distillation. In particular, the description of homogeneous azeotropy is essential for the selection of strategies in shyntesis, design, and operation of separation units. This description consists of establishing the temperature, pressure, and composition as well as the component number of the azeotrope. An azeotrope can occur in reactive and nonreactive mixtures. For nonreactive systems, several methods for finding homogeneous azeotropes have been proposed (Fidkowski *et al.*, 1993; Harding *et al.*, 1997; Maier *et al.*, 1998). However, for systems subject to chemical reactions, only few robust methods have been developed to address the problem of locating all reactive homogeneous azeotropes (Harding and Floudas, 2000; Maier *et al.*, 2000).

Although these methods have demonstrated to be very promising, some of them are model dependent, may require problem reformulations or a significant computational time for multicomponent systems. In this context, stochastic optimization methods offer some advantages for the calculation of homogeneous azeotropes and may overcome the difficulties described above. Specifically, these methods are robust numerical tools that present a reasonable computational effort in the optimization of multivariable functions; they are applicable to ill-structure or unknown structure problems; they are efficient if properly implemented requiring only calculations of the objective function and can be used with all thermodynamic models. Many thermodynamic problems that are very difficult to solve by conventional techniques can be solved by using stochastic methods. In Thermodynamics, Simulated Annealing and other stochastic methods have been successfully applied in phase equilibrium and stability problems, critical point calculations, and parameter estimation. To the best of our knowledge, these methods have been not applied in the calculation of homogeneous azeotropes in both reactive and nonreactive mixtures.

Considering this fact, in this work we introduce an alternative technique to find homogeneous azeotropes in reactive and nonreacitve mixtures which is based on the application of Simulated Annealing optimization technique. Specifically, this stochastic optimization method is used to robustly solve a system of nonlinear equations that results from the equalities of the orthogonal derivatives of the Gibbs energy, and the Gibbs energy of mixing in the vapor and liquid phases. For nonreactive systems, this equations system is solved by considering conventional composition variables plus the unknown temperature or pressure of the azeotrope; whereas for reactive cases, the transformed composition variables proposed by Ung and Doherty (1995) are considered. In fact, if the Gibbs free energy, or transformed Gibbs free energy, is plotted for both liquid and vapor phases against the corresponding composition variable, at the homogeneous azeotrope pressure and temperature, the Gibbs curves of each phase will intersect at one point satisfying the equality criterion of the tangent plane, and the equality of chemical potentials.

Our systems of equations are an alternative set of thermodynamic conditions to locate all homogeneous nonreactive and reactive azeotropes in multicomponent systems

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which can be applied with any thermodynamic model. Also, they are nonlinear and may have one, more than one or no solutions (one azeotrope, several azeotropes or the nonexistence of a homogeneous azeotrope). In these circumstances, conventional root-finders cannot be used robustly to solve these equations. So, a reliable and efficient numerical strategy must be employed for the calculation of homogeneous azeotropes. Therefore, we utilize an optimization approach to locate all solutions of these system of equations. Thus, the system of equations is formulated as a single objective function that should minimize by a suitable optimization technique. The stochastic optimization method Simulated Annealing (SA) is used to globally minimize this objective function. In general, SA method overcomes most of the numerical difficulties that local optimization methods show. Due to the statistical nature of the SA, a local minimum can be hopped much more easily than in conventional methods. At the homogeneous azeotrope condition, the global minimum of our objective function must be zero, but we assume that an azeotrope is found when we find the composition variables (conventional or transformed), and temperature or pressure that make the value of this function less than or equal to 1×10^{-9}; otherwise, we consider that an azeotrope does not exist in the mixture at the given conditions. For polyazeotropy systems, the objective function will show several optimums where $\leq 1\times10^{-9}$.

Explicitly, the following procedure is used to locate all homogeneous reactive or nonreactive azeotropes. First, we define the initial search intervals for the composition variables and the temperature or pressure. In these intervals, the objective function is minimized using Simulated Annealing method and, once a minimum is found, we create new search intervals using the found solution for the temperature or pressure. In these new intervals, we again minimize the objective function. This procedure is repeated until the convergence criterion cannot be satisfied. We penalize the objective function in the limits of the intervals obtained from segmentation to avoid convergence to the last solution when system shows polyazeotropy. Usually, the value of the objective function is greater than 1×10^{-4} if one interval does not contain an azeotrope.

Finally, we have calculated the homogeneous azeotropes for binary, ternary, and quaternary reactive and nonreactive mixtures that appears in the literature to test the numerical performance of our strategy. In these calculations, solution models and equations of state were used. Our results show that proposed method is a suitable strategy for the calculation of homogeneous azeotropes in mixtures with or without chemical reactions.

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A portable library for equilibrium and thermodynamics properties calculations based on object oriented paradigms

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Keywords: Vapor-liquid equilibrium, thermodynamic library, object oriented, Thermolib

This work presents the development of a portable and embeddable numerical library aimed at the calculation of equilibrium and thermodynamics properties. This library, identified as ThermoLib, is being developed using object-oriented concepts. It was created with part of the core code from an arcane application called Ekilib (Báez, *et al.*, 1990; Vieira, 2007). That application was compressed by a group of six thermodynamic modules assembled with a user friendly interface, using classical procedural programming in Turbo-Pascal, being free distributed in the academic community. At that time, the availability of easy to use thermodynamic number cruncher applications was rare and commercial software only ran in huge mainframes. The portability of the code was difficult by tough limitations in hardware. Fortran 77 language was the “king of hill” in the scientific computing area and C language was directed to operating system development.

Nowadays, computer power and its availability are fairly common and software development has been revamp with object-oriented paradigms, allowing the management of complexity with well established patterns. The object-oriented paradigm is well supported in languages like C++ and Java, which in last years have been shifting Fortran from scientific programming crown. In this work, we have chosen Java as developing language platform, because it adopts a shorter but a flexible sub-set of C++ powerful characteristics, due to its omnipresence, even increasing performance and acceptation by scientific computing community (Pang, 2006).

The scenario in thermodynamic libraries for process modeling continues to be limited. It has raised recently a free object-oriented library developed in C++ (Landschützer, 2007), which seems will become in a large open source process simulator application, shareware packages callable from inside worksheets or thermodynamic packages with too narrow application. However, a portable, embeddable, easy to use, command driven, wide thermodynamic numerical library continues to be scarce or expensive, most of them restricted to commercial applications environments or not easily portable.

General propose computing environments as Matlab or Octave are used in academy and research, normally solving problems with thermodynamic requirements. It is always hard to include in dynamic models, rigorous thermodynamic treatment for determining properties and/or solving equilibrium equations. This library will try to fulfill this gap, both pedagogically and as a research working toolbox. The new library uses a database with critical data for pure components, easily accessible with single commands. The possibility to have a live object in memory for direct inspection with the “pure” component data and behavior, ready to use “mixture” objects allocating all data and methods for computing mixture properties and “mixture-stream” objects with vapor-liquid capacities are the main add-ons of the library we are developing.

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Figure 1 shows a textbook problem (Smith, *et al.*, 2005) solved with our library, using Matlab. The plots are obtained from standard commands, while the thermodynamic calculations where carried out with Thermolib embedded or imported directly in the computer environment. Figure 2 shows how to create an instance of a pure component directly from Matlab console window, after three commands, and a simple vapor pressure calculation. The scroll-window over the white pane shows the internals of the pure component available in memory at a finger distance.

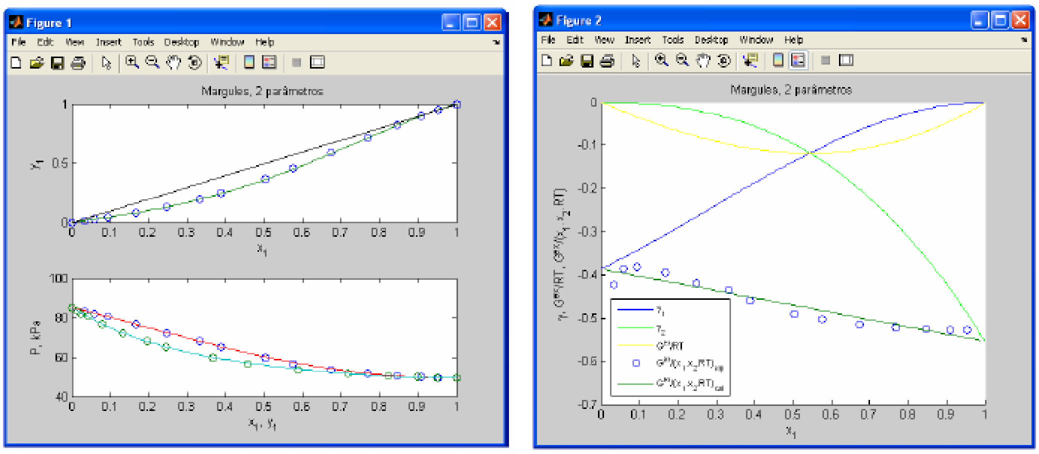
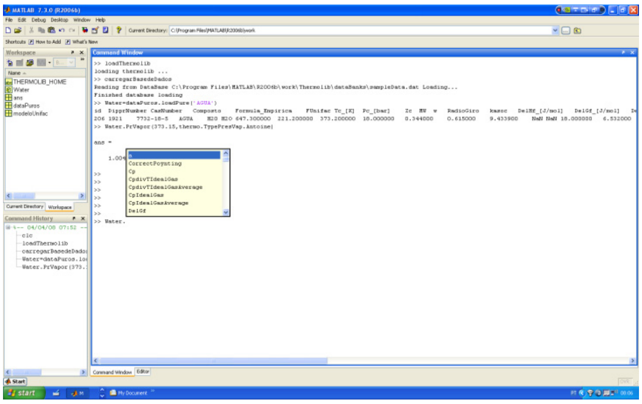


Figure 1: VLE data for *tert*-butyl ether(1)/dichloromethane(2) at 308.15K and equilibrium calculations using two-parameters Margules equation (A_{12} = -0.3861, A_{21}=-0.5534. Problem adapted from Smith *et al.*, 2005.



```
>> loadThermolib
loading thermolib ...
>> carregarBasedeDados
Reading from DataBase C:\Program Files\MATLAB\R2006b\work\Thermolib\dataBanks\sampleData.dat Loading...
Finished database loading
>> Water=dataPuros.loadPure('AGUA')
id  DipprNumber CasNumber   Composto    Formula_Empirica    FUnifac Tc_[K]  Pc_[bar]    Zc  MW  w   RadioGiro   kasoc   DelHf_[J/mol]   DelGf_[J/mol]
206 1921     7732-18-5    AGUA    H2O H2O 647.300000  221.200000  373.200000  18.000000   0.344000    0.615000    9.433900    NaN NaN 18.000000   6.532000
>> Water.PrVapor(373.15,thermo.TypePresVap.Antoine)

ans =

    1.00

>>
>>
>>
>>
>>
>>
>> Water.
```

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CorrectPoynting
Cp
CpdivTIdealGas
CpdivTIdealGasAverage
CpIdealGas
CpIdealGasAverage
DelGf
```

Figure 2: Using ThermoLib in a direct command drive session, showing how to get a “water molecule”, its internal class data and methods.

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Thermophysical properties of aqueous solutions of CNTs in the temperature range (298.15-343.15) K

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Keywords: Nanofluids; CNTs; Surface tension; Viscosity; Density

Nanofluids are liquid suspensions containing nanometer-sized particles and have recently been demonstrated to have great potential for improving the heat transfer properties of fluids (Ko G. H. *et al*). The conventional heat transfer fluids have low heat transfer characteristics which reduce the performance enhancement and the high compactness of heat exchangers which gives low costs of the thermal apparatuses.

This work is inserted in the research project from FCT "Studies of heat transfer characteristics of nanofluids under horizontal, vertical and inclined flow conditions". The relationship among dynamic viscosity, thermal conductivity and heat transfer coefficient of nanofluids are to be studied in detail where the nanofluid is passing through a circular tube under laminar or turbulent conditions in horizontal, vertical and inclined tube. As the derivation of the Nusselt numbers from the operating conditions requires the knowledge of transport properties of nanofluids (Y. Ding *et al*), this work constitutes a contribution in this direction. The viscosity, density and surface tension of aqueous solutions with different CNTs compositions have been studied, in the temperature range (298.15 - 343.15) K.

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Modelling solubility of amino acids in aqueous solutions

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Keywords: Amino Acids, Activity Coefficient, Solubility, UNIFAC, Debye-Hückel

Many processes in biotechnology industry produce dilute biochemicals in reactors which imply their subsequent separation from impurities and excess reagents. In addition, a large number of commercially interesting biomolecules are solids at ambient conditions, hence crystallization is generally one of the final unit operations of purification.

Therefore, it is fundamental for the development of more efficient separation methods (concentration and purification) the knowledge of the equilibrium of systems containing small biomolecules, like amino acids and peptides, since they can represent up to 50% of the total cost production. The amino acids (AA) are zwitterionic substances and exist in solution as charged $\left(AA^{+} \text{ and } AA^{-}\right)$ and neutral forms $\left(AA^{\pm}\right)$.

The correct representation of the solubilities and activity coefficients of amino acids in aqueous solutions is a good starting point for the knowledge of more complex biochemical systems, owing to their similarity towards more complex biomolecules as peptides and antibiotics.

There are several models in literature to correlate and predict amino acids solubilities (Nass, 1988; Gupta and Heidemann, 1990; Pinho et al., 1994; Gude et al., 1996; Soto et al., 1999). Most of them embody short-range physical interactions and chemical equilibrium between molecules. The activity coefficients are frequently calculated by Wilson, NRTL, UNIQUAC or UNIFAC models. Other authors couple a long-range contribution to the non-ideal behaviour of solutes, introducing interactions represented by Pitzer-Debye-Hückel like terms.

Pinho et al. (1994) have accounted for chemical and physical equilibrium simultaneously, where the UNIFAC model is combined with a Debye–Hückel term to describe activity coefficients.

In this work we are combining both long-range and short-range interactions for the activity coefficient, using Debye-Hückel and UNIFAC contributions too. Nonetheless, the impact of the electrostatic interactions with or between the neutral dipolar zwitterions $\left(AA^{\pm}\right)$, which carries dual electric charges, is also taken into account due to the powerful fields surrounding charged groups.

The results obtained by our model and other expressions taken from literature are compared with experimental values from a collected database.

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Liquid-liquid equilibria, surface and interfacial tension for water+n-butyl acetate+1-propanol at 323.15 K

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Keywords: LLE; Interfacial tension; Surface tension; Water ; N-Butyl Acetate; 1-Propanol

The importance of liquid-liquid extraction in separation technology has increased over the last 30 years. It is now usually used in pharmaceutical and food industries for recovering valuable organic materials for example the alcohols from aqueous solutions. The problems associated with the development and construction of industrial extraction units are the complexity of the materials to be extracted, the heterogeneity of the liquid medium and the necessary low separation time required. The published studies on liquid-liquid equilibrium and on the thermophysical properties data of ternary systems of practical interest are scarce when compared with the wide variety of binary systems which have been investigated. The surface and interfacial tensions of solutions has a great effect on the efficiency of the extraction. In the last years we have made measurements of this properties and we have correlated the data for systems of the type water+alcohol+ester.[1-3] The aqueous solutions of alkanols can be efficiently extracted with esters.

The purpose of this study is to determine the liquid-liquid equilibria for the water+1-propanol+N- butyl acetate at 323.25 K. For this system the surface tension was measured in the homogeneous region and the interfacial tension was determined in the immiscible range at the same temperature. The binodal curve was found by the cloundpoint method, using a solubility cell assembled for this. The tie-lines were determined by sampling the liquid phases from a cell carefully designed for LLE and the composition was determined in a TRE METRICS 9001 gas chromatograph with a J & W Scientific DB-1 column. N-buthanol was used as internal standard for the calibration of the detector (FID). The LLE data for the system water+ N- butyl acetate+1-propanol at 323.25 K is shown in Figure 1. The dependence of binary surface tension γ on composition was described by

$$\gamma = \sum_{i=1}^{2} x_i \gamma_i + \gamma_{ij} \tag{1}$$

were γ_i is the surface tension of pure component i which molar fraction is x_i, and γ_{ij} is a rational function

$$\gamma_{ij} = x_i x_j \frac{\sum_{k=0}^{p} B_k z_{ij}^k}{1 + \sum_{l=1}^{m} C_l z_{ij}^l} \tag{2}$$

where $z_{ij}=x_i-x_j$. B_k and C_l are adjustable coefficients obtained from the fitting of Eq. (1) to binary γ. The ternary data were correlated by the following equation

$$\gamma = \sum_{i=1}^{3} x_i \gamma_i + \gamma_{123} \tag{3}$$

where γ_{123} is a ternary contribution expressed by

$$\gamma_{123} = \sum_{i<j}^{3} \gamma_{ij} + x_1 x_2 x_3 \left(\frac{B_0 + B_1 z_{13} + B_2 z_{23}}{1 + C_1 z_{13} + C_2 z_{23}} \right) \tag{4}$$

In Figure 2 we present the function γ_{123} of the surface tension of the ternary system comparing the experimental results with the fitted data. The interfacial tension γ' data was correlated with the Li and Fu model [4]

$$\gamma' = \gamma_0' \left(\frac{X}{X_0} \right)^k \tag{5}$$

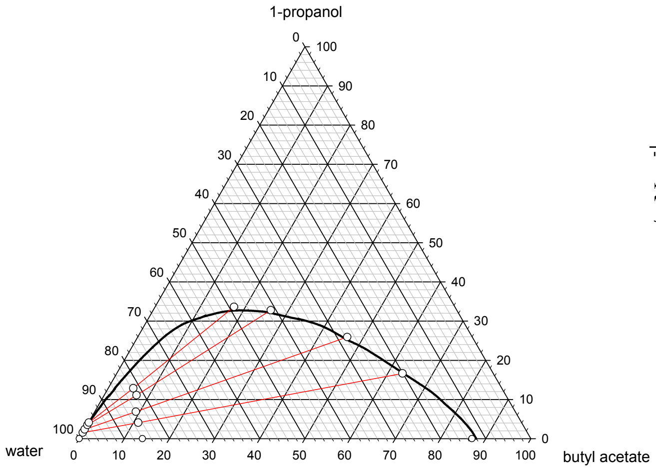

Figure 1. Binodal curve (—)and tie-lines(–○–) for LLE of water + 1-propanol+N- butyl acetate at 323.25 K.

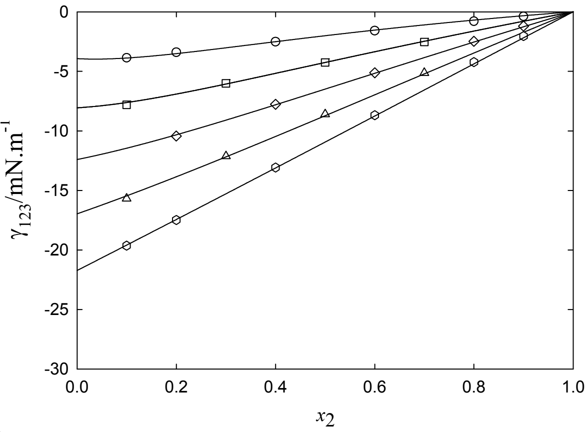

Figure 2. Function, γ_{123} for the ternary system water(1)+N-butyl acetate(2)+1-propanol(3), at 323.15 K and atmospheric pressure, along the curves of constant ratio $z = x_1/x_3$ as a function of the ester composition,x_2:(○), z = 0.111; (□), z = 0.250; (◊), z = 0.429; (Δ), z = 0.667 and (○), z = 1.000. (—) Correlation with eq (4).

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Thermodinamic analysis of leather tanned with different tanning agents

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Keywords: leather, tanning agents, DSC

Traditionally, quality, cost and yield are the three main concerns of leather manufacturers. However, the issue of environmental impact is now a worldwide concern. This environmental concern, combined with consumer preference, particularly in the European market, for chromium-free leather now makes the development of new tanning methods.

It is well known that collagen cross-linked with various cross-linking agents such as metal ions and aldehydes is made resistant against the degradation by collagenase and also that the thermal stability of collagen is increase owing to the cross-links formation. This cross-linking is the main objective of tanning, a process that converts raw hide/skin into leather. Understanding the effect of them on the dimensional stability of collagen will also aid in understanding the process of tanning.

The thermal stability of collagen has been investigated by measuring the hydrothermal shrinkage of collagen fibres, the characteristic temperature at which collagen fibre shrinks to one-third its original length and by differential scanning calorimetric (DSC) measurements. DSC allows the study of enthalpy changes, which are associated with the denaturation of collagen (Komanowsky 1992).

In this work the effect of different tannig agents in the stabilization of collagen was evaluated by DSC. The alternative agents selected were: mimosa extract, acrylic resin, gluteraldehyde, silicate, genipine. Chromium at 7% as been also tested as tanning agent, in the same scale, to be used as a reference.

The leather tanned with 7% of cromium was undoubtely the best results about themodynamic stability but several tanning agents can be choosed for and a good themodynamic stability was achieved. The DSC data of leather thermodynamic stability were correlated with test of leather shrinking temperature described in ISO 3380.

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Effect of isooctane on vapor-liquid equilibria of water and TEG solution

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Keywords: Experimental data; Activity Coefficient; Virial; NRTL; UNIQUAC

Experimental Vapor-liquid equilibriua have been measured for systems water-triethylene glycol (TEG) and water-TEG-isooctane (2,2,4 trimethylpentane) at atmospheric pressure and various temperatures. NRTL and UNIQUAC models were correlated for liquid phase behavior. It was observed that NRTL and UNIQUAC can provide a good correlation. The results demonstrate the increase of the volatility of water in water-triethylene glycol solutions and increasing the purity of the dehydrated TEG by the addition of isooctane. Using this method, the more concentrated TEG can be obtained in the stripping column of the natural gas dehydration unit.

1. Introduction

The natural gas dehydration is an important operation in the gas processing and conditioning industry. The standard method for natural gas dehydration is by absorption of water using TEG. An alternate approach for the enhancement of reconcentrator performance is the use of stripping agent. The hydrocarbon increases the volatility of water in the solution. Smith and Skiff (1990) reported that this type of processes can achieve concentrations of over 99.99% with TEG. Isooctane is used as entrainers in the stripping column of natural gas dehydration unit (Manning et. Al,1991). In this work, experimental procedure was done in order to determine vapor-liquid equiliria data for the systems water-TEG and isooctane. Modified Othmer still was used for taking samples and they were analyzed by gas chromatography (GC). The results were used to predict the interaction parameters of the NRTL and UNIQUAC equations.

2. Experimental section

The materials were purchased from Merck with nominal purities that are TEG with > 99 % purity and Isooctane > 99.5 % purity. The VLE data were determined at atmospheric pressure in a modified Othmer still which is the continuous-distillation still that recirculates the vapor phase (J.F. Morrison, 1990). The still comprises a distillation flask, a reflux condenser and condensate return. The all seal glass recirculating still with two magnetic homogenizers was used. Analysis of samples was carried out by gas chromatography (GC) on a Varian coupled with a personal computer by use of relative program. The GC apparatus equipped with 2m×1/4 in column packed with Propack Q. Helium was used as a carrier gas. Two different detectors were used to analyze the amounts of components. FID was applied to obtain the concentration of Isooctane and TCD was chosen to detect the water content. The concentration of TEG was determined using titration method (Fritz and Schenk, 1959).

3. Results and discussion

Experimental vapor-liquid data for binary system water-TEG and ternary system containing water, TEG and isooctane are taken at atmospheric pressure. Water volatility as a function of isooctane mole fraction is shown in Fig 1(a). Addition of isooctane causes enhancement of water volatility and acts as enterainer. Also the equilibrium temperature of solution declines with addition of isooctane which is demonstrated in Fig. 1(b).

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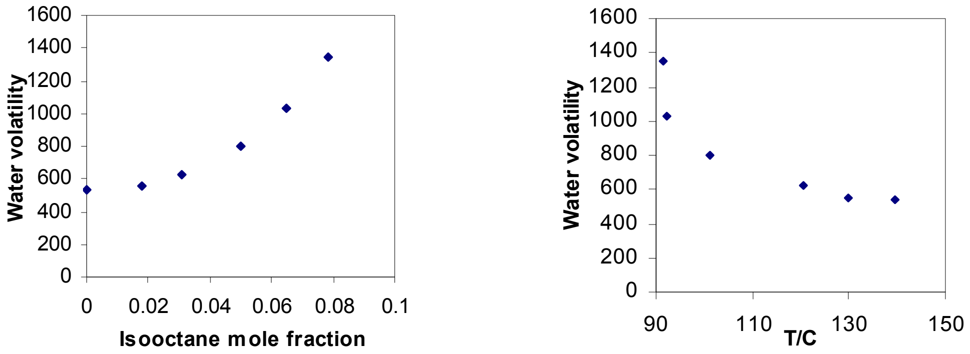

(a) (b)

Fig. 1 Relative volatility of water in water-TEG solution with addition of Isooctane, (a) as a function of Isooctane mole fraction, (b) as a function of temperature

The liquid-phase activity coefficients of the components in the mixtures were calculated from UNIQUAC (Abrams and Prausnitz, 1975) and NRTL (Renon and Prausnitz, 1968) equations and the vapor-phase fugacity coefficients were calculated from the virial (Smith et al, 2001) equation. Estimation of the binary interactions of this model was based on minimization of the objective function in terms of calculated and experimental activity coefficient values. The binary interaction parameters are listed in Table1.

Table 1. Binary interaction parameters between water (1)-isooctane (2)-TEG (3)

	Interaction Parameters (UNIQUAC)			Interaction Parameters (NRTL)	
Water+TEG					
Water-TEG	$A_{13}=-428.627$	$A_{31}=331.663$	$A_{13}=248.949$	$A_{31}=-172.965$	$\alpha_{13}=2.366$
Water+TEG+Isooctane					
Water- Isooctane	$A_{12}=-647.140$	$A_{21}=-464.858$	$A_{12}=-72.078$	$A_{21}=314.051$	$\alpha_{12}=-2.060$
Water-TEG	$A_{13}=-99.311$	$A_{31}=151.290$	$A_{13}=-155.068$	$A_{31}=-67.819$	$\alpha_{13}=-4.103$
Isooctane-TEG	$A_{23}=-608.41$	$A_{32}=52.198$	$A_{23}=1081.279$	$A_{32}=1181.917$	$\alpha_{23}=0.501$

4. Conclusions

Isobaric vapor-liquid equilibria of water-triethylene glycol and the effect of Isooctane on this solution have been investigated. The experimental data of the binary system (water+TEG) and ternary systems (water+TEG+isooctane) were correlated using UNIQUAC and NRTL activity coefficients models. Good agreements were obtained. The results demonstrate the volatility of water increases in water-TEG solution and the purity of residual TEG increase by the addition of Isooctane. By use of this approach, the separation of TEG and water is better performed in a stripping column of natural gas dehydration.

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Optimized force fields for predicting vapour-liquid equilibria of binary mixtures involving alkanes and xenon at low temperatures

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Keywords: Monte Carlo, Alkanes, Xenon, Vapour-liquid equilibria, Force field

The lightest alkanes are still the object of much scientific work for both practical and fundamental reasons. As main components of natural gas and LPG, the measurement, estimation and prediction of their properties, both as pure fluids and as mixtures with other substances have a great technological relevance. On the other hand, alkanes constitute a family of compounds whose properties vary regularly through the homologous series and their molecules can be viewed as chains of the equal units, which are good attributes to be used as models to test the theories of liquid state. However, it is not yet clear, at least in terms of mixture and mixing thermodynamic properties, what kind of regularity (if any) the lightest alkanes show and if there is any relation with the regular behaviour displayed by the heavier partners.

We have recently carried out a theoretical study on mixing properties of the lightest alkanes (excluding methane) with a noble gas (xenon), where heats of mixing and excess volumes as a function of composition at low temperatures were obtained by computer simulation (Monte Carlo)[1]. The agreement between simulation results simulation and those from experiment was satisfactory. The force fields used (Bohn *et al.*[2] for xenon and TraPPE-UA for alkanes[3]) were chosen because of their good performance in predicting liquid densities of pure components. Besides the mixing quantities at liquid state and constant pressure, it is important to predict also vapour-liquid equilibrium for this kind of systems. Although Bohn et al. and TraPPE-UA force fields were able to predict isobaric densities for pure xenon and light alkanes (ethane, propane and butane) respectively, the agreement between simulation and experiment was poor for vapour pressures. For this reason, we have been doing a reparameterization of both force fields in order to match the results of Gibbs ensemble simulations of vapour-liquid equilibrium to experimental data specifically for the lightest alkanes and xenon at temperatures between 160 K and 245 K. For xenon, the Lennard-Jones parameters were optimized by a simple trial and error procedure. In the case of the alkanes, we have optimized the Lennard-Jones parameters of CH_3 unit by trying to match experimental and simulation results for density and vapour pressure for ethane. Then, using the optimized parameters for CH_3, the CH_2 ones were fitted to vapour pressures and densities of pure propane. In order to test the transferability of the potential parameters, vapour pressures and densities of butane were predict in the same temperature range.

Finally, the vapour-liquid diagrams of mixtures involving xenon and the lightest alkanes were obtained by computer simulation at temperatures for which experimental results are available (in the same temperature range) using the optimized force fields.

The computer simulations both for pure components and mixtures have been done by the Monte Carlo method in the Gibbs ensemble using the MCCCS Towhee Monte Carlo molecular simulation package.

Since the vapour pressures and saturated liquid densities of butane were reasonably predicted, we concluded that the Lennard-Jones parameters are transferable among the lightest alkanes in this range of temperature (figure 1). The vapour-liquid coexistence diagrams for the mixtures xenon/ethane, xenon/propane and xenon/butane are also well predicted by this model (figure 2: for clarity, only the former two systems are shown).

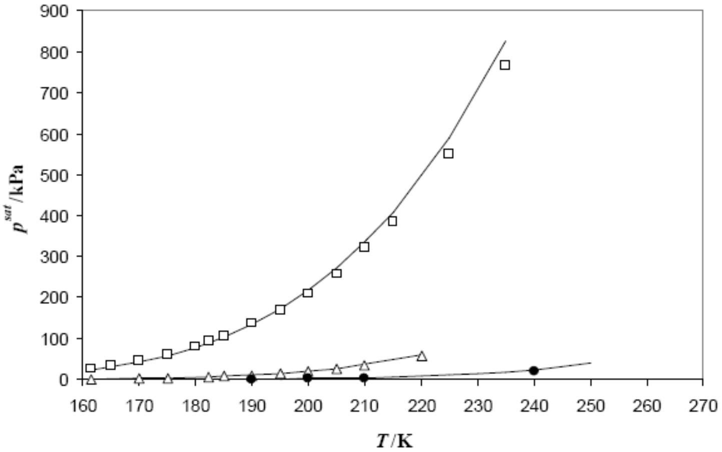

Figure 1. Vapour pressure curves as a function of temperature from simulation (ethane: open squares; propane: open triangles; butane: filled circles and form experimental correlation (lines)[4]

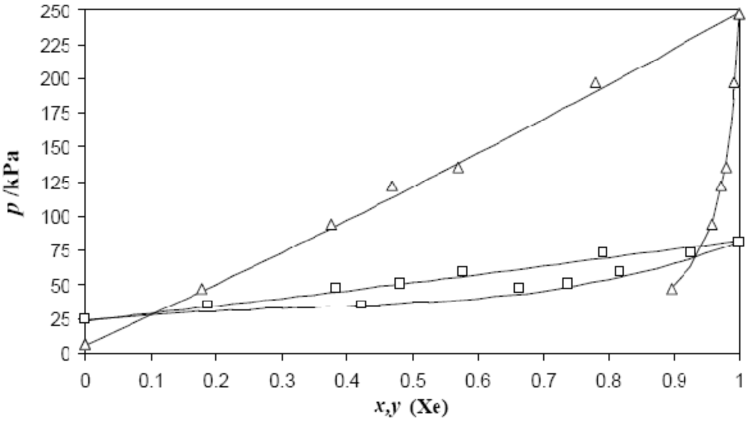

Figure 2. Coexistence curves from simulation results (marks) and correlation from experimental data[5] (lines) for systems xenon/ethane at 161.4 K (open squares) and xenon/propane at 182.34 K (open squares).

This work is a part of a larger project in progress concerning the thermodynamic properties of systems involving light alkanes and noble gases, where one of the main objectives is to test the predicting capability of vapour-liquid equilibria and excess properties in a wide range of temperatures by theoretical modelling.

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Kinematic viscosity of ternary solutions of ethanol, glycerol and water from 293.1 to 318.1 K

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Keywords: Capillary viscosimetry, Concentration, Temperature, Modeling.

Abstract

The purpose was to determine, using a capillary viscosimeter, kinematic viscosity data of aqueous solutions of ethanol and glycerol, at several temperatures (from 20 up to 45 ºC) and concentrations. Kinematic viscosity modelling with solution concentration and temperature simultaneously, based on previous models for respective binary systems, was successfully carried out. The estimated values by this model gave low deviations from the experimental data.

Introduction

Viscosity is an important transport property for equipments design and for evaluation of operational costs. Glycerol is very employed in the pharmaceutical and food industries because is nontoxic and can be considered as good solvent for organic and inorganic compounds. Aqueous solutions of glycerol with presence of alcohols are also frequently found in several industrial processes. Nowadays, these systems have also specially importance in the production of biofuels from vegetal oils and ethanol where glycerol is a by-product (Ito et al., 2005).

One of the aims of this work is to determine experimentally the kinematic viscosity of glycerol/ethanol/water ternary solutions. Viscosity of the corresponding ethanol/water and glycerol/water binary solutions are already studied in wide range of temperatures and concentrations (Chenlo et al., 2004; Moreira et al., 2007). From results corresponding to these binary solutions a model to estimate the kinematic viscosity of the ternary solutions is proposed and validated.

Materials and methods

The solutions were prepared by weight, with a Mettler AJ150 balance with a precision of ± 0.0001 g using degassed distilled water, commercial ethanol (Panreac, 96%) and glycerol (Merck > 99%).

Kinematic viscosities were determined by using a Schott-Gerate AVS 350 automatic Ubbelohde viscosimeter with the procedure previously described (Vázquez et al., 1997). All measurements were made at least in five replicates. Temperature control was assured by inserting the capillary viscosimeter into a water thermostatic bath with a Selecta Digiterm heating system (precision of ± 0.1ºC). The temperature was varied from 20 ºC up to 45 ºC by steps of 5 ºC. Upper temperature was limited by the presence of ethanol. The uncertainty in the measurements was within $\pm 0.001 \cdot 10^{-6}$ m^2/s and capillaries were periodically calibrated by determination of tri-distilled water at several temperatures.

In terms of concentration, the ternary system was generated from ethanol/water binary solutions with concentrations ranged from 0 up to 75% molar fraction adding different amounts of glycerol up to 60% molar fraction.

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Results and discussion

In all cases, kinematic viscosity decreased with temperature at fixed concentration, and increased with glycerol concentration at constant temperature. At same glycerol concentration, kinematic viscosity decreased with ethanol concentration.

The modelling of kinematic viscosities corresponding to these ternary solutions, ν (m^2/s), was carried out applied the following equation:

$$\nu = \nu_{eth/water} + a\left[\exp(bx_{gly}) - 1\right]$$

where x_{gly} is the molar fraction of glycerol in ternary solution, a (m^2/s) and b are parameters of fitting and $\nu_{eth/water}$ (m^2/s) is the kinematic viscosity of ethanol/water binary solutions previously modelled employing Redlich-Kister equation (Redlich and Kister, 1948) for the concentration effect and, for the dependence on temperature, empirical Vogel-Fulcher-Tamman (Cocchi et al., 1999) relation was selected. The model is given by:

$$\upsilon_{eth/water} = x\upsilon_{eth} + (1-x)\upsilon_{water} + x(1-x)\left[\exp\left(\frac{3255}{T} - 9.41\right) + \exp\left(\frac{-3917}{T} + 11.44\right)(1-2x) + \exp\left(\frac{5113}{T} - 16.06\right)(1-2x)^2\right]$$

where ν_{eth} and ν_{water} (m^2/s) are the kinematic viscosity of pure ethanol and water, x is the molar fraction of ethanol and T (K) the absolute temperature.

Parameter a was correlated simultaneously to T and x (ethanol concentration corresponding to binary system).

$$a = 1.39 + 5.64\left[\exp(-T/62.03\right] + \left[3.56 - \frac{89.18}{T^{1.5}}\right]x - 8.80x^2 + 5.91x^3$$

and parameter b showed only dependence on temperature according to:

$$b = 4.11 + 5.54\left[\exp(-T/25.03\right]$$

Using these equations is possible to reproduce satisfactorily the kinematic viscosity of ternary solutions of glycerol, ethanol and water in the range of concentrations and temperatures assayed with deviations less than 4%.

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Aqueous biphasic systems with ionic liquids

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Keywords: Ionic liquids, aqueous biphasic systems, inorganic salt, chaotropic, kosmotropic

Ionic liquids (ILs) have garnered increased attention recently due to their potential environmental benefits as “greener” replacements over conventional volatile organic solvents (VOCs), especially because they present negligible vapour pressures and therefore they do not evaporate at atmospheric pressure. ILs can, among others applications, replace VOCs in the liquid-liquid extraction providing a clean manufacturing and being environmental benign. ILs are composed by ions and it is possible to adjust the anion and/or the cation fine-tunning their chemical and physical properties (Huddleston et al., 1998).

Aqueous biphasic systems (ABSs) are very interesting in the biological field for the extraction, recovery and purification of biomolecules, such as proteins, enzymes and nucleic acids (Zhang, et al., 2007). Zhao et al. (2006) showed that enzymes could maintain their activity and stability in certain ionic media including ionic liquids. Hydrophilic or water miscible ILs can be induced to form ABSs by the addition of an aqueous salt solution, forming an upper IL rich-phase and a lower inorganic salt rich-phase improving the biomolecules extraction in liquid-liquid systems (Gutowski, et al., 2003). Nevertheless, there are many ILs intrinsic factors,, such as polarity, hydrogen-bond basicity, anion nucleophilicity and chaotropicity/kosmotropicity nature, that have shown to have a significant influence in the stability and activity of enzymes. In aqueous environment it was shown that enzymes are stabilized by the kosmotropic (anion) – chaotropic (cation) combination (Zhao et al., 2006).

The main goal of this study is to determine ABS phase diagrams at 298.15 K to evaluate the chaotropicity/kosmotropicity strength of the ILs ions. The turbidimetric titration method was used with K3PO4 and hydroplilic imidazolium-based ILs aqueous solutions. The ILs studied are preferentially based on kosmotropic anions and chaotropic cations. The selected ILs combination allowed the study of the cation alkyl chain length, the number of alkyl groups at the cation, the type of functional groups at the cation and the anion identity impact in their water-structuring ability. Other benign systems were also studied using the water-structuring sucrose and it was compared with the inorganic salt results. The coexistence curves were determined using an empirical mathematical description from relationships from volume and density data collected for each aqueous phase (Merchuk et al.,1998).

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Hydrophobic ionic liquids and water mutual solubilities

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Keywords: solubility measurements, ionic liquids, water.

Ionic liquids (ILs) belong to the molten salts group and are usually composed of large organic cations and organic or inorganic anions what allow them to remain liquid at or near room temperature. Their ionic nature afford them with an unusually and unique set of characteristics and properties such as negligible vapour pressures, a wide liquid range, a wide electrochemical window, non flammability at room temperature and high thermal stability. Furthermore, ILs present physicochemical properties that can be finely tuned by the appropriate selection of the cation and/or anion, which determine their high potencial to be exploited as "green solvents" (Freire et al. 2007). The ILs particular properties allow their use in several applications in the chemical industry such as solvents in organic synthesis, as homogeneous and biphasic transfer catalysts, and in electrochemistry. Another important field is the use of ILs for separation processes as extraction media where, among others, ILs have shown promising in the liquid-liquid extraction of organics from water. For the extraction of organic products from chemical reactions that proceed in aqueous media and for liquid-liquid extractions from aqueous phases, ILs with lower solubility in water are required, so the knowledge of their mutual solubilities with water is very important. While ILs vapour pressures are negligible it decreases the possibility of air pollution, but they could cause water contamination because of their potencial toxicity and limited biodegrability (Freire et al. 2008).

In this work a systematic study of the mutual solubilites between ILs and water from (288.15 to 318.15) K and at atmospheric pressure was conducted for imidazolium, pyridinium, piperidinium and pyrrolidinium-based cations and bis(trifluoromethylsulfonyl)imide, hexafluorophosphate and tricyanomethane-based anions ILs. The main goal of this work is to determine the impact of several IL structural modifications in their liquid-liquid phase behaviour with water keeping the possibility to design a solvent for a specific application, in this case to fine-tune a particular IL with known mutual solubilities with water. Moreover, from the experimental results the ILs hydrophobic tendency and thus their toxicity impact can be predicted.

The huge number of possible combinations of binary mixtures of water and ionic liquids make the exhaustive experimental measurement of all these systems impracticable requiring the use of a predictive model for their study. In this work, the predictive capability of COSMO-RS, a model based on unimolecular quantum chemistry calculations, was evaluated for the description of the liquid-liquid equilibria binary mixtures of water and the several family-based ILs experimentally studied.

One of the main contaminants of ionic liquids is the chloride ion that arises from their preparation. Seddon et al. (2000) have demonstrated the chloride ion influence through

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several ionic liquids physical and chemical properties such as density, viscosity and 1H NMR chemical shifts showing to have significant impacts. In this work the effect of the chloride ion was also evaluated through the ionic liquid solubility in water and vice-versa.

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Carbon dioxide solubilities in [$C_n$mim][Tf_2N] ionic liquids at high-pressure

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Abstract:
Ionic liquids (ILs) are a class of "*neoteric*" solvents commonly composed of relative large, asymmetrical and flexible organic cations and organic or inorganic anions that cannot form an ordered crystal and thus remain liquid at or near room temperature. Unlike molecular liquids, the ionic nature of these compounds results in an unique combination of intrinsic physical properties such as high stability, large liquid temperature range, nonvolatility, high ionic conductivity, nonflammability and good solvation properties for both polar and nonpolar compounds.

These outstanding characteristics, along with their easy manipulation, due to the possibility of interchangeability of thousands of cations and anions, cataloged ILs as "green" and "designer" solvents. Among the wide range of applications foreseeable for ILs such as solvents in organic synthesis, homogeneous and biphasic transfer catalysts, electrochemistry, chemical separation and extraction, absorption cycle processes, and gas storage, ILs great potential caught the attention of the industry and academia alike.

The solubility of gases provide useful information on the fundamental knowledge for physical and chemical interactions between these compounds on the molecular level. Thus, to evaluate the applicability or even establish proposals of a specific IL for a target application the knowledge of phase behavior (solubility) is of most importance.

In this work, solubility data of carbon dioxide (CO2) in the ionic liquids 1-alkyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([$C_n$mim][Tf_2N], n=2, 3, 4, 5, 6, 7, 8, 10) are investigated at pressures up to 45 MPa and at temperatures between 288 K and 343 K. The ability of the Cubic-plus-Association equation of state (CPA EoS) for modeling the gas solubilities in ionic liquids, in the temperature and pressure range studied, is also evaluated.

Physicochemical characterization of surface sized paper surfaces

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Keywords: Contact Angle, PCA, PLS, Printing quality, Profilometry, Surface sizing

At present, there is an increasing demand concerning the quality of printing and writing paper grades. Printing quality strongly depends on the properties of the fibrous matrix and on the characteristics of the paper surface, which are influenced by the quality of the pulp fibers, the refining process, the chemicals added in the preparation of the furnish, the operations at the paper machine and the modifications of the paper surface. These surface modifications include calendering and/or chemical treatments such as coating and surface sizing. Nowadays, chemical modifications of paper surface for improving printing quality is a common practice in papermaking for controlling paper surface characteristics, being surface sizing a common operation in many paper mills.

Surface sizing is affected by the sizing formulation properties (composition, viscosity, pH, and temperature) as well as the paper properties (basis weight, bulk, internal sizing, water content and surface energy, porosity and roughness). However, these properties must be adequately controlled in order to avoid too much penetration of the surface sizing agent in the sheet structure.

Typically, only starch is used as sizing agent, but there is an increasing tendency for combining this compound with synthetic polymers, as a consequence, there is a large increase in the production of new chemicals that meet specific end-use paper requirements.

For selecting the most appropriate surface sizing agent for a specific paper it is essential to perform tests at lab scale, since the surface sizing film changes the number and size of pores as well as paper roughness, and changes paper surface energy, so that paper ink interactions, that roll the printing process and the printing quality are modified.

The present study is mainly focused on the mathematical of the changes caused by the application of different surface sizing formulations, both in paper surface properties and in the printing quality

For that, several paper samples prepared with different surface sizing formulations were characterized by using profilometry and contact angle measurements. Additionally, a mask was printed onto the paper samples, by inkjet printing, and optical densities, Gamut areas and line quality were computed.

Two mathematical tools were applied to analyze the results: first Principal Component Analysis (PCA) was performed for each set of results (topographical parameters, properties achieved by contact angle measurements and printing quality parameters) in order to isolate inside each set the variables that better describe the paper samples. After, Partial Least Squares (PLS) was used to correlate the printing quality parameters with the physical and chemical paper surface properties.

Sub-micron size aragonite synthesis by double-jet precipitation

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Keywords: Aragonit, Precipitation, Ultrasonic field, Size distribution

Calcium carbonate displays three main morphologies, rhombohedral calcite, orthorhombic aragonite with whiskers appearance and spherical vaterite Aragonite, calcium carbonate high pressure polymorph is becoming more interesting due to its possible applications in biotechnology. Properties like purity, morphology, dimensions and particle size distribution, porosity, aggregation degree must be carefully controlled.

Aragonite with sub-micron size particles was synthesized in a liquid-liquid reaction by the controlled double jet precipitation method.

The reagents used in the synthesis were analytical grade calcium nitrate and potassium carbonate which were introduced into the reactor at the same time by means of peristaltic pumps. The reaction medium was an ethanol-water solution. The precipitation process depends on many parameters like supersaturation, pH, temperature, reaction time, agitation speed, feeding order of the reacting solutions, the nature and the presence/absence of the additives. This parameters influence the final solid phase morphology and also the particle sizes. Studying the temperature dependence we found that pure aragonite was formed at 50-55°C. Below 50°C a mixture of aragonite with calcite is formed.

The precipitation occurred in a mixed vessel using a magnetic stirrer and in ultrasonic field. The reagent solution concentrations were varied between 0.1M and 1M. All samples were characterized by FT-IR spectroscopy, XRD, optical and electron microscopy, particle size distribution by laser granulometer, microscopic measurements and dynamic light scattering method. The results proved that a very small amount of the precipitated particles were agglomerated and the mean particle sizes were around 1μm.

The population balance equation describing the precipitation process was integrated using the method of classes (Marshal P. et al, 1988). The kinetic parameters were estimated using a random search optimization method. The results obtained were in good agreement with experimental data and proved that in the present working conditions nucleation and growth are the main mechanisms while agglomeration is inhibited.

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Response surface methodology applied to the study of temperature and concentration effect on the rheology of cupuassu pulp (*Theobroma grandiflorum* Schum)

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Keywords: Cupuassu pulp, rheology, gum, temperature, concentration

Introduction

The cupuassu tree *Theobroma grandiflorum* is a typical Amazonian plant. Its fruit, the cupuassu, is one of the most popular fruits in the region with an increasing demand due to its organoleptic characteristics and multi use by the agro industry as juice, ice cream and jam. (Silva et al., 2003). Gums and other macromolecules are frequently used to control the texture and structure of food or other oil-in-water emulsions and dispersions. Another function of these macromolecule additives is control of crystallization, inhibition of syneresis, to prevent the disperse drops or particles from settling or creaming due to the increased viscosity and yield stress (Dickinson, 2003). Xanthan gum is microbial heteropolysaccharide produced by the aerobic fermentation of *Xanthomonas campestris*. The gum shows excellent stability in heat and acid systems (Casas et al. 2000). Knowledge of the rheological properties of fluid and semisolid foodstuffs is important in the design of flow processes in quality control, in storage and processing stability measurements, and in understanding and designing texture.

The aim of this research was to determine the effect of xanthan gum, addition at levels commonly used in food formulations on the rheological behavior of cupuassu pulp in steady shear.

Materials and methods

The cupuassu pulp was supplied by Cupuassu Cooperative from Pará, Brazil The xanthan gum was donated by CP KELCO BRAZIL S/A, São Paulo, Brazil

The cupuassu pulps (9 ± 0.03 ºBrix, pH, 3.3 ± 0.01, a_w, 0.99) .were prepared by mixing three concentrations of xanthan gum (0.3, 0.7 e 1) %, range frequently used in food systems.

The rheological measurements were made the 10, 20, 30, 40, 50 and 60ºC temperature range. The measurements were carried out with a concentric cylinder Thermo Haake rheometer, model Rheo Stress1, with measurement system Z20 Din. Each experimental run to the upward curve had duration of 2 min with shear rate range from 0.3 to 300 s^{-1} and 2 min to the downward curve with shear rate range from 300 to 0.3s^{-1}. The experiments were repeated twice, at least, always using new sample.

Results and discussion

Arrhenius and power law relationships were proposed (Eq.1) to fit apparent viscosity as a function of temperature and concentration at 100 s^{-1} and 300 s^{-1}, respectively.

$$\eta_a = 83.91 \times C^{0.71} \exp(1.25/RT) \qquad \eta_a = 32.29 \times C^{0.61} \exp(1.30/RT) \qquad (1)$$

Figure 1 presents response surfaces of apparent viscosity at these shear rates indicating a decrease of apparent viscosity of pulp at higher shear rates and with

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temperature and increase in the apparent viscosity at higher concentrations, as also shown by Assis et al. (2005), Haminiuk et al. (2006), Toralles et al. (2006).

The constants of equation 1 (R^2= 0.96) were obtained by non-linear regression, through Quasi-Newton procedure, from the Statistical program v. 7.0.

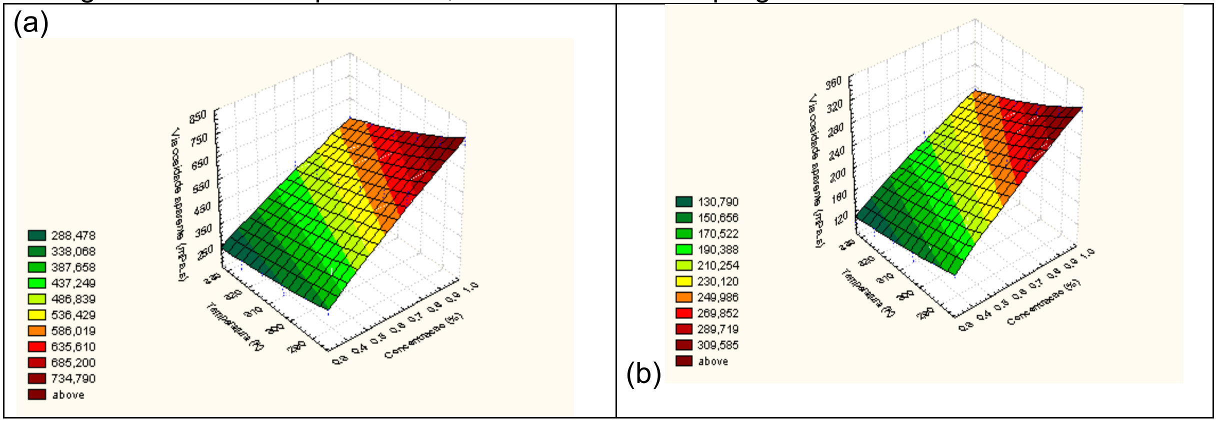

Figure 1: Response surfaces representing the effect of temperature and concentration on the apparent viscosity at 100 s^{-1}(a) and 300s^{-1} (b).

Conclusion

Equations derived from Arrhenius and power law relationships described very well the apparent viscosity at selected shear rates as a function of temperature from 10 to 60ºC and of concentration from 0.3 to 1%. The values of viscosity strongly depend on temperature and concentration of xanthan gum.

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Effect of temperature on rheological properties of Surinam cherry pulp

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Keywords: Pitanga, *Eugenia uniflora* L., Rheology

1 Introduction

The Surinam Cherry (*Eugenia uniflora* L.) is a tree widely distributed in South American countries, mainly in Brazil, Argentina, Uruguay and Paraguay (Consolini & Sarubbio, 2002), but it has been disseminated in other tropical and subtropical regions of the world, where it is valued by its fruit (Bezerra, Silva Junior and Lederman, 2000). Pitanga (Portuguese common name for fruit) is appreciated in nature, juices, ice creams, jellies and liquors being rich in vitamins A and C (Franzão & Melo, 2007).

Rheology is the science of the deformation and flow of matter and has many applications in the fields of food acceptability, processing, and handling (Barbosa-Cánovas, kokini, Ma & Ibaz, 1996). Rheological measurements are quite relevant in the food industry.

The objective of this work was to investigate the effect of different temperatures on the rheological properties of Surinam Cherry pulp.

2 Material and Methods

The Surinam Cherry used in this work was obtained from a single batch from Campina Grande, Paraíba State, Brazil The fruits were chosen based on their skin color, appearance and ripeness. The fruits were processed through a pulping machine.

The rheological behavior of pulp was measured with the use of a controlled stress Rheometer Haake RS 100, in the temperature range 10–50°C were conducted over a shear rate range of 0.1 - 300 s^{-1}.

3 Results and Discussion

The whole pulp was adequately described by the Ostwald-De-Waele or Power Law model ($\tau = K \dot{\gamma}^n$), showed values of R^2 closer to one and values of χ^2 closer to zero (Table 1).

Table 1. Flow parameters of the pitanga pulp determined by Power Law equation

T, ºC	K_H, Pa.s^n	n_H	χ^2	R^2
10	0.146	0.702	0.335	0.996
20	0.267	0.569	0.246	0.996
30	0.273	0.548	0.269	0.995
40	0.287	0.520	0.214	0.995
50	0.327	0.479	0.190	0.994

Figure 1 represents the relation between shear stress and shear rate at all the temperatures studied with whole Surinam Cherry pulp.

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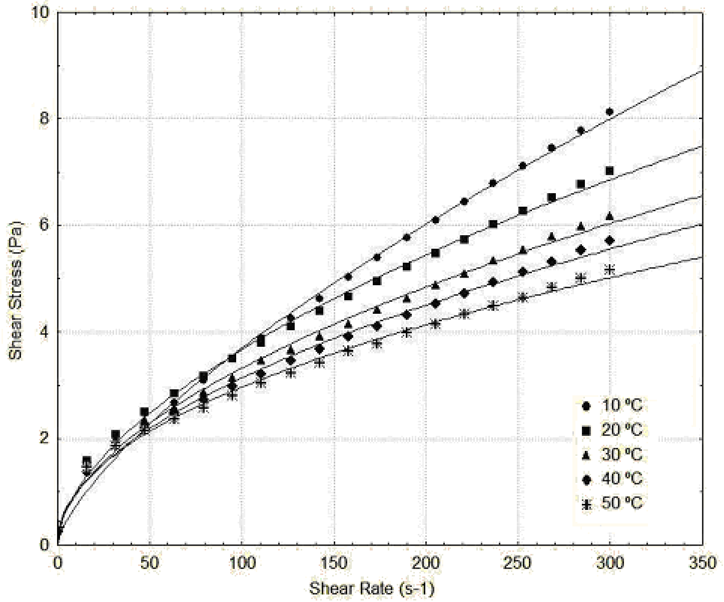

Figure 1. Shear stress-shear rate plots for pitanga pulp

The sample exhibited shear thinning behavior, The whole pulp was adequately described by the Ostwald-De-Waele or Power Law model and exhibited shear thinning behavior, that is, an increase in temperature causes a decrease in apparent viscosity and a increase in consistency coefficient, as expected for a fruit pulp.The effect of temperature on the viscosity can be described by means of an Arrhenius-type equation ($\eta_a = \eta_o \exp(E_a/RT)$ and the Ea (activation energy) determined at a shear rate of $100s^{-1}$ was 1.082 Kcal/gmol.

Conclusion

Rheological analysis showed that pitanga pulp exhibited pseudoplastic behavior (flux index of n< 1). The Arrhenius model gave a good description of temperature effect on apparent viscosity of the pulp.

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Change on rheological properties of mushroom cream due to freezing process

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Keywords: Rheological properties, mushroom cream, freeze-thaw treatment.

This study was carried out to determine the effect of the freeze-thaw treatment on rheological behaviour and the activation energy in mushrooms cream. Freeze-thaw treatment was made at different times of frozen storage; it was within a range of 0 to 6 weeks. For each freezing cycle, the sample was submitted to rheological measurements within a range of temperature between 20 and 40°C.

A rotational, concentric cylinder viscosimeter (Viscotester VT550) was used in this study. In this type of viscosimeter, the outer cylinder is fixed and the inner cylinder rotates. This apparatus measures the shear rate and the apparent viscosity of a fluid at a certain temperature. The apparent viscosity, η, is defined as the ratio of shear stress, τ, to that of shear rate, γ, (η = τ/ γ), which was varied between 17.8 and 445 s-1. The SV1 sensor system was used. The amount of sample to be used must be adjusted in order for the top surface or the inner cylinder to be just covered. The temperature of this system was kept constant by means of a thermal liquid circulator.

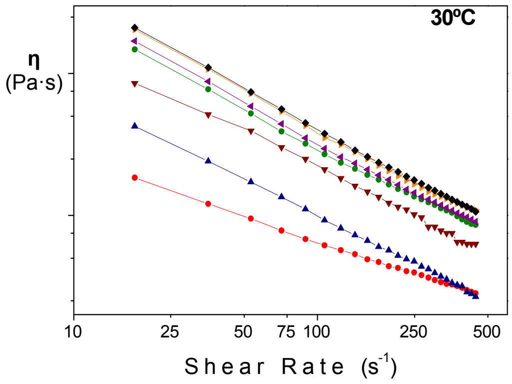

Figure 1. Viscosity vs. shear rate for all time of frozen storage: (–●–) 0 Week, (–▲–) 1st Week, (–▼–) 2nd Week, (–●–) 3rd Week, (–◄–) 4th Week, (–►–) 5th Week, (–◆–) 6th Week.

All Flow curves were fitted to the Power Law or Ostwald de Waele model. Since, this model described very well the flow behaviour of analyzed samples, for each temperature and time of frozen storage, the adjusted determination coefficient being in all cases higher than 0.999.

Trypsin purification by using *Cratylia mollis* trypsin inhibitor immobilized on paramagnetic particles

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Key Words: *Cratylia Mollis* Trypsin Inhibitor, Paramagnetic Particles, Fish Trypsin, Tilapia, *Oreochromis.*

Introduction

The application of trypsin inhibitors immobilized in insoluble supports is widely employed to purify trypsin by affinity chromatography (El-Shemy and Levin, 1997). Seeds of Leguminosae family are rich in serineproteinase inhibitors. Paiva et al. (2006) has isolated a trypsin inhibitor from *Cratylia mollis* seeds (CmTI), a Brazilian native bean. Comparison of its primary structure revealed homology with plant inhibitors of the Bowman Birk family. Magnetic materials as matrices for enzyme immobilization have been extensively used because the advantages of water insoluble enzymatic derivatives are combined by their ease removal from the reaction mixture by a magnetic field (Amaral et al., 2006). In this work, CmTI was covalently immobilized on paramagnetic particles and the derivative was used to purify a trypsin from tilapia (*Oreochromis niloticus*), a worldly important freshwater fish.

Material and methods

The Bowman-Birk-type *C. mollis* seeds trypsin inhibitor (CmTI) was purified according to Paiva et al. (2006). Fish trypsin was obtained from the crude extract of tilapia (*O. niloticus*) intestine (20 mg of tissue/mL). Paramagnetic particles-PMP, a derivative of silica, (20.0 mg) was activated for 2h at 25°C with 2 mL of 5% (v/v) glutaraldehyde solution in 0.1 M Tris-HCl buffer, pH 8.0, under rotatory agitation of 20 rpm. The particles were collected under a magnetic field (6,000 Oe) and washed 10 times with 2.0 mL of Tris-HCl buffer. The activated support was incubated with 1 mL of CmTI solution (0.662 mg/mL), for 16h at 4°C, under rotatory agitation (20 rpm). Then, the support was washed 6 times with 2.0 mL of Tris-HCl buffer. Afterwards, the CmTI-PMP was incubated with 1.0 M glycin solution (2 mL) for 16h at 4°C, under rotatory agitation (20 rpm) and washed 10 times with Tris-HCl buffer. A solution (2 mL) containing ovalbumin (100 µg) and porcine trypsin (100 µg), prepared in Tris-HCl buffer, was incubated with CmTI-PMP (20.0 mg) for 30 min at 25°C under stirring (20 rpm). Similar incubation was also carried out with the 30-80% ammonium sulfate fraction of the tilapia intestine. The particles were collected and washed 5 times with Tris-HCl buffer to remove unbound proteins. Then the particles were washed 5 times with 0.5 M KCl/HCl buffer, pH 2.0, to elute eventual protein specifically complexed to the immobilized CmTI. The elutes pH values were immediately raised to 8.0 by adding 1.0 M Tris-HCl buffer. The proteolytic activities were determined according to Erlanger et al. (1961), using benzoyl-DL-arginine-p-nitroanilide – BAPNA (Sigma) as substrate. Protein was spectrophotometrically measured at 280 nm. Polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to Laemmli (1970), using 6% (w/v) stacking gel and 10% (w/v) separating gel.

Results and Discussion

The synthesis of CmTI-PMP yielded a preparation containing an amount of 33.0 µg protein per mg of PMP. Its use to purify trypsin was firstly investigated by using a mixture of ovalbumin and porcine trypsin (31.5 mU/mg protein). Unbound proteins (ovalbumin and free trypsin) were washed out during the five washings carried out with Tris-HCl buffer. No enzymatic activity and 11.3 µg of protein were detected in these washings. However, the presence of enzyme and protein were detected in the 1st KCl-HCl washing, presenting specific activity of 117.1 mU/mg, i.e., a purification of 3.7 folds. It is important to register that further Tris-HCl buffer washings of the KCl-HCl treated CmTI-PMP allowed this derivative to be reused. An enzyme 3-fold purified was collected presenting a yield of 7 % and, a total purification of 33.4 times was attained by using heat treatment, ammonium sulfate precipitation and CmTI-PMP affinity binding. The SDS-PAGE electrophoresis of the KCl-HCl fraction revealed a unique protein band (Figure 1) with an apparent molecular weight of 23.5 kDa. Similar molecular weights have been reported in the literature for trypsin from other sources (Rafiq and Bailey,1996).

Conclusion

Trypsin inhibitor from *Cratylia mollis* seeds covalently immobilized on paramagnetic particles, via glutaraldehyde, was successfully used to purify porcine trypsin and fish intestine trypsin. The magnetic derivative makes the washing procedures easier and faster under a magnetic field. Furthermore, its reuse showed to be feasible after final washing with Tris-HCl buffer.

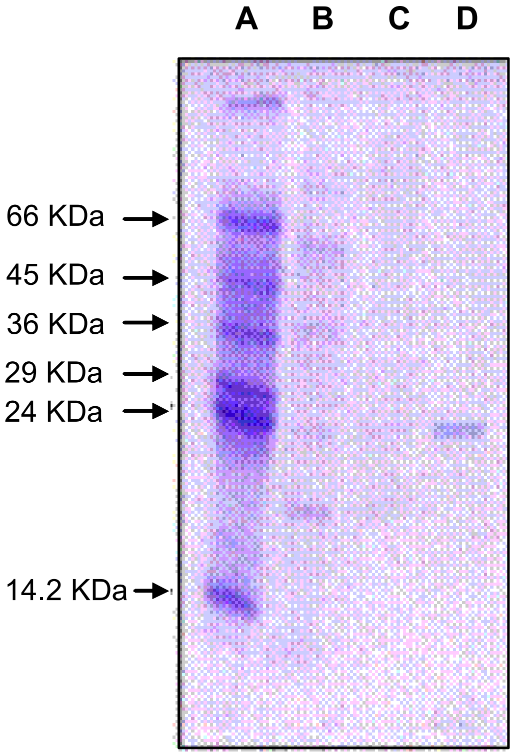

Figure 1 SDS-PAGE electrophoresis of trypsin from tilapia intestine. A: molecular weights; B: crude extract; C: 30-80% ammonium sulfate fraction; D: purified fish trypsin.

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The effect of operating conditions on styrene bulk polymerization in a spinning disk reactor

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Keywords: bulk polymerization, polystyrene, spinning disk reactor, stirred batch reactor

In this work the bulk polymerization of styrene with 2,2-Azobisisobutyronithrile as an initiator in a Spinning disk reactor(SDR) has been studied.

The reactor consisted of 2 surfaces that polymerization was carried out on the underneath surface. The upper surface keeps nitrogen gas on the reaction place. Experiments were carried out in 70 °C by 0.5 weight % to monomer.

Free radical polymerization technique is widely used by industry for polymerization of styrene. Industrial polymerization process has been traditionally carried out in large stirred tanks. In the market world, quality is one of the most important parameters of competition. SDR reactor technology uses centrifugal accelerations to create thin highly sheared films on rotating surfaces so it provides good mixing as well as high mass and heat transfer.These characteristics can solve problems of bulk process significantly in high polymer concentration and viscosity during polymerization and it can be reached to the uniformity in the mixture. Excellent mixing as well as plug flow characteristics should aid in producing polymer products characterized by narrow molecular weight distribution. In the application of the spinning disk technology time scale is the other benefits in polymerization process. The reactor set-up that used in this study is shown in figure1.

Figure1.SDR reactor

Various parameters for flow on the spinning disk surface have been derived on the basis of centrifugal model.The SDR parameters of interest in this study are the disc residence time t_{res} and the average shear rate $\dot{\gamma}_{ave}$ as expressed in eqs (1), (2) respectively.

$$t_{res} = \left(\frac{81\pi^2\nu}{16\omega^2 Q^2}\right)^{1/3}\left(r_o^{4/3} - r_i^{4/3}\right) \quad (1)$$

$$\dot{\gamma}_{ave} = 1.5\left(\frac{Q\omega^4 r}{18\pi\upsilon^2}\right)^{1/3} \quad (2)$$

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The mean residence time represents the contact time between the various reacting species on the rotating disc while the average shear rate characterizes the degree of mixing achieved in the reacting film.

In this study different feed conversions are led to disk. The conversion results of runs in SDR can be seen in fig1.as it has shown by increasing feed conversion, there is an increase in product conversion. As we know by proceeding polymerization the viscosity of mixture will be increased so at fixed rotating speed the residence time will be increased so the liquid has higher time exposure on disk and polymerization proceed to higher conversions.The batch time means the time that need to reach to the same conversion in the batch.

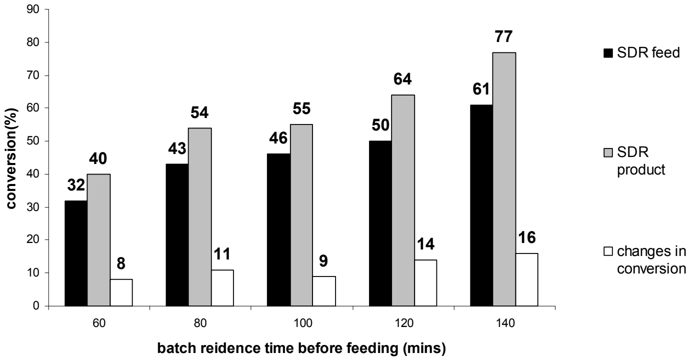

Figure2. Effect of pre-polymer conversion at fixed speed of 1500rpm in SDR

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Synthesis of crosslinked poly(vinyl chloride): Study of polymer properties

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Keywords: Poly(vinyl chloride), copolymerization, crosslink, polymer properties.

The world consumption of poly(vinyl chloride) (PVC) in 2007 was around 33.5 million of metric tons, only second considering the different types of poly(ethylene) (Bryen, 2007). Although the vast majority of applications are linked directly with its excellent mechanical properties, compatibility with additives, recyclability and good cost to performance ratio, the major disadvantages comes from its lower heat stability and limited range of temperatures on the final applications (Beltran *et al*, 1998; Yong-Zhong *et al*, 1999). To overcome these deficiencies, the application of the copolymerization technique intends to incorporate a new monomer on the poly(vinyl chloride) growing chain, in order to provide new properties or improve the original ones (Burgess, 1982)

A particular type of copolymerization, consists in the application of a small amount of a difunctional (divinyl or diallyl) monomer to prepare a chemically crosslinked PVC (Burgess, 1982). Besides other properties, this technique has been continuously studied and developed for the production of special polymers for matte surfaces in top coated finished products (Amano *et al*, 1992; Yong-Zhong *et al*, 1999; Koga *et al*, 1997).

The crosslinked polymer normally follows significant changes on the structure and final behaviour of the material. If previous soluble in the presence of a solvent, the crosslinked polymer swells as the solvent penetrates the network, producing a solvent-swollen crosslinked polymer so called a gel (Stevens, 1990).

The denominated crosslinking agents, such as divinylbenzene, glycol dimethyl acrylate, diallyl maleate or phthalate, have been reported on the literature as efficient gel promoters (Burgess, 1982). Nevertheless, a new investigation was carried out with new difunctional monomers, like ethyl glycol dimethyl acrylate (EGDMA), polyethylene glycol dimethyl acrylate (PEG Mn: 550) and the correspondent high molecular weight (PEG Mn: 750). Also, besides the study of the effect of co-monomer dosage on the obtained gel fraction, the study of the correspondent thermostability and influence on Tg, can give a valuable information over the produced copolymer and potential market applications.

Moreover, the application of a given standard formulation and procedure for the processing of the final crosslinked polymer, can provide a further evaluation of its processability, mechanical toughness and type of obtained top surface (matte/bright).

Figure 1, shows the polymer gel fraction *versus* the EGDMA dosage on a vinyl chloride suspension polymerization. Also, in figure 2, the obtained matte top effect of a crosslinked polymer dry blended with a homopolymer product in different mass fractions.

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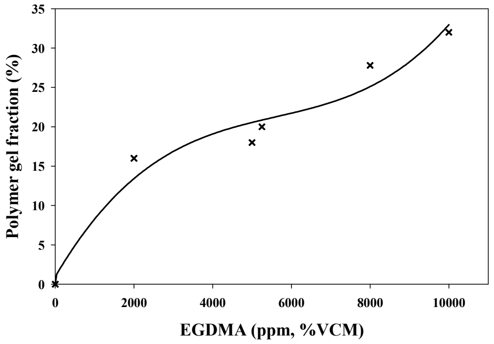

Figure 1: Polymer gel fraction *versus* the EGDMA dosage on the vinyl chloride suspension polymerization.

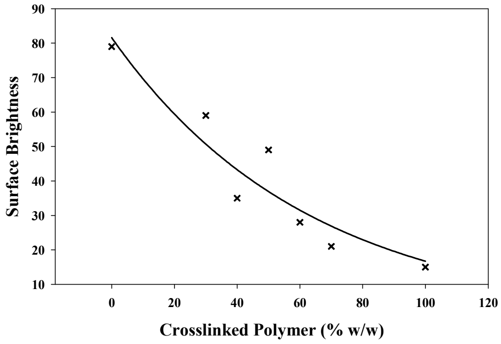

Figure 2: Matte top effect of a crosslinked polymer dry blended with a standard homopolymer in different mass fractions.

Acknowledgments

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Experimental study of the TEMPO mediated copolymerization of styrene with divinylbenzene

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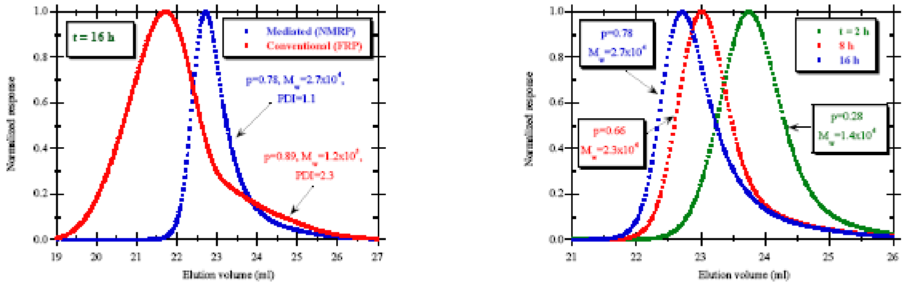
Keywords: Crosslinking, Nitroxide-Mediated, Kinetic, Modeling, Radius of Gyration.

Since the discovery of controlled radical polymerization (CRP) in the early nineties (Georges et al., 1993), an ever increasing activity has been focused on the production of polymers with narrow molecular weight distributions and well-defined architectures (such as block copolymers, stars or brushes). This work describes an experimental research on the nitroxide-mediated radical polymerization (NMRP) of styrene (S) and divinylbenzenes (DVB) in xylene (X) solution at 130 °C, using the stable radical TEMPO (2,2,6,6-tetramethylpiperidinyl-1-oxy) as mediator and AIBN (azobisisobutyronitrile) as initiator. Measurements of absolute molecular weights and *z*-average radius of gyration of the produced copolymers have been performed for different polymerization times using a SEC system with coupled refractive index (RI) and multi-angle laser light scattering (MALLS) detectors.



(a) (b)

Figure 1: (a) Observed chromatograms (RI) of polystyrene (PS) samples synthesized at 130 °C using conventional free radical polymerization (FRP) and NMRP. In both cases the polymerization time is t = 16 h. Polymerizations in S/X solution (50 % v/v) using the same initial ratio S/AIBN in both runs: $S_0 = 4.4$, $AIBN_0 = 0.0086$ and $TEMPO_0 = 0.0096$ mol dm^{-3} (NMRP experiment). (b) Observed chromatograms of NMRP synthesized PS samples (same conditions as (a)) with different polymerization times showing the growth of MW without concomitant broadening of the distribution (PDI around 1.1).

Experimental measurements are compared with the predictions of a recently developed general kinetic approach for dealing with very complex generic irreversible polymerizations (Costa and Dias, 2007, 2005; Dias and Costa, 2007, 2006). A kinetic scheme comprising a total of 36 chemical species and 125 different chemical reactions is considered in the modeling studies. Besides multiple living/dormant species exchange reactions, other pecularities such as the thermal initiation of styrene (Fu et al., 2007; Zhang and Ray, 2002) and the different reactivities of the pendant double bonds of the two isomers of DVB are included in these studies. Figure 1 shows in detail the improved control of the molecular architecture achievable in NMRP synthesized PS samples as compared to the FRP products. Figure 2 (a) shows the impact on the polymer molecular sizes of the presence of a small amount of DVB (about 0.5% mole fraction) in the NMRP polymerization system. Figure 2 (b) compares the observed and

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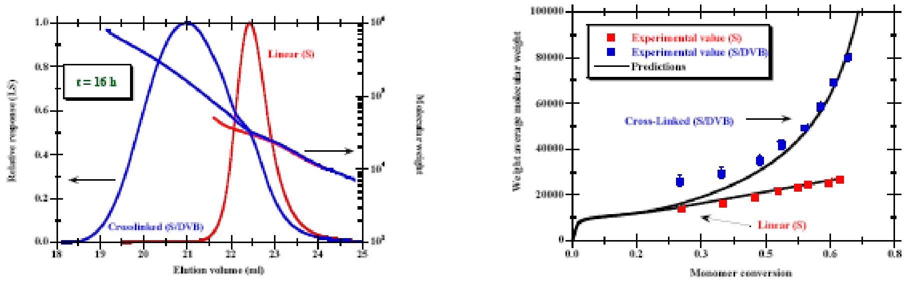
predicted evolution of $\overline{M}_w$ for linear and non-linear polymer formation. The well-known linear growth of $\overline{M}_w$ for linear PS does not occur with S/DVB copolymerization owing to the development of branching.



(a) (b)

Figure 2: (a) Observed chromatograms (MALLS) of PS and S/DVB samples synthesized using NMRP (see also Fig. (1)). Copolymerization S/DVB was performed with $\mathrm{DVB}_0 = 0.022\ \mathrm{mol\,dm^{-3}}$. (b) Predicted and measured $\overline{M}_w$ in linear (S) and non-linear (S/DVB) NMRP polymerizations.

With this study it is intended to improve the knowledge about the synthesis of non-linear polymers of controlled molecular architecture having nowadays important applications in different fields such as Biomedicine and Pharmaceutical industry.

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The effect of shear on styrene polymerization

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Keywords: polymerization, polystyrene, shear, AIBN

The bulk polymerization is an industrially used procedure that produces million tones of polystyrenes. Due to increasing market competitiveness there is a growing need for industry to produce high quality polymer product as well as reducing process time. Commercial polymerization processes have been carried out in large tanks. One problem encountered is the inadequate heat transfer surfaces for the removal of the heat generated during the polymerization reaction. This problem is more sever in bulk process where the viscosity of the mixture increases during the course of the polymerization. The spinning disk technology uses the centrifugal accelerations to create thin, highly sheared films on rotating surfaces.within this films result in significant enhancement in heat and mass transfer rates.

Methods. Styrene monomer and AIBN as initiator from Merck Co are being used for experiments. Our procedure of styrene polymerization included 2 stages. Firstly, a pre polymer is prepared in batch reactor. Tests were carried out to investigate the effectiveness of the spinning disc on bulk polymerization of styrene. 200 ml styrene polymerized with 1 % w/w AIBN as initiator at 70 °C. Samples were taken every 5 minutes for determining the conversion.

In second stage hot Pre-polymer from batch stage is fed into the center of disc through a feeder. centrifugal forces of rotating disc force the liquid to flow over the disc from the center to edge of surface in form of very thin film, the sheared film gets thrown off the surface, hits the cold walls and polymerization is being stopped. The product collected and conversion were determined. A schematic of SDR reactor is shown in Fig 1. It consists of a chamber enclosed in water cooled housing; the chamber is located on a double pipe rotating shaft driven by an electric motor. The rotating disc system is made of stainless steel 316 and was 20 mm in diameter. The surface of disc is smooth. The disc is rotated by an electromotor positioned on the central shaft, which supported double disc arrangement. The speed of disc is variable by inverter system that is connected to the rotating shaft. The system can used to vary the disc speed in the range 0-3600 rpm. Several copper coils are around the out side of reactor for preventing further polymerization and cooling the product. The temperature of disc is controlled by continues flow of water through a narrow channel underneath it. The water is pumped from a temperature – controlled water bath up the central shaft pipe and is returned to the bath through the outlet shaft pipe.

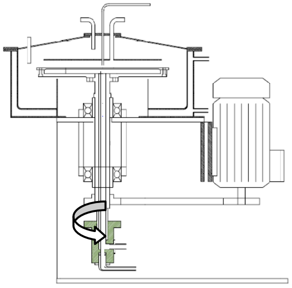

Fig1. Schematic of Spinning disk Reactor

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Effect of disk speed. The changes in conversion of the polymer collected from spinning disk surface at disk rotational speeds in the range 400-1500 rpm and fixed SDR feed conversion of 46% can be seen in Fig 2. The maximum increase in conversion of 16% can be obtained at disk speed of 1500 rpm. It is believed that by changing the disc size the optimum speed can change. By increasing the speed, the residence time will be decreased but shear will be increased and the shear has more influential role for increasing the conversion.

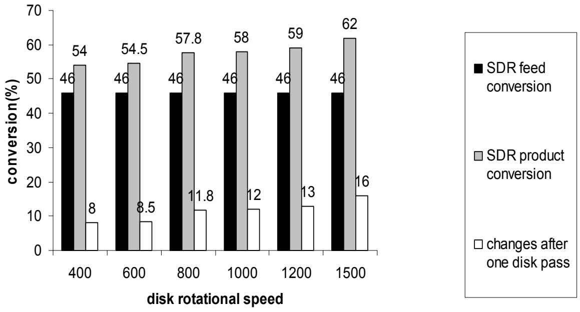

Figure2. Effect of disk speed at fixed pre-polymer conversion of 46%in SDR

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Novel stimulus responsive coating for polystyrene particles

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Keywords: Stymulus-Responsive Polymers, Poly(N,N-dimethylaminoethylmethacrylamide), Poly(N-methylmethacrylate), Rheology Behaviour

Smart or intelligent (co)polymers are materials which exhibit reversible changes in properties, in response to small physical or chemical changes in their environment. The stimulus can be the exposure to the light (UV irradiation), a mechanic constraint, an application of an electric or magnetic field, changes in environmental conditions (like pH, ionic strength, temperature) or the polymer molecular weight and its polydispersity and tacticity. The applications of these kind of materials have wide ranging uses in areas such as: environmental (depollution of water), biomedical (implants), biological molecules recognition, pharmaceutics (drug-delivery systems), personal care products (shampoos, cosmetics) and agrochemical formulations.

The aim of this project is to characterize a novel stimulus responsive particle coating, polystyrene, based upon poly (N, N-dimethylaminoethylmethacrylate) (PDMAEMA) and poly (N-methylmethacrylate) (PMMA). The styrene block is an excellent anchor point for linkers. However, it has the disadvantage of being quite hydrophobic. Thus, coupling PDMAEMA-PMMA to the styrene polymer should provide a greater range solvent compatibility over pure grafted DMAEMA or MMA. To characterize the PS/(PDMAEMA-PMMA) suspension, a BI-200SM Laser Light Scattering Goniometer was used to measure the hydrodynamic diameter and size distribution as function of the temperature pH and of the KNO_3 concentration. A Zetasizer Nano ZS was used to measure the zeta potential as a function of the pH and of the KNO_3 concentration. Finally, a Bohlin C-VOR Rheometer was used to study the fluid behaviour at different pH ranges.

PDMAEMA has a hydrophilic amine group and two hydrophobic methyl groups. At very low temperatures, lower critical solution temperature (LCST), the solution is stabilized by the presence of hydrogen bonds between the amine groups and water and by ice-like structures which water molecules form around the hydrophobic groups. As the temperature increases, the hydrogen bonding weakens and the attraction between hydrophobic groups increases leading to eventual demixing of the PDMAEMA chains, above the LCST. The loss of entropy by demixing is more than compensated by the gain of entropy from the water molecules that lose their ice-like structure in the presence of the dimethyl. The same situation can be observed with the change of pH. The interest in LCST behaviour is very important, because it is possible to adjust the final material properties by a single temperature change.

We observed that the diameter of PS/(PDMAEMA-PMMA) particles (i) for a low pH shows a monotonic rise with increasing pH, and then reaches a peak value of ≈ 380 nm, (ii) from pH = 5 to 6, the hydrodynamic diameter stays constant and (iii) from pH = 6 to 8.40 it decreases gradually to ≈ 230 nm. For pH with values above 8.40, the hydrodynamic diameter increases, due to the flocculation. This phenomenon, in this pH range, is stronger. Under pH 6, the PDMAEMA are protonated and the repulsion between NH^+ tends to swell the polymeric chains grafted to the polystyrene surface. A decrease in pH (pH< 5) leads to a higher protonation degree of PDMAEMA chains and thereby a stronger repulsion, increasing the hydrodynamic diameter. However, the decrease of the pH also results in adverse effects of swelling. Indeed, for a lower pH range, the concentration of NO_3 ions increase (from HNO_3 solution) and this tends to disrupt the hydrophobic hydration, therefore the chains collapses due to the polymer-polymer interactions. In collapse state polystyrene rule the suspension and the zeta potential decrease. A higher concentration of HNO_3 induces a higher ionic strength;

hence a higher concentration of counterions induces a smaller repulsion between the polymeric chains on the particles surface as the protonated nitrogen ions are screened from each other. The [NO_3^-] is enough to penetrate into the brushes of the polymers chains, to push over the water molecules and to relocate, between NH^+ and the adjacent polymer chains. In this case, we suggest that this mechanism is the same as the one acting in the presence of high salt concentrations. As salt concentration rises, the isoelectric point (IEP) and hydrodynamic diameter average of PS/(PDMAEMA-PMMA) suspension decreases.

Another process that can also occur is the cationic flocculation. We realize that the H^+ from the acid solution may establish bonds between nitrogens. When the samples are dipped in an acidic concentrate solution for equilibrium, the band N-H becomes more intense than when dipped to equilibrium in pure water or alkali solutions. Hence, the hydrogen bonding can be a non-negligible force in this system and can act to facilitate aggregation of the PDMAEMA chains. At pH = 8.40 the flocculation occurred, as we mentioned, in DLS measurements. A KOH solution was used to increase the pH, which resulted in the deprotonation of nitrogen atoms. The attraction force between hydrophobic groups increases and the PDMAEMA chains start to collapse and the aggregation between the chains of different particles occurs (pH> 8.50).

In order to understand the behaviour of PS/ (PDMAEMA and PMMA) fluid, it was performed rheology measurements, Flux and Oscillation tests. Six samples with different concentrations, 1, 5, 10, 23, 29 and 40% (w/w) were studied. For 5< pH< 7, the fluid has a Newtonian behaviour and for pH values under 5 and above 7, the PS/(PDMAEMA and PMMA) presents as Shear Thinning fluid. The aggregation phenomenon occurs in this pH range. For pH's 7.31 and 7.84, the viscosity is larger than for basic pH, because it is near of the IEP. The Van der Walls forces are higher than the repulsion forces between the electrical double layers and flocculation occurs.

The gyratory movement of the bob propitiates an increase in collision between the particles, which favours the flocculation process. However, the shear provokes the rupture of the weak flocs and the viscosity decreases. At around pH 7 the flocs are more resistant, but less dense compared with the basic pH values. If we observe the DLS measurements, it is possible to establish a relation between the swelling and shrinking of polymer chains with the flocculation phenomenon, and thus with rheology. For pH's above 7 the PDMAEMA collapse and, if the flocculation occurs, it forms hard flocs - Charge Patch Flocculation. For 5< pH< 7, the polymer swells and therefore the chains aggregate - Bridging Flocculation. In this last flocculation mode, the flocs have no resistance and they are destroyed when exposed to the bob movement. It was to expect for pH 4.28 the same rheological behaviour, Newtonian behaviour, that we were observed at pH 6.04. As previously mentioned, it was suggested that H^+ and NO_3^- played bonds between the amine group nitrogens and that is why the polymeric chains shrunk. If we plot high or low shear viscosity and hydrodynamic diameter average versus pH, we can observe that the ranges, where the diameter and viscosity decrease, are the same. The same transition occurs with different properties.

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Molybdenum η^3-allyldicarbonyl complexes as a new class of precursors for highly reactive epoxidation catalysts with *tert*-butyl hydroperoxide

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Keywords: Molybdenum complexes, N ligands, allyl, catalytic epoxidation

η^3-Allyldicarbonyl complexes of Mo and W play an important role in both coordination chemistry (Yamaguchi et al., 2004; Krafft et al., 1999; Barrado et al.,1998) and catalytic organic transformations, and their reactivity depends on the nature of the ligands completing the coordination sphere (Krska et al., 2002; Trost et al., 2002; Luft et al., 2002).
In the present work, we set out to prepare complexes of the type $[Mo(\eta^3\text{-allyl})X(CO)_2(N\text{-}N)]$ (X = Br, Cl; allyl = C_3H_5 or C_5H_5O) by reaction of the parent species $[Mo(\eta^3\text{-allyl})X(CO)_2(CH_3CN)_2]$ with several bidentate chelating 1,4-diazobutadiene ligands and to examine, for the first time, how the η^3-allyldicarbonyl Mo(II) complexes perform as catalyst precursors for the epoxidation of olefins using *tert*-butyl hydroperoxide (TBHP). For comparison, two different allyl ligands were used in the organometallic complexes.
All Mo complexes were catalytically active catalysts for epoxidation of several cyclic and linear olefins. The initial rate of olefin epoxidation depends on the nature of the X, N-donor ligands and π-allyl ligands of the original compounds. The acetonitrile precursors were generally more active than the diimine complexes, and the chloro derivatives more than the bromo ones. No major decrease (or none at all) in olefin conversion was observed for two consecutive catalytic runs.

Combined experimental (vibrational spectroscopy) and theoretical (DFT) studies carried out for one of the diimine complexes suggest that a probable intermediate is a dimer of the type $[\{MoO_2(N\text{-}N)\}_2\text{-}(\mu\text{-}O)_2]$. This proposal is also supported by the fact that similar olefin conversions are achieved after a relatively short period of time for complexes with the same N-donor ligands, independently of X and allyl ligands.

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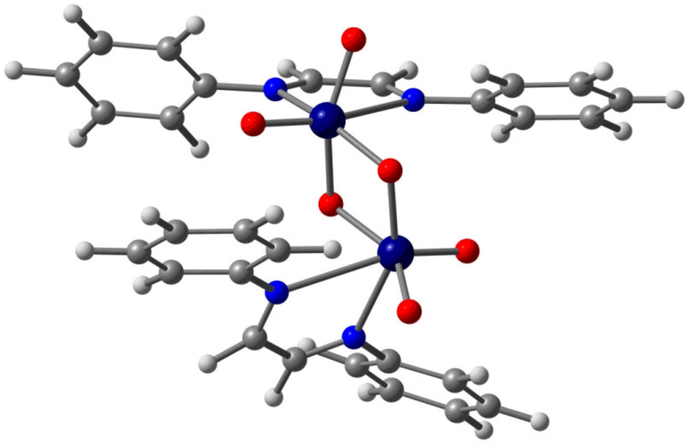

Figure 1. DFT optimized geometry of a probable intermediate Mo(VI) dimer [$\{MoO_2(N\text{-}N)\}_2\text{-}(\mu\text{-}O)_2$].

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Cadmium (II) removal from aqueous solution using microporous titanosilicate ETS-4 and ETS-10

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Keywords: ETS-4, ETS-10, Ion Exchange, Batch experiments, Nernst-Planck equations

It is well known that heavy metals are very toxic elements and their discharge into receiving waters causes detrimental effects on human health and the environment. For this reason, there is a constant need to remove heavy metals from industrial effluents and other wastewaters (Biskup and Subotic, 2004). Cadmium is one of the most toxic non-essential heavy metals present in the environment, even at low concentrations. Therefore, removal of trace levels of cadmium is required.

Crystalline microporous titanosilicates have been recently recognized by their interesting adsorption properties and ion exchange capability, as well as relatively easy catalytic activation (Lin et al., 2004; Rocha and Anderson, 2000).

The ability of microporous titanosilicates ETS-4 and ETS-10 to uptake the Cd^{2+} ion from aqueous solutions has been investigated, assessing the potential of these materials in water remediation. With this purpose, batch stirred tank experiments have been carried out by contacting a fixed volume of solution with known masses of ETS-4 or ETS-10. The evolution of the cadmium concentration with time has been monitored by Inductively Coupled Plasma Mass Spectrometry. pH is an important variable in on-exchange processes, hence it has been studied in detail.

In Figure 1 some experimental results for ETS-4 are presented. In the case of ETS-10 it has been found that the kinetics of removal is extremely fast.

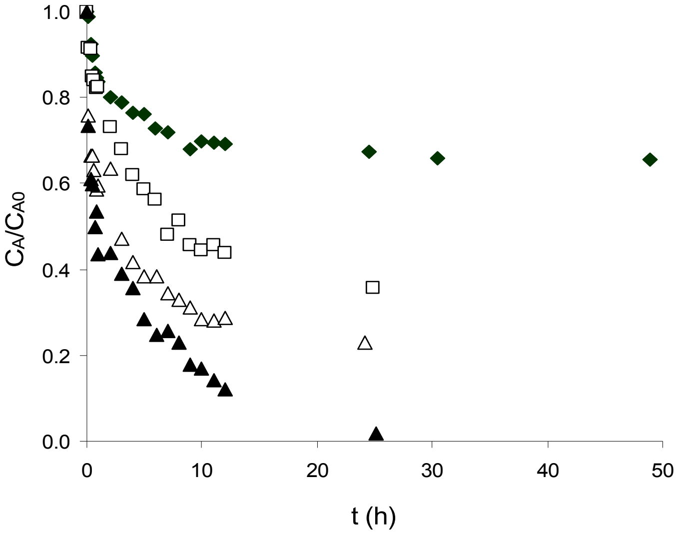

Figure 1 – Experimental data for ETS-4: ◆, $C_0(Cd^{2+})$=1.215 ppm, pH=3.50; □, $C_0(Cd^{2+})$= 0.797 ppm, pH=3.50; △ $C_0(Cd^{2+})$=0.769 ppm, pH=6.00; ▲, $C_0(Cd^{2+})$=0.752 ppm, pH=8.50; $V_{solution}$ = 2 dm^3; m(ETS-4) = 50mg

Results show that the ion-exchange and the uptake kinetics increase with increasing pH. It has been also verified that the Freundlich isotherm provides good representation

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in the range of experimental conditions studied. For ETS-4 at pH=4 it is given by the following equation:

$$\left(q_{A,eq} \big/ eq.m^{-3}\right) = 2639.37 \left(C_{A,eq} \big/ eq.m^{-3}\right)^{\frac{1}{5.51}} .$$

A Nernst-Planck based model combining internal and external diffusion resistances has been used to describe the process along time. The model accomplishes good representation even in the transition from the steep descent to the horizontal branch of the normalised bulk concentration *versus* time curve.

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Poly(vinyl alcohol) with sulfonic acid groups as catalyst for the alpha-pinene alkoxylation

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Keywords: a-pinene, alkoxylation, PVA, sulfonic acid groups

Alpha-Pinene is a renewable raw material usually obtained from pine gum or as a waste from the Kraft process. Its acid catalysed methoxylation yields a complex mixture of monoterpenic ethers, being alpha-terpinyl methyl ether the main product. The alpha-terpinyl methyl ether smells grapefruit-like and might be used as flavour and fragrance for perfume and cosmetic products, as additive for pharmaceuticals as well as in food industry. Strong homogeneous acid catalysts, e.g sulphuric acid, have been used, but the effluent disposal leads to environmental problems and economical inconveniences. These problems can be overcome by the use of solid acid catalysts. Beta zeolite (Hensen et al., 1997) and acidic cation exchange resins have been used for the α-pinene alkoxylation.

Homogenous and heterogeneous catalysts could be immobilized in polymeric membranes in order to improve their selectivity or activity, thanks to environment created by the polymeric matrix around the catalyst (Ozdemir et al., 2006, Vankelecom, 2002). In a previous work, it was observed that PVA membranes with $–SO_3H$ groups have catalytic activity in esterification of acetic acid by isoamylic alcohol (Castanheiro et al., 2006).

In the present work, we report the apha-pinene alcoxylation over poly(vinyl alcohol) containing sulfonic acid groups. These groups were introduced by the direct reaction between -OH of PVA and sulfosuccinic acid.

PVA membranes were prepared by dissolving PVA (MERCK, average molecular weight: 72000) in water at 90ºC, during 6 h. Aqueous 10 wt.% PVA solutions were mixed with the appropriate amounts of sulfosuccinic acid (SSA) and then the mixtures were vigorously stirred at room temperature for 24 h. After that, the homogeneous solutions were poured and cast on a Teflon plate. The cast membranes were allowed to dry at 60ºC during 24 h. The dried membranes were heated at 120ºC, during 2 h (Rhim et al. 2004). The code PVA_SSAx means a membrane containing 2x% of its –OH groups esterified.

The amount of sulfonic acid groups in PVA_SSA membranes was measured by a classic titration. The samples were equilibrated with 10 ml of 0.1 mol/L NaOH solution for 24 h. Then, the amount of H^+ was calculated from the reduction in alkalinity determined by titration.

The FTIR spectra were recorded in a Bio-Rad FTS 155 instrument.

Catalytic experiments were carried out in a batch reactor equipped with a stirrer, at 60ºC. In a typical experiment, the reactor was loaded with 1 ml of α-pinene, 50 ml of methanol and 0.5 g of catalyst. Samples were taken periodically and analysed by GC, using a Konic HRGC-3000C instrument.

Figure 1 shows the FTIR spectra of PVA and the PVA crosslinked with sulfosuccinic acid, PVA_SSA40. The absorption band at 1730-1735 cm^{-1}, in the spectrum of PVA_SSA40, usually assigned to the ester group (-CO-O-), suggests that the crosslinking with SSA was succeeded. The absorption band at 1037 cm^{-1} in the

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spectrum of PVA_SSA40, which does not appear in the spectrum of PVA, indicates the presence of the sulfonic acid groups by the introduction of SSA (Rhim et al., 2004).

The α-pinene concentration profile over PVA_SSA5, PVA_SSA20 and PVA_SSA40 membranes were shown in figure 2. It was observed that the conversion increases when the amount of sulfosuccinic acid used in the polymer cross-linking is increased from 5% to 20%. This behaviour can be explained by the increase of the amount of sulfonic acid groups in the PVA matrix. However, when crosslinking degree increases from 20% to 40%, the conversion of α-pinene increases only slightly. This is, probably, due to the increase of restrictions on the mobility of molecules, in the polymer matrix.

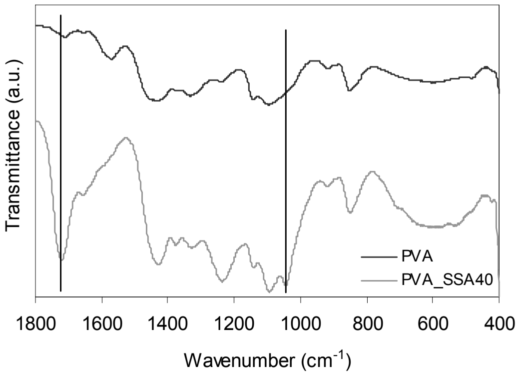

Figure 1 – FTIR spectrum of PVA membrane with and without sulfonic groups.

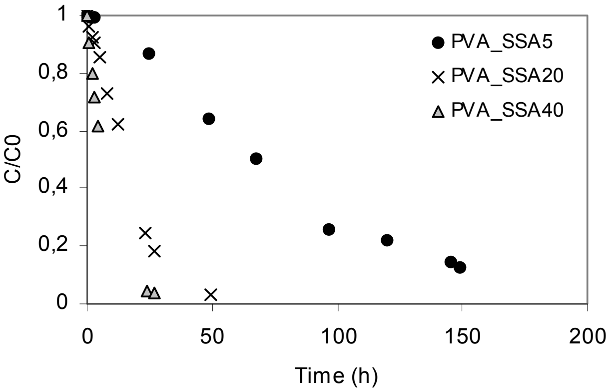

Figure 2 – Metoxylation of α-pinene over PVA membranes. α-Pinene profiles concentration.

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Production by hydrogen reduction and characterization of nano-structured materials

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Keywords: Synthesis, Characterization, Nano-materials, Nano-composites, Alloys

Introduction

It is well recognized the importance of nano-structured materials in the present technological stage. Due to their unique properties these materials can be used in a large number of applications. It is also well established that materials properties can be severely altered by giving them a nano-structured condition (Cheary at al, 1992). In that sense, some materials can even be designed in order to fulfill the requirements for high performance in some specifics functions (Lieberthal et al, 2001). One example is the growing interest in nano-composites, in which a very fine dispersion of a ceramic phase in a metal matrix will significantly improve the material properties. In view of that, extensive studies have been carried out on a variety of materials such as alloys and different types of composites (Sakino et al, 1997). Recently, the authors have developed an alternative chemical based synthesizing route to obtain nano-structured materials such as in-situ formations of nano-scale composites (Cu-Al_2O_3 and Ni-Al_2O_3). It has been based on the decomposition of their mixed nitrate solutions to co-form the nano oxides, followed by preferential reduction of CuO or NiO by hydrogen at low temperature. This approach can be applied to the production of oxides, metals, composites and alloys, in the last two cases with a high level of homogeneity. Studies carried out by the authors on the kinetics of reduction of such fine oxides indicated that under low partial pressure of hydrogen (0.25 atm) in argon, the oxides of Ni (Jena et al, 2001) and Cu can be reduced completely, in a low temperature range of 523 to 623 K. The composites containing nano-sized metal-metal oxide particles have been found to be quite homogeneous in nature. In view of this, Cu-Ni alloy was produced by mixing the aqueous nitrate solutions of copper and nickel, followed by decompositions of their nitrates to their mixed oxides and subsequent low temperature hydrogen reduction (Jena et al, 2004). The alloy powder has been characterized and cold pressed and sintered to determine the density, hardness and workability. In that context, the purpose of the present work is to address the fundamental aspects of the synthesis procedure, emphasizing the thermodynamics background of the two steps involved. Also, the work aims to illustrate the outcome, by presenting experimental conditions and providing relevant characterization of the obtained nano-materials, by means of electron microscopy and micro-analytical techniques. Examples are given in terms of individual and co-formed oxides, composites and alloys.

Theoretical Considerations

The thermodynamics aspects of the reactions involved in the synthesis are discussed. Initially, it has been considered how the nitrate behaves when they are subjected to high temperatures. This study is based on the equilibrium constant as function of temperature for a number of nitrates dissociation reactions. It was noticed that, although the zinc nitrate requires a higher temperature, above 650 K all the nitrates will have their dissociation as a thermodynamic favorable reaction. In fact, it also happens in kinetics terms as confirmed by the experimental tests. Conversions up to 100% could be identified by relating the sample weight to a general reaction stoichiometry, such as:

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$2M(NO_3)_2 = 2MO + 4NO_2(g) + O_2(g)$.

The next step is related to the oxides reductions with hydrogen. The system reactivity was estimated by thermodynamic measurements of the following general reaction:

$MO + H_2(g) = M + H_2O(g)$

As expected, copper and nickel oxides can be reduced while the aluminum oxide is very stable in the presence of hydrogen. Experimentally, it was also observed that copper and nickel oxides are readily reduced at temperatures about 550K and 700K, respectively.

Experimental Procedure

By dehydrating a water dissolved homogeneous mixture of more than one nitrate it was possible to obtain co-formed oxides with a complete homogeneity. Then, the co-formed oxides were subjected to hydrogen reduction in order to obtain an alloy or a composite. The material to be obtained is established by the choice of the reactional system as well as the experimental applied conditions. The procedure which has been adopted in this paper allowed the synthesis of the following types of nano-scale powder: i)co-formed binary oxides, ii) metal-matrix ceramic nano composites and iii) alloys.

Conclusions

Thermodynamics studies and kinetics results support the idea that certain materials such as oxides or co-formed oxides and composites can be obtained by nitrates dissociation and preferential reduction with hydrogen, respectively.

Copper and nickel oxides and co-formed oxides (CuO-NiO and $CuO\text{-}Al_2O_3$) were obtained and their crystallites, estimated from X-Ray diffraction analysis and directly observed in detail by TEM techniques, covering a significant range of particle sizes. In the lower range NiO with 20 nm average size followed by co-formed NiO-CuO particles with 50 nm sizes and larger particles around 200 nm in the CuO powder. It has also been observed that particles smaller than 20 nm are defect-free.

Nano-composites of $Cu\text{-}Al_2O_3$ and $Ni\text{-}Al_2O_3$ were obtained by selective hydrogen reduction and their microstructures showed a high homogeneous distribution of the elements. While the matrix copper crystals are about 50 nm in average, the alumina particles are one order of magnitude smaller.

X-ray diffraction studies indicate that, co-formation of the oxides through low temperature decomposition of their nitrates, followed by hydrogen reduction at 623K would lead to the formation of homogeneous Cu-Ni alloy powder. SEM and TEM characterization confirm the purity and homogenization of Cu and Ni in the alloy on nano-scale.

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Carbon aerogel supported platinum catalysts for selective hydrogenation of cinnamaldehyde

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Keywords: carbon aerogels, surface groups, cinnamaldehyde hydrogenation

The synthesis of polymeric carbon forms was first reported by Pekala in the late 80's and two types can be distinguished according to the drying step: (i) aerogels, when the solvent is removed with supercritical CO_2 and (ii) xerogels, when solvent removal is performed under normal conditions. These and other carbon materials (like nanofibers, nanotubes or fullerenes) can be used to support noble metals providing very efficient catalytic systems. One important application of heterogeneous catalysis is the selective hydrogenation of unsaturated aldehydes to the corresponding unsaturated alcohols, which are important intermediates in the preparation of fine chemicals for fragrance, pharmaceutical and agrochemical industries.

In this work, we prepared several carbon aerogel supported Pt catalysts, using supports with different textures and surface functionalities, seeking to establish appropriate structure/activity relationships, which are useful for smart catalyst design.

Organic aerogels were prepared by polymerization of resorcinol (R) and formaldehyde (F) in aqueous solution (Pekala 1990), using different alkali carbonates (M_2CO_3; M= Li and Cs) as polymerization catalysts. This process yields organic RF-aerogels which were carbonized in N_2 flow at 900ºC. The obtained carbon aerogels will be here referred as Li900 and Cs900 indicating the alkaline metal and the carbonization temperature used. The Li900 carbon aerogel was further oxidized (1 g carbon/10 mL of solution) with concentrated H_2O_2 (9.8 M) and with a saturated solution of $(NH)_4S_2O_8$ in H_2SO_4 (1 M) for 48 h at ambient temperature (Moreno-Castilla, 1995). The samples oxidized with H_2O_2 and $(NH)_4S_2O_8$ will be here referred as Li900H and Li900S, respectively. Supported 1 wt. % Pt catalysts, using $Pt(NH_3)_4(NO_3)_2$ as precursor, were prepared by the incipient wetness method over the above described materials and were treated at 400ºC in N_2 and reduced at 350ºC in H_2 prior to reaction. A post-reduction treatment in N_2 was performed at 700ºC to remove part of the surface groups.

Hydrogenation of cinnamaldehyde was carried out in a 100 mL well-stirred stainless steel reactor at 90ºC and 10 bar (total pressure). The reaction mixture contained heptane (solvent), cinnamaldehyde, decane (internal standard for gas chromatography) and the catalyst. As the reaction proceeded, samples were withdrawn to determine conversions and product selectivities. The analysis was performed in a DANI GC-1000 Gas Chromatograph, equipped with a WCOT Fused Silica column (length 30 m, 0.32 mm i.d., film thickness 1μm).

The type of polymerization catalyst was found to strongly influence the porous texture of the support (Table 1). Smaller cations like Li produce materials with significant mesoporous volume, whereas aerogels catalyzed by larger Cs particles have mainly macropores. Oxidation treatments allowed surface groups like carboxylic acids,

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lactones and phenols to be successfully introduced without any significant compromise of the initial textural properties of the aerogels (Moreno-Castilla, 1995). The surface acidity of the materials, as determined by pH_{PZC} measurements, varied in the following order: Li900 (10.22) < Li900H (4.94) < Li900S (3.16).

Table 1. Textural properties of the carbon aerogel supports.

Support	S_{BET} ($m^2 \cdot g^{-1}$)	$W_0^{(1)}$ ($cm^3 \cdot g^{-1}$)	$S_{ext}^{(2)}$ ($m^2 \cdot g^{-1}$)	$V_{MESO}^{(2)}$ ($cm^3 \cdot g^{-1}$)	$V_{MACRO}^{(2)}$ ($cm^3 \cdot g^{-1}$)	$d_{pore}^{(2)}$ (nm)
Li900	750	0.31	191	1.06	0.00	12
Cs900	658	0.26	59	0.07	1.20	48

(1) By application of Dubinin-Radushkevich equation to N_2 adsorption data.
(2) By mercury porosimetry.

Oxidation of the carbon materials had a marked effect over the selectivity towards cinnamyl alcohol (Table 2). Selectivity increased by a factor of 4.8 for Li900HPt (53% at 50% conversion of cinnamaldehyde) and 3.3 for Li900SPt (36%), compared with the non-treated catalyst (11%). A thermal treatment in N_2 at 700ºC shifted selectivity to the C=C bond. The selectivity towards the carbonyl group appears to be related to the surface chemistry of the material, as a more acidic surface favors the interaction with the C=O bond while a more basic surface favors the adsorption of the olefinic bond.

Table 2. Pseudo first-order kinetic constant and selectivities towards cinnamyl alcohol (C=O), hydrocinnamaldehyde (C=C) and hydrocinnamyl alcohol (C=O, C=C) measured at 50% conversion of cinnamaldehyde.

Catalyst	k_{APP} ($g\ g_{Pt}^{-1}\ s^{-1}$)	$S_{C=O}$ (%)	$S_{C=C}$ (%)	$S_{C=O,\ C=C}$ (%)
Li900Pt	14.8	11	54	20
Li900Pt700	18.9	12	54	25
Li900HPt	13.1	53	18	20
Li900HPt700	19.0	21	41	28
Li900SPt	18.0	36	23	25
Li900SPt700	22.1	21	44	27
Cs900Pt	11.6	24	34	35
Cs900Pt700	17.0	12	54	19

Surface properties of carbon aerogels can be finely tailored by adjusting the texture (choosing the polymerization catalyst) and the surface chemistry (type of liquid-phase oxidation). The increase in surface acidity can explain the higher selectivity towards cinnamyl alcohol of Li900HPt and Li900SPt compared with the Li900Pt catalyst. A thermal treatment at 700ºC removed most surface groups and shifted selectivity back to the hydrogenation of the C=C bond.

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Extraction of nickel from spent nickel catalyst with inorganic acid

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Keywords: Nickel recovery, Leaching, Hydrometallurgical, Spend catalyst

This study examined the recovery of nickel from spent nickel catalyst ($NiO/CaAL_4O_7$) by hydrometallurgical technique with sulfuric acid. The influences of temperature, acid concentration, solid: liquid ratio and contact time has been studied. Under optimum conditions (temperature: 120Ċ, acid concentration: 70% (v/v), solid: liquid 1/20, time of reaction: 220min) 99% Ni were extracted as sulfate phase. In addition to the economical advantages the proposed procedure will be helpful in saving the wastage of metal and protecting the environment from metal pollution.

The increasing demand for metals in the world has required intensive studies for the extraction of metals from low-grade ores and/or secondary resources. Extraction of nickel can be performed from secondary resources like spent catalysts. Regeneration of spent catalysts after many cycles of use is not possible by ordinary techniques and it poses a significant waste disposal problem for most of the hydrogenation and fertilizer plants.

Increasing environmental concerns and legislation regarding the disposal of hazardous residues are forcing companies and countries to process their own waste products and residues, Spent hydro processing catalysts have been classified as hazardous wastes by the Environmental Protection Agency in the USA (Rapport, 2000). So the treatment of this waste is compulsory due to its toxicity and polluting character. This situation has resulted in increasing emphasis on the production of nickel from secondary sources like spend catalysts.
Spent nickel oxide catalyst used in this study was kindly provided by Arak petrochemical complex (Iran). We can find out, structure of nickel catalysts by X-ray diffraction (XRD). Figure1 shows presence of NiO and $CaAL_4O_7$ as the major phases and $CaAL_2O_4$ as the minor phase. The content of nickel in the catalyst sample was 14.06% as indicated by X-ray fluorescence analysis.

The recovery of nickel was more than 99 percentages during the leaching process. The optimum experimental parameters achieved under the following conditions: temperature 120Ċ, acid concentration 70% (v/v), solid: liquid 1: 20 and contact time 220 minute. Although in all these experiments eliminate the stirring rate and particle size diameter factors.

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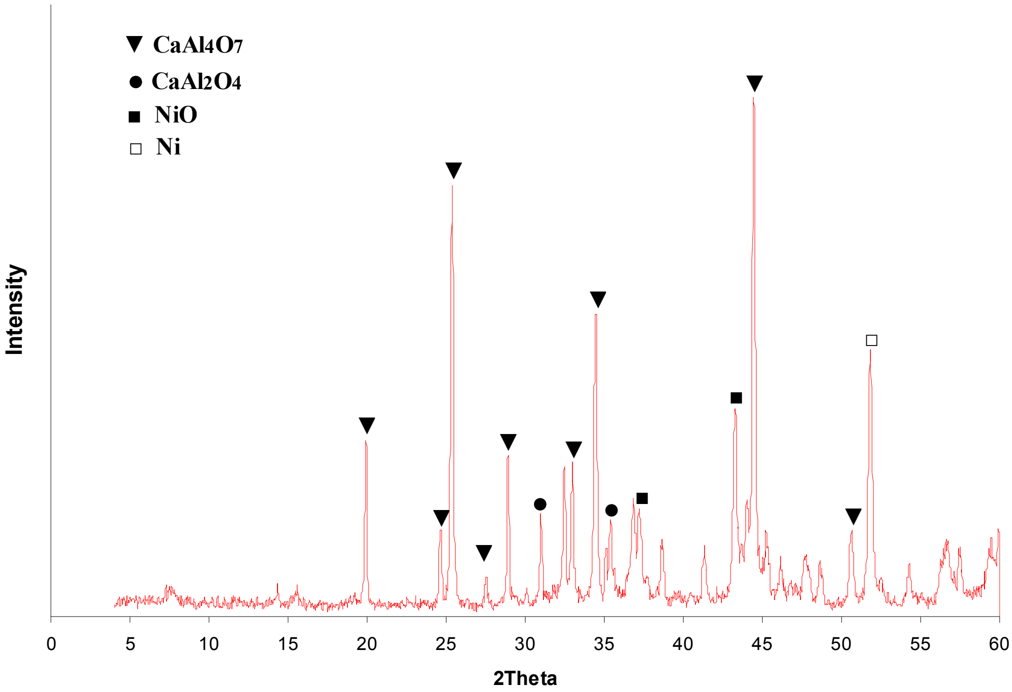

Fig. 1. The X-ray diffraction result of spent nickel catalyst

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Oxidation of Fe(II) in sulphuric medium

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Keywords: Iron oxidation, Leaching, zinc concentrate

Ferric ion is one of the most important oxidants used in leaching processes. As an example, the leaching reaction of zinc sulphide with ferric ion follows the equation (Aydogan et al., 2005; Dutrizac et al., 2003):

$$ZnS + 2Fe^{3+} \rightarrow Zn^{2+} + 2Fe^{2+} + S^{0} \qquad (1)$$

The reaction consumes the ferric ions, thus leading to the decrease of the redox potential and to the observed reduction of the leaching rate. There are several advantages of performing the oxidation of Fe(II) to Fe(III) during the leaching by the addition of external oxidants. In fact, this procedure would allow maintaining the concentration of the oxidant during the leaching thus minimizing the consumption of fresh reagent. Oxidant agents such as air, oxygen (O2) and hydrogen peroxide (H2O2) may be used to promote the oxidation of ferrous ions in the aqueous solution.

In this study, oxidation tests using synthetic solutions containing only ferrous sulphate and sulphuric acid at atmospheric pressure were performed in a five-necked round bottom reactor (1 L, 13 cm diameter) heated by a thermostatic oil bath. For stirring, a single round paddle (6.5 cm diameter) was used. Also, some autoclave tests were carried out using a Hastelloy C-276 autoclave (Parr, model 4843). In both cases samples were collected at regular time intervals and analysed for total iron by Atomic Absorption Spectroscopy. The concentration of the Fe(II) was assessed by a standard volumetric method (Vogel, 1972).

The results show that at atmospheric pressure (Figure 1), hydrogen peroxide was more effective than air. In fact, the addition of 14 mL of H2O2 130 V (2 mL after each sample) to 800 mL of solution allowed the complete oxidation of Fe(II), whereas after 46 h of air injection, only 30% of the ferrous was oxidised.

At 80 ºC and 2 bar of oxygen, the autoclave experiments showed that after 5 h, 44 % of the ferrous ion was oxidated. The results obtained were improved when the temperature was increased to 95 ºC (Figure 2). In fact, 60% of the ferrous ion was in its trivalent form after 5 h of reaction. An increase of the O2 pressure up to 6 bar resulted in 80% of the ferrous oxidation in 5 h.

Stirring speeds of 500 and 1500 rpm were used in the autoclave to test the effect of this variable on the oxidation rate but the results showed that ferrous ion oxidation rate was not significantly affected by the increase of the stirring speed.

The results showed that H_2O_2 was the most effective oxidant agent. However, it is a very expensive reagent to be used in a leaching process at industrial scale. As an alternative, ferrous ion oxidation may be carried out in an autoclave, where it is possible to have more than 20% of the total iron in its ferric form at 95 ºC and $P(O_2)$=6 bar in 1 h, with the expected beneficial effects on the leaching rates of sulphide minerals.

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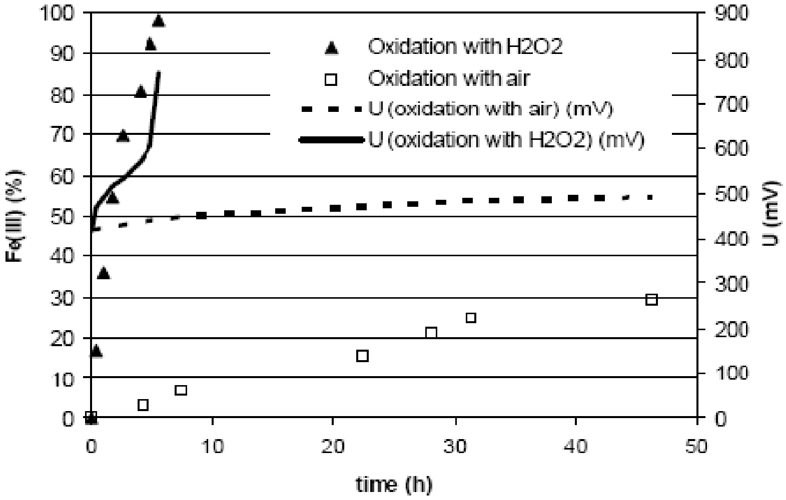

Figure 1: Effect of the addition of air and H_2O_2 on Fe(II) oxidation. Experimental conditions: solution with $[Fe(II)]_{initial}$=15 g/L, $[H_2SO_4]$=0.5 M, T=80 ± 3 °C, stirring speed=500 rpm, air=64 L/h, VH_2O_2 =14 mL ($V_{solution}$=800 mL). Reference electrode: silver/silver chloride.

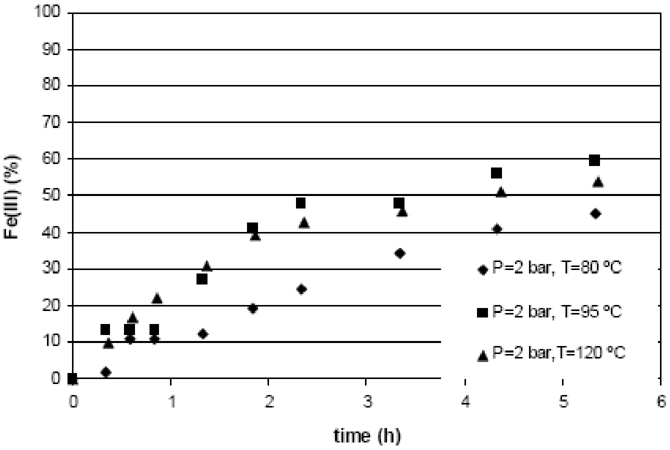

Figure 2: Autoclave oxidation of Fe(II). Experimental conditions: solution with $[Fe(II)]_{initial}$=15 g/L, $[H_2SO_4]$=0.5 M, stirring speed=500 rpm, $P(O_2)$=2 bar.

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Separation of a nickel/cobalt mixture by using a neutral bifunctional extractant

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Keywords: Liquid-liquid extraction, Nickel, Cobalt, Cyanex 272, Aliquat 336

Solvent extraction has played an important role in the development of processes to recover metals from various sources. The phosphinic acid reagents CYANEX 272 and CYANEX 301, have allowed the direct application of solvent extraction for the separation of cobalt from nickel at very low pH what has resulted in minimal caustic addition during the extraction process. Recent works have demonstrated that working in neutral media the quaternary amines can be used in the organic phase as pH controllers.

The mixture of Cyanex 272 (HA), of acid character, and Aliquat 336 (R_4NCl), of basic character, in the organic phase and their subsequent washing with an aqueous $NaHCO_3$ solution allows the formation of a new neutral extractant:

$$\overline{R_4NCl} + \overline{HA} \longrightarrow \overline{R_4NA{\cdot}HCl}$$

$$\overline{R_4NA{\cdot}HCl} + HCO_3^{-} \longrightarrow \overline{R_4NA} + Cl^{-} + CO_2 \uparrow$$

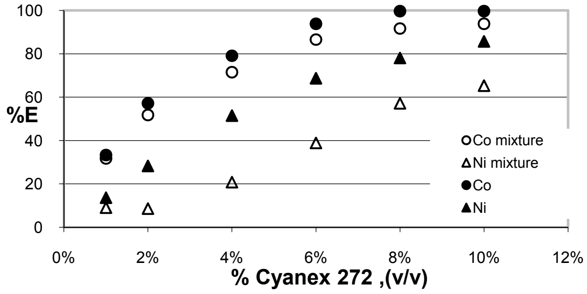
In these conditions, the extraction reaction of the metal present in the aqueous phase is:

$$2\ \overline{R_4NA} + Me^{2+} + 2\ Cl^{-} \longrightarrow \overline{MeA_2} + 2\ \overline{R_4NCl}$$

Therefore, the pH in the aqueous phase after the extraction should remain almost constant.

In order to prove it, liquid-liquid extractions were carried out under different experimental conditions such as concentration of Cy272 and Aliquat 336 in the organic phase, concentration of metal ions and the chloride concentration. Under optimised conditions, in order to determine the stages required to achieve maximum recovery of these metals in multi stage mixer-settler apparatus, extraction isotherms of a mixture of Co(II) and Ni(II) and of both of them separately were obtained.

In all experimental studies, stequiometric ratio of the extractants has been used. The most favourable condition in order to separate a mixture of 1 g/L of Cobalt and 1 g/L of Nickel was 2% (v/v) Cyanex 272 plus 3% (v/v) Aliquat 336 as it is given in figure 1 and figure 2 where the separation factor has been named as β.



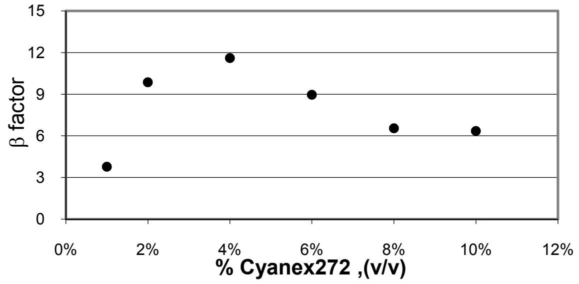


Figure 2. Separation factor evolution

with the Figure 1. Influence of the extractant on the extraction extraction concentration

Equilibrium pH has been measured and only a slight increase in the relation to the initial pH has been observed.

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Different stripping solutions have been tried and the results obtained have led to use EDTA of 0.02 M in all the experiments. The metal recovered has been almost quantitative. To wash the organic phase before a new contact is recommended to use a solution 0.25 M of $NaHCO_3$.

The feasibility of the reuse of the extractant has been evidenced and after six contacts the extraction decreases only about 5% .

The influence of the Cl^- ions in the media has been studied and the results are given in the figure 3. An increase in the anion content produces an increase on the extraction extension.

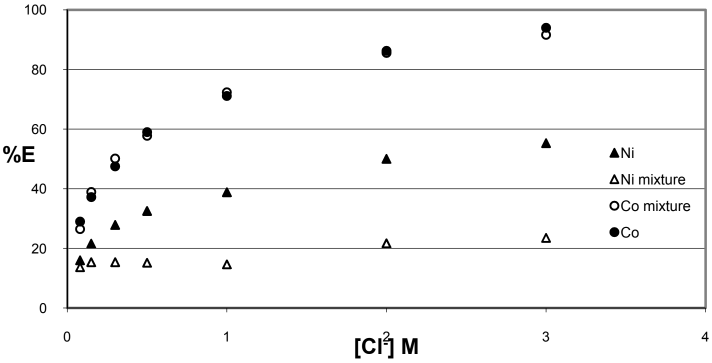
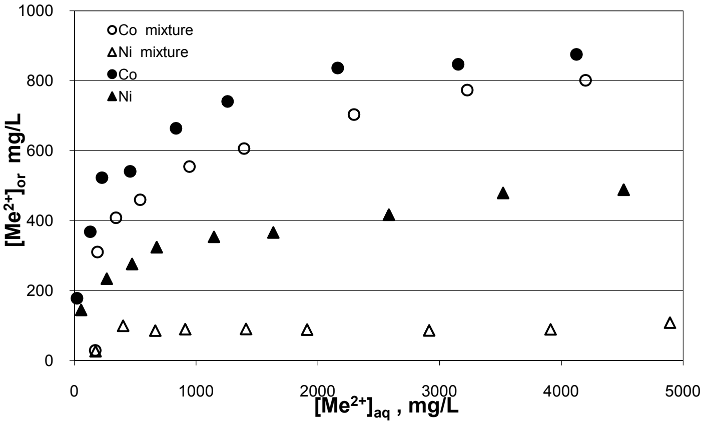


Figure 3. Extraction extension with chloride anion



Fiqure 4. Isotherms for individual metals and for the

The extraction isotherms for individual metals and for the mixture of cobalt and nickel have been obtained. The organic phase used for these experiments, where different concentrations of the metal (200 mg/L-5000mg/L) in 0.4M Cl^- have been tested, was 2% (v/v) of Cyanex 272 plus 3% (v/v) of Aliquat 336 in D-100. In figure 4 it can be seen that the capacity to extract cobalt is better than that obtained for nickel. The value obtained for the nickel decreases significantly in the presence of cobalt.

The equilibrium distribution of cobalt in the mixture and the mass balance equations allow knowing the theoretical number of contacts needed for a nickel/cobalt separation in a multi-step counter-current extraction process. By McCabe-Thiele method, four steps of equilibrium will be enough working with a flow ratio (Aq:Or) of 0.6:1.

Four continuous counter current contactors were used to simulate the separation of a solution of 1000 mg/L of Co(II) and 1000 mg/L of Ni(II) in 0.4M Cl^- media using the organic phase chosen. The scheme of the experimental setup of the process, including the values of the concentrations for each metal, can be seen on figure 5.

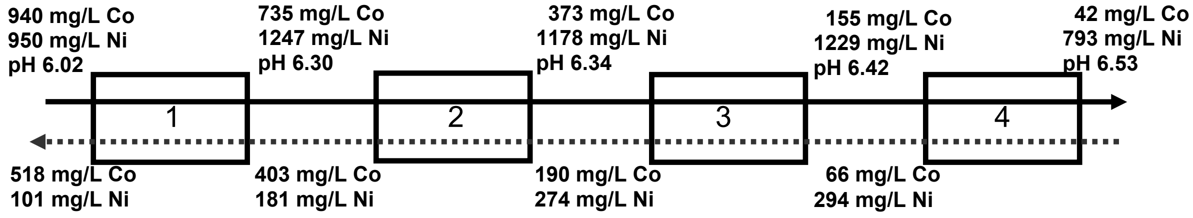


Figure 5. Scheme and results for countercurrent mixer-setler system

Acknowledgments

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Influence of temperature and composition on density of binary mixtures of alkanolamines

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Keywords: Density, Alkanolamines, Temperature

Physical properties of pure amines and their mixtures are needed by different chemical engineering operations for the design of processes. The most important use of this kind of compounds is for removal of sour gases from natural gas and petroleum streams. Other uses of these compounds are such as surfactants, additives in detergents, agriculture products. The aqueous solutions of blended amines have received increasing attention in the past few years. They have been demonstrated to be highly efficient in the absorption of acid gases because by utilizing the advantages of each amine and varying the relative concentrations of the amines, an optimum absorption system can be designed for a specific application.

On the other hand, knowledge of the physical properties of process solutions is necessary for the operation of process equipment, solution density and viscosity are also important in the mass transfer rate modeling of absorbers and regenerators because these properties affect the liquid-film coefficient for mass transfer.

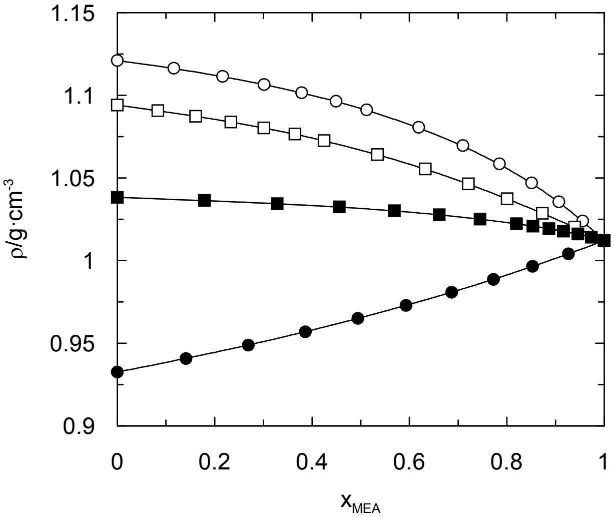

Figure 1. Influence of mixtures composition on density value at 293 K. (○) MEA+TEA; (●) MEA+AMP; (□) MEA+DEA; (■) MEA+MDEA.

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Influence of temperature and internal devices on the drying of fine particles in a conical spouted bed

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Keywords: Conical spouted bed, Non-porous draft-tube, Open-sided draft-tube, Moisture content, Drying time

The applicability of the spouted bed technique lies in its ability to treat (drying, coating, encapsulation and so on) granular products that are too coarse for fluidized beds, or solids that are heat sensitive (Brennan, 1989; Ando and Maki, 2002; Rosiane et al., 2000). This is the case in the food and pharmaceutical industries. Thus, the spouted bed regime is an alternative contact method that is especially interesting when the conventional regimes have limitations imposed by the physical characteristics of the solid and by gas residence time (Rosiane et al., 2000; Olazar and San José, 1992).

Spouted beds with fully conical geometry combine the features of the conventional spouted beds (cylindrical with conical bottom) with those inherent to their geometry, such as stable operation in a wide range of gas flow-rates (Olazar et al., 1999). This versatility in the gas flow-rate allows for handling particles of irregular texture, fine particles and those with a wide size distribution and sticky solids, whose treatment is difficult using other gas-solid contact regimes.

A crucial parameter that limits the scaling up of spouted beds is the ratio between the inlet diameter and particle diameter. In fact, the inlet diameter should be smaller than 20-30 times the average particle diameter in order to achieve spouting status. The usual solution in conventional spouted beds is the use of a draft-tube.

The main aim of this paper is to study the effect of temperature on the drying of fine particles using two different types of draft-tubes (open-sided and non-porous draft-tubes) and without draft-tubes. The material used for drying is construction sand whose average particle size is 0.5 mm. In all runs, a bed with 200 g of sand has been used. The initial moisture content of the sand is about 1%, and the specification is that it should be dried to approximately 0.005 kg of water/kg of dried solid for subsequent use. This solid is usually dried in rotary driers where mass transfer and efficiency are low.

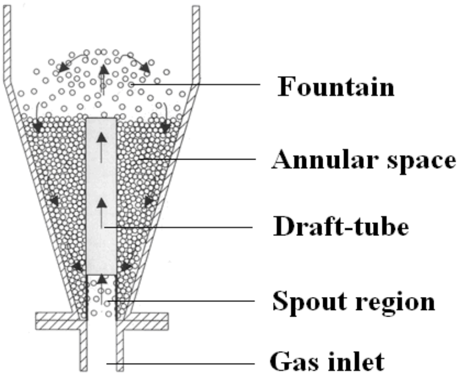

Figure 1. Zones in the conical spouted bed with draft-tube.

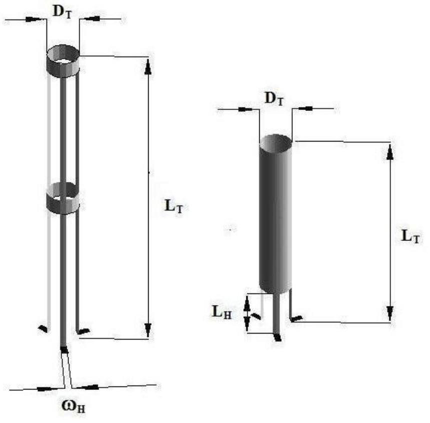

Figure 2. Scheme of open-sided and non-porous draft-tubes.

Batch runs have been carried out in a conical spouted bed with and without draft-tubes at four different temperatures: 25, 100, 200 and 300 ºC. The evolution of the air moisture content with time has been monitored at the bed outlet. In the experimental

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study, an air flowrate 75% higher than the minimum one has been used in order to achieve a stable performance of the spouted bed.

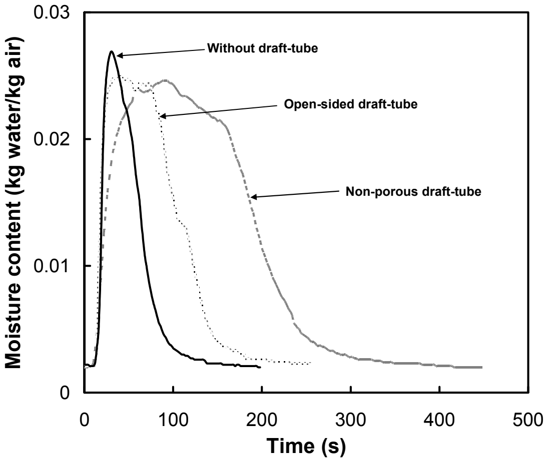

Figure 3. Evolution of air moisture content with drying time at 100 °C.

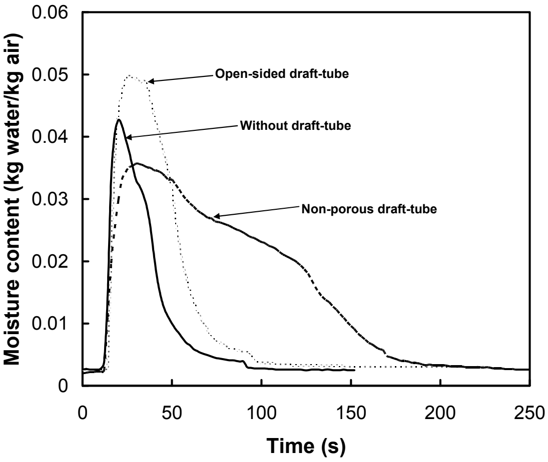

Figure 4. Evolution of air moisture content with drying time at 200 °C.

Figures 3 and 4 show that the time required for drying decreases when the drying temperature is increased. As observed, the drying time required without using any draft-tube is shorter than using draft-tubes. The reason for that is that the air required to achieve a stable performance of the spouted bed is much higher (2 or 3 times) and consequently the gas-solid contact is more vigorous. When draft-tubes are used, the drying time required with open-sided draft-tube is shorter than with non-porous draft-tubes, although the air flowrate required is slightly higher. The reason for that lies in the much higher solid circulation rate, which helps air percolation to the annulus and provides a better gas-solid contact.

On the other hand, runs carried out at temperatures above 200 °C show that the time required for drying does not decrease, which is evidence that the process is controlled by the internal diffusion in the particle.

Furthermore, conical spouted beds are more stable at high temperatures than at ambient conditions, because under the latter conditions wet sand behaviour is much worse and the bed is more unstable. In addition, much more time is required for the drying at ambient conditions.

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Control and minimization of crud in a shallow layer gravity settler

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Keywords: liquid-liquid systems, crud, shallow-layer gravity settler

In large-scale liquid-liquid extraction installations (facilities, plants) that use mixer-settler set-ups, correct and careful design and construction of the settler units are critical both to the overall economy of the process and the smooth operation. Experimentation-based modelling of shallow-layer (horizontal-flow) gravity settlers is thorny because the depth and composition of the dispersion band vary along settler length and because biological/impurity interfaces (crud) may mechanically hinder the longitudinal development of the band and chemically alter (modify) the interfacial tension and significantly affect the time-scale of the coalescence process. The main future objective is the acquisition of accurate experimental data about the geometry (length and thickness) of the dispersion band as well as its structure (drop-size distribution and dispersed phase hold-up) along the length of a shallow-layer gravity settler, under both steady and transient states, for different operating conditions (agitation power input, phase flow rate and feed hold-up). These data, together with the appropriate value of the interfacial tension, are critical for ascertaining the optimal values of the phenomenological parameters of the model describing the behaviour of the settler.

Previous work (Baptista *et al*. 2005) in pilot plant experiments using pure water and kerosene phases, in closed circuit and under steady operating conditions has shown that dispersion band length stabilization was not attained, even at long experimentation times. Crud formation at the interface was observed. Crud analysis, by fluorescent microscopy using Propidium Iodide as a probe, suggested the presence of bacteria in the gelatinous-like material (crud) (Ribeiro *et al*., 2008). The use of a biocide resulted in a dramatic decrease in crud formation and allowed the dispersion band stabilization.

In order to make these data reliable and reproducible, the effects of the formation and accumulation of crud at the water-organic interface must be taken into consideration, controlled and minimized by using appropriate biocides, the side effects of which on the interfacial tension must also be acknowledged. The appropriate techniques for these measurements have been developed and tested in previous work.

In the present work batch scale experiments are performed over kerosene-water agitated mixtures with and without biocide addition. Working cycles of several agitation/ decantation times are acomplished in jar tests and diferent operating conditions are tested: phase ratios, agitation intensitiy, cycle time and quality/quantity of biocides added. In order to acelerate the crud formation in these tests the use of kinetic acelerators (nutrients) is envisagead. The effects of crud and biocide presence over the interfacial tension are measured using a new volume drop method tensiometer.

The results obtained in the present work are of critical importance for a future use of biocides in pilot and industrial equipment utilization plants.

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Visualization of the concentration boundary layer in a cross-flow rectangular thin channel with a soluble wall by holographic interferometry

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Keywords: CFD, concentration boundary layer, holographic interferometry, mass-transfer

In membrane-based processes, the mass-transfer resistance in the liquid feed solution / membrane interface is usually high and controls the process performance with associated high pressure losses. One of the main roles of the membrane modules is to provide optimal hydrodynamics to minimize the mass-transfer resistance. Among the several types of membrane modules that have been developed in the last decades, spiral-wound modules combine high mass-transfer coefficients in the feed stream and high membrane packing density. These modules have spacer-filled channels, each of them comprising two membrane leaves and a feed-spacer which are spiral wounded around a central tube. The feed-spacers strongly increase the mass-transfer coefficient because they promote secondary flows and reduce the critical Reynolds number above which the flow becomes unstable.

Unlike other modules with simpler feed channels geometries, the design of spiral-wound modules is more complex. The three-dimensional flow patterns and earlier unsteady flow promoted by the feed-spacers cannot be predicted by simple momentum transport models. Thus, the design of spiral-wound modules has been performed mostly based on experimental empirical data. In the last decade, the increase of computer power and the emergence of robust computational fluid dynamics (CFD) software has made possible to predict the flow structure and concentration distribution in membrane spacer-filled channels (Ghidossi et al., 2006). However, experimental measurements of solute concentration distributions inside these channels, which are essential to validate CFD predictions, are still scarce. To our knowledge, determinations of concentrations distributions in membrane spacer-filled channels have not been reported so far. These measurements are fundamental to determine the critical Reynolds numbers and to enlighten whether it is meaningful using turbulence models in CFD predictions for these systems (Cao et al., 2001, Karode and Kumar, 2001). Furthermore, the analysis of experimental concentration distributions allows to identify regions of poor flow and mass-transfer characteristics and, thereby, design optimized spacer geometries and/or pulsating flow strategies.

The holographic interferometry has already been used to visualize the concentration polarization in unstirred reverse osmosis cells (Fernandez-Sempere et al., 2006, 2007). In the present work, we intend to extend the application of this technique to a cross-flow rectangular thin channel bearing a soluble wall, inside which a very thin concentration boundary layer develops in the vicinity of the soluble wall. The ultimate goal is to use the experimental data collected in this cell in a subsequent work involving a spacer-filled channel with a nanofiltration membrane instead of a soluble wall.

The cell comprises an open channel of 15 mm wide, 20 cm long and 2 mm height. The channel top consists of a soluble wall casted in benzoic acid. Two lateral glass windows placed in the middle of the cell and with a diameter of 1 cm enable the

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visualization of the concentration boundary layer by using a holographic interferometer, as described elsewhere (Fernandez-Torres et al., 1998). Pure water is pumped through the cell with a pulsation-free gear pump (Eclipse Model 05 Pump, Pulsafeeder, Rochester, New York) and the Reynolds number (based on the channel height) varies from 50 to 1000. The concentration boundary layer is visualized for several Reynolds numbers and the experimental concentration distributions, determined through image analysis of the interference fringes, are compared with the numerical predictions achieved by computational fluid dynamics.

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Removal of 17*β*-estradiol from water solution

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Keywords: 17 *β*-estradiol, adsorption, thermodynamic parameters

Summary

Removal of 17*β*-estradiol (E2) from aqueous streams by two polymeric resins XAD-7 and XAD-16 and Granulated Activated Carbon (GAC) was investigated. Adsorption equilibrium isotherms of E2 from water were measured in a batch adsorber at different temperatures, ionic strengths, and pH of the external phase. The experimental data was correlated with the Langmuir isotherm, and thermodynamic parameters for adsorption process were determined. Mass transfer kinetics were investigated in a batch adsorber and correlated with a Fickian diffusion model. It was found that adsorption capacity increases in the following order: XAD-16 > GAC > XAD-7.

Extended Abstract

Recently, the presence of harmful endocrine disrupting chemicals (EDCs) in the water and wastewater has attracted an interest of many environmental scientists. EDCs have been detected on surface, in drinking water, as well as in wastewater. Due to they are potent at nanogram concentrations and increase of their consumption with growth of population, as well as development synthetic hormones, the efficiency of their removal from wastewaters is very important.

Polymeric resins have recently been widely investigated as an alternative to activated carbon for the removal of organic pollutants from wastewater (1,2). Their benefits include more uniform surface chemistry, pore structure, and the possibility of regeneration under mild conditions. In this work, adsorption of 17*β*-estradiol onto non-ionic polymeric resins and GAC used as reference was investigated.

Adsorption equilibrium isotherms were measured at various ionic strengths (0.1, 10 and 100 mM NaCl) and pH (4, 5.7, 10). For estimation of thermodynamic parameters, the equilibrium experiments were conducted at three temperatures 23 ^{o}C, 37 ^{o}C, and 50 ^{o}C. Adsorption kinetics experiments were conducted in a batch adsorber at pH 5.7, an ionic strength of 0.1 mM NaCl, and a temperature of 23 ^{o}C. Removal of E2 from aqueous streams in a column adsorption process was also studied.

The adsorption equilibrium isotherms were successfully correlated with the Langmuir isotherm. It was found that the adsorption efficiency was strongly dependent on the ionic strength and pH of the external phase in the case of GAC. For the polymeric adsorbents influence of ionic strength and pH was not detected. Higher sorption capacity per mass of adsorbent was obtained in the case of the hydrophobic XAD-16 polymer and GAC.

Considering the chemical structures of the adsorbents, it seems probable that, in the cases of XAD–16 and GAC the adsorption mechanism involves hydrophobic interaction. The interaction observed with XAD–7 stems most from hydrogen bounding interaction. Thermodynamic parameters for the adsorption process were determined using adsorption equilibrium parameters calculated for different temperatures. The relatively low values of Gibbs energy (–33 kJ mol^{-1} for XAD-7, -17 kJ mol^{-1} for XAD-16, –22 kJ mol^{-1} for GAC) indicates that adsorption has physical character. The enthalpy of adsorption onto polymeric adsorbents was found to be relatively low (26 J mol^{-1} for XAD–7 and 29 J mol^{-1} for XAD–16) and had negative value that indicate exothermic character of adsorption. The positive enthalpy of adsorption for the GAC (2.5 J mol^{-1})

indicates that adsorption has endothermic character. The positive adsorption entropy for all adsorbents (21 J $mol^{-1}K^{-1}$ for XAD-7, 20 J $mol^{-1}K^{-1}$ for XAD-16, and 80 J $mol^{-1}K^{-1}$ for GAC) indicates an increased randomness at the solid/solution interface during E2 adsorption.

Removal of E2 from aqueous streams in a single-column adsorption process was studied experimentally and by means of mathematical modeling. As expected from the batch adsorption kinetics data, intraparticle mass transfer resistance was found to be significant, reducing the working capacity of the column.

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Decolourization of reactive red 180 by laccase: optimization by response surface methodology

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Keywords: Optimization, Laccase, Reactive dye, Enzymatic catalysis, Decolourization

Reactive dyes are the main class of dyes employed in textile industries to colour cellulosic fibres. However, around 30% of the applied reactive dyes are discarded causing serious environmental problems and the conventional treatments of wastewater are not efficient to completely remove the residual colour (Vandevivere et al., 1998). A number of biotechnological approaches have been suggested to fight this pollution source in an eco-efficient manner. Different studies show that ligninolytic enzymes (laccase) can degrade a wide variety of recalcitrant compounds, such as dyes (Abadulla et al., 2000; Soares et al., 2002a). Its substrate range can be extended by addition of a redox mediator (Bourbonnais and Paice, 1990).

The experimental design and Response Surface Methodology (RSM) are useful statistical techniques that can be used in a decolourization process to identify and optimize the relevant factors that influence the colour removal (Myers and Montgomery, 2002; Hanrahan and Lu, 2006).

In this study a three-level Box-Behnken full factorial design and RSM were applied to optimize the decolourization conditions (temperature, pH and enzyme concentration) of C.I. Reactive Red 180 (RR180) dye by enzymatic catalysis with a commercial laccase (DeniLite IIS). This formulation includes a buffer and an enzyme mediator. To study the decolourization, 50 mg/L of RR180 was incubated in 25 mL Erlenmeyer flasks at different temperatures, pH values and enzyme concentrations under stirring during one day. Dye decolourization was determined by monitoring the decrease in the absorbance peak at the maximum absorbance wavelength of RR180 (540 nm) and calculating the total area under the plot (integration of absorbance between 350 and 700 nm).

The decolourization of RR180 by commercial laccase was optimized leading to dye degradation above 92%. The Pareto chart and the ANOVA results indicate that pH and enzyme concentration were the principal factors that affect the decolourization whilst the temperature did not affect the degradation. The decolourization increased with increasing of pH and enzyme concentration up to approximately 7.5 and 85 U/L, respectively. Then the decolourization decreased slowly. Validation experiments were carried out in order to check the suitability of the model. The results showed that the predicted decolourization was in good agreement with the experimental value.

A comparison between dye decolourization by absorbance reduction at the maximum absorbance wavelength of the dye and the total decolourization based on all visible spectrum range shows that the first decolourization is higher than the second. The decolourization of RR180 causes a good reduction of absorbance at the maximum absorbance peak, but not fully decolourization considering the entire visible spectrum.

These results are very promising and reveal the high potential of commercial laccase to RR180 decolourization (> 92 %). Therefore, dye degradation by commercial laccase proved to be a suitable alternative treatment for reactive dyes decolourization.

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Modeling orange II azo dye degradation by Fenton's reagent

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Keywords: Orange II, Kinetics, Fenton's Reagent, Hydrogen Peroxide, Oxidation.

The oxidation using Fenton's reagent has proved to be a promising and attractive treatment method for the effective decolourization and degradation of dyes, as well as for the destruction of a large number of hazardous and organic pollutants (Dutta et al., 2001; Malik and Saha, 2003; Lin and Lo, 1997). Besides, the process is simple, and takes place at low temperatures and atmospheric pressure (Bigda, 1995). Oxidation with Fenton's reagent is based on ferrous ion and hydrogen peroxide, and exploits the very high reactivity of the hydroxyl radical produced in acidic solution by the catalytic decomposition of H_2O_2 (see eq. (1)) (Walling, 1975).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^- + HO^\bullet \qquad k_1 = 51\text{-}100\ M^{-1}s^{-1} \qquad (1)$$

The $HO^\bullet$ species produced through eq. (1) will then attack the organic matter present in the reaction medium (eq. (2)), because the hydroxyl radical is a powerful inorganic oxidant that reacts non-selectively with numerous compounds (Walling, 1975; Haag and Yao, 1992):

$$Orange\ \ II + HO^\bullet \rightarrow \mathrm{Pr}\,oducts + H_2O \qquad (2)$$

The dye (orange II - also called acid orange 7) concentration history in a batch reactor showed a change in the kinetics, typical of this process, being initially very rapid (Fenton stage) and afterwards the slower Fenton-like stage proceeds, where iron is mostly in the 3+ oxidation state. Although the process is considerably complex, with numerous consecutive and parallel reactions that involve different radicals, irons species, complexes, etc., a simpler approach was used, based on the pseudo steady state assumption concerning the hydroxyl radical concentration (Sun et al., 2007) in the Fenton-like stage (the longer and last one). It was found that in such phase the kinetics follows a pseudo-first order with the respect to the dye concentration, wherein the apparent kinetic constant (k_{ap}) depends only on the initial conditions.

Therefore, a kinetic model was developed regarding the degradation of the azo dye orange II (OII) using Fenton's reagent, at the optimum pH of 3.0. The effect of initial concentrations of OII, hydrogen peroxide (H_2O_2) and ferrous ion catalyst (Fe^{2+}) on the degradation rate has been investigated in a batch reactor, being the apparent kinetic constant hence determined in a wide range of experimental conditions. The rate equation deduced was found to fit well with the data, k_{ap} depending on the initial conditions following a power-law dependency and on the temperature with the typical Arrhenius dependency (apparent activation energy of 58.1 kJ mol^{-1}).

This rate equation, without further fitting parameters, revealed to be reasonable useful to predict dye concentration histories in the batch reactor (based on the known initial conditions), but was especially useful for predicting the steady-state dye conversion in a continuous stirred tank reactor. In fact, a good agreement between experimental and model results was noticed, even for experiments performed out of the range used in the batch kinetic study, in a wide range of dye conversions values.

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Viscosity of heavy oil and distillation residues with diluents

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Keywords: Heavy Oil, Distillation Residues, Viscosity Correlations, Viscosity Reduction, Solvent Dilution

Introduction

The production and processing of heavy crude oil is constantly increasing as more heavy and viscous oil resources are being exploited to satisfy rising world demand for petroleum. The principal difficulty in recovery, refining and transport heavy oils is related to its high viscosity. The heavy oils also generate large amounts semi-solid residues of distillation in the refineries. The dilution with a solvent, can be used in conventional technologies of recovery, processing and transport of heavy oils and the residues

The mixture can be modeled as a homogeneous solution or a non-homogeneous mixture with a dispersed phase made of heavier components. This study aims at selecting a simple model that can quantify the dilution and the reduction of viscosity, as well as getting a good correlation between the viscosity of the mixture with temperature and concentration of diluent. We measured the kinematic viscosity of solutions of a crude oil, two atmospheric and one vacuum residues of distillation in toluene for different concentrations of diluent at several temperatures. We identify the literature models that best correlates the variation of viscosity with temperature and the variation of viscosity with low and high concentrations of dispersed phase.

Materials and methods

For this study a stable medium heavy naphthenic crude oil (**OIL**), a very light atmospheric residue similar to several heavy oils (**AR I**), a typical atmospheric residue (**AR II**) and a typical vacuum residue from same oil (**VR II**) were selected . The samples were characterized by chemical analysis (elemental and SARA) and several relevant physical properties were measured. Table 1 lists molecular weight obtained by cryoscopy, density by Anton-Paar densimeter and molal average boiling point Tb_{50} obtained by simulated distillation. Toluene was chosen as diluent as it dissolves all the asphaltenes present in chosen residues. Toluene high purity "HPLC / Spectra grade "was purchased from TEDIA Inc of United States and was used with out further purification.

Table 1

	Oil	AR I	AR II	VR II
Molecular Wt.	340	565	604	1037
Density (ºAPI)	20.2	26.7	12.6	7.9
Avg. Boiling Pt.Tb_{50}	373.3 ºC	490 ºC	383 ºC	518 ºC

The kinematic viscosity is determined experimentally by the method ASTM D 445 (Brazilian standard MB-293), where a given volume of liquid sample is introduced into a standard viscometer and viscosity is determined by the time of flow by gravity through a capillary, under precise and reproducible control of temperature. We used calibrated Cannon-Fenske reverse flow viscometers for opaque fluids The calibration factor was obtained by the standard procedure ASTM 446. This analysis takes about 60 minutes, with a reproducibility of approximately 0.35% of the average value, and a repeatability of 0.7% of average.

Analysis of results and conclusions

There are several studies in the literature of viscosity of crude oils, in general lighter than a typical Brazilian crude, in lower range of temperatures, below 80°C. In the present work, the behavior of viscosity with the variation of the temperature of a Brazilian crude oil is studied experimentally at higher temperatures up to 200°C. The ASTM method correlates the viscosity as a function of temperature better than the method of Eyring, especially in the higher temperature range. For the temperature range of 120 to 200 ºC, the average absolute deviation (AAD) of the estimated viscosity is 0.33 cSt for the ASTM method and o.62cSt for the Eyring´s method. The ASTM and Eyring equation correlate the viscosity with temperature for all solutions for fixed concentrations in this study. However the parameters vary non-linearly with concentration. The viscosity has to be measured at least at two temperatures for every concentration.

The variation with concentration of residues correlates accurately with literature correlations for colloids and emulsions. Krieger correlation with one empirical parameter correlates at all concentrations of residues. The parameter depends linearly on temperature. At low concentrations, the relation of Phan-Thien e Pham correlates the viscosity data effectively. (figure, Table 2 and Table 3)

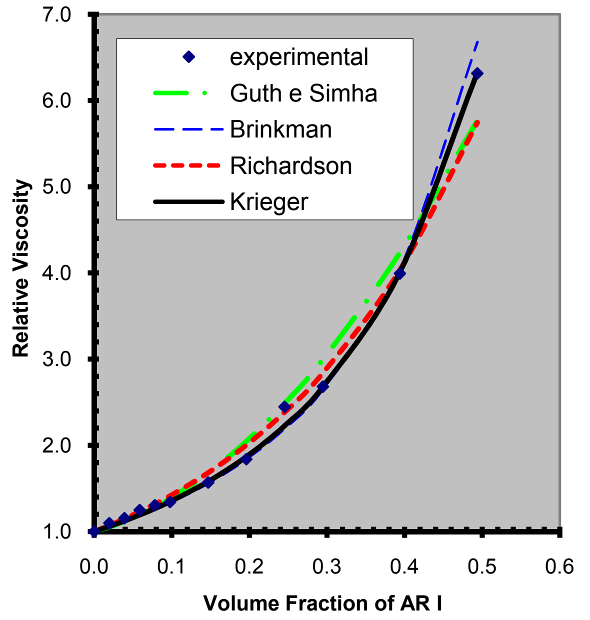

Table 2 Average Absolute Deviations of Relative Viscosity (**AAD**) for concentration<10%

Correlation	**AR I**	**AR II**	**VR II**
Taylor (1932)	0,0737	0,0991	0,0985
Mooney (1951)	0,0833	0,1146	0,1186
Roscoe (1952)	0,0952	0,0982	0,0529
Yaron & Gal-or (1972)	0,0838	0,0964	0,0841
Choi & Schwlater (1975)	0,0647	0,0692	0,0762
Phan-Thien & Pham (´97)	0,0465	0,0588	0,0586

Table 3 Deviations of Relative Viscosity for concentration<50%

Correlation	AAD	Maximum
Guth & Simha	**0,233**	1,547
Brinkman	**0,129**	0,869
Richardson	**0,067**	0,570
Krieger	**0,055**	0,305

Considering as a non-homogeneous mixture, we can estimate the viscosity reliably at all concentrations with solvent viscosity and two parameters for a wide variety of temperatures.

Acknowledgements

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Possibility of productivity increase for distillation column

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Keywords: distillation, combined column, nozzle mass-transfer arrangement, separation possibility, distillate selection percent

Distillation plants for separation of multicomponent mixture represent the equipment complex involving the main units, which are essential for product production, and auxiliary ones intended for the waste processing. Productivity of the main and auxiliary units must be balanced. The corresponding modernization auxiliary units are obligatory under the capacities expansion of the main equipment.

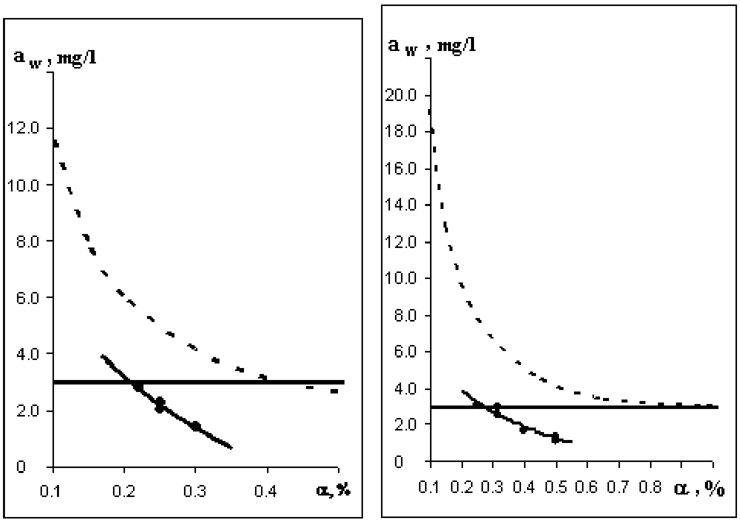
If under the productivity increase of the main equipment the capacities of the auxiliary columns to keep the same, then there will be the increase of the consumption of the liquid and vapor phases compare to the calculated. At the columns with plate mass-transfer arrangements this situations leads to the increase of the droplets entrainment and to reverse mixing at liquid phase, which consequently leads to the effectiveness reduction of plates. As a result, with the productivity increase of the auxiliary columns without modernization connected with extension of the plate's diameter, their separation ability declines.

A relatively inexpensive modernization method of the auxiliary plate's columns is considered below, this method is conclude in the replacement of some plates with more effective nozzle mass-transfer arrangement at the upper part of columns. At the same time feeding should be given to a higher plate than before. Such combined columns provide higher separation ability with the increase of the productiveness than before modernization. This is achieved due to the significant expansion of the number of theoretical plates at the lower part of columns, in spite of their efficiency reduction, and to the increase mass transfer at the upper part of columns through the utilization of nozzle mass-transfer arrangement instead of plate ones.

Verification of the offered method to the modernization of the auxiliary distillation column was executed at industrial column of ethyl alcohol rectification from impurities of volatile ethers, aldehyde and methyl alcohol. The content of these impurities at the original product usually does not exceed 5 % vol. Originally the column was projected for the initial mixture capacity of 7.0 m^3 the per day. Two possible options of its work with heightened initial mixture capacities like 8.10 and 11.25 m^3 per day were studied experimentally.

The principal impurity is crotonic aldehyde, the selection of which is determined the work quality of this column. As an intermediate impurity it is badly separated from the ethyl alcohol and can be accumulate in the distillation plant. It is known from the exploitation experience that it's concentration at ethyl alcohol taken from column should not exceed 3 mg/l. Moreover, the impurities concentrate, produced on the column, ought to contain as much as possible impurities and as less as possible the ethyl alcohol.

The influence of the volumetric rate expansion of feeding on the compositions of distillate and boiling fluid under the increase of percent of distillate selection α from 0.4 % up to 1.0 % from the main equipment productivity is studied. On basis of the chromatographic data determine the crotonic aldehyde concentration dependence at the product taken from boiler a_w for various percent of distillate selection α. These are shown by dotted line on Fig. 1. It is clear that the column productivity of 11.25 m^3 initial mixture per day is the highest possible, at which the required product quality on the crotonic aldehyde can be guaranteed, distillate selection must be 0.8÷0.9% from the initial material quantity, processed on the main equipment. Furthermore there is too much ethyl alcohol in such distillate.



a b

Fig. 1. The crotonic aldehyde concentration dependence at boiling fluid on α and lines of its highest possible concentration: a – the productivity is 8.10; b – 11.25 m^3 per day. Solid lines are experimental data for the modernized column, the dotted line – before modernization

After the described modernization the new column separation ability was studied experimentally for the same productivity 8.10 and 11.25 m^3 of initial mixture per day. The results are shown by solid lines on Fig. 1. The minimal distillate selection percent at given productivity corresponds to the cross point of the corresponding crotonic aldehyde concentration curve at product with the highest possible concentration line.

The minimal distillate selection dependence on the total productivity of the main equipment at which the no hit crotonic aldehyde into ethyl alcohol is shown on the Fig. 2.

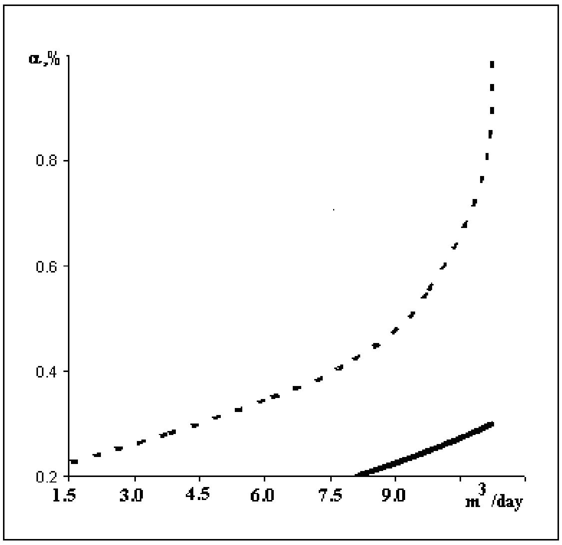


Fig. 2. The dependence of minimal distillate selection on column productivity, where the highest possible crotonic aldehyde concentration is achieved at boiling fluid, solid line – after modernization, the dotted one – before modernization

It is important to notice, as a result of modernization not only the distillate selection percent decreased but also the ethyl alcohol content in it decreased too.

Solving population balance models in precipitation using orthogonal collocation and stochastic optimization

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Keywords: population balance, precipitation, collocation, evolutive algorithms

The general dynamic population balance (PB) equation for precipitation reflects the time variation of the number based density function n (L, t):

$$\frac{1}{V(t)}\frac{\partial[n(L,t)\cdot V(t)]}{\partial t}+\frac{\partial[G(L,t)\cdot n(L,t)]}{\partial L}=B^0\cdot\delta(L-L_0)+B(L,t)-D(L,t) \qquad (1)$$

In eq. (1) L is the particle characteristic length, V(t) is the reaction volume, $B^0(t)$ stands for the net nucleation rate considered to occur at the critical size L_0, G(L, t) is the size dependent growth rate: $G(L)=G_0*(1+aL)^b$. G_0 is the growth rate at nucleus-size while a and b are the parameters in the size dependent growth model. B(L, t) and D(L, t) represent the "birth" and "death' functions characterizing the agglomeration process.

$$B(L,t)=\frac{1}{2}\int_0^L \beta(L,y,t)\cdot n(L-y,t)\cdot n(y,t)dy \qquad (2)$$

$$D(L,t)=\int_0^\infty \beta(L,y,t)\cdot n(L,t)\cdot n(y,t)dy \qquad (3)$$

where β(L,y,t) is the agglomeration kernel representing a rate constant for the agglomeration process.

The difference between B(L, t) and D(L, t) represents the net number of particles that reach the characteristic size L at moment t by agglomeration.

The mathematical model for the semi-continuous precipitation includes the reaction kinetic characterization together with the mass and particle number conservation laws. Additional relations to describe the crystallization processes: nucleation, growth and agglomeration must be considered and corresponding kinetic parameters must be calculated (Mersmann et al, 2002). Therefore, experimental investigations are a must.

The present work aims to solve the mathematical model of precipitation using the population balance (PB) technique, considering homogenous nucleation, size dependent growth and agglomeration and estimate the parameters that describe the crystallization kinetics. The method of choice is the orthogonal collocation, since it transforms the integro-differential equation into a system of ordinary differential equations.

The parameter estimation was performed minimizing, using genetic algorithms, the sum of squares of the residual experiment - calculated density function in the collocation points:

$$\text{Fob}=\mathit{min}\sum_i\left[\frac{\ln n(L_i)_{calc}}{\ln n(L)_{\exp}}-1\right]^2 \qquad (4)$$

The methodology was applied for data obtained in the precipitation of $CaCO_3$ by slowly pouring into a $CaCl_2$ solution, initially placed in a well stirred vessel, an equivalent volume of K_2CO_3 solution.

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Analyzing regimes in chemical reactors with allowing for the multistage kinetics and non-perfection of a system

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Keywords: reactor, multistage kinetics, non-perfect systems, stability, wave front.

Theoretical details

Multistage kinetics and non-perfection of reaction-diffusion systems have an essential influence on the process regime in chemical reactors. At the same time engineers often don’t give due attention to these aspects of chemical processes and apparatuses design. In our work this problem has been theoretically investigated and illustrated by means of two model auto-catalytic reactions as examples.

Firstly we consider the following model scheme with main reagents X, Y and intermediate products A, C, despite the first stage is supposed to be an auto-catalytic reaction (Saul and Showalter, 1985):

$$X + Y \xrightarrow{k_1} X\,,\; X \xrightarrow{k_2} A\,,\; Y \xrightarrow{k_3} C\,, \tag{1}$$

where k_1, k_2, k_3 are the reactions rate constants.

It can be concluded that under the low rate *q* of the reagent Y feed the system (1) has one stable rest point. But when this rate exceeds the critical value

$$q > \frac{k_2 k_3}{k_1} \tag{2}$$

the system acquires yet another rest point.

The regimes analysis gives the folowing results:

1) for $(k_3/k_2) \geq 1$ at any q the rest points are stable nodes, and oscillations don’t exist;

2) for $(k_3/k_2) < 1$ at q exceeding the critical value rest points can be unstable, and transition regimes can exist in the form of oscillations with frequency w and decrement ν:

$$\omega = \sqrt{\frac{2q}{k_3} - \frac{2k_2k_4}{k_1k_3} - \frac{k_3k_4^2}{4}}\,, \tag{3}$$

$$\nu = -\frac{k_4}{2} \tag{4}$$

The second example is the auto-catalytic reaction of the following type (Saul and Showalter, 1985; Kudryavtsev, 1987):

$$A + X \underset{k_2}{\overset{k_1}{\rightleftarrows}} 2X + C \tag{5}$$

Let’s consider the case of non-perfect reaction-diffusion system, where the chemical potentials of reagents read (Karapetianz, 1975; Jou et all, 2001)

$$\mu = \mu^* + RT \ln X + \omega_{AX}(1 - X)^2\,. \tag{6}$$

Here

$$\omega_{AX} = \kappa\left[2\varepsilon_{AX} - \left(\varepsilon_{AA} + \varepsilon_{XX}\right)\right], \tag{7}$$

and ε_{AX} , ε_{AA}, ε_{XX} are energies of an interaction between molecules of reagents, κ is parameter depending on the model of liquid state.

According to thermodynamics of diluted solutions the diffusion coefficients determine by the derivatives of chemical potentials over the concentrations. Thus we obtain:

$$\frac{\partial \mu}{\partial X} = \frac{RT}{X} - 2\omega_{AX} = \frac{RT}{X}\left(1 - 2\omega_{AX} X\right), \tag{8}$$

$$D = D_i\left(1 - 2\omega_{AX} X\right), \tag{9}$$

where D_i is the diffusion coefficient in perfect system, D is the one in a real system.

From detail analysis which is analogous with the analysis of system (1) we conclude that non-perfect kinetics in the case of high diluted solutions doesn't change types of rest points and reactor regimes. At the same time concrete values of regimes characteristics have undergone changes.

Particularly, the velocity of wave front which generates from transient oscillatory regimes differs from the velocity of wave front in perfect system. The appropriate relation reads

$$c_{0r} = c_0 + \gamma\sqrt{k_1 D_i A} \tag{10}$$

Here c_0 is the wave front velocity calculated for the perfect system:

$$c_0 = \left|V - 2\sqrt{k_1 D A}\right|, \tag{11}$$

and $\gamma < 1$ is the special amendment coefficient for which we obtain the following relation

$$\gamma = 2\omega_{AX}\frac{k_1}{k_2}A. \tag{12}$$

Conclusion

As a result of our investigations we can conclude that multistage and non-perfect kinetics can essentially affect reactor regimes and change their important characteristics. Moreover we submit the set of parameters governing the regime stability and describing the regime bifurcations. The main types of possible dissipate structures induced by these factors as well as conditions of their formation have been determined. The results of investigations are likely to be useful for calculating intensity of mass transfer processes in chemical reactors.

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Optimisation of a package production process for the implementation of a "Total Productive Maintenance"

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Keywords: Lamination, TPM, packages, factorial design, optimization

This research consists of the detailed study about powder detergent packages production process, focusing especially on optimal control variables levels' selection and stipulation, with the purpose of minimizing the number of defective board boxes.

In spite of the fact that the production process has four stages, this study falls mainly upon the second stage's events, where the laminated board boxes are produced.

Among the several defects these boxes may have, one in particular was studied, as it is responsible by the major problems that have happened, since the company started producing these boxes. This defect is the curling of the laminated board boxes, which by going to the third stage cause the machine to stop, as well as a high level of waste.

Performance's characteristic is defined as the maximum deformation height reached by the board boxes, in cm, being the maximum of 2 cm from the upper and lower left corners of the box.

From all the variables selected, only seven were considered critical. Those are: velocity (v); fibre breaker's position (QF1 and QF2); paper's tension (tp); wax temperature (Tw); board grammage (Gc) and the board humidity (Hc).

By using a factorial design, one has concluded that the variables which influence the boxes curling the most are tp, QF2 and Gc.

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Technology prospecting for a model of industrial ecology based on microalgae biomass in Rio de Janeiro

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The converging problems of climate change, energy security and environmental degradation are the result, for the most part, of the permanent growth in the conversion of natural resources into waste, which began with the Industrial Revolution and which has had a profound effect on the environment. To the current picture of environmental degradation is now added an energy scenario marked by increases in the price of a barrel of oil and the threat of reserves running out. Alternatives to oil are a strategic problem for industry, and the only plausible alternatives appear to be renewable raw materials, i.e. biomass and its derivatives (byproducts).

In recent years, much interest has been focused on the biotechnological potential of microalgae, due mainly to the environmental diversity and physiological adaptability of these organisms. This diversity underlies the fact that all those involved in microalgae cultivation have their own technical solutions, ranging from ponds to reactors, either open or closed. In every case, effort is aimed at guaranteeing the introduction of luminous radiation in the dispersion of microalgae, a technological challenge which demands approaches involving biological knowledge and engineering procedures for photobioreactor projects and scale-up. Cultivation of microalgae have been undertaken with the aim of producing biomass for the manufacturing of food and chemical products with higher added values, as well as for the production of biofuels and CO2 capture.

The present study, which is part of a multidisciplinary project composed of various research groups at the Federal University of Rio de Janeiro, Brazil, proposes a model of industrial ecology based on microalgae for the state of Rio de Janeiro. The objectives of the model are an increased participation of renewal energy sources in the composition of the energy grid, generating conditions for regional economic development in Brazil, based on the addition of value to the productive process, and the consequent creation of opportunities for increased employment, income and royalties.

One stage of this study deals with the concept of technology prospecting in the sense of indicating opportunities and threats with regards to technological, sectoral and regional development, identifying bottlenecks, limitations, opportunities and demands for technologies. Within the context of innovation, technology prospecting is of use in the senses of, 1) promoting channels and common language for the circulation of information and knowledge strategic for innovation; 2) supplying more timely intelligence for decision making in science, technology and innovation; 3) shaping visions of the future in the thoughts of those making decisions and creating networks; and, 4) supporting decisions on establishing priorities for R&D, managing the risks of technological innovation, and improvements in the technological competitiveness of products, processes and services. At this stage of the study, information is being gathered in patent databases such as the USPTO and Derwent, and in scientific and technology databases such as Science Direct, Web of Science, Scirus, and ScFinder Scholar. Analysis of the data, based on keywords, covers several levels of knowledge, from the type and/or author and country of the patent depositor through to a deeper analysis, with the creation of taxonomies and knowledge maps. The study aims to validate the technological, economic and social importance of a model of industrial ecology based on microalgae biomass for the State of Rio de Janeiro.

Automatic complex installation to ensure the microclimates in grafting some friut-growing, dendrological and forest species with difficult propagation

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Keywords: product innovation, microclimat, hot callusing

The article is the result of the accomplished researches for the developing of an efficient and modern method to ensure the best microclimate for the achievement of a high performance in the vegetative propagation for some species that have a difficult vegetative propagation. The installation conceived and accomplished by our team contributes to the percentage's increase of grafting attaching by achieving a proper callusing, it avoids the challenge of a metabolic symbionts imbalance, thus increasing the possibilities of attachment when plating in ground and a high functioning capacity of the ones presently existing. Our purpose was to determine the grafting behaviour in protected spaces during the vegetative rest for walnut tree and hazelnut tree by using the grafting methods: perfective copulation, in slash and steps (mechanized). The automatic complex installation is based on "hot callusing" method and consists in the achievement, only at the grafting point level, of the best temperature according to the species (approximately 260-280C). The installation has the role to accelerate the cell division phenomenon only at the grafting point (in the combining area of the two symbionts, the graft and the stock), while the graft and the stock with its radicular system, remain in a still controlled environment that does not allow them to reproduce the metabolic processes and do not start in vegetation (the ensuring of the temperature below biological zero according to the species with the purpose to maintain the latency state).

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Rheological and thermodynamic study on a biodiesel production process

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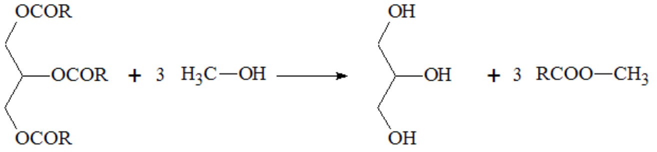
Keywords: Biodiesel, Rheology, Surface Energy, Thermogravimetry, Differential Scanning Calorimetry.

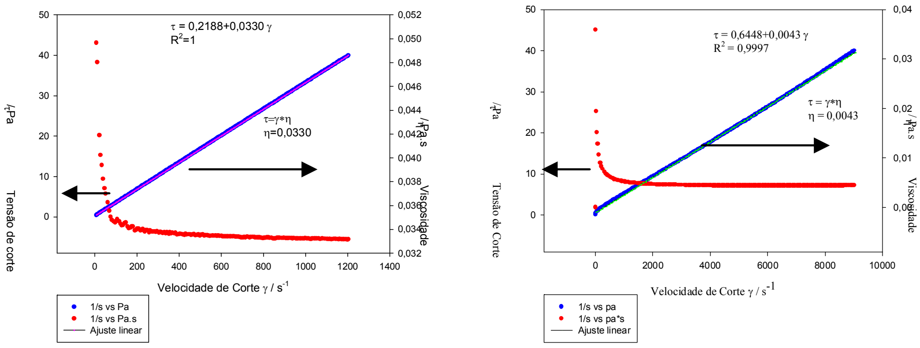
Most of the world energy consumption is based on oil, coal and natural gas. The rise in energy demand related with new global players resulted in a surge of the conventional energy sources prices. The global concern on environmental issues such as greenhouse effect and threats related with future supply shortage have opened the way for new sustainable alternatives based on renewable sources, and the conversion of natural oils into biodiesel. Biodiesel is a fatty acid mono-alquil esther derived from vegetable oils and animal fats obtained by a transestherification process with an alcohol (methanol or ethanol), as represented by the equation (Knothe et al.):



(Equation 1)

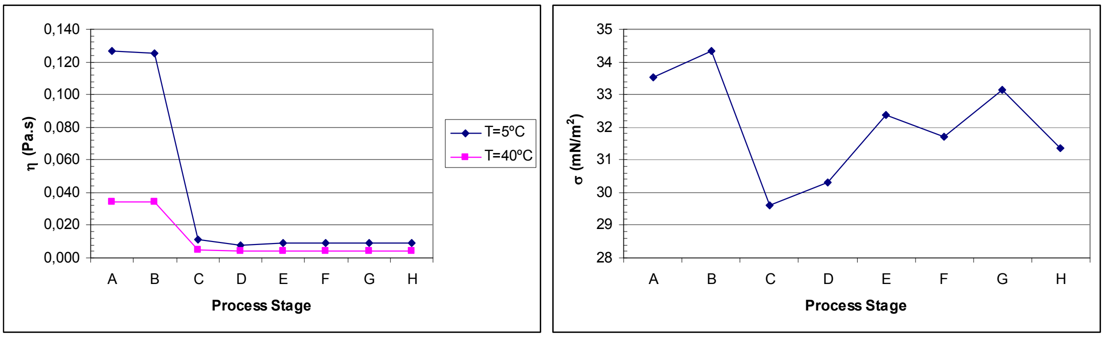
The oil in the industrial process passes trough a pre-treatment (such as filtering, degumming, free fatty acids removal and catalyst mixing), a transestherification reaction, a phase separation from glycerol, a washing step, a purification step and a drying step. This work characterizes different vegetable oils such as rape seed, sunflower seed and soybean, and biodiesel produced industrially by methanolic via. All samples were furnished by PRIO, from an industrial site. The scope of this study embraces rheological and surface tension characterization and a thermodynamic study on thermogravimetry and differential scanning calorimetry (DSC). The reason behind these options is the importance of these itens on the biodiesel flow, atomization and proper combustion through the injector (Ejim et al.). The evolution troughout a PRIO industrial process was the goal of this study, represented by steps A trough H.

The oil and biodiesel viscosity evaluation was made on a HAAKE RS150 rheometer with baths at 40ºC and 5ºC. The surface tension was made using a DATAPHYSICS Contact Angle System OCA. Both oils and biodiesel have shown pseudoplastic responses, as exemplified in Graphic 1. The value of the apparent viscosity can be considered constant for high shear rates.



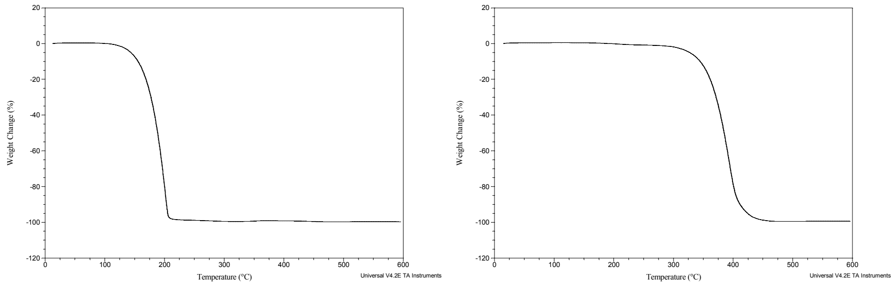
Graphic 1: Oil and Biodiesel pseudoplastic responses.

Graphic 2 shows the evolution during the process of the apparent viscosity (at 5ºC and 40ºC) and surface tension. It can be seen that there is a steep viscosity reduction after the transestherification step. This can be justified by the molecular length reduction and a more linear structure in the biodiesel. The surface tension similarly reduces after the transesthterification step, but it increases regularly in the subsequent steps.

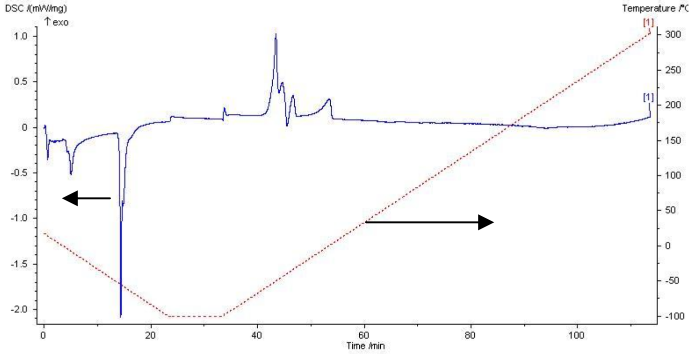


Graphic 2: Process viscosity (Pa.s) at 5ºC and 40ºC and surface tension (mN/m^2) evolutions.

Thermogravimetry is a technique which obtains a sample mass variation as the temperature increases regularly. Graphic 3 represents thermogravimetric results for a sample of oil and sample of biodiesel, where evaporation took place at close to 175ºC for the oil and close to 375ºC for the biodiesel. Differential Scanning Calorimetry compares the heat flow to a sample and a standard material, with a programmed temperature evolution. It permits to study phase transitions and reactions. A study has already been made between -100ºC and 300ºC in a nitrogen inert atmosphere. As seen in Graphic 4, three phase transitions where obtained in the -100ºC–0ºC interval. Further studies with non-inert air atmosphere are being made. A comparison of these results with a representative petrodiesel sample will also be made.



Graphic 3: Oil and biodiesel thermogravimtric evolution.



Graphic 4: Differential scanning calorimetry for a biodiesel sample.

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Optimal parameters to produce microbial biomass to degrade hydrocarbons

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Keywords: bacteria, bioremediation, fungi, hydrocarbon-degrade

There are many alternatives to deal with a petroleum contaminated soil; one of the most recommended is the bioremediation process. It uses microorganisms to transform or destroy contaminants (Oudot *et al.*, 1987). A great number of microorganisms capable of utilizing petroleum hydrocarbons as carbon and energy source have been identified (Davis, 1967). This work shows the optimal parameters to produce the maximum growth of a bacterium (*pseudomona*) and a fungus (*penicillium sp*) which have been confirmed as consumers of petroleum. An *in vitro* bioassay was established to evaluate the microbial growth. A factorial design AxBxC was used, where A prevails for temperature (room temperature, 35°C and 40°C), B stands for pH (for bacterium: 4.0, 6.0 and 8.0; for fungus: 3.5, 5.0 and 6.0), and C corresponds to nutrients (three types of commercial nutrients: N1, N2, N3 and a combination of mineral salts). The whole experiment consisted in 72 experimental units (Erlenmeyer flasks of 250 mL). A continuous flow of filtered air was bubbled through the cultures. The set of optimal parameters for bacterium was with nutrient N2, room temperature (28~30°C) and a value of pH of 4.0. After nine days of treatment, the maximum growth was obtained during the third day (9.52E+09 FCU-mL^{-1}), Fig 1. Fungus was followed for 37 days and the best growth was showed after 25 days (1.50E+6 FCU-mL^{-1}), Fig 2, the set of optimal parameters was nutrient N2, room temperature (28~30°C) and a pH equal to 3.5. Once the optimal growth parameters were found, a second experiment was performed with this set of conditions in a 2L-reactor and bacterium had a maximum growth after four days (4.6E+10 FCU-mL^{-1}). In order to observe the maximum growth of fungus in a shorter period of time, nutrient concentration in the culture medium was reduced in 50%, respect to the concentration in the Erlenmeyer flasks, and exhibited its maximum after ten days (1.15E+5 FCU-mL^{-1}).

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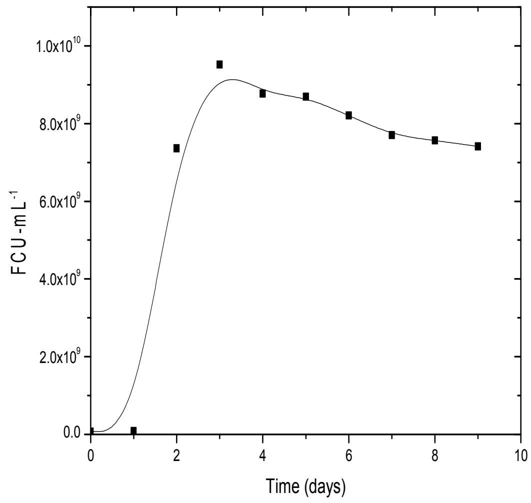

Figure 1. Growth of bacterium with the set of optimal conditions.

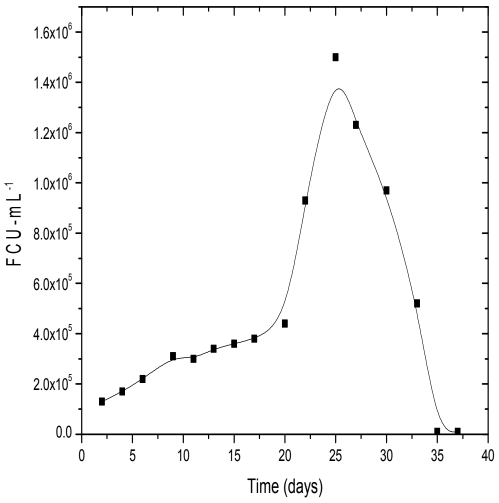

Figure 2. Growth of fungus with the set of optimal conditions.

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Comparison of ozonolysis and steam explosion of wheat straw to obtain bioethanol

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Keywords: bioethanol, lignocellulosic biomass, steam explosion, ozonolysis

Introduction

Bioethanol is one of the best renewable fuels to reduce the present dependence on crude oil because it's a renewable combustible, non toxic, highly soluble and quickly biodegradable. Ethanol can be used as fuel for automobiles either alone or blended with gasoline to reduce the consumption of petroleum fuels. The National Renewable Energy Laboratory in the USA establish that the use of bioethanol blended gasoline results in fall of around 1.85-2.65 Kg CO_2 emissions /L of gasoline. It can also be used as an additive for standard gasoline as ETBE (ethyl tertiary butyl ether), being a replacement for MTBE (methyl t-butyl ether), the most common additive, which use is being limited in the United States owing to its probed groundwater contaminant effect.

Nevertheless, despite the efforts made to decrease the production cost of ethanol, this is still too high compared with petroleum derived fuels. To reduce this cost, lots of researchers have focused on the exploitation of lignocellulosic materials which abundance would guarantee process self-sufficiency. The main obstacle for the better use of lignocelluloses to produce ethanol is their low digestibility because of the tight association between its components: cellulose, hemicellulose and lignin.

In this work ozonization and steam explosion of wheat straw have been compared as adequate pretreatments before enzymatic hydrolysis, leaving cellulose accessible to enzymes. Hydrolysis tests for each sample were performed in order to determine the improvement produced in the enzymatic saccharification under the different pretreatment conditions used.

Material and Methods

Raw material. Wheat straws were kindly donated by Institute of Technological Agriculture of Castilla y León. The straw was ground in a blender, sieved to obtain the size desired and kept in an oven at 45ºC.

Ozonolysis Pretreatment. The ozonolysis was performed in a fixed bed reactor under room conditions. Ground material was firstly hydrated to the required value and exposed to an ozone/air gas stream in the fixed bed reactor (glass column 50 cm in heigh and 2.7 cm in diameter). The ozone production was controlled by varying either the oxygen flow rate or the electrical power supply and its concentration was measured according to the iodometric method. Reactor's outlet went past through a 2% KI solution in order to remove the unreacted ozone from the gas stream. The resulting ozone-treated substrate was dried in an oven at 45ºC, stored in a freezer and used for analysis.

Steam explosion Pretreatment. The steam explosion is carried out in a 5L stainless steel batch reactor in which the straw is loaded at the top and heated to the desired temperature with saturated steam. When the pre-set residence time has concluded, the steam-treated biomass is released from the reactor by rapid depressurization of the vessel. After the pretreatment, the product is washed with warm water and the residual solid is separated by filtration and used for analysis. The liquid portion is also employed to analysis.

Enzymatic Hydrolysis. The enzymatic hydrolysis was carried out using a mixture of cellulose complex (NS50013) and β-glucosidase (NS50010), both enzymes kindly donated by Novozymes (Denmark). The tests were performed with 0.3 g of freeze stored biomass suspended in 5 mL acetate buffer 0.1 M (pH 4.8), containing 10 FPU g-1 and 10 CBU g-1 of substrate (dry basis) at 50 ºC for 96 h. The flasks were shaken in a rotary incubator at 150 rpm. After the hydrolysis, liquid portion is analyzed for carbohydrates.

Analytical methods. Acid insoluble lignin, acid soluble lignin and carbohydrates were estimated according to NREL laboratory analytical procedures Lap 003, 004 and 002 (10, 11, 12) respectively, except that a Bio-Rad HPX-87H ion-exclusion column was used to measure sugar concentration. The mobile phase was 5 mM H_2SO_4 at a flow rate of 0.6 mL min-1 at 60 ºC. The detector was based on the refraction index measurement. Carbohydrates after enzymatic hydrolysis were analyzed by HPLC with the same method indicated before.

Results and Discussion

Experiments carried out in the ozonolysis of wheat straw took into account the main operating parameters, e.g. sample moisture, particle size, ozone concentration and ozone flow. Results from the statistical analysis showed that the higher percentage of lignin removed after the ozone treatment corresponded to the higher flow and/or concentration of ozone. On the other hand, the higher size particle lead in the higher yield on glucose after enzymatic hydrolysis of solid pretreated.

The operating conditions leading the maximum yield on enzymatic hydrolysis were: 40 % of moisture, 3-5 mm of particle size, 90 L/h of ozone and a ozone concentration corresponding the 76% of ozonizator power. These values agree with the higher lignin removal observed.

Steam explosion experiments took into account the residence time of solid into the reactor, temperature and particle size as the main operating conditions. The statistical analysis of the results showed that delignification is not influenced by the parameters considered: higher the residence time, higher the delignification obtained but always in an extent lower than 6 %. The yield of enzymatic hydrolysis increased when the highest temperature (210 ºC) and particle size (20 mm) was tested. The influence of the residence time is also positive, the highest residence time lead the higher yield on enzymatic hydrolysis, but the influence is lower that the observed with the other parameters tested. From these experiments, it can be concluded that the maximum yields (87,1 % on glucose) on enzymatic hydrolysis were obtained when wheat straw is ozonized, operating with 40 % of solid moisture, 3-5 mm of particle size and 90 L/h of ozone, whereas a 80,9 % of glucose after enzymatic hydrolysis was obtained when the raw material was steam exploded at 210 ºC, 20 mm of particle size and 10 min of residence time. Moreover, ozonolysis has demonstrated to be a very suitable pre-treatment for wheat straw to be used for bioethanol production. More research must be done in this way, in order to have a costly effective pretreatment.

Acknowledgements

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The vegetative cycle of *Lavandula luisieri* – influence on essential oils and antioxidants

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Keywords: *Lavandula luisieri*, essential oil, antioxidant, Rancimat, peroxide value

The *Lavandula luisieri* was studied in terms of yield and composition of essential oil and antioxidant activity during the vegetative cycle of life. The essential oil was obtained by hydrodistillation (Clevenger apparatus), and the liquid and the solid residues were solvent extracted to obtain extracts, which were studied in terms of antioxidant activity. The composition of essential oils was determined by gas chromatography and confirmed by GCMS.

The main components found included α-pinene, 1,8-cineole and α-terpineol. The antioxidant activity was determined in terms of induction time by the Rancimat method and the peroxide value. The highest value of essential oil yield was 0.59 %, obtained from the plant harvested in September. The extracts with the highest antioxidant activity were obtained from the plant harvested in May.

Pectin extraction from guavas using response surface methodology

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Keywords: Pectin, Extraction acid, Guava

Abstract

Extraction of natural hydrocolloid carbohydrate polymers, such as pectin, from de surplus of the crop of fruits becomes interesting commercially. Thus, the extraction of pectin from guavas pulp and pulp with peel was carried out in an aqueous acid medium under different concentrations using a experimental design for two extraction parameters (acid concentration and time). Considering the efficiency, the economization of energy and the feasibility of experiment, the optimum conditions of pectin extraction were extraction time 60 min and citric acid solution 5%, getting up yield of extracted pectin from guavas pulp and pulp with peel the range of 12–13.65% and pectin of low degree of esterification.

1 Introduction

Pectin obtained from fruits has different characteristics, which vary according to its origin, allowing for wider use in industry (Willats et al., 2006). The raw material commonly used is the citrus peel, apple or crushed. Studies of new sources have been the target of several searches, especially of fruits and agro-industrial waste. The guava fruit is considered that are high in pectin, however, is not used for that purpose. The taking of pectin from the pulp and the pulp with peel can be alternative marketing and minimization of losses during harvest.

The method of extraction of pectin in fruit is chemical extraction and alcohol precipitation. The factors that influence the yield of extraction are type and concentration of acid, temperature and time of extraction. The most common method is the use of solutions of inorganic acids under high temperatures (May, 1990). However, the use of inorganic acids generates waste pollutants. Organic acids become an alternative. This work was extract pectin from the pulp and the pulp with peel of guavas and characterized by degree of esterification.

2 Materials and methods

Red guavas (Psidium guajava L.) *cv* Pedro Sato were acquired in the district of Santa Terezinha, Itaporã city, MS state, Brazil The fruits were washed, separated into two lots and broke up pulp and pulp with peel. Fractions of pulp and pulp with peel were dried at 55 ° C until final moisture of 13%. The fractions were ground and stored until their use.

The extraction of pectin was performed in acidic medium. Next is a composed central rotational design 2^2, whose independent variables were: concentration of citric acid (g $L)^{-1}$ and time of extraction (min). The dependent variable was the yield of extraction. Sample of each flour were dissolved in distilled water before extraction. The extractions were performed to 97 ° C under conditions of concentration of citric acid in the extraction of time pre-set by design. After extraction, samples were cooled, filtered and pectin was precipitated with ethanol in the ratio of 1:2. The pectin precipitated was separated by filtration and dry in stove at 55 ° C until constant weight. The yield of pectin was calculated from the initial amount of the raw material used. In samples of pectin obtained on the condition chosen by the result of the planning, was quantified

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the content of acid galacturonic and the degree of esterification. The results were analyzed by ANOVA and Tukey test.

3 Results and discussion

Table 1 presents the means yield of pectin extracted from flour pulp and pulp with peel guava.

Table 1. Yields of extraction of pectin obtained from the flours of pulp and pulp with peel guava by experimental planning.

Experiment	Pulp	Pulp with peel	Independent variables	
	[g (100 g)$^{-1}$]	[g (100 g)$^{-1}$]	Acid [g (100 g)$^{-1}$]	Time (min)
1	7,83^{c}	7,31^{e}	3,5	35
2	13,64^{a}	10,57^{b}	6,5	35
3	9,38^{b}	8,87^{c,d,e}	3,5	85
4	12,90^{a}	10,64^{b,c,d}	6,5	85
5	9,56^{b}	8,49^{d,e}	3	60
6	13,66^{a}	13,24^{a}	7	60
7	7,91^{c}	5,91^{f}	5	25
8	12,77^{a}	12,85^{a,b}	5	95
9	12,52^{a}	11,41^{a,b}	5	60
10	12,75^{a}	11,81^{a,b}	5	60
11	12,44^{a}	11,36^{a,b,c}	5	60

*Letters equal columns do not differ statistically between them ($p>0,05$).

The experiments performed with concentrations of citric acid from 5 to 7 g (100 g)$^{-1}$ provided higher income for the extraction of pectin both in the flour pulp when the flour pulp with peel guava. Analysis of variance results showed that the concentration of 5 g (100 g)$^{-1}$ citric acid in the extraction time of 60 min was obtained better training of the gel, noted for its firmness. So, as this condition, there was no significant difference in mean of income obtained from the use of higher concentrations of citric acid and times longer, it was concluded the combination be more conducive to obtaining pectin from flours.

Pectin obtained from the flours showed degree of esterification less than 50%, and characterized with pectins low esterification; compared with pectins, high-power esterification, are less sensitive to changes in pH, they can form in gels range pH 2.6-6.0 and be used as a thickener and stabilizer in food emulsions (Yapo et al. 2007). Despite the low degree of esterification, pectins showed concentration of acid galacturonic next to the standard commercial.

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Study on caqui (*Diospyrus Kaki L*) fruit osmotic dehydration treatments

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Key words: Osmotic dehydration, caqui (*Diospyrus Kaki L.*), mass transfer, Reynolds Number, response surface methodology

Introduction

In Brazil, great part of fruits and vegetables produced is lost in the production centers for not using efficient techniques, which assure a longer time of shelf life. The non-processed fruits are sufficiently perishable, their losses postharvest, in the developed countries, not exceed 20% of total production. On caqui fruit production, especifically, the high perishability of these fruits and the local market, make the demand lower than the offer, causing collapse of prices and diverse losses for the farmer, in the short period of harvest. Thus, the search of alternative methods to minimize these losses is constant. The effect of osmotic dehydration, as pre-treatment, on fruits conservation methodology, is mainly related to the improvement of its nutritional, sensorial and functional properties. The type and degree of alteration in the composition of the osmodehydrated fruit depend, specially, on: the intrinsic characteristics of the fruit, the posterior process of conservation and the desired qualitative characteristics. The knowledge of mass transfer rates on caqui osmotic system between the fruit and the dehydrated solution can improve nutritional and sensorial properties to the fruit.

Objective

The goal of this work was to: construct a system of osmotic dehydration with recirculation of the drying solution; dehydrate slices of caqui osmotically in the system with recirculation; determine the mass transfer parameters and calculate the apparent diffusivity of water and solute.

Material and methods

Caquis (*Diospyrus Kaki* L.) of variety Fuyu, from the same orchard, were supplied in the local market by the same provider. They were chosen in accordance to size, appearance (healthy fruits), shape and degree of maturation (mature, however firm), in order to get more homogeneous samples. Sugar (sucrose) was commercial provided by Açúcar União (Brazil). Caqui samples had the rind and seeds removed, cut into slices of 1 cm of thickness, and then washed with water and dried with absorbent paper, before being submitted to the osmotic process. The samples were weighed, placed on polypropylene trays and put into the osmotic solution (60 ºBrix), previously warmed at 40 ºC. The flow of the solution was established by a peristaltic bomb with outflow of 0,003 L/h, to guarantee the contact of the sample with the solution. Analytical methods were determined according to AOAC (1996). Soluble solids content was determined by refractometric index using refratometer (Zeiss West Germany). Reynolds number calculation was performed according to Mavroudis, Gekas, and Sjoholm (1998). The experimental data obtained were analyzed for regression and graphical analysis by the software STATISTICA version 5.0 (Statsoft, USA). A full 2^2 factorial design with replicate at the center point was used to study the effect of the two variables of process: temperature (40, 50 e 60 ºC) and period of immersion time (60, 180 e 300 min) on water loss (WL), weight loss (WEL), solid gain (SG) ratio. The flow type in the system was determined by the Reynolds Number.

Results and conclusion

The model designed for the osmotic dehydration showed to be efficient to dehydrate caqui. The fluodynamic of the osmotic system was conducted by the laminar draining, and the Reynolds Number determined was 3.33. The water loss was influenced mainly by the period of time of immersion and had little influence by temperature. The polynomial model for water loss had a satisfactory value of R^2 with 99% at significant at 5% level, showing that this polynomial model is adequate for describing the relationships between the response under study and the experimental factors. The solute gain was significantly influenced by the temperature and time of immersion; however, the analysis of variance demonstrated that the polynomial model did not have good adjustment for the experimental data. The osmotic dehydration of sliced caqui at 40 °C in sucrose solution (60 ºBrix) for 4 hours was chosen as the best condition for water loss (53.5%), weight loss (38.5%) and solid gain (15%).

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Purification of ascorbate oxidase from *Cucurbita maxima* in perforated rotating disc contactor using PEG/citrate system

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Keywords: Aqueous two-phase system, Ascorbate oxidase, PRDC, Continuous extration

1. Introduction

Ascorbate oxidase is largely used in the pharmaceutics industry and in the determination of ascorbic acid concentrations in diverse products, such as natural juices and wines (Fatibelo-Filho e Vieira, 2002). The development of techniques and methods for the separation and purification of proteins and other biomolecules has been of paramount importance for many of theses advances in biotechnology industry. The aqueous two-phase system (ATPS) offers the advantages of gentle environment, favorable processing time, low cost and easy scale-up (Cavalcanti et al., 2008). This work describes the purification and recovery of ascorbate oxidase from *Cucurbita maxima* extract by ATPS using polyethylene glycol (PEG) and sodium citrate system in perforated rotating disc contactor (PRDC).

2. Materials and Methods

2.1. Enzyme extract: The extract containing the ascorbate oxidase (EC1.10.3.3.) was obtained by extraction using the pumpkin Cucurbita maxima, according to the methodology described by Carvalho et al. (1981).

2.2. Analytical determinations: The ascorbate oxidase activity was performed using methods described by Carvalho et al. (1981) in citrate-phosphate buffer pH 6.0, 0.1 M, which uses ascorbic acid 50 μM as substrate. The concentration of total protein in these phases was determined by the Bradford method (1976).

2.3. Preparation of the phase system: The phase system (700 g) was prepared from stock solutions of 50% (w/w) PEG and 30% (w/w) sodium citrate/citric acid. The latter solution consisted of a mixture of sodium citrate salt and citric acid at pH 6.0. The system contained PEG (20000 g/mol, 10% w/w) and sodium citrate (25% w/w), was stirred for 4h at 25°C, and the phases were separated by funnel overnight. After separation, the phases were introduced into the column by means of peristaltic pumps.

2.4. Perforated rotating disc contactor: The perforated rotating disc contactor (PRDC) was made of a Perspex column with 32 mm internal diameter and 160 mm height. Six perforated discs with 30 mm diameter (disc free area of about 9%) were mounted on a central shaft and equally separated.

2.5. Experimental design: The influence of the rotational speed of disks (ν_r) and dispersed phase flowrate (F_D) on the four responses was evaluated from the results of experiments performed according to a 2^2 factorial experimental design (Bruns et al., 2006), plus four central points (Table 1). All statistical and graphical analyses were carried out using the Statistica 6.1 program (Statsoft, 2004).

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3. Results and Discussion

As the results of Table 8 shows, the enzyme preferentially partitioned to the dispersed PEG rich phase in all experiments. After the statistical analysis of the data, considerations were made for each response studied (K-Partition coefficient, PF-Purification factor, K_Da-mass transfer coefficient, Es-Separation efficiency and H- Hold up)

Table 1 - Results of the 2^2 design for the extraction by PRDC using PEG/Citrate system.

runs	ν_r (rpm)	F_D (mL/min)	K	*PF*	K_Da(min^{-1})	Es (%)	H
1	35	1	7,70	2,00	0,010	73,4	0,256
2	140	1	7,34	2,13	0,018	73,3	0,302
3	35	3	3,03	2,24	0,005	55,0	0,324
4	140	3	16,08	2,20	0,018	96,8	0,362
5 (C)	80	2	1,82	2,71	0,059	26,9	0,382
6 (C)	80	2	3,91	2,29	0,040	53,7	0,400
7 (C)	80	2	4,59	3,10	0,037	22,7	0,290
8 (C)	80	2	3,12	1,17	0,047	71,7	0,254

After the analysis of results presented, we found that the central points were those that provided better performance in extracting continuous process by using two-phase aqueous systems. The best results in mean values were: partition coefficient of 3.36 ± 1.19, purification factor 2,31 ± 0,83, separation efficiency 43.7% ± 12% and Hold up 0.33 ± 0.07. Similar results were presented by Cavalcanti et al., (2008). So the best operating conditions selected in this study for the extraction of ascorbate oxidase in the PRDC, was obtained with the rotational speed of disks of 80 rpm and dispersed phase flowrate of 2 mL / min.

4. Acknowledgment

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High pressure treatment of grape seed to enhance the yield of oil extraction

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Keywords: High pressure processing, grape seed oil, extraction.

The increasing consumer demands for high quality, fresh-like, safe foods minimally processed, incited the food industry to continuously improve the existing technologies and gave birth to many research efforts on novel technologies (Ludikhuyze et al., 2003).

In addition to food preservation, high-pressure processing (HPP) can result in food products acquiring novel structure and texture, and hence can be used to develop new products or increase the functionality of certain ingredients (Saraiva et al., 2002; Castro et al., 2006). The application of high pressure leads to rearrangement of tissue architecture and eventually to cell disruption, increasing extractability even at ambient temperature. The combination of very high pressures and lower temperatures could become a viable alternative to conventional industrial practices (Rastogi et al., 2007). Furthermore, used in the pre-treatment of natural matrices, it not only induces higher yields of extraction, but also reduces the treatment times needed.

This essay embodies a larger research on the maximization of the yield and functionality of grape seed products by using technologies such as HPP, enzymatic treatments, and supercritical fluid extraction.

In this work, experiments are carried out to study the effect that several variables have upon the yield, such as: pressure (1000 to 5000 bar), processing time (5 to 15 min), and grinding range of the particles.

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Relations between oxidative stability and antioxidant content in vegetable oils using an accelerated oxidation test –Rancimat

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Keywords: Antioxidant activity, Rancimat Method, vegetable oil, antioxidant extract

In vegetable oils, particularly virgin olive oil and sunflower oil, the effects of temperature and antioxidant extracts concentration on the induction time were investigated using a sequence of simple factorial designs. An empirical equation was derived.

The antioxidant extracts were obtained from aromatic plants by hydrodistillation followed by liquid-liquid extraction using diisopropyl ether as a solvent (Bernardo Gil et al., 2004).

Vegetable oils were spiked with those extracts in a range of concentrations from 250 to 2500 ppm and then subjected to oxidation in a 679 Rancimat apparatus (ISO 6886:2006).

Using water and diisopropyl ether an odourless extracts were obtained, which possesses a great antioxidant activity in vegetable oils. Another important property, good thermal stability, was provided by these extracts, indicating that they can be used in food products.

In the sequence of factorial designs, two blocks of 2^3 design were run. In the first block only peppermint extract was used in two different matrices (variable A). In the second block the antioxidant extract was introduced as a new variable but keeping the same matrix (sunflower oil). The variables and levels are summarized in table 1.

Table 1: Variables and levels of factorial designs

Variables	Level	
Block 1: Peppermint extract	-	+
A: Vegetable Oil	Sunflower	Virgin Olive Oil
B: Temperature, ºC	110	130
C: Extract Concentration, ppm	250	2500
Block 2: Sunflower oil		
A: Extract	peppermint	rosemary
B: Temperature, ºC	110	130
C: Extract Concentration, ppm	250	2500

By using factorial design, one can conclude that the variables that strongly effect the induction time are extract concentration, vegetable oil, and temperature. A simple equation was derived trying to relate the induction time with extract concentration:

$$T_I / T_{I_0} = 1 + k^{\alpha} C_{Ext} \tag{1}$$

where T_I and T_{I0} are the induction time of the mixture and of the control sample respectively, C_{ext} is the concentration of the extract in the mixture, k and α are constants that should depend on the vegetable oil and on the temperature.

The model was validate with data obtained from rosemary (Rosmarinus officinalis), peppermint (Mentha piperita), lemon balm (Melissa officinalis) and marjoram (Origanum majorana) in virgin olive oil and sunflower oil at temperatures of 100, 110, 120, 130, 140 and 150 ºC.

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Experimental sorption isotherms of loquat and quince fruits in the range from 20 to 65 ºC.

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Keywords: Water activity, Gravimetric method, Equilibrium moisture content, Isosteric heat.

Loquat is an edible fruit (*Eriobotrya japonica Lindl.)* that belongs to the Rosaceae family. Its reported composition (wet basis) refers water 78.0%, carbohydrates 10.6%, fiber 10.2%, fat 0.5%, protein 0.4% and other components above 0.3% (Mataix *et al.*, 1998). The world production of loquat in 2006 is estimated at 550,000 t (Soler *et al.* 2007).

Quince (*Cydonia Oblonga Mill*) belongs to the pome fruit family. The main constituents of quince are water 84.0% and carbohydrates 15.0% (wet basis). Other small constituents include proteins 0.4% and fats 0.6%. It is also considered a good source of fibre, potassium and vitamin C. The world production of quince in 2006 is estimated at 490,000 t (FAO, 2007).

The difficulties of hand harvesting and the damage during transport limit the commercial expansion of edible fruits as quince and loquat. Data on moisture content and water activity is important to predict the physical, chemical and biological changes occurring during storage and processing of food materials. The water sorption isotherm represents the equilibrium relationship between moisture content of the food sample and water activity at constant temperature and pressure.

Sorption isotherms of loquat and quince fruits were determined by a gravimetric method at different temperatures, in the range from 20 to 65ºC and water activities (0-0.9). As expected, the equilibrium moisture content increased with water activity at constant temperature. There is also an increase in moisture content with decreasing temperatures, at constant water activity. This can be explained because at higher temperatures, the activation of the water molecules changes to higher energy levels and the links become less stable.

The curves obtained can be considered as type II at 20ºC and type III at higher temperatures according to the Brunauer–Emmett–Teller (BET) classification (Brunauer *et al.*, 1940).

Equilibrium moisture content data were correlated by different mathematical models usually applied to foodstuffs (GAB, Oswin, Henderson, Halsey and Peleg). The coefficients of the five sorption models tested at different temperatures were obtained, and shown in Table 1. The parameters of the models used were estimated by nonlinear regression procedure employing Table Curve software (Jandel Scientific), in order to select the best correlation and to improve the analysis of the experimentaldata.

Most of these models showed high coefficients of determination ($R^2 > 0.943$), acceptable standard error ($E < 18$ %) and low root mean square error ($E_{RMS} < 0.147$). These results indicate that all the models can be considered as acceptable for predicting the equilibrium moisture content of these fruits. However, the Peleg and the GAB models (four- and three-parameters, respectively) were found to have the lowest standard error and the highest coefficient of determination (R^2). It is expected that the models with more parameters give best results; comparing only the two-parameters

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models, the Oswin model gives the best adjustment and the Henderson was the model that worst fitting of the experimental results.

Table 1. Sorption isotherm models applied to the fitting of experimental data of loquat (pulp and seeds) and quince

Model	Parameter	Loquat								Quince		
		Fruit				Seed				Fruit		
		20ºC	35ºC	50ºC	65ºC	20ºC	35ºC	50ºC	65ºC	20ºC	45ºC	65ºC
GAB	X_M	0.163	0.136	0.121	0.0989	0.0620	0.0550	0.0484	0.0450	0.115	0.0802	0.0435
	K	0.986	0.981	0.979	0.976	0.971	0.962	0.933	0.929	0.985	0.968	0.960
	C	8.58	2.70	1.07	0.795	22.5	9.59	3.39	1.88	24.9	10.0	7.39
	R^2	0.998	0.993	0.989	0.988	0.997	0.995	0.999	0.998	0.984	0.961	0.977
	E (%)	3.95	4.39	8.37	8.67	7.84	7.39	4.82	7.01	6.11	11.7	7.35
	E_{RMS}	0.0207	0.0264	0.0145	0.00738	0.0147	0.00731	0.00267	0.00334	0.00379	0.04483	0.0120
Oswin	A	0.302	0.192	0.119	0.0871	0.133	0.0998	0.0685	0.0507	0.219	0.143	0.0747
	B	0.620	0.797	0.931	0.950	0.579	0.618	0.665	0.874	0.685	0.653	0.652
	R^2	0.994	0.996	0.992	0.990	0.990	0.994	0.999	0.999	0.987	0.985	0.969
	E (%)	7.07	4.97	9.35	9.51	8.58	8.17	4.94	7.55	13.78	10.82	8.58
	E_{RMS}	0.109	0.0282	0.0167	0.0111	0.0209	0.00860	0.00282	0.00420	0.0489	0.0297	0.0109
Henderson	A	2.08	2.19	2.46	2.79	6.26	6.70	6.97	7.35	2.48	3.74	6.81
	B	0.981	0.627	0.545	0.520	1.140	0.989	0.855	0.813	0.766	0.887	0.895
	R^2	0.983	0.996	0.996	0.995	0.995	0.993	0.994	0.997	0.943	0.983	0.976
	E (%)	17.9	17.6	13.8	13.9	17.4	15.3	15.2	12.4	17.9	18.4	12.9
	E_{RMS}	0.147	0.0338	0.124	0.0618	0.0416	0.0129	0.00658	0.00685	0.0677	0.0232	0.0108
Halsey	a	0.1599	0.1199	0.1049	0.0836	0.0402	0.0359	0.0244	0.0722	0.0968	0.0565	0.0222
	r	1.17	1.04	0.833	0.769	1.33	1.26	1.23	0.701	1.25	1.32	1.35
	R^2	0.998	0.981	0.992	0.985	0.995	0.990	0.992	0.989	0.985	0.969	0.978
	E (%)	7.34	11.4	9.43	11.1	8.91	11.7	8.8	12.3	6.9	16.9	7.94
	E_{RMS}	0.126	0.0325	0.0294	0.0238	0.0251	0.00924	0.00639	0.00625	0.0338	0.0353	0.0114
Peleg	m_1	0.660	0.289	0.114	0.0544	0.257	0.194	0.115	0.0546	0.427	0.0533	0.0732
	n_1	0.951	0.941	0.734	0.710	0.925	0.919	0.889	0.876	0.778	0.103	0.411
	m_2	3.31	1.60	1.46	1.10	1.39	1.22	0.339	0.236	4.01	0.690	0.368
	n_2	13.3	6.64	6.33	5.42	16.0	13.1	6.29	3.47	18.8	2.99	4.46
	R^2	0.997	0.999	0.998	0.995	0.993	0.998	0.999	0.999	0.992	0.996	0.991
	E (%)	6.89	4.79	5.30	8.09	7.65	6.85	4.76	5.01	11.24	10.0	4.99
	E_{RMS}	0.0304	0.0246	0.0133	0.00678	0.0112	0.00548	0.00280	0.00317	0.0277	0.0150	0.00760

To sum up, after testing five equations describing moisture sorption isotherm in the temperature range investigated (20-65ºC), the kinetic three-parameters GAB model and the empirical four-parameters Peleg equation were found the best correlations for the experimental sorption data for quince and loquat throughout the range of water activity studied (0-0.9).

A more detailed analysis of the GAB parameters can provide further valuable information as the monolayer moisture content (X_M). Estimated values for X_M value are within the reported values for fruits, which vary between 4% and 15% (dry basis) (Moraga *et al.*, 2006).

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The Influence of sucrose osmotic pretreatment on the kinetics of rehydrated dried chestnuts

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Keywords: Dehydration, Water sorption, Rehydration indexes, Peleg's model

Rehydration process is an typical stage in the use of dried food products. The study of the rehydration kinetics is very important to improve the global food processing. In this way, it is necessary the knowledge on the food product composition, pre-drying treatment, drying techniques and conditions, composition and temperature of rehydration media (Funebo and Ohlsson, 1998; Lewicki, 1998a). The determination of rehydration indexes is useful to quantify the degree of cellular and structural disruption caused during processes previous to rehydration so as to establish the market acceptability (Lewicki, 1998b). The water absorption capacity (WAC) index provides information on the ability of the material to absorb water and increases with water absorption. WAC value can be calculated according to equation:

$$\mathrm{WAC} = \frac{M_r X_r - M_d X_d}{M_0 X_0 - M_d X_d} \qquad (1)$$

where M is the dry mass (kg d.b.), X is the moisture content (kg water/kg d.b.), and subscripts d, o and r refer to after drying (or before rehydration), before drying and after rehydration, respectively.

The dry matter holding capacity (DHC) index is a measure of the ability of the material to hold soluble compounds and represents the extent of tissue damage and its permeability to soluble compounds. The DHC was calculated according to equation:

$$\mathrm{DHC} = \frac{M_r\ s_r}{M_d\ s_d} \qquad (2)$$

where s is the solid content and subscript r and d are refer to after and before rehydration.

Finally, rehydration ability (RA) index is a measure of ability of the product to rehydrate and shows the damage of the tissue by drying and rehydration processes. The RA was calculated according to equation:

$$RA = (WAC)\,(DHC) \qquad (3)$$

The rehydration kinetics of dehydrated chestnut was obtained and the resulting data were fitted to the Peleg's model (Peleg, 1988) defined by:

$$X = X_0 + \frac{t}{K_1 + K_2 t} \qquad (4)$$

where K_1 (s·kg d.b./kg water) is a kinetic parameter and K_2 (kg d.b./kg water) is other parameter related to equilibrium moisture content.

The effect of the previous dehydration steps (osmotic treatment and convective air drying) on rehydration kinetics of chestnuts was evaluated. Chestnuts with rectangular

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prismatic geometry (10x10x15 mm) were carefully prepared. The dehydration of samples were firstly carried out using osmotic solutions of sucrose (60 % w/w) at 25 ºC for 8 hours. After, convective air-drying was carried out at 65 ºC during various times (obtaining samples with different moisture content). After drying, samples of chestnuts were immersed into distilled water at 25 ºC during different periods of time.

Figure 1a,b shows, as examples, rehydration kinetics of chestnuts with or without sucrose osmotic pre-treatment partially air-dried at 65 ºC up to 0.33 and 0.15 kg of water/kg d.b. Rehydration kinetics showed horizontal asymptotes, with an initial step increase in water absorption followed by a decrease in rehydration rate. Samples previously dried above longer periods showed minor rehydration, indicating the presence of modified structures. The osmotic pre-treatment increased the rate and the amount of water gained during rehydration process regarding to chestnuts without pretreatment. Initial moisture content is also a significant variable in rehydration kinetics. In this manner, the amount of water imbibed during drying depends on the extension of the drying step. Peleg's model was satisfactorily applied to describe rehydration kinetics of chestnuts. Relate to rehydration indexes, DHC index decreases and WAC and RA indexes increase with time. These results confirm that the rehydration ability is reduced due to the drying modifies the structure of the chestnut.

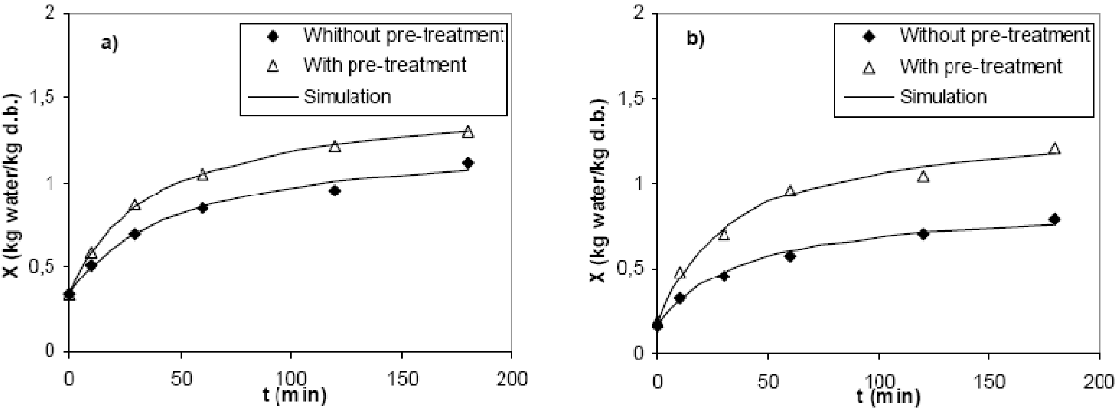

Figure 1. Rehydration kinetics at 25 ºC of chestnuts, with and without sucrose pretreatment, and dried at 65 ºC up to a) 0.33 and b) 0.15 kg water/kg d.b.

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Production of polygalacturonase from *Coriolus versicolor* grown on tomato pomace and its chromatographic behaviour on immobilized metal chelates

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Keywords: Tomato pomace; polygalacturonase production; IMAC purification; *Coriolus versicolor* ; lignocellulosic enzymes.

Tomato pomace and pectin were used as the sole carbon sources for production of polygalacturonase from a strain of *Coriolus versicolor* in submerged culture. The culture of *C. versicolor* grown on tomato pomace exhibited a peak of polygalacturonase activity (1427 U/L) on the 3rd day of culture with a specific activity of 14.5U/mg protein. The production of polygalacturonase by *C. versicolor* grown on pectin as sole carbon source, increased with the time of cultivation, reaching a maximum activity of 3207 U/L of fermentation broth with a specific activity of 248 U/mg protein. Differential chromatographic behaviour of lignocellulosic enzymes produced by *C. versicolor* (i.e polygalacturonase, xylanase and laccase) was studied on immobilized metal chelates. The effect of ligand concentration, pH, the length of spacer arm and the nature of metal ion were studied for enzyme adsorption on immobilized metal affinity chromatography (IMAC). The adsorption of polygalacturonase as well as other enzymes to immobilized metal chelates was due to coordination of histidine residues which are available at the protein surface since the presence of imidazole in the equilibration buffer abolished the adsorption of the enzyme to immobilized metal chelates. A one-step purification of polygalacturonase from C*. versicolor* was devised by using a column of Sepharose 6B-EPI 30-IDA-Cu(II) and purified enzyme exhibited a specific activity of about 150 U/mg protein, final recovery of enzyme activity of 100% and a purification factor of about 10. The use of short spacer arm and the presence of imidazole in equilibration buffer exhibited a higher selectivity for purification of polygalacturonase on this column with a high purification factor. The purified enzyme preparation was analysed by SDS-PAGE as well as by "in situ" detection of enzyme activity.

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Biological deacidification of wine – must

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Keywords: Maloalcoholic Fermentation, *Schizosaccharomyces pombe*, Immobilized yeast, wine deacidification

Total acidity, a key parameter for wine's organoleptic equilibrium and stability during ageing, depends essentially on the relative amounts of two organic acids: tartaric and malic acid (Garcia et al, 2006). Total acidity can be reduced by biological, chemical or mechanical methods but biological deacidification is usually preferred (Garcia et al, 2006). Traditionally biological deacidification involves the growth of lactic acid bacteria, which can be a spontaneous process or induced by inoculation methods depending on the wine's microflora.

Malolactic fermentation (MLF) is a naturally occurring biological process where lactic acid bacteria transform malic acid (a dicarboxylic acid) into lactic acid (a carboxylic acid) and CO_2. This transformation reduces the wine's total acidity but it also changes the wine's aromatic characteristics. Furthermore, MLF is often difficult to occur because during wine fermentation the pH, the temperature and the concentration of SO_2, ethanol and micronutrients can reduce the activity of lactic acid bacteria (Silva et al, 2003).

Maloalcoholic fermentation (MAF) is an alternative to MLF that uses yeast species such as *Schizosaccharomyces pombe* to transform malic acid into pyruvic acid which is further metabolised to ethanol and CO_2 (Fig. 1). The transformation of a dicarboxylic acid (malic acid) into an alcohol reduces acidity and increases ethanol yield. Another advantage of MAF is the fact that yeasts are more tolerant to the above mentioned factors that inhibit lactic acid bacteria (Silva et al, 2003).

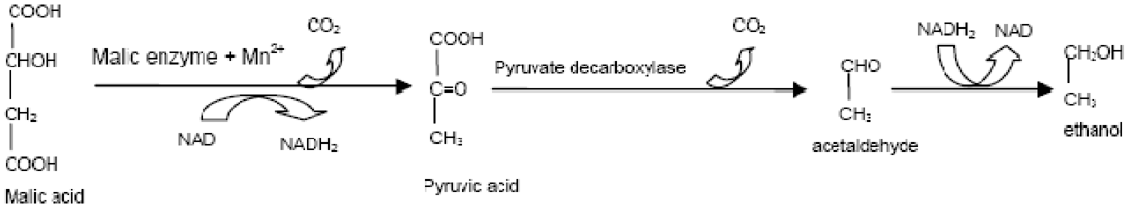

Figure 1 – Schematical representation of maloalcoholic fermentation (Garcia et al, 2006).

S. pombe can be used either as free cells or immobilized cells with similar malate fermentation activity (Silva et al, 2003). The latter have the advantage of beeing easier to recover and re-use. The purpose of our work was to study the activity of *S. pombe* yeast encapsulated in double-layer alginate beads (diameter ~2 mm) for the biological deacidification of wine-must in a batch recycle fixed-bed reactor.

First batch assays were performed to evaluate the influence of malic acid initial concentration and temperature on MAF rate. The results showed that the conversion of malic acid into ethanol is a first-order reaction (Fig. 2) with an activation energy aprox. 15 Kcal/mol.

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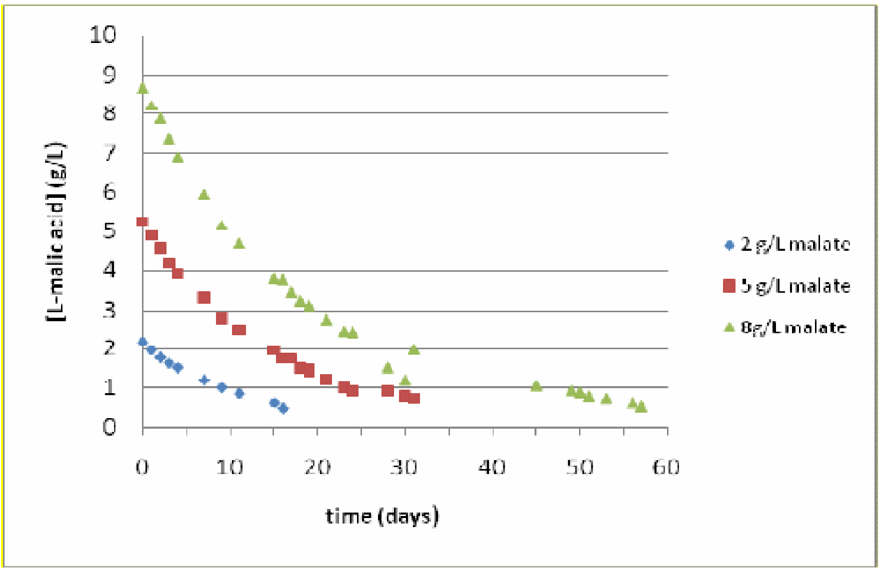

Figure 2 – Degradation of L-malic acid using immobilized *S. pombe* (15ºC, pH ≅ 3).

Further experiments were performed in a recycle fixed-bed reactor operating in batch mode: the fermentation solution flows through a fixed-bed of immobilized cells alginate beads (~20 g), at a constant flow rate (51 mL/min), connected to a 5-L tank. MAF rate was higher in this reactor in comparison with the batch reactor (Fig.3), but slightly lower than in the batch process using free cells. Due to the liberation of some cells into the medium the reaction was stopped and the target value of 0,5 g/L of malic acid was not reached. Modeling and optimization of reactor operation will be presented.

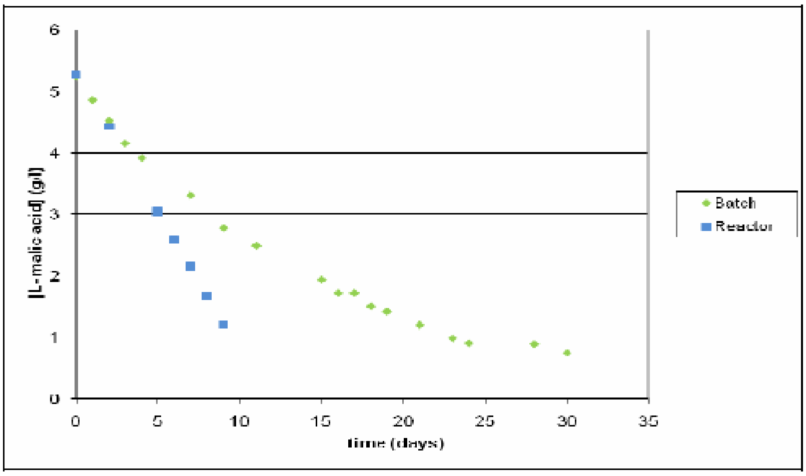

Figure 3 – Comparasion of MAF rate in batch and recycle fixed-bed reactors (15ºC, pH ≅ 3).

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Using GC-MS and multivariate statistics to safeguard the identity of Madeira wine: a preliminary study

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Keywords: Madeira wine, GC-MS, principal component analysis, maximum likelihood principal component analysis

Our image as a country producing distinctive wines brings the responsibility and need for conducting accurately studies aimed at sustaining and encouraging their production, taking advantage of the great evolution in time that they do suffer, in order to ensure the identity and required quality of such wines. Madeira Wine is a good example of this need and potential. Its exceptional longevity makes out of it a product with high economic value, which, on the other hand, also makes it attractive for possible fraudulent copies. For this reason, a reproducible method, able to provide a credible evaluation about authenticity and identity of Madeira Wines, is of utmost relevance and opportunity. The results presented here do regard preliminary developments of such a methodology, involving the screening of volatile compounds by gas chromatography, coupled with mass spectrometry detection (GC–MS), in order to perform the necessary wine flavour fingerprinting that does support the process of wine authenticity evaluation. Such an analytical measurement procedure generates large amounts of experimental data, with strong correlations among them. Therefore, such data are well suited for being analyzed through multivariate statistical techniques, in order to efficiently extract useful information from the data sets available.

After optimizing and establishing raw information about volatile compounds, results obtained so far (in terms of relative amounts) were analyzed using multivariate statistical techniques. Data were organized under a matrix of 24 rows (samples analysed) by 37 columns (variables), where it is very difficult to find out significant trends in the behaviour of samples as such. Consequently, we proceeded by reducing the number of relevant dimensions present in these data sets, in order to highlight the analytical information that is relevant for characterizing the wine's flavours. This task was carried out through the application of unsupervised multivariate statistical techniques, leading to a two or three dimensional space that effectively summarizes the structured variations amongst data sets.

Two groups of samples were considered here: one group collected after 5 years of ageing in casks (five year old wines), and another group collected after 10 years of ageing in casks (10 year old wines). All samples within each group do correspond to the same ageing conditions and were submitted to the same vinification process. In each group, samples were collected for the most important and recommended white wine varieties used in Madeira Wine production, namely: "Malvasia", "Boal", "Verdelho" and "Sercial".

In this work, two different methods for the dimensional reduction of the original data set were applied: principal component analysis (PCA) and maximum likelihood principal component analysis (MLPCA). These two PCA methods estimate subspaces that explain the maximum variance present in the original data sets, differing about the information they use while solving their related objective functions. While in conventional PCA (Johnson and Wichern 2002) only the (scaled) raw data are used, in MLPCA (Wentzell, Anderws et al. 1997) one further considers their associated uncertainties, which are used to weight the relative impact of each data point in the estimation of the PCA subspace (compounds containing smaller experimental errors are more influential in the MLPCA estimation process). In our study the weights used are the uncertainties of each measurement, given by the standard deviation of the GC-MS replicates. Using both approaches, two PCA models were estimated, as well as their associated loadings and score vectors The score and loading values confirm that there are indeed differences between the MLPCA and PCA models. Analysing the scores plot shown in Figure 1, for the first and second principal components, one can see that samples from each variety, with different aging times in casks, appear well separated under the conventional Principal Components of Analysis representation (Figure 1a). Results regarding the "Malvasia" variety seem a bit distinct in this analysis, but they follow the same general trend identified for the remaining varieties: the value of PC2 decreases with wine aging. The coupled analysis of the score and loading values does allow one to identify which set of volatile compounds are responsible for this trend, as well as for the initial deviation between Malvasia and the other varieties.

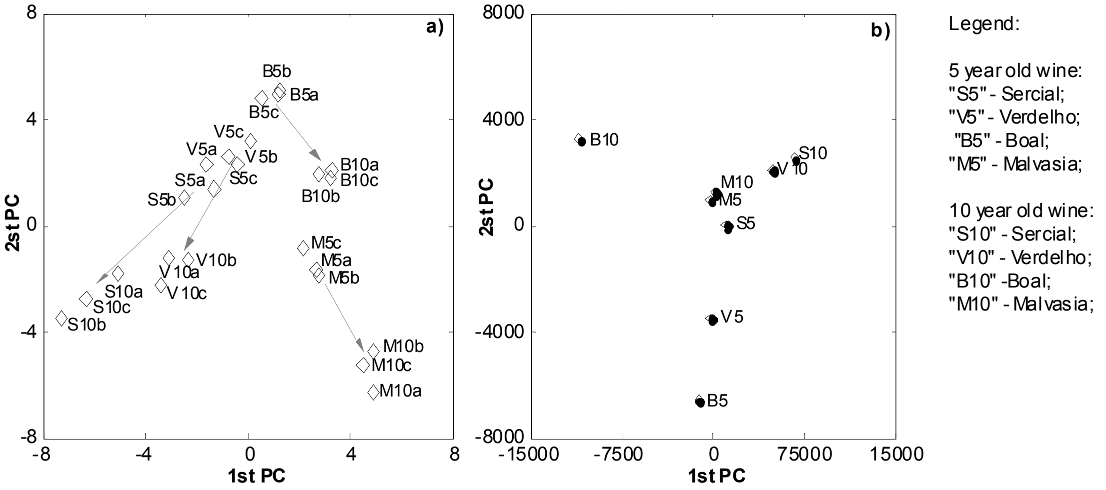

Fig. 1- Scores plots for the 1st and 2nd principal components, with ageing signature for samples from different wine varieties: a) Principal Components of Analysis; b) Maximum Likelihood Principal Components of Analysis.

When using rather MLPCA, the separation obtained is not so clear (maybe due to the fact that the uncertainty estimates employed may not be reliable enough). We also analysed each group of aged samples separately (i.e., the 5 years old group and the 10 years old group). In this case, the two first components do show good separation capabilities for all of the four varieties, explaining approximately 80% of the overall original data variance (Johnson and Wichern 2002). Therefore, using PCA, one is indeed able to identify: 1) the year the wines were produced (using the PCA model estimated using all the groups) and 2) the wine variety (using the PCA models for each group).

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A new strategy for using banana as an ingredient in the brewing process

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Keywords: Beer, Adjunct, Banana, Brewing, Fermentation

Beer is a traditionally fermented beverage made from malted grains of barley, hops, yeast, and water. After wort preparation and during the alcoholic fermentation phase, fermentable sugars present in the wort are transformed to ethanol and several aroma compounds important for the final beer quality are produced. In this phase, the contribution of aroma compounds from other ingredients to the final beer flavour depends on the wort composition (*e.g.* adjunct types), on the yeast strain and on the process conditions. Nowadays, the brewing industry has applied a whole spectrum of technical, biochemical, microbiological and genetic innovations. Furthermore, in today's competitive beer-market it is very important to develop different types of beers, e.g. obtained by the use of new ingredients as adjuncts.

On the other hand, banana, an important crop in tropical and sub-tropical regions and one of the most consumed fruits in the world, can be a raw material favorable to alcoholic fermentation being rich in carbohydrates, minerals and providing low acidity. Its sensorial attributes, such as flavour, taste, texture and colour are significantly influenced by the chemical composition, mainly by acids, sugars and phenolic compounds. Thus, new strategies are now being considered for the use of banana as adjunct or industrial supplement in brewing processes. Its use as an adjunct in food and beverage industries can also be considered because of its low price and high level of production in specific regions of producing countries like Brazil or African countries.

In this work, the objective was to evaluate the performance of banana as adjunct to increase the fermentable sugars and to supply a specific aroma, in pilot-plant brewing experiments. Thus, the addition of banana was evaluated when changing the concentration of the wort from 10 to 12 ºP and from 10 to 13.5 ºP (ºP is the weight of sugar extract equivalent to the weight of sucrose in a 100 g solution/20 ºC), fermented under a constant temperature of 10 and 12.5 ºC, respectively.

For this, static fermentations were conducted in a 180 L cylindrical-conical reactor (Pilot-Scale, Microbrewery Pilot Plant of the Department of Biotechnology, Engineering School of Lorena, University of São Paulo USP/Brazil) using 140 L as working volume. The initial wort was prepared according to conventional brewing techniques (also at Pilot-Scale). Banana juice of variety Prata was used to increase the sugar concentration of the wort. The typical profile of carbohydrates in the ripe banana used in this procedure was: sugars as glucose and fructose (12.4 %ww^{-1}), sugars as sucrose (3.2 %ww^{-1}) and starch (0.5 %ww^{-1}).

The banana juice was produced in solution of 0.3 gL^{-1} by enzymatic treatment (43.2 ºC during 1.3 h, with agitation of 32 rpm, pH 5 and 8.4x10^{-7} L of Ultra Pectinex SP-L enzymatic pectinolytic solution by gram of medium) and by thermal treatment (84 ºC during 0.7 h). The yeast strain employed was a bottom fermenting *Saccharomyces cerevisiae* (commercial lager brewing strain). For initial inoculation in the fermentation reactor, this biomass was cultivated on a rotary shaker and then in a 40 L stainless

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steel vessel under aerobic conditions. The initial cell concentration on the pilot reactor was of 1.19 g.L^{-1} (equivalent to viable cells concentration in suspension of 3.93 x 10^{7} cell.mL^{-1}). Static fermentations were carried out at initial pH adjusted at 5.0 (by lactic acid or ammonium bicarbonate) and concentration of dissolved oxygen of 8 mg.L^{-1}. The fermentations run until an attenuation of 70 %, approximately, was achieved.

During fermentation, samples were taken in duplicate at specified intervals. After centrifugation, the apparent extract and ethanol concentrations in the supernatants were measured with an automatic beer analyser. The yeast cell number was determined using a Neubauer counting chamber and the viability was determined by methylene blue staining.

In both experiments, the final time was achieved at 120 h. Figure 1 shows the profile of the glucose and fructose concentrations as a function of the time of fermentation. For the fermentation conditions, an increment in the initial sugar concentration (12 to 13.5 ºP) and in the temperature (10 to 12.5 ºC) due to the use of the banana as adjunct, increased approximately 17% the volumetric productivity. The final ethanol productivity of the 12 ºP/10 ºC process was 0.29 g.L^{-1}, while in the 13.5 ºP/12.5 ºC process was 0.34 g.L^{-1}. Ethanol yield values were close at the end of each experiment (approximately 0.45 g.g^{-1}). As a complement, the authors suggest the use of statistical designs to evaluate the optimization conditions of the variables simultaneously considered in this work (temperature and concentration). Besides, it also was concluded that, by using of simple preparation techniques of banana juice, the banana fruit can be used as adjunct in brewing processes, helping in the development of new products as well as in the elaboration of more concentrated worts when compared the traditional brewing wort (11-12 ºP).

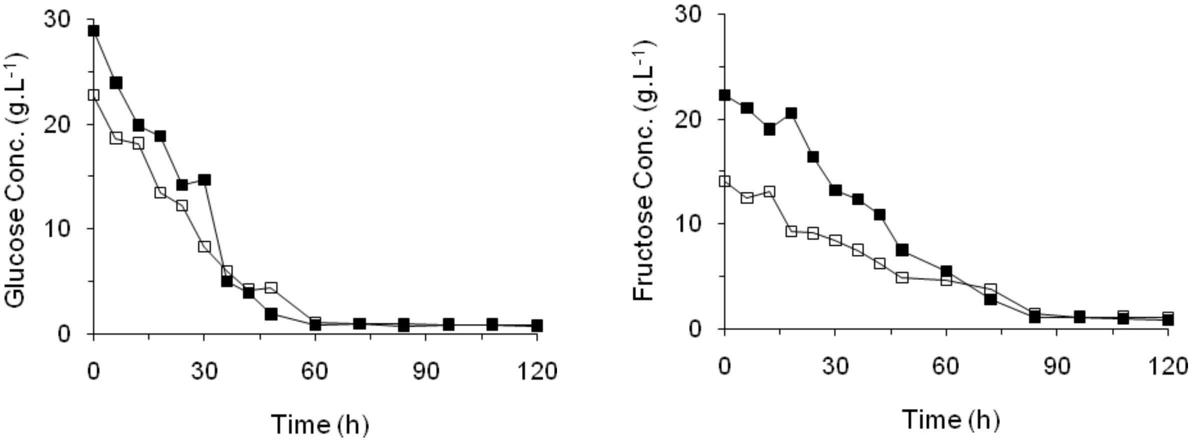

Figure 1. Fermentation performance of glucose (A) and fructose (B) at wort with banana as adjunct under the following conditions of concentration and temperature: (■) 13.5 ºP/12.5 ºC and (□) 12.0 ºP/10.0 ºC.

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β-Galactosidase from *Aspergillus oryzae* immobilized onto different magnetic supports: A comparative experimental and modeling study of the galactooligosaccharides production

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Keywords: β-galactosidase, immobilization, magnetic supports, GOS, mathematical modeling**.**

β-Galactosidase from *Aspergillus oryzae* is an enzyme with a wide industrial application, mostly in the hydrolysis of lactose and, more recently, in the synthesis of oligosaccharides. Galactooligosaccharides (GOS) are non-digestible oligosaccharides, comprised of 2-20 molecules of galactose and one glucose (Miller and Whistler, 2000). GOS are prebiotics that can stimulate the proliferation of lactic acid bacteria and bifidobacteria in the human intestine (Sako *et al*, 1999).

Several advantages are associated with the application of immobilized enzymes on magnetic supports. In this work, β-galactosidase was covalently immobilised onto a Polysiloxane-Polyvinyl Alcohol Magnetic Composite (mPOS-PVA), Magnetic Polysiloxane with Polyaniline (mPOS-PANI), Magnetized Dacron (DACRON) and Magnetite with Polyaniline (MAG-PANI) using glutaraldehyde as activating agent being the synthesis of GOS evaluated and compared at different temperatures (30, 40, 50 and 60 ºC) and various initial lactose concentration (50, 100, 200, 300, 400 and 500 g/L). The support that presents the highest productivity of GOS at various temperatures was mPOS-PANI and the DACRON was the less performant. In all supports the production of GOS increased with increasing initial lactose concentration. With an increase in the initial lactose concentration from 50 g/L to 500 g/L, the maximum GOS content in the product increased from 10.1-10.8% (at 29.5-38.2% conversion) to 25.7-26.2% (at 48.6-55.1% conversion) for the different supports. The immobilization also proved to have an advantageous effect as, after ten reutilisations for the different supports, the immobilized enzyme retained from 84 to 90% of its initial activity.

In addition, a kinetic model for the interpretation of lactose hydrolysis and oligosaccharides synthesis by free and covalent immobilized β-galactosidase on the various supports was used. The kinetic parameters obtained by fitting the experimental data were compared in order to determine the effect of the immobilization process with different supports on the synthesis of oligosaccharides.

These results clearly demonstrate that all supports may be used for β-galactosidase immobilization as, besides improving the enzyme hydrolytic and GOS synthesis properties, its separation from the obtained reaction products is easy to accomplish.

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Microencapsulation of thyme oil by coacervation

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Keywords: Polylactide, thyme oil, coacervation, microencapsulation.

Microencapsulation is a technology that includes several processes to cover an active agent with a protective wall material. There are various industrial applications, such as carbonless paper, “scratch and sniff” fragrance sampling, “intelligent” textiles, controlled release of drugs, pesticides and cosmetics. A wide range of core materials have been encapsulated, including adhesives, agrochemicals, live cells, active enzymes, flavours, fragrances and pharmaceuticals (Gosh, 2006). Microencapsulation techniques can be as diverse as coacervation (Jégat, 2000; Soper et al., 2000), atomization (Ascheri et al., 2003), interfacial polymerisation (Mizuno et al., 2005; Hirech et al., 2003) and “in situ” polymerisation (Bang et al., 2005). The choice of the appropriate technique depends on the core material properties, the involved manufacturing conditions and the product end user requirements.

Complex coacervation was the chosen technique to produce polylactide (PLA) microcapsules using *Thymus Vulgaris* L. (thyme oil), an antioxidant and antimicrobian agent as the core material. This technique consists in the separation of an aqueous polymeric solution into two immiscible liquid phases: a dense coacervate phase and a dilute equilibrium phase. The dense coacervate phase wraps as an uniform layer around suspended core materials.

Biodegradable microcapsules of PLA have received extensive attention as delivery systems for drug encapsulation. This type of biodegradable polymeric carriers can be hydrolyzed in the body to form products that are easily reabsorbed or eliminated (Huang et al., 1997). The core material, thyme oil, is extracted from an aromatic plant of increasing economic importance in North America, Europe and North Africa, so it has an important and growing place in the world market (Badi et al., 2004).This essential oil is widely used in the flavour and food industries. As a pharmaceutical compound, thymol and carvacrol are used in mouthwashes, soaps and creams. Thyme oil is also used in manufacture of perfumes and cosmetics.

In this work we have developed a process to encapsulate thyme oil using PLA as the wall material. Firstly, an oil-in-water (o/w) emulsion stabilized with tween® 20 (HLB of 16,7) and a solution of PLA in dimethylformamide (DMF) have been prepared.

Thereafter, the PLA solution was added to the previously prepared o/w emulsion. The o/w emulsion was obtained by dispersion with an ultraturrax and the encapsulation process proceeded under stirring using an impeller stirrer in a batch actor. The microcapsules formed were hardened by adding octamethylcyclotetrasiloxane and allowed to stand during one hour. After this step, they were decanted and washed with pluronic® F68 solution (0.1% w/w), an ethanol solution (30% v/v), and hexane. Finally, the microcapsules were freeze-dried during 24h.

Microcapsules particle size distributions were determined by laser dispersion using a Coulter LS230. It was observed a bimodal distribution in volume with a mean particle size of 40µm. Microcapsules analysis by optical microscopy (Leica DM 2000

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microscopy equipped with software Leica Application Suite Interactive measurement) and by cryogenic scanning electron microscopy (JEOL JSM-6301F/Inca energy 350/Gatan alto 2500) have confirmed the spherical shape, the rough surface, and allowed to estimate the wall thickness around 5μm. Moreover, it was observed two predominant sizes of microcapsules, compatible with a bimodal distribution and the absence of agglomerates. Quantification of the encapsulated thyme oil was performed by gas chromatography GC/FID using the thymol peak area. The chromatogram analysis also allowed to inspect for the quality of the encapsulated oil and have shown that apolar compounds of thyme oil were preferentially encapsulated in detriment of the polar ones.

This work intends to develop a coacervation technique to produce microcapsules of PLA to encapsulate thyme oil that will be used on cosmetics or drugs. Control of size and wall thickness of and encapsulation efficiency will be the subject of future work.

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Multivariate analysis of DNA microarrays

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Keywords: DNA Microarrays, Gene expression, Multivariate data analysis, Fisher discriminant analysis, PLS-DA

Introduction

The analysis of processes going on at the cell scale plays a central role in bioengineering activities. In this field, fundamental knowledge about the complex behaviour of such systems is continuously increasing through the application of the scientific method to well defined biologic problems, through iteration between data analysis stages and hypothesis testing stages, until a mechanistic explanation is found that is consistent with all known results. Between the wide range of currently available data gathering methodologies from biologic systems operation, DNA microarrays have become a standard technique, since all cellular processes originate at the transcription stage of cell operation (Stephanopoulos et al., 2002), enabling the simultaneous screening of the expression level of thousands of genes. Therefore, this technique provides fundamental data-driven information over which explanations about system operation can be elaborated, or through which one is able to characterize a variety of physiologic states (classification) or discover new ones (discovery).

DNA microarrays result from the hybridization of target samples of labelled mRNA representative of different cells states, with genomic DNA sequences immobilized in a solid matrix (probes). The magnitude of signal intensity at each probe location is then interpreted as a measure of the expression level of that particular gene, at the state corresponding to the label being analyzed.

Data from DNA microarrays have been analyzed through univariate techniques, where genes that most differentiate between the states under analysis are identified through F and t statistics, or through other techniques, such as the signal to noise ratio (Golub et al., 1999) and the SAM method (Tusher et al., 2001). The simplicity of these methodologies enables the adequate control of classification error rates, such has the False Positive Rate (FPR), Family-Wise Error Rate (FWER) and the False Discovery Rate (FDR). However, they disregard the cooperative behaviour of gene expression.

Multivariate approaches, on the other hand, are more suitable for taking into account gene co-expression, but still present some methodological limitations. For instance, Fisher Discriminant Analysis (FDA) requires the number of variables (genes in microarray data) to be less than the number of observations, a condition not met in practice. Therefore, such multivariate techniques do require a preliminary stage of variable selection, usually based on univariate approaches.

In this work, an intrinsic multivariate approach is presented where such preliminary univariate variable reduction stage is not needed, but that can still be conducted after a first run of the proposed methodology, on the basis of multivariate information thus generated, namely the genes VIP's, for which a formula is proposed. The approach combines PLS-DA and FDA, and has incorporated a "non-classification" analysis, enabling the assessment of the uncertainty for each class prediction, according to two distance measures of the expression profile under analysis to training dataset entities.

Illustrative example

In order to illustrate the proposed methodology, a well known data set was used (Golub et al., 1999), where different expression phenotypes were measured in samples from patients with different types of leukaemia: acute lymphoblastic leukaemia (ALL), subdivided according to their lineage (ALL-B and ALL-T) and acute myeloid leukaemia

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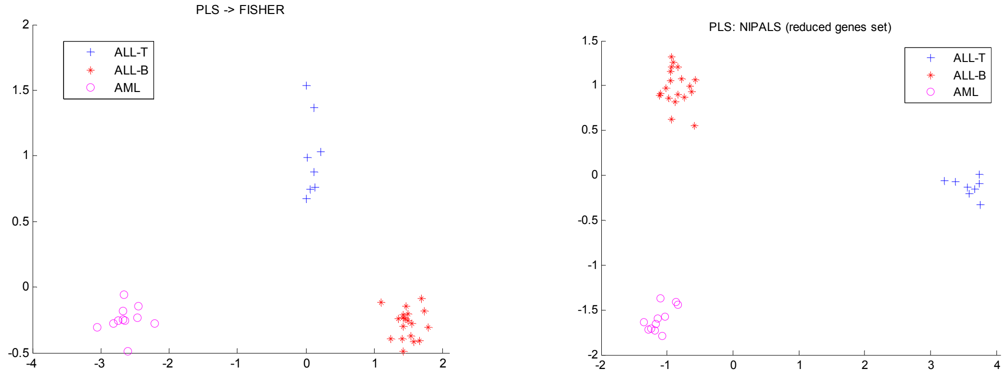
(AML). Applying the combined multivariate approach to the training set, results in the plot shown in Figure 1-a), where a good class separation is immediately observable. Such a separation can still be improved by selecting the most important genes, according to the proposed VIP criteria, as a large number of genes do not carry relevant information for classification purposes (Figure 2), leading to the results presented in Figure 1-b).



a) b)

Figure 1. Scatter plots for the several leukaemia classes in the reduced dimensional space after a first run analysis (a) and after gene selection using the VIP criteria (b).

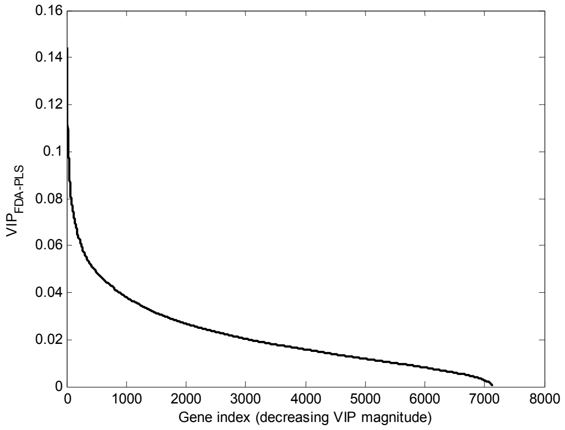


Figure 2. Profile of gene VIPs, by decreasing order of magnitude, in the leukaemia case study.

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Evaluation of olive oil-lemon juice emulsion stability through digital image analysis

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Keywords: Emulsion, image analysis, xanthan gum, starch modified, maltodextrine

Emulsions are thermodynamically unstable systems because of the positive free energy needed to increase the surface area between oil and water phases and because oil and water have different densities. To form emulsions kinetically stable for a reasonable period of time (a few weeks or months), chemical substances known as emulsifiers must be added prior to homogenization (McClementes and Decker, 2000). Several kinds of physicochemical mechanisms contribute to the instability of an emulsion, including gravitation separation (creaming), coalescence, and flocculation.

The Mediterranean diet has been associated with greater longevity and quality of life in epidemiological studies (Majem-Serra, Roman and Estruch, 2006). “Greek salad dressing” is, by definition, mixtures of virgin olive oil and lemon juice instantly prepared before use. Due to their compositions, they are a good source of biophenols as well as lipid-soluble and watersoluble vitamins (tocopherols, beta-carotene, ascorbic acid). Besides, virgin olive oil, thanks to its balanced fatty acid composition, has highly appreciated nutritional characteristics (Paraskevopoulou, Boskou and Paraskevopoulou, 2007).

Polysaccharides are usually added in food emulsions to improve creaming stability (Quintana et al, 2002).They are generally odorless, colorless and tasteless, have low energy and digestibility. The majority of such polysaccharides show little surface activity and their incorporation into oil-in-water emulsions is aimed inhibiting droplet creaming by increasing the viscosity of the aqueous phase thus preserving the desired textural properties of the emulsion (Paraskevopoulou, Boskou and Kiosseoglou, 2005). Starch, similar to other major polysaccharides, has low surface activity and is often used as a thickening agent or stabilizer in food emulsions (Ye, Hemar and Singh, 2004). Hydrophobically modified starch, such as octenyl succinate anhydride starch, is an amphiphilic macromolecule. It offers properties that have many applications within the formation and stabilization of dispersed food systems such as emulsions.

The objective of the present work was to develop oil-in water emulsions containing two basic ingredients, olive oil and lemon juice, added by different combinations of xanthan gum with modified starch or maltodextrine that would exhibit reasonable stability over prolonged storage. Digital image processing was used to evaluate the stabilization of the emulsion by distribution of the droplet sizes along storage time at room temperature (± 27 ºC).

Oil-in-water emulsions (50% v/v) were prepared as follows: a lemon juice polysaccharide solution was first prepared by slowly dispersing 1% w/v arabic gum and 0.5% w/v xanthan gum (control), 1% w/v starch modified (or maltodextrine) and 0.5% w/v xanthan gum, 1% w/v starch modified (or maltodextrine) and 0.6% w/v xanthan gum, 2% w/v starch modified and 0.6% w/v xanthan gum. After, the olive oil was added stirring it with a vortex (2200 rpm) for 2 min. The resulting crude emulsion was then

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homogenized for 1 min at 9500 rpm using Ultra-Turrax T25 homogenizer (IKA Instruments, Germany) equipped with a dispersing tool.

Benzoic acid was added at a concentration for 1% w/v in the continuous phase. The droplet size distribution of the emulsion during storage period was measured.

Images acquisition was conducted using an optical microscope (Eclipse E200, Nikon) with 400× magnification coupled with digital camera (Media Cybernetics, QImaging) linked to a personal computer. The images were focused in such a way as to enhance droplet contours (Fig. 1a). Afterwards, a step to suppress objects connected to the image border, and a holefill procedure were performed. Application of operations, such as erosion (to remove small debris) and reconstruction, produced the final binary image (Fig. 1b). These image processing were developed using the Matlab v.7.0.4 (The Mathworks, Natick, Mass.) package and were fully automated. The image processing procedure herein described showed to be a good method to the evaluation of oil in water emulsions stability (Freire et al., 2005).

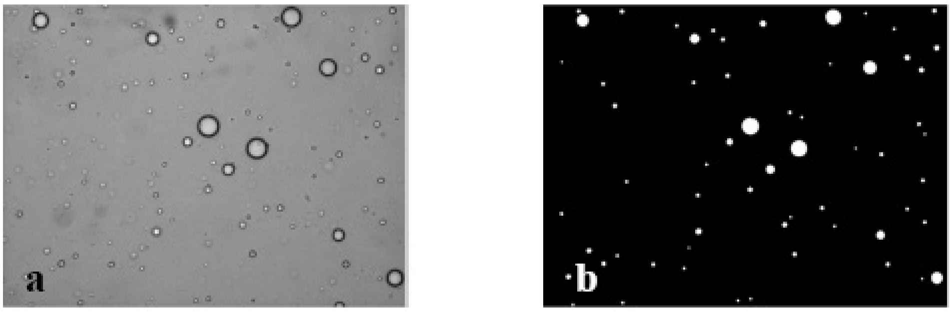

Fig. 1: (a) Image of a control emulsion. (b) Image obtained after digital processing

To exemplify the results of this work, the emulsion displayed in figure 1a and b was obtained soon after preparation. This emulsion contained equal ratios of olive oil and lemon juice, 1% w/v arabic gum and 0.5% w/v gum xanthan (control) according to method described above. The image 1b presents different droplets diameters and its behavior with time is still being analyzed. Therefore, the emulsifiers influence in emulsion stability loss will allow the suitable characterization the system during a long time besides to contribute development of more consistent formulations.

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Integration of a Digital Holographic Microscope in a single use bioreactor for animal cell culture: the promising benefits of an original multidisciplinary approach for bioprocesses

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Keywords: Bioreactor, Animal Cell Culture, Microcarriers, Vaccine, Digital Holographic Microscope

Biotechnological productions especially in the biomedical field require more and more clearly a multidisciplinary approach. An adequate bioprocess depending on animal cell culture for the production of cells themselves as needed for cell grafting therapy, for the production of recombinant proteins of therapeutical interest, or for the production of a vaccine, needs an appropriate bioreactor.

The full process should be easily scalable, and when used in a biomedical environment, like an hospital for example, it should be safe and secure, and easy to run. It must also allow an easy on-line follow-up.

The present work was undertaken in order to design such a friendly process, using single-use bioreactor vessels (“VueLife” Teflon bags) (Mosbeux et al., 2006) to grow cells in a more or less classical CO2 incubation condition (either in suspension or on microcarriers). Follow-up of cell growth was operated with a new kind of microscope, the Digital Holographic Microscope (DHM), developed and patented at our University (Dubois et al. 2006).

The DHM allows a direct observation of cells in 3D conditions. Appropriate image treatment may provide not only counting of cells but also, detection of intracellular infectious organisms (as virus or rickettsiae) cytopathogenicity allowing a precise determination of the most appropriate time for harvesting the vaccine produce. DHM allows also the observation of bead to bead transfer of cells when grown on microcarriers, an unsual way to scale-up cell production avoiding trypsinisation, that we have tested with different cell systems as reported here.

The DHM could be instrumental also to detect the appropriate time for collecting stem cells before they differentiate during their production, by observing their morphology in real time.

Different aspects of these biomedical fields have been approached here using model cellular and infectious microorganisms systems, as Vero cells, endothelial cells or Mesenchymal Stem cells, and VSV or *Ehrlichia ruminantium*.

In conclusion, along the present line, future work, (within the Waleo2 DECISIV project) will deliver shortly, a fully integrated, new, easy and relatively unexpensive process for animal cell culture, a field that is still at this time one of the most important engineering challenge for the biotechnological industry.

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Looking toward new biomolecules: IR and ^{1}H NMR spectroscopies and DFT calculations on N_α-benzoyl-L-Arginine ethyl ester hydrochloride

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Keywords: Biomolecules, N_α-Benzoyl-L-Arginine Ethyl Ester Hydrochloride (BAEE·HCl), IR, NMR, DFT calculations.

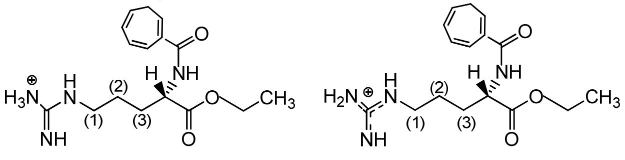
In recent years, there has been a growing interest in developing new materials for biomedical applications, which mimics some of the functions of biomolecules (Zhang *et al.*, 2007). Polydepsipeptides, copolymers of α-hydroxy acids and α-amino acids, are promising materials for that end, with potential application in controlled delivery systems and tissue engineering. These copolymers contain ester (-COO-) and amide bonds (-CONH-) in the main chain, which can be degraded by hydrolytic scission into non-toxic compounds (John and Morita, 2000, Zhang *et al.*, 2007). It is of great importance the investigation of their properties, not only at the macroscopic, but also at the molecular level. In this field, vibrational and NMR spectroscopies, combined with molecular modeling calculations, have been shown to be a promising approach in the development of new materials. (Strittmatter and Williams, 2000, Zhang *et al.*, 2007).

Despite the promising capabilities of polydepsipeptides, these materials have only been studied very scarcely. Moreover, they were essentially investigated as models to predict some of the properties of polypeptides and proteins, due to their structural similarity. For example, the study of polydepsipetides allows the investigation of the role of hydrogen bonds in the helical structure of peptides (Ingwall *et al.*, 1976, Zhang *et al.*, 2007).

In this work, high level molecular modeling and experimental studies by IR and ^{1}H NMR spectroscopies on an L-arginine derivative, N_α-Benzoyl-L-Arginine Ethyl Ester Hydrochloride (BAEE·HCl) are being carried out. This compound is an appropriate model to better understand some of the polydepsipeptides properties, since its structure comprises an amide and an ester bond. Moreover, the study of quite small systems can stimulate and provide vital data for the development of better, more efficient and/or reliable computational methods to be applied for more complex systems. To the best of our knowledge, no studies have been reported on this compound hitherto.

In what respects to α-amino acids, L-arginine is one of the most studied. Its conformational study is very challenging since it has many degrees of freedom. Besides this, there is some speculation around the tautomeric state of arginine in the gas phase. In solution, arginine exists in the zwitterionic form, but it is believed that this form also occurs in the gas phase (Ling *et al., 2006,* Schlund *et al.*, 2007).

In this study, we performed an investigation on two tautomers of BAEE·HCl, whose structures are represented in Figure 1.



(A) (B)

Figure 1 – Tautomers of BAEE·HCl considered for conformational search.

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For each tautomer we considered fourteen different internal rotation axes that can give rise to conformational isomers.

Since these compounds have a great amount of possible conformers, our first approach was the systematic conformational search by Molecular Mechanics (MM) and semi-empirical PM3 methods. This approach allowed a selection of the most stable conformations, which were afterward submitted to optimization through quantum chemical methods [DFT(B3LYP)/6-311++G(d,p)]. In order to support the experimental findings (Figure 2), IR and NMR spectroscopic properties were also calculated at the same level of theory.

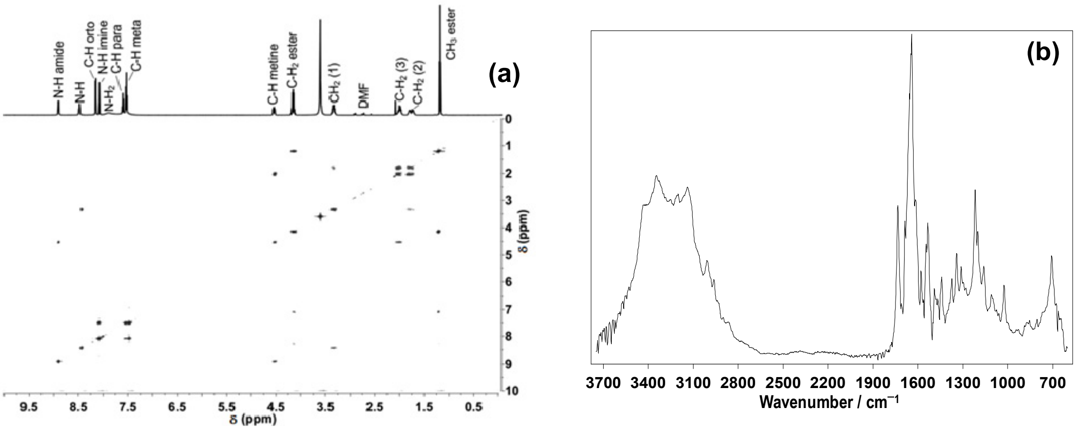

Figure 2 – N$_\alpha$-Benzoyl-L-arginine ethyl ester hydrochloride (BAEE·HCl): (a) ^{1}H NMR unidimensional and COSY spectra; (b) FT-IR spectrum.

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CFD analysis of blood rheology

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Keywords: Blood rheology, Numerical modelling, non-Newtonian

Atherosclerosis is a very common disease characterized by the deposition of fatty material, in the internal walls of blood arteries, causing their thickening and reduction of the available artery cross section. It is often associated with abnormal flow, happening mostly in regions of flow separation, recirculation-stagnation zones, turbulence and low wall shear stress which are believed to be atheromatic factors (Papaioannou, 2006). The blood flow behaviour, or hemodynamics, is dependent on different factors, as the artery geometry (Carneiro et al., 2008) and blood rheology.

In most of CFD (Computational Fluid Dynamics) simulations, blood is modelled as a Newtonian fluid, particularly in large arteries, where the influence of shear thinning properties is not significant. The blood is a complex medium composed by plasma, a Newtonian fluid, and a suspension of various cells, such as erythrocytes, leucocytes and platelets, which are related to its microscopic structures, as aggregation, deformation and alignment of the erythrocytes (Chen and Lu, 2006).The blood cells are about 45% of blood by volume, forming a non-Newtonian fluid, with shear-thinning viscoelastic behaviour.

The rheological model for blood viscosity is a key factor in the simulation of hemodynamics flows. Gijsen et al. (1999) proved experimentally that the blood flow should be simulated by the non-Newtonian Carreau-Yasuda model in the carotid arteries, at both low and high shear stress. It was suggested that the non-Newtonian properties of blood are an important feature in the hemodynamics, which affect the interaction between the fluid and the vessel. This interaction plays an important role in vascular biology and pathophysiology.

The present paper reports the numerical modelling of two blood fluids, Newtonian and non-Newtonian, in the cardiovascular system. The effect of blood rheology, on the flow dynamics, was studied in a three-dimensional model of the abdominal aorta bifurcation into the iliac arteries. The geometry and the computational mesh were created using the GAMBIT software (13376 hexahedral elements). Blood was modelled as an incompressible fluid, with the density of 1057 kg/m^3. The uniform inlet velocity is 0.234 m/s. The inflow is laminar and the turbulence generated was simulated by the k-ε model with enhanced wall treatment. The Navier-Stokes equations were solved using the FLUENT package. To study the influence of blood rheology on the atherosclerosis development the flow was modelled either as a Newtonian fluid or as a non-Newtonian fluid described by the Carreau model. The influence of blood rheology was analyzed in terms of the recirculation zone size, velocity profiles and wall shear stress (WSS) distribution, which are believed to play an important role in the atherosclerosis lesions. The velocity distribution, in the vicinity of the bifurcation, is shown in figure 1.

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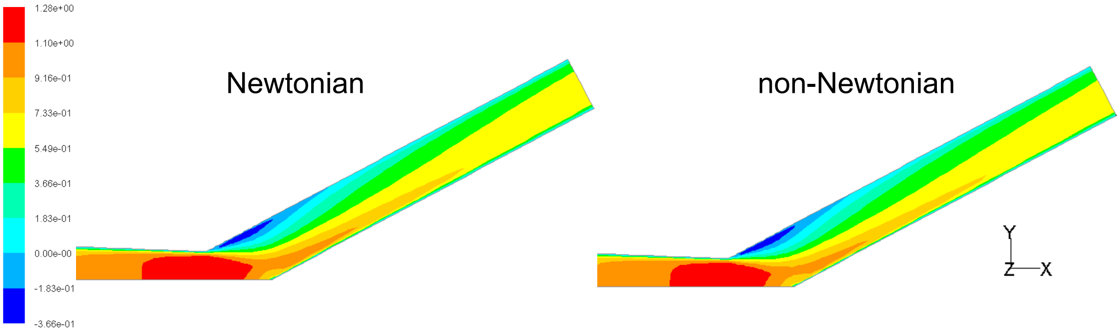

Fig. 1 Velocity contours for the Newtonian and non-Newtonian fluids, at the plane z=0.

Both simulations showed flow separation and a recirculation zone, in the vicinity of iliac bifurcation, characteristic of atherosclerosis lesions. Similar velocity profiles are obtained, but the velocity contours of non-Newtonian fluid are flattened compared with the Newtonian fluid. Table 1 includes the velocity and the wall shear stress (WSS) peak and the recirculation size of recirculation zone near the wall, for the Newtonian and non-Newtonian simulations.

Table 1. Velocity and WSS peak values and recirculation length

	Newtonian	non-Newtonian
Minimum velocity (m/s)	-0.3544	-0.3663
Maximum velocity (m/s)	1.262	1.240
Recirculation length (mm)	24.5	24.5
Recirculation width (mm)	15.7	13.7
Maximum WSS (Pa)	30.9	22.3

The maximum velocity is obtained just upstream the bifurcation, but the maximum value is 1.7% lower for the non-Newtonian fluid. The minimum velocity is located downstream the bifurcation near the outer wall, 4.34 mm in radial direction. Although, for the non-Newtonian fluid, the minimum velocity is higher in magnitude (3.3%), the recirculation width on the wall is smaller (-12.5%). The higher WSS differences are observed for the maximum peak at the iliac inner wall, with a decrease of 28% for the non-Newtonian fluid. This atherosclerosis lesion factor, downstream the iliac inner wall, that is also observed clinically, is more evident with the Newtonian solution. The WSS values are always lower for the non-Newtonian fluid, which is at odds with the higher velocity gradients near the wall. This is may due to the lower viscosity of non-Newtonian simulation, particularly near the wall. This work is a part of a wider effort to couple experimental data with numerical simulations for the knowledge improvement of the hemodynamic in arteries bifurcations.

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Study of cerebrospinal fluid dynamics in a computational model of stenosed aqueduct

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Key words: Computational Fluid Dynamic (CFD), Cerebrospinal Fluid (CSF), Stenosed aqueduct,

Abstract

In this study we evaluate the cerebrospinal fluid (CSF) dynamic using computational fluid dynamic (CFD) analysis in an anatomical 2D model of a ventricular system. Stenosed aqueduct, leading to obstructive hydrocephalus was modeled and the results of CSF velocity in the aqueduct and pressure in the lateral ventricle were compared. Considering CSF pulsatile flow and modeling the ventricles as flexible components based on solid-fluid interaction analysis for hydrodynamic study is a new approach in our work, enabling a more accurate simulation of CSF flow. The anatomical basis of our simulation was generated from Magnetic Resonance Imaging (MRI) scans. Increase of velocity and an up to two-fold pressure drop were observed due to aqueduct stenosis.

Introduction

The aqueduct of sylvius is a narrow channel connecting the third and fourth ventricle (Fig 1). It is a narrow pathway for CSF flow and hence may be an important site for development of hydrocephalus.

3D Modeling of the human ventricular system (HVS) from MRI data, though yielding a precise geometry of the brain and the HVS, is a very complicated process and renders the CFD simulation analysis almost impossible. These challenges are compounded by meshing and loading problems in the presently available software packages. All these factors strengthen the need for 2D or simple 3D models of HVS. Such models can not take into account the elastic and compressive properties of the surrounding brain tissue [1,2], although Fin and Grebe have published a deformable three-dimensional CFD model of the aqueduct of Sylvius based on MRI scans and solid fluid interaction [3]. None of the present models consider the real pulsatile flow pattern, synchronized with the heart beat. Evaluating the CSF pulsatile hydrodynamic in a stenosed aqueduct in a 2D model based on fluid–solid interaction (FSI) analysis to simulate a kind of obstructive hydrocephalus is a new approach adopted in our study.

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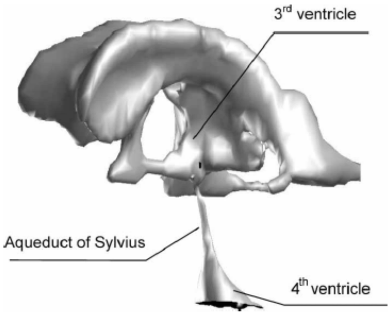


Fig 1: Three- dimensional representation of the HVS

Materials and methods

Our aim is to use the physical principles of fluid flow and solid material to quantify intracranial CSF hydrodynamics in the hydrocephalus condition. The procedure is performed in three stages. In step one, MRI techniques are used to accurately define

the ventricle geometry. In step two, MR image is converted into an accurate two-dimensional surface with two models of normal and stenosed aqueduct that are imported into CFD analysis software. Mesh generation is then used to partition these spaces into a large number of small finite elements. In step three, CFD software solves the equations of fluid motion and solid tissue numerically over the brain geometry.
Figure 2 shows the 2D model of HVS with and without stenosis. The JPEG pictures were imported to the CATIA 5.16 software. The models in IGES format were meshed in ADINA software (ADINA^{TM}, version 8.2, Automatic Dynamic Incremental Nonlinear Analysis, Watertown, MA). Solid components have been constructed in ADINA and the fluid segment in ADINA-F domain. The values of physical constants used in our model are the CSF viscosity (μ=0.008 Pa.s), density (ρ=10^{3}kg/m^3). Young modulus (E=15 kPa), Poisson ratio (ν=0.49) and density of brain parenchyme (SP=1.02).

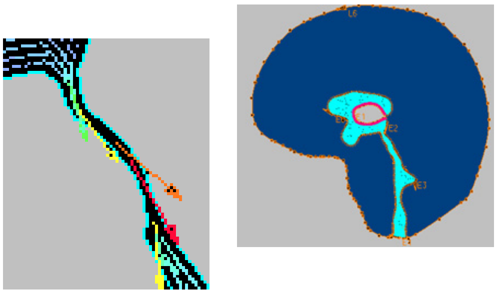


Fig 2: 2D model of HVS of normal case (right) and model of 50% stenosed aqueduct (left)

CSF motion is described by the equations of mass and momentum conservation for an incompressible Newtonian fluid. These conservation balances lead to a system of partial differential equations known as the continuity and the Navier-Stokes equations. The surrounding tissue was modeled as an elastic compartment.

Results

For the solid a rule-based mesh of 10065 point and 3665 rectangular solid elements of the plane strain type with 4 nodes in each element was used and for the fluid a rule-based mesh of 1734 points and 3060 elements. Fig 3 shows the pressure contour for both cases. The intracranial pressure (ICP) is highest in the lateral ventricle (LV) and lowest in the Magendie foramina during systole. The highest CSF velocity occurs within the cerebral aqueduct. In the hydrocephalus case, the results of pressure drop and velocity magnitude of the aqueduct region are almost two times larger than in the normal condition. Volume changes of the lateral ventricle of about 5% were observed in the case of hydrocephalus. Moreover the stenosis also influenced the CSF flow pattern. While clinical evidence also validates the reverse flow of CSF in aqueduct in diastolic time, the observation showed that there is no backflow in diastolic time in the case of hydrocephalus.

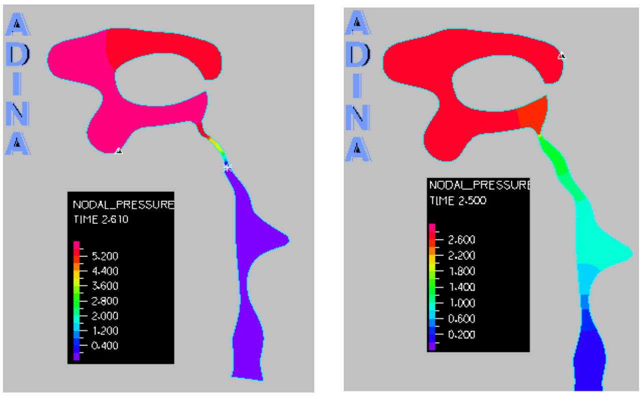
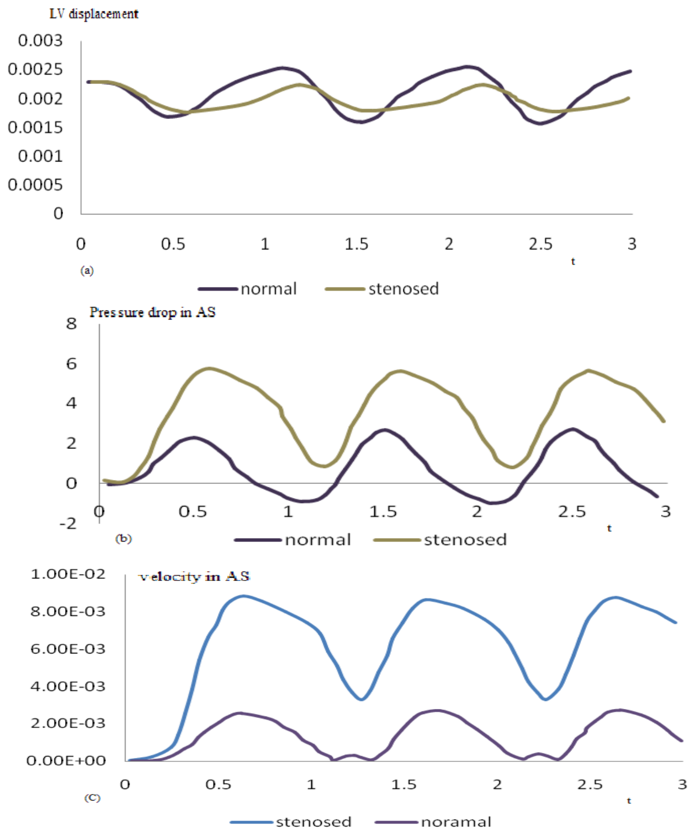


Fig 3: Pressure distribution in normal (left) and stenosed model(right)

Discussion

The main difference between the model with and without stenosis lies in the amplitude of the lateral ventricle (LV) displacement, which decreased while the LV volume increased. In the present configuration with aqueduct cross-sectional area reduced 50% a maximum relative pressure increase of 46% is observed. (Fig 4).
The stenosis also led to increased velocity in the aqueduct. At the smallest cross section, the maximum velocity increased by 50%. Hence, aqueduct stenosis might not only indirectly damage the ventricular walls through the development of hydrocephalus, but also directly through increased amplitude of the CSF pressure oscillation and velocity magnitude. This shows that single ventricles or pathways should, in general, not be modeled detached from the remainder of the system.



Fig(4): (a):LV displacement, (b) pressure drop in AS, (c) velocity magnitude in AS

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Multiphase flow inside the Volumatic® spacer: a CFD approach

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Keywords: Asthma, Respiratory flow, Volumatic® spacer, Computational fluid dynamics.

Introduction

Over the last years, allergic diseases, particularly asthma, have been acquiring an increasing importance in the developed countries, due to the rising of its prevalence and morbidity and the economic burden associated. In the preventive and urgent treatment of asthma, the oral pharmacological therapeutics assumes a major role. For children with less than seven years, the main mechanism of drug distribution has been the use of pressurized metered-dose inhalers (pMDIs) coupled with spacers, since it minimizes the problems of a poor technique of inhalation associated with the use of pMDIs. Indeed, they are often reported to achieve a better drug delivery to the lungs than pMDI alone (O'Callaghan and Barry, 2003). Spacer devices have a port at one end to which the pMDI is attached and a mask or mouthpiece fitted at the other end. These devices constitute a volume into which the patient actuates the pMDI and from which the patient inhales, without necessarily having to coordinate both actions. By acting as an aerosol reservoir, these devices slow the aerosol velocity and increase transit time and distance between the pMDI actuator and the patient's mouth, allowing particle size to decrease (Newman, 2005).

Thus, it is essential to identify all the spacer characteristics that may optimize the drug distribution to the low respiratory tract combined with a reduced drug loss inside the spacer, in order to achieve more efficient treatments in young children.

The purpose of the present work is to use CFD tools in order to develop a tridimensional computational model of the Volumatic® spacer able to predict the flow patterns during the respiratory cycle, in order to compare with the experimental results.

Numerical Study

The geometric model used in the simulations were created in *Gambit™*. A computational mesh of finite volumes was created for the spacer, and the boundary conditions were defined: inlet, outlet and wall. The mesh generated in the Volumatic® spacer contains 65326 hexahedric elements. This was imported to *FLUENT™*, where the continuity and momentum equations were solved, as well as, the turbulence equations. The air inlet velocity in the spacer was calculated based on the respiratory cycle performed by the patient during the inhalation technique (Boron, 2003). Figure 1 (a) shows the sinusoidal function and also the time instants considered for the study of the periodic air inlet, namely 0.4 s (ascending phase), 1 s (maximum velocity inlet), 1.6 s (descending phase), 2 s (beginning of expiration), and 4 s (beginning of inspiration). After, the dispersed phase was defined in the mathematical model as a continuous injection of particles with an inlet velocity of 40 m/s.

The profiles of axial velocity along the centerline associated with the monophasic and biphasic flow are practically overlapped, with only a small difference on the initial part of the spacer, where particles are injected. The profiles of axial velocity inside the Volumatic® spacer for the evaluated five instants of the respiratory cycle are presented in Figure 2.

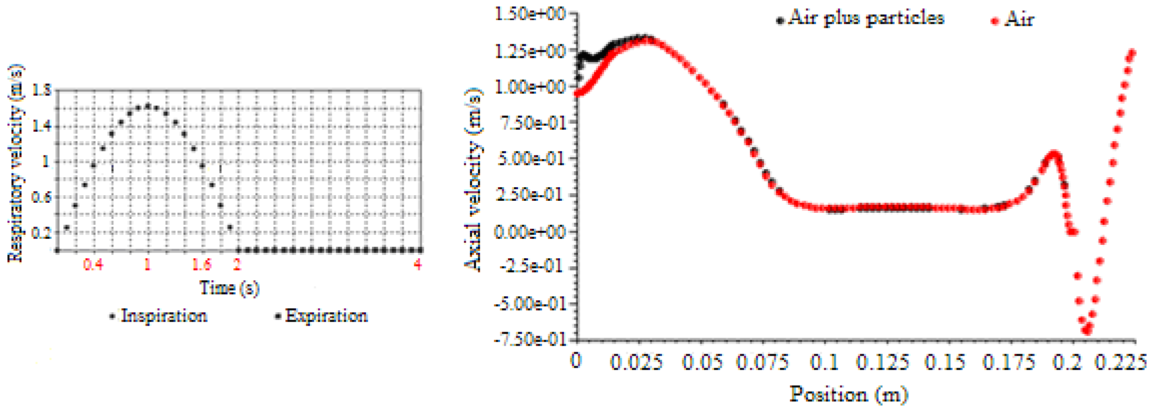

Figure 1. (a) Representation of the periodic velocity in a respiratory cycle, defined in the inlet face of Volumatic®, focussing the (five) time instants studied. (b) Comparison between the profiles of axial velocity along the centerline, considering only the continuous phase (black profile) and the biphasic flow (red profile), respectively, for the instant of 0.4 s.

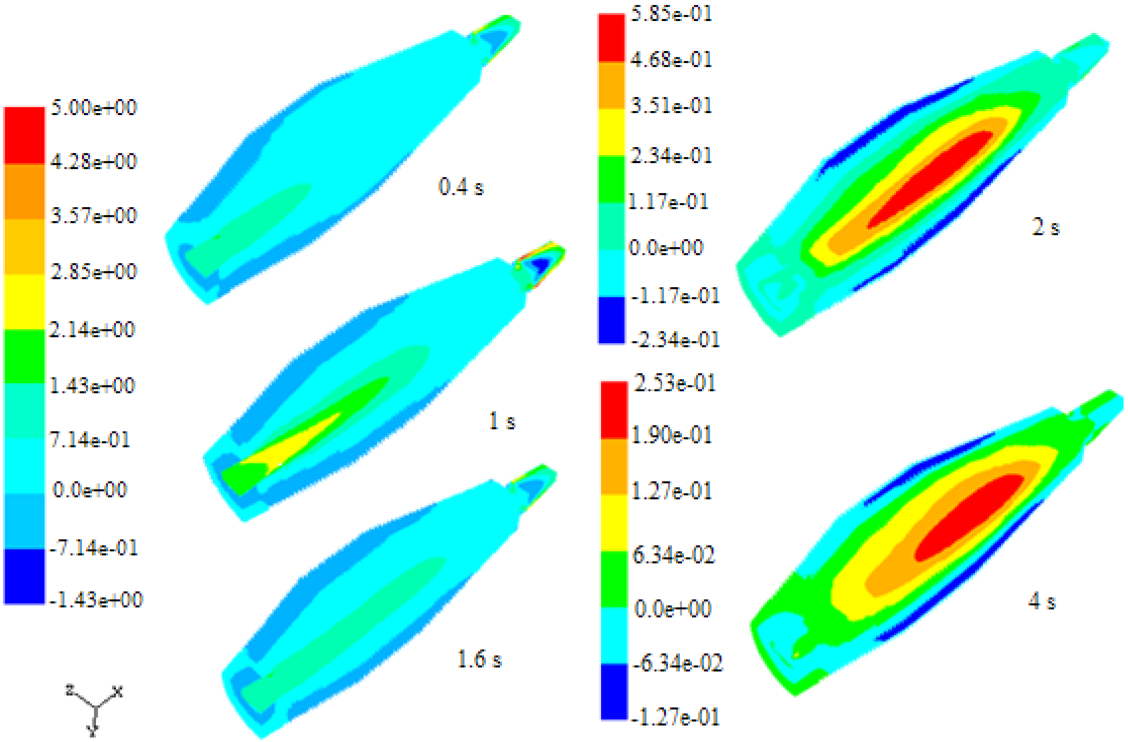

Figure 2. Isokinetic profiles of axial velocity (m/s) in the Volumatic®.

These numerical results are being compared with the experimental results obtained *in vitro* concerning the deposition of salbutamol sulphate in the spacer, through a simulator of the respiratory tract (Abreu *et al.*, 2008).

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Computational Fluid Dynamics (CFD) behavior of water flow in a packed column filled with spherical particles

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Keywords: Simulation, Computational Fluid Dynamics, Packed Column, Porosity.

Abstract

Water flow behavior in a packed column was modeled based on computational fluid dynamics. The parameters of Ergun's equation were modified by applying the CFD results into the empirical Ergun's equation. The range of Reynolds number considered in the present CFD work was far wider than that already obtained experimentally that are reported in literature.

Introduction

Nowadays expensive process operations can be simulated by Computational Fluid Dynamics (CFD) with considerable reduced expenses. The modeling of flow patterns in porous media has attracted many attentions due to the complication of simulation (Puncochar et al., 2002, Nemec et al., 2005). Ergun's equation (1952) explains the pressure drop in a random structured bed. He showed that the equation satisfies the experimental data for cylindrical, spherical and crushed packing in a wide range of Reynolds numbers. In the present work, the pressure drop of fluid water was investigated in a packed column of spherical particles as well as the effect of roughness of the wall. Also, validity of experimental parameters of Ergun's equation was verified using CFD. Finally, the analytical data were compared to experimental data.

Modeling and Simulation

The geometry of porous media was generated in Solid Works software as the preprocessor. Figure 1 (a) depicts the geometry of porous media. It is composed of a column packed with spherical particles with free spaces at the beginning and the end of the column.

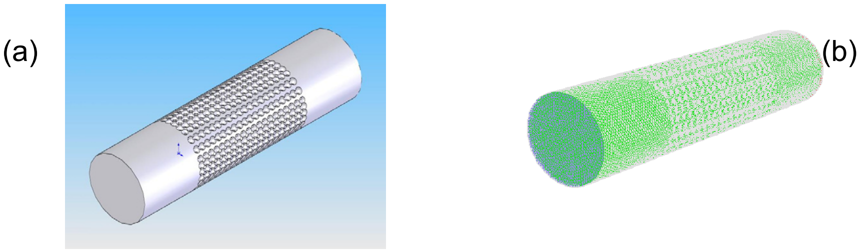

Figure 1. The geometry (a) and meshing (b) of column packed with spherical particles

The free space at the beginning makes the inlet stream to be uniform and such a space at the end of column prevents the formation of vortices. The mechanical characteristics of the model is as follows: total length: 0.6 m, porous length: 0.298 m, Inlet (outlet) empty space: 0.151 m, bed to particle diameter (D/dp): 9.625 and porous volume of the bed: 46.9 %. The arrangement of the packing is a state between random arrangement and ordered one. The contact area between the spherical particles is defined to be a circle i.e., the packing is not complete spheres in agreement with experimental conditions.

After meshing of such a complicated volumetric model (see Figure 1 (b)), the meshed geometry was examined to ensure the agreeable quality of the meshing. Then, it was

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exported to Fluent software as the processor. The solver was three-dimensional double precision (3ddp) and due to incompressibility of the water, the solving method was selected as implicit segregated. The flow calculation was done using $K-\varepsilon$, laminar and Realizable $K-\varepsilon$ models. After determining the physical properties of the fluid and the boundary conditions, the problem should be initialized. The solving procedure was iterated until the convergence criteria were obtained.

Results and Discussion

The present simulation results in 28 descritized points that half of them are related to the smooth wall condition and the others are related to the glass lined wall with roughness height of 0.3 mm. The solver took 5 h to converge for any of these points. Experimental parameters of Ergun's equation were modified by the CFD. Also, the analytical data were compared to experimental data based on a previous work done by Rumpf et al. (1971). As observed in Figure 2, the analytical data fits well the experimental data. It is noticeable that Ergun's equation overestimates the pressure drop.

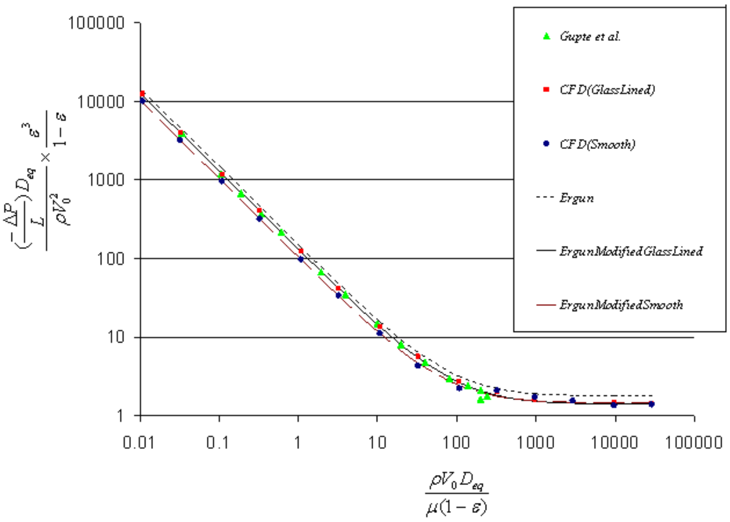

Figure 2. Comparison between experimental (Rumpf et al., 1971) and analytical data

Conclusion

Water flow behavior in a packed column was modeled based on computational fluid dynamics. The parameters of Ergun's equation were modified by applying the CFD results into the empirical Ergun's equation. The range of Reynolds number considered in the present CFD work was far wider than that previously existed in literature. The obtained CFD results were in good agreement with the available experimental data in literature.

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Boundary conditions effect on CFD simulation of heat transfer in plate type heat exchanger

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Keywords: CFD, Heat transfer, Plate heat exchanger, Boundary conditions

Abstract:

Acurate heat transfer simulation is essential to modeling fouling, friction factor and prediction of thermal efficiency of plate heat exchangers. One of the main important steps in CFD simulation of heat transfer is proper selection of boundary conditions (B.C.s). Three B.C.s (convective, heat flux and temperature) which are implemented in commercial CFD code (CFX 11) are employed. Deficiencies are found in all three B.C.s. Becuase of the very complex geometry, the values of some parameters in B.C.s are function of the space and can not be defined unambiguously. However, the convective B.C. describes most reliably the physical situation of heat transfer in plate heat exchangers.

1 Introduction

New heat exchanger geometries are traditionally developed by the trial and error method using some kind of heuristics. Theoretical predictions of the thermal efficiency of plate heat exchangers would facilitate design of new heat exchangers. Also accurate prediction of reactions, heat transfer and fluid flow in different heat exchanger geometries would help to minimize fouling of heat exchanges by geometrical changes. Accurate heat transfer modeling is an essential part of fouling models, because temperature has a considerable effect on many fouling mechanisms. Without physically correctly defined boundary conditions neither heat transfer nor fouling can be modeled reliably. Selection of boundary conditions is complicated especially in the case of complex geometry of corrugated heat exchangers where distribution of the local heat transfer coefficient fluctuates (Heggs et.al., Zettler et.al.).

The objective of this work is to model fluid flow and heat transfer in the corrugated 3D plate heat exchanger geometry with a commercial computational fluid dynamics (CFD) program, CFX 11, and to find the most realistic heat transfer boundary conditions for a plate heat exchanger and to evaluate the limitations of different boundary conditions. For the verification of the model flows with Reynolds numbers between 1000 and 4000 are investigated and results of those simulations are compared to the experimental correlations.

2 Results and Discussion

The Fanning friction factor, which indicates the pressure drop in the geometry, is calculated from the results and plotted as a function of Reynolds number (Figure 1). The simulated results are compared with the experimental correlation found in the literature. It can be seen that both simulation models under predict the fanning factor and thus the pressure drop. The best agreement between the correlations and simulations was achieved when comparing simulations to the correlation of Tribbe (M6) in which only corrugated section was considered and the dimension of the wavelength was closest to the simulated geometry. Difference between the laminar and the RNG k-ε model is quite small, but the laminar model under predicts more the Fanning friction factor. The difference between laminar and turbulent models may indicate that the flow is quite perturbed in the corrugated flow field and inadequate for the laminar model. This result is consistent with the results of Ciofalo et al.

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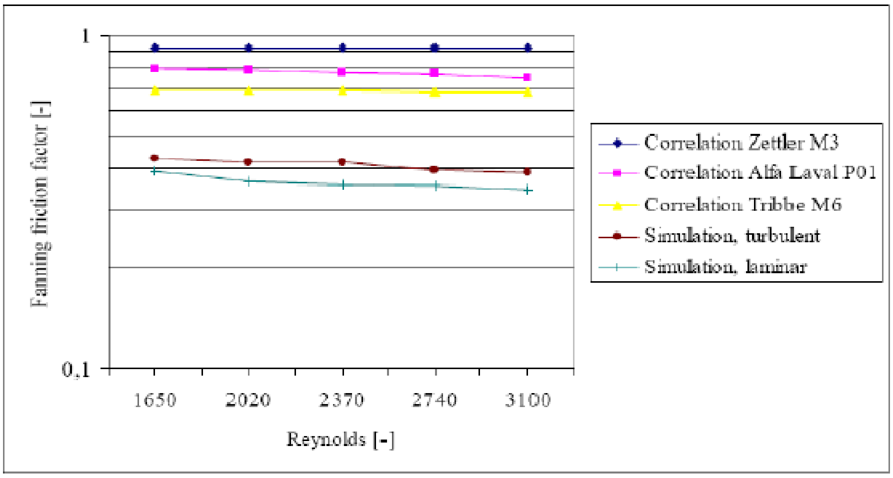

Figure 1. Comparison of the Fanning friction factors of the experimental correlations and the CFD simulations with different Reynolds numbers

The results of the heat transfer simulations calculated with different boundary conditions are collected to Figure 2.

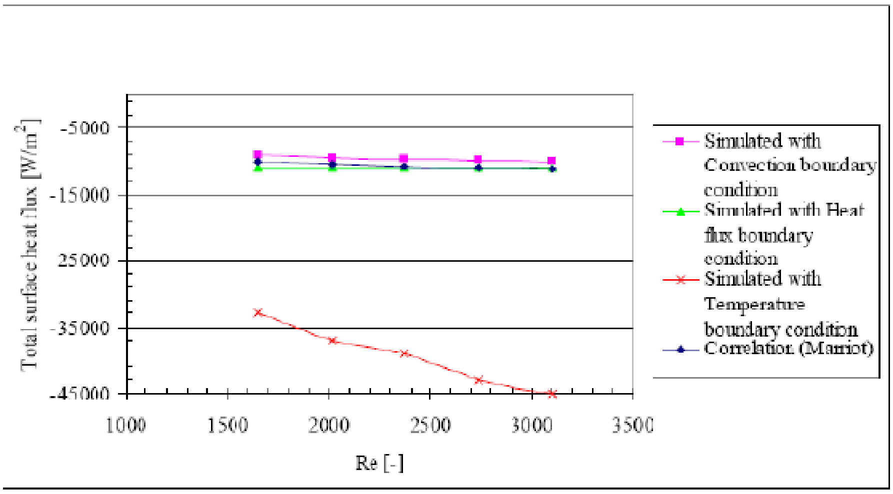

Figure 2. Comparison of the total heat flux of the Marriot's correlation and the CFD simulations with Convection, Heat flux and Constant wall temperature boundary conditions as a function of Reynolds number

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Application of CFD in the study of supercritical fluid extraction with structured packing – pressure drop calculations

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Keywords: Pressure drop, CFD modelling, structured packing, supercritical fluids

Liquid phase SFE has been carried out mainly in packed extraction columns, with structured packings particularly of the gauze type. Structured packing performs very well during extractions under these conditions, mainly due to their relatively large surface area and free volume. Nevertheless, there are also important disadvantages such as high cost, low capacities at high flow rates, premature flooding, and entrainment of the liquid phase due to low density differences [1-7]. The assessment of the real efficiencies of these packings poses extreme difficulties related to the moderately high pressures involved in these processes. Computational Fluid Dynamics (CFD) can be used to characterize the complex multiphase flow inside the packed bed and evaluate the influence of the shape and geometry of the packing on the hydrodynamics and mass and heat transfer rates in the SFE packed column[8]. The final objective of our work is to model the complex transport phenomena present in SFE columns with structured packing in order to predict rates and to optimize the process. The first stage, presented here, consists in an accurate modelling of the hydrodynamics inside the relatively complex geometry of the structured packing. The current paper presents a CFD model to predict the pressure drop in these columns; the results are compared to a wide variety of experimental data available in the literature.

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Application of CFD techniques in a high-flux riser-reactor flow prediction

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Keywords: CFD, simulation, riser FCC, gas-solid, catalytic cracking

The application of Computational Fluid Dynamics (CFD) in industrial operations has been increased not only in the project of new equipments but also in the optimization of the existing ones.

The present work shows a set of tridimensional two-phase and adiabatic conditions, using the two-fluids model for the calculation of gas-solids flow in a high-flux risers of two different geometries, one with 10m of height and 76mm of internal diameter, and the second with 18m of height and 50mm of internal diameter. The k-epsilon model was applied to consider the influence of turbulence over the gas phase.

The four-lump and ten-lump schemes kinetic models were used to predict the catalytic cracking reactions in the two different reactors. The riser with 10m of height and 76 mm of internal diameter had two entrances, one for particles inlet and one for the organic materials. The size of mesh for this case was 584.000 control volumes. The second riser studied with geometry of 18m of height and 50mm of internal diameter had only one entrance for vapor. The mesh generated over this geometry was considered 650.000 control volumes.

The numerical results of gas-solids dispersion flow in a high-flux were compared with the experimental data from the work of Pärssinen and Zhu (2001) and the four and ten lump schemes for the mass concentration of chemical species were compared with the work of Ali et al. (1997).

The catalytic cracking reactions models show an important role in the prediction of all phenomena present in the reactor. The simulated results show that the kinetics models are important not only because of the variation of the gas phase and the temperature profiles inside the reactor but also because these parameters interfere directly in the dynamic behavior of the gas-solid flows.

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Gas holdup in laboratory scale bubble column: CFD simulations vs. measurements

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Keywords: CFD, Euler-Euler, rectangular bubble column

This contribution deals with CFD Euler-Euler simulation of laboratory scale rectangular bubble column. Horizontal profiles of gas holdup obtained from simulations are compared with experimentally measured profiles using optical fiber probe.

Goal

The goal of the presented contribution was two-fold. Goal 1 was to examine effect of interphase forces and turbulent models on simulation results and effect of front-rear flow field symmetry assumption. Goal 2 was to compare simulated horizontal profiles of gas holdup with profiles measured with optical fiber probe.

Experiments

Experimental column width was 20 cm, measurements were done for column thickness 4 and 10 cm. Ungassed liquid height was 25 and 50 cm. Water and air were used as liquid and gas phase. Gas distributor consisted of one line of 33 orifices (0.5 mm orifice diameter, 5 mm distance between two neighbouring orifices). Experiments were done for air flow rates 800, 1200 and 1800 l/h. Gas holdup profiles were measured at height 11, 21, 31 and 41 cm above the gas distributor. There were 19 measuring points in horizontal profile with distance 1 cm. Local gas holdup at each point was measured for 2 minutes - the obtained values are 2 min time averages.

Simulations

Euler-Euler model was used for the simulation of gas-liquid flow in the bubble column. Model equations were solved using commercial code Fluent 6.3. In Euler-Euler model all involved phases (gas, liquid, (solid)) are treated as interpenetrating continua (see e.g. [1], [2], [3]). There is continuity and momentum equation for each particular phase. These equation sets for each phase are coupled together through pressure, phase volume fractions (which sum to unity) and interphase force terms like drag, lift, added mass force. Closure relations for the interphase forces are needed to solve the equations. Also turbulence has to be modelled.

We did 3-dimensional transient simulations of the flow field in the bubble column. One set of simulations was done assuming front-rear symmetry of the flow field, another set was done without this assumption (full 3D). Three version of k-ε turbulence model were tested (standard, RNG and realizable). Also simulations with different combinations of enabled interphase forces (drag, drag + lift, drag + added mass, drag + lift + added mass force) were run to see how they influence the output. Influence of the lift coefficient value (from -0.3 to 0.5) on results was examined.

Time averaged horizontal profiles of gas holdup were compared with experimental data. Profiles obtained from simulations were averaged over 60s time interval. Time averaging was started after 30s to let the flow field reach a pseudosteady state.

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Results

Goal 1. The difference in gas holdup profiles calculated with and without the front-rear symmetry assumption was often larger than 20 %, thus the symmetry assumption is not recommended for column simulation even when the column thickness is only 4cm. Each of the simulations using different version of k-ε turbulence model predicted qualitatively different results – two different steady flow fields in the case of standard and realizable model, unsteady flow field in the case of RNG model. RNG k-ε model gave best agreement with experiments. Lift force (lift coefficient values) significantly influenced simulation output, influence of the added mass force was smaller.

Goal 2. Qualitative agreement between simulations and experiments was reached, when drag, lift and added mass force were accounted for, see Fig. 1.

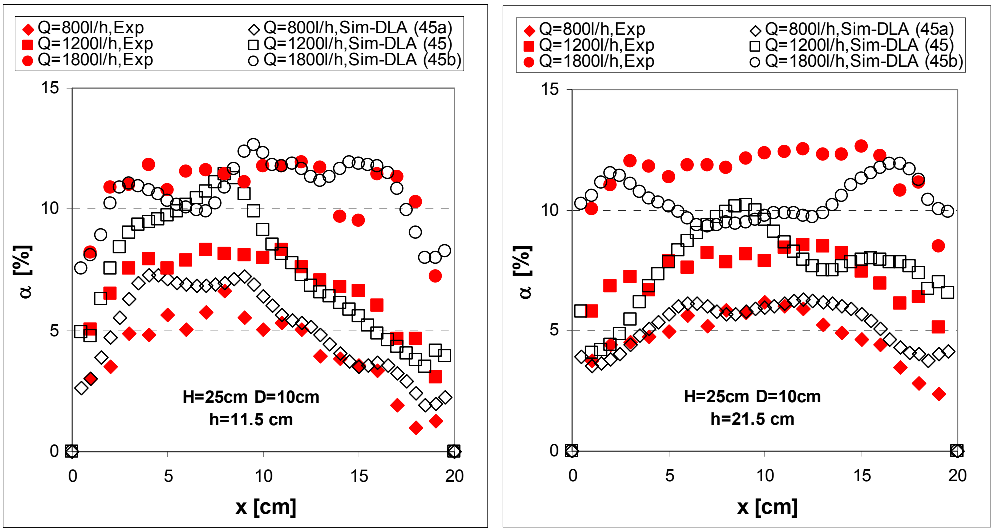

Fig. 1: Simulated (white marks) and experimental (red marks) holdup profiles at two heights h above gas distributor for air flow rates 800, 1200 and 1800l/h. Ungassed liquid level 25 cm. column thickness 10 cm.

Acknowledgements

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Healthcare Decision Support System

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Keywords: Healthcare Management, Decision Support System, Job-Shop Scheduling

Throughout the last decades, the world had being experiencing massive changes in demography, economy and social, being the healthcare industry one of the most affected. As healthcare becomes more accessible and effective people are living longer. On the other hand, the needs for healthcare services increase with people's age. This conjuncture creates an exponential demand for healthcare services that are intrinsically finite. If a valuable utilization of limited resources has always been a major challenge for healthcare managers, this problem is now critically urgent (Propper et al., 2006).

Assuming that episodes such as external and internal appointments, lab requests, diagnostic imaging prescriptions, etc., are the link between the provider and the patient, a feasible scheduling tool is necessary to improve and control the care delivery. Scheduling tools represent an opportunity to optimize the service quality delivery and minimize costs. The architecture of these tools has to be transversal to the healthcare delivery combining the administrative, clinical and financial processes, embodying the demands of all players - patient, healthcare organization and provider.

In this paper a decision support system, based in a scheduling model, is presented. A case study is described based on an imaging clinic of a private hospital. The case study is descritized in a set of jobs J and resources R, based on Job-Shop Scheduling Problem (JSSP) methodology (Brucker, 2004). Jobs are composed by a sequence of tasks that follow precedence relations, according to the job requirements, which restrain some tasks to start until others are completed. It is considered that tasks cannot be interrupted (non-preemptive scheduling) and resources can perform more than one task simultaneity (cumulative scheduling). Furthermore, it is considered in this research that each task establishes restrictions in the resource allocation and may have to be processed in more than one resource, denoted as multiprocessor tasks. These restrictions impose limits in resources selection according to their capabilities (each resource may have more than one capability, denominated as multipurpose machines) and their use in the job (the selection of the resource may be limited to one that was used in a previous task). Additionally, the set of resources have elements that exhibit the same capabilities - machine repetition. Resources may also have different processing requirements for different tasks - multimode machine (Brucker and Knust, 2006). The selection of a resource may restrict the choice of another resource in the subsequent tasks. The goal of the developed model is to describe accurately a clinical workflow.

A comprehensive validation of clinical workflows and model results has been performed. It was concluded that the developed model describes accurately the operations of a chosen healthcare provider. The model has proved to be a useful tool in the decision making process, since it was able to predict the impact that the change of different resources have in the operations of a healthcare provider.

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Investigation of the drug delivery behavior of porous hydroxyapatite crystals

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Keywords: hydroxyapatite, crystal, drug delivery

In the developing medicine industry, the drug delivery is being studied upon instead of producing new active substances. As a result of these studies, the drug delivery systems, which mostly are based on polymers, have been developed. However, the fact that most of the polymers are not biocompatible or biodegrading resulted in searching new materials that can replace these polymers. The most important bioadapting material that can be used in drug delivery is hydroxyapatite (HAP) (Kim et al., 2004). Studies on HAP as drug carriers for antibiotics, Ibuprofen and other agents have deserved a great interest. HAP has almost the same chemical composition as human bone therefore it has enhanced as a drug delivery carrier due to its osteoconductivity and biocompatibility.

In the present work, porous apatite crystals to be used as drug delivery systems were produced by wet chemical method. HAP crystals were prepared from calcium chloride, dipotassium hydrogen phosphate solution in the presence of latex particles (Dogan and Öner, 2006). The ratio of calcium to phosphate ions in the reactants was equal to 1.67. This is the same as the ratio of the respective ions in the molecular formula of HAP crystals (Ca/P=1.67). The crystals precipitated in the temperature range of 70ºC. During precipitation, presaturated nitrogen gas was introduced into the stirred solution to ensure a CO_2 free atmosphere. The obtained body was heat treated at a heating rate of 10°Cmin^{-1} to 600°C to burn out the organic compounds. The results showed that the release was mainly controlled by the open porosity of the HAP.

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Optimization of hydroxyapatite synthesis

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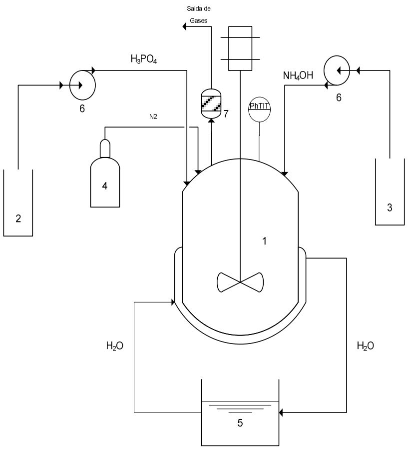
Keywords: Optimization, Hydroxyapatite, Chemical Reaction

Hydroxyapatite (HAP), is represented by the formula $Ca_{10}(PO_4)_6(OH)_2$, and is one of the inorganic components of the hard tissues of living bodies such as bones and teeth. HAP is a calcium phosphate based bio-ceramic, which has been used for several years in medicine and dentistry because of its excellent biocompatibility with human tissues. The success of its application in these fields depends upon factors such as the composition, crystallinity, size and morphology of HAP particles. This paper describes the work performed regarding the synthesis of this compound by a wet method comprising the direct precipitation of orthophosphoric acid solution to a calcium hydroxide solution, the process being described by the following reaction: $10\ Ca(OH)_2 + 6\ H_3(PO_4) <=> Ca_{10}(PO_4)_6(OH)_2 + 18\ H_2O$. Synthesis was performed in a laboratory reactor, 700 mL in capacity, instrumented and controlled using a computer interface, so that the influence on process variables such as reaction temperature, pH, medium inertization by N_2, velocity of stirring and the flow rate input of $H_3(PO_4)$, could be assessed. The influence of these parameters was, therefore, evaluated in terms of the required composition and morphology of HAP formed particles, analysing them by FTIR, XRD, SEM and EPMA for determination of the Ca/P ratio. From the obtained results, it can be concluded that HAP particles having suitable properties for use in medicine, could effectively be prepared by this technique, provided that a good control of the involved process variables is maintained.



1 – Stirred reactor;
2 – Container with orthophosporic acid (H_3PO_4), 0,3M;
3 – Container with ammonia (NH_4OH) a 25%;
4 – Nitrogen cylinder (N_2);
5 – Heated water bath;
6 – Pumps;
7 – KOH Filter.

Experimental synthesis apparatus:

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Investigation of Silica Particle Structure Containing Metallocene Immobilized by a Sol-Gel Method

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Keywords: metallocene; sol-gel method; SAXS; polymerization; polyethylene.

Introduction

Several methods have been proposed in the literature dealing with supporting methodologies for metallocene catalysts. As a matter of fact, silica has largely been the most employed material for grafting metallocenes in the studies (Severn et al., 2005).
In a different approach, we have proposed the immobilization of zirconocene into silica matrix concomitant to the oxide synthesis obtained by non-hydrolytic sol-gel method (Fisch et al., 2006). According to this method, metallocene complexes are immobilized among the aggregates of primary structures which form the silica particle. The resulting catalyst was shown to be active for ethylene polymerization. As an extension of this previous work, the present study deals with the immobilization of different metallocenes (Cp_2ZrCl_2, Cp_2HfCl_2, Cp_2TiCl_2, (*n*BuCp)$_2$ZrCl$_2$, (*i*BuCp)$_2$ZrCl$_2$, (*t*BuCp)$_2$ZrCl$_2$, $EtInd_2ZrCl_2$, $Et(IndH_4)_2ZrCl_2$) within silica by the proposed method and characterization of the resulting silica by Small Angle X-Ray Scattering (SAXS) in order to get information about the silica particle structure and possible interactions with the metallocene complex. In addition, the catalysts were evaluated in ethylene polymerization in an attempt to obtain some correlation with the silica structural parameters.

Results

SAXS measurements:

A typical SAXS profile found in this study is shown in Figure 1. Changes in the slope profile are related to the presence of distinct structures in the material. Figure 1 shows two regions with different slopes, D_1 for $q<q_b$ and D_2 for $q>q_b$. A characteristic length (ξ), corresponding to the spatial scale of the structure change presents in the q_b point (see Scheme 1), could be estimated as $\xi= 2\pi/q_b$. Taking into account that silica is formed by agglomeration of primary particles, and that the organometallic complex is entrapped within the interstices, q_b could be assigned to the spatial scale of such a primary particle (Scheme 1). Assuming the model depicted in Scheme 1, information about the influence of the metallocene on the silica structure could be found in D_2 region. Besides, the curve slope (α_1 and α_2 corresponding to D_1 and D_2 region, respectively) is usually related to a fractal dimension in the region.
All α_1 values were found to be between 3.1 and 3.4, which is the interval attributed to surface fractal structures. Furthermore, no clear trend can be established between α_1 values and the metallocene structures. Conversely, α_2 values lie between 3.0 and 3.8. In addition, it was found a correlation between α_2 values and the ligand-metal-ligand angle of the metallocenes, which is a steric hindrance measurement of its structure. The spatial scale of the primary particles estimated as $\xi= 2\pi/q_b$ was found to be about 160 Å for all samples. Therefore, no influence of the metallocene nature on the primary particle dimension (ξ) was clearly observed.

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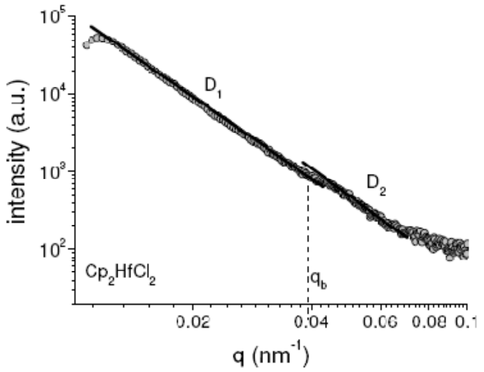
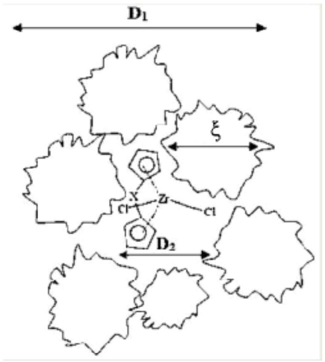


Figure 1: Typical scattering profile found to supported metallocene (Cp_2HfCl_2/SiO_2).

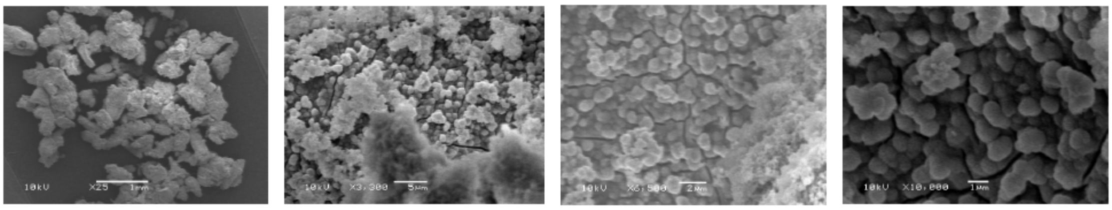


Scheme 1: Particle structure and dimension related to the SAXS scattered profile.

Polymerizations:

In a general way, the catalysts showed a poor activity with exception of those systems containing Cp_2ZrCl_2, Cp_2HfCl_2, and $(nBuCp)_2ZrCl_2$. In addition, it was not found any evident correlation between activity and fractal dimensions as well. Due to the deep location of the metallocene complex into the oxide matrix, the poor catalytic activity could be attributed to restrictions of monomer access to the active sites hindering the chain propagation. This is attributed to an inadequate particle fragmentation during the early moments of the polymerization as shown in Figure 2. In this sense, a huge shell of oxide matrix around the metallocene hinders the breakup of the particle leading to a poor activity. This problem is worse for metallocenes exhibiting lower propagation rate.

In an attempt to solve the described diffusional problems, new catalysts were synthesized with higher metallocene content (from 0.5 to 1.5 w.% Zr). Increasing the load of metallocene allows the catalysts to breakup adequately during the early moments of the polymerization avoiding monomer accessibility problems to the active sites.



25× 3300× 6500× 10000×

Figure 2: Micrographics of polymer particle obtained in the ethylene polymerization with Cp_2ZrCl_2/SiO_2 of particle diameter between 53-100 μm.

Conclusion

The immobilization of metallocenes within silica matrix using a non-hydrolytic sol-gel route shows that the silica particle is formed by aggregation of the primary structures with diameter about 160 Å. This result suggests that the organometallic complex is surrounded by primary particles having a deeper location in the oxide matrix. Some metallocenes, which in homogeneous systems are less active, showed a neglected activity when entrapped within the oxide matrix by the proposed method. This result could be related to problems of catalyst fragmentation leading to monomer mass transfer limitation along the partially fragmented particle. In order to solve this problem, the metallocene amount in the catalyst should be increased.

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Electronic transference assessment in a *Geobacter sulfurreducens* fuel cell

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Keywords: Microbial Fuel Cell, *Geobacter sulfurreducens*, Electricity, Wastewater, Cyclic voltammetry.

Microbial Fuel Cell technology (MFC) is attracting growing interest as an environmentally friendly energy production system. Bacteria can be used in MFCs to generate electricity, while accomplishing the biodegradation of organic matters present in different kinds of wastewater (Logan, 2006). In this context, the electrochemical properties of rigid graphite and carbon Toray electrodes at pH 7, are investigated in experiments using medium prepared in anaerobic conditions inoculated with *Geobacter sulfurreducen*s (DSM 12127).

The voltammograms obtained in assays with carbon Toray in growth medium (acetate concentration: 60 mM), with and without bacteria, are shown in Figure 1. An important increase of the current intensities between 0.4 and 1 V vs. SCE is noticed after the addition of bacteria.

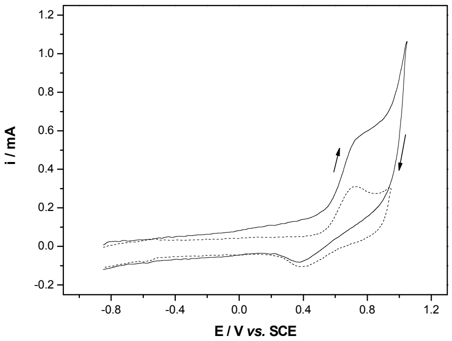

Figure 1. The voltammograms of carbon Toray electrodes in growth medium, with (—) and without (---) *Geobacter sulfurreducens* (75 mg/L) at 37° C (v = 50 mV s-1).

In order to assess the electronic transfer processes associated with the identified oxidation peaks in cyclic voltammograms, a cyclic voltammetric study was carried out. The influence of potential sweep rate on peak potentials and current densities allows to determine the reversibility of the process and the limiting step.

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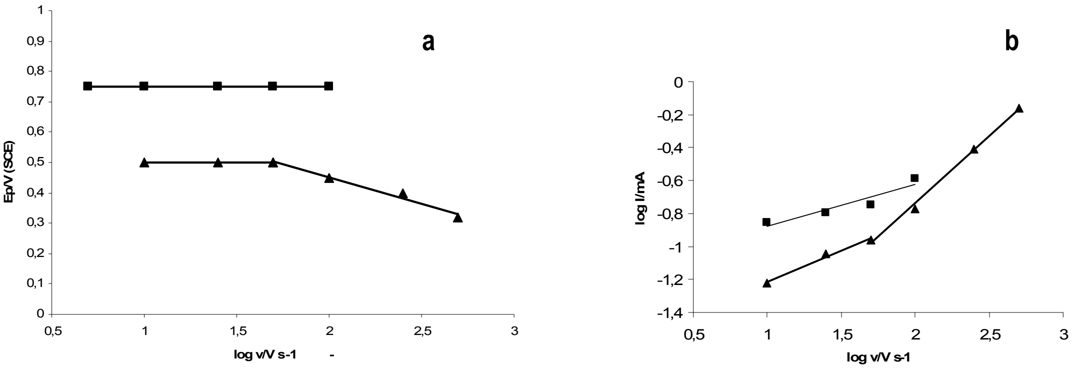

Figure 2. Graphic representation of (a) Ep vs. log v and (b) log I vs. log v whit carbon Toray (■) and rigid grafite (▲).

From the Figure 2 (a), it can be seen that for carbon Toray the potential of the oxidation peak (Ep) is independent of the sweep rate (v) showing a reversible process. Regarding the rigid graphite, between 10 and 50 mV s^{-1} the process seems to be reversible while from this last value up to 500 mV s^{-1} the dependence of Ep on v indicates an irreversible process.

Figure 2 (b) represents the dependence of log i on log v. The slope of the straight lines gives information about the nature of the limited step. With carbon Toray, the slope of 0,26 suggest a mechanism with numerous adsorbed species and products. No simple kinetic laws can be proposed in this case. For rigid graphite two distinct slopes are observed: 0.38 (between 10 and 50 mVs-^{1}) and 0.81 (between 50 and 500 mVs-^{1}). Considering this results it can be concluded that at low sweep rates the mechanism is governed more by diffusion, while adsorption takes more importance when increasing the sweep rate.

Other kinetic parameters of the reactions were also determined, namely the number of exchanged electrons.

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Studies on developing the red mud capability of binding the tannery residual chromium

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Keywords: red mud, chromium, adsorption, levigability

90 % of hides processed currently in the world are subjected to tannage with basic chromium salts that give rise unavoidably to a great amount of chrome leather wastes containing about 75-85 % organic matter and not less than 2,5 % Cr_2O_3. Leather wastes containing chromium (LWCC) reach about 25 % of the whole weight of salted hides to be processed, that is a burden for the leather sector performances and success related to the environment protection [1].

Waste processing by hydrolysis and protein reclamation for a variety of viable applications (biofertilizers, surfactants, eco-adhesives etc.) [2] cause the chromium compound transfer (with economically non-convincing applications) into the effluents and sludge, and their treatment and disposal has to be considered from the environmental point of view.

This work suggests an adsorbing material prepared particularly by neutralization of the residual red mud, collected in large ponds from the alumina refining plants [3], to be used in binding the tannery residual chromium in the solid phases and stabilizing it with the view of long term storage.

The environmentally compatible mineral complex resulting from the two noxious wastes is a potential source to obtain embedded compounds or for other applications [4].

This study has involved the investigation of how the sulphuric acid and alkaline-earth metals neutralize the red mud and measurement of zero charge points (ZCP) in the neutralized red mud with the aim of assessing their neutralization level [5].

Phase composition was not changed by neutralization with sulphuric acid, the major participation of every red mud constituent being kept unchanged when pH was decreased from the initial one of 12,0-12,5 to 8,0. Sulphuric acid has neutralized the sodium ions and as removed as soluble sodium sulphate from the red mud.

No red mud constituent containing calcium dioxide and silicon dioxide (calcium aluminates, calcium silicates, calcium alumino-silicates etc.) has been decomposed and changed molar ratios to other constituents. Though, the neutralization process has stabilized the material and increased its ion exchange capability.

Red mud neutralized by sulphuric acid down to pH = 8 was used as adsorbent for Cr^{3+} ions found in tannery effluents. Subsequently the leaching of the chromium bound to the red mud was tried by levigation tests at pH = 3,5.

Data in Table 1 have revealed the strong Cr^{3+} binding on the solid adsorbing phase and reasonable chromium amounts released in the leaching water.

Even though there was a good chromium adsorption onto the red mud neutralized with sulphuric acid, this has resulted from an reversible interaction. Under such conditions, other neutralization types were tried by means of alkaline-earth metals.

Table 1. Data for chromium leaching at pH=3,5.

Sample	**Cr in washing I effluent, mg/g**	**Cr in washing II effluent, mg/g**	**Cr in washing III effluent, mg/g**
1	33×10^{-5}	37×10^{-5}	0
2	42×10^{-5}	45×10^{-5}	26×10^{-5}
3	13×10^{-5}	40×10^{-5}	80×10^{-5}
4	15×10^{-5}	58×10^{-5}	68×10^{-5}
5	23×10^{-5}	12×10^{-5}	56×10^{-5}

The common calcium and magnesium salts can remove alumina from the diluted alumina solution by binding it as insoluble aluminates and aluminate-silicate compounds with or without captured sodium ions. Such compounds give a high specificity for the heavy metal adsorption. Trials for red mud neutralization from pH=12,5 down to pH=11 and 9 by means of $CaCl_2$, $MgSO_4$, $MgCl_2$ and combinations of these have led to sludges showing ZCP values below the pH values for the neutralized sludge, in the range of negative charges, proper for positively charged ion adsorption, as revealed in Table 2. ZCP was measured by plotting the surface charge σ = f (pH), being the cross point of the curve with the pH axis as shown in Figure 1 (ZCP = 6,45). Data obtained from the potentiometric titration (acid/alkali) were used to calculate the surface charge σ.

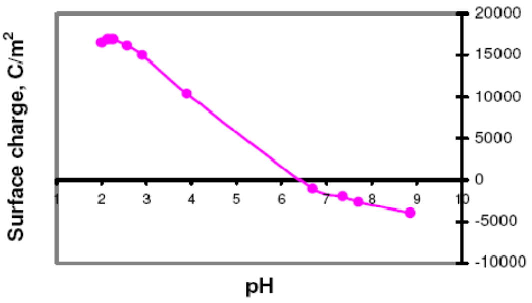

Figure 1. ZCP measurement for the red mud neutralized by $CaCl_2 + MgSO_4$ at pH=9, in the electrolyte solution of 0,1M NaCl

Table 2. ZCP values obtained by trials

Ref. No.	**Neutralizing agent**	**Neutralizing pH**	**ZCP**
1.	- (namol brut)	12,5	6,28
2.	$CaCl_2$	11	6,44
3.	$CaCl_2$	10,5	6,39
4.	$MgSO_4$	11	6,24
5.	$MgSO_4$	9	6,24
6.	$CaCl_2 + MgSO_4$	11	6,44
7.	$CaCl_2 + MgSO_4$	9	6,45
8.	$Mg Cl_2$	11	6,42
9.	$Mg Cl_2$	9	6,13
10.	$MgCl_2 + CaCl_2$	11	6,72
11.	$MgCl_2 + CaCl_2$	9	6,00

Conclusions

Red mud neutralization by sulphuric acid is an outstanding solution recommended for its treatment previously to storage in ponds.

Neutralization by alkaline-earth metals has resulted in new solid phases of higher chemical stability, able for chromium seizing.

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Statistics model and optimization of uranium extraction in ultrasounds field in sulphuric acid medium

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Keywords: uranium, statistics model, ultrasound

This paper provide an experimental study based on mathematical statistics that is the factorial experiment designs regarding the effects of ultrasounds power on the extraction efficiency of acid dissolution of uranium.

The operation parameters varied using a 2^{5-1} factorial experiment were the temperature, the pH of the reaction medium, the operating time and the ultrasound power (Şayan and Bayramoğlu, 2004). The mathematic model obtained through the experimental results processing was determined using the least squares method and it describes dependency of the extraction efficiency on the technical and economical significant operation parameters. Mathematical model optimization was performed using a maximum slope algorithm and it points out the major factors that influence the uranium extraction efficiency, namely: the temperature, the oxidant amount and the ultrasounds power within the range of studied experimental values.
Uranium dissolution experiments were performed on samples of 50 g (0.03% uranium) with particles of 0.100 mm grinding size, the solid/liquid mass ratio was 1:1 and the stirring velocity was maintained invariable at 500 min^{-1}(Panţuru et al., 2006).
In order to intensify the uranium extraction the ultrasound field (480 W and 35 kHz) is used beside the mechanical stirring (Margulis et al., 2003).

When the experiments were performed the sulfuric acid 96% was used as extraction reagent and potassium chlorate 0.5-1% as the oxidant reagent providing a oxidation potential between 450 and 500 mV necessary for uranium dissolution.

Experimental results represented in Figure 1 points out the efficiency surface, which is obtained by rising of a vertical line at the intersection point between the temperature and ultrasounds power values.

It can be observed that the extraction efficiency increases with the temperature and ultrasounds power increasing while the duration, the medium pH and the oxidant amount are maintained invariable.

It is important to point out the following two aspects:

- approximately equal values are obtained for the extraction efficiency even only when the mechanical stirring is used but the operation parameters are different; for example the efficiency of 97.36 % when the ultrasound field is used for 10 minutes per stage at a temperature of 40ºC, for mechanical stirring time of 6 hours, pH 1 and an amount of oxidant reagent of 10 kg/t solid, is obtained even when only the mechanical stirring is used but at temperatures of 80ºC, operation time 10 hours at the same pH and with the same amount of oxidant reagent;
- under similar operation conditions, temperature of 40ºC, mechanical stirring time 6 hours, pH 1 and an amount of oxidant reagent of 10 kg/t solid, are used and the ultrasound field is missing the extraction efficiency is 85.47%.

These results point out the efficiency of ultrasound field applied with a view to intensifying the mass transfer process beside the improvement of working conditions.

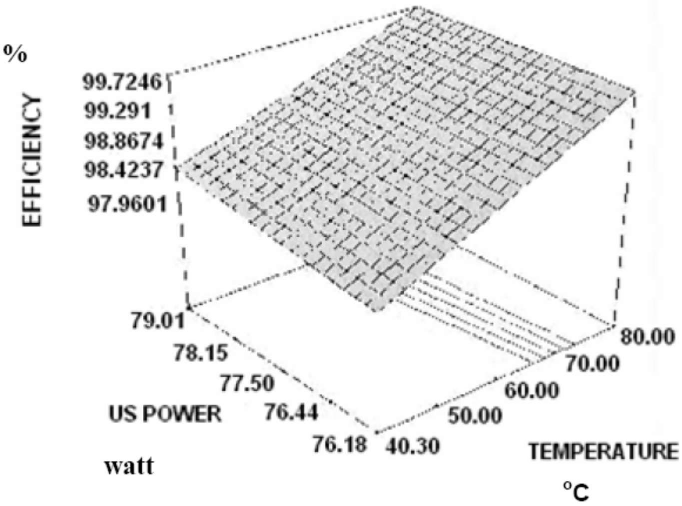

Figure 1. 3D representation of temperature/US power/efficiency interaction

Ultrasound utilization has positive effects on radioactive pollution diminution, obtaining extraction efficiencies above 96% respectively 10% much higher values than those obtained in identically conditions but in the absence of the ultrasonic field.

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Stability of binary mixtures in a novel combustor conical spouted bed with a draft tube of different length

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Keywords: spouted bed, conical spouted bed, draft tube, combustor, mixtures.

The introduction of a central draft tube in a spouted bed provokes great changes in the hydrodynamics of the spouted beds and provides operating advantages on using of wastes energy. Certain advantages for the use of this central draft tube are: greater flexibility in the operation; lower pressure drop; solids of any size or nature may be treated; narrower residence time distribution; lower flow rate; lower pressure drop; better control of solid circulation; avoids maximum spoutable bed height. Consequently, solid circulation may be controlled by changing independently column diameter, stagnant bed height or particle diameter. Of the disadvantages the following are worth mentioning: lower mixing degree; complexity of design; risk of tube blockage.

The experimental unit design at pilot plant scale is provided with conical contactors of poly(methyl methacrylate) of cone angle between 28 and 45°. The values of length of the central draft tubes, l_d, located centrally at a height from the bottom of the contactor, have been varied, between 0.010 and 0.30 m. The maximum value corresponds to the location of the draft tube at the same level that the upper bed surface, H_o-l_d (San José et al., 2007). The distance between the base of the contactor and the lower level of the device, h_d, is between 0.01 and 0.10 m, respectively. The beds studied are composed of mixtures of glass spheres of different particle diameter between 1 and 9 mm.

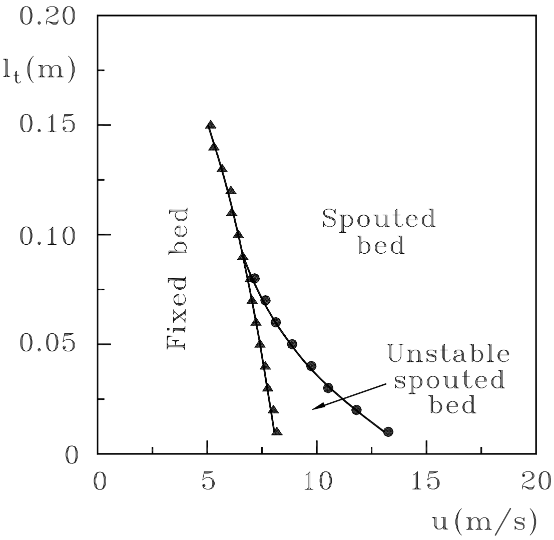

Figure. Operating map. System: γ= 33°, D_o= 0.04 m, beds of glass spheres of Sauter average diameter $\overline{d_S}$ = 2.5 mm. Draft tube of d_d= 0.04 m and h_d= 0.05 m.

In order to design the appropriate length of the non-porous draft tube to operate without any instability or operating drawbacks in conical spouted beds, the effect of draft tube length on bed stability has been studied in beds consisting of binary mixtures of solids of different particle diameter. A novel diagram has been proposed in this paper to provide the effect of the length of the central draft tubes on bed stability. In this diagram the length of the draft tube, l_d, has been plotted against the gas velocity, u. corresponding to a system taken as an example with a bed of mixtures of glass spheres of Sauter average diameter, $\overline{d_S}$ = 2.5 mm. The borders between the different regimes, drawn with solid lines, have been obtained experimentally (the points drawn are the experimental base for tracing these borders).

As is observed, as the length of the central draft tube is increased, the stable spouted bed regime is reached and the minimum spouting velocity decreases. For small lengths of the draft tube there is a slight instability zone. Therefore, the increasing in the draft tube length enhances the stable operating conditions. Thus, with the longest draft tube the maximum range of stable operating conditions is reached.

Acknowledgements

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Simulation of maleic anhydride production from n-butane in a two moving med reactors system

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Key words: Maleic Anhydride, Riser Reactor, Regenerator Reactor, VPO catalyst

Abstract

Modeling and simulation of production of Maleic Anhydride (MA) with a two fluidized bed reactors system have been studied. Effects of riser's hydrodynamic conditions on n-butane conversion and MA selectivity have been investigated. Finally, influence of operational parameters on catalyst oxygen re-loading in regenerator reactor was simulated.

Introduction

MA as an important industrial material used in various applications is basically produced via heterogeneous reactions. Vanadium- Phosphorus- Oxide (VPO) catalysts are the most effective systems used to convert n-butane to MA, selectively (Dente et al., 2003). Up to now, based on diversity of feeding methods and reactor type several processes have been suggested (Strangio et al., 2002). Fixed-bed and fluidized-bed reactors, operating with assist of a Gas-Phase Oxygen stream, are two of the most important processes (Alenzo et al., 2001). In 1994, Dupont announced commercialization of moving-bed recycled-based technology in which MA is produced through a high velocity plug-flow in absence of oxygen stream by use of a promoted VPO catalyst. Having noticeable amount of lattice oxygen for partial oxidation of n-butane and removing of the main oxygen stream (Golbig et al., 1997, Roy et al., 2000), being more selective than old type catalysts and finally outstanding capability of being regenerated are the notable advantages encountered in this method (Fogler, 1999, Golbig et al., 1997).

At this work the effects of hydrodynamic conditions such as temperature, n-butane concentration, inlet solid particles velocity, and feed gas velocity on conversion and Maleic Anhydride selectivity in the riser reactor and modeling parameters on simulation results and reloading of catalysts' lattice oxygen have been evaluated, respectively.

Modeling and Simulation

In this paper, Berruti-Kalogerakis's model is the basic model for simulation of riser hydrodynamic (Berruti et al., 1989), in which the riser is divided into two sections in the reactor's radial direction. Voidage and Mass balance equations are solved simultaneously to achieve the simulation results.

Mathematical modeling of catalyst oxidation in the regenerator reactor is based on the study on residence time instead of studying over transient state of the reaction reported by Chen et al. (2002).

Results and Discussion

As it is shown in Figure 1, which depicts n-C4 conversion and MA selectivity vs. temperature variation, the higher level of energy consumed, the lower time is needed to reach the certain conversion.

To represent oxygen loading of the catalysts in Figure 2 depicts weight gain of catalyst versus residence time for different θ^o_L values, where θ^o_L is the fraction of total removable oxygen capacity of the catalyst, in a specified duration of 30 min. Modeling results represent the limitation of catalyst capacity in oxygen loading process. As it is

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obvious, in higher amounts of θ^o_L the lower amounts of oxygen would be loaded in catalyst structure as lattice oxygen. In fact, less atomic oxygen find their ways into the catalyst bulk as it contains a portion of oxygen in the form of crystalline atoms before getting involved in the re-oxidation process.

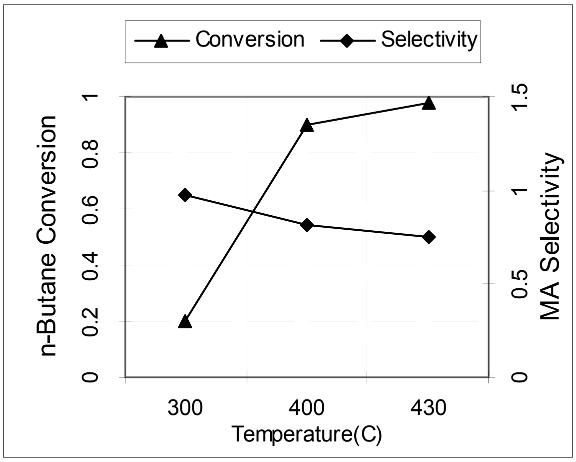

Figure 1: Xn-C4 & SMA vs. temperature

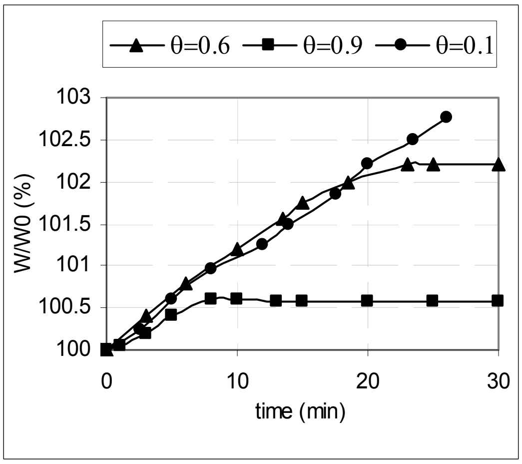

Figure 2: VPO catalyst weight gain percentage vs. catalyst residence time at different catalyst lattice concentrations **(θ^o_L)**

Conclusion

Operation parameters have crucial effects on both riser and regenerator performance and should be chosen precisely for each of which. A good choice is 416 °C for the riser reactor and 580 °C for the regenerator. In addition, the higher inlet solid particles velocity applied, the lower residence time and consequently the lower oxygen load on catalyst would be. Also it was deduced that feed gas velocity should be limited into two specified bounds to control bypassing and back mixing, respectively.

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Production of hydrogen by methane steam reforming coupled with CO_2 sorption

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Keyword: Sorption-enhanced reaction process, steam methane reforming, Carbon dioxide, hydrogen production

1. Abstract

Hydrogen is becoming the fuel of the future. The most important technology for the production of hydrogen is methane steam reforming (SMR) (Twigg 1989). However, there are high costs associated, both in energy and materials, as the reaction has to be carried out at high temperatures (973K) to achieve high methane conversions.

The combination of reaction and adsorption in the reactor displaces the equilibrium maintaining a high conversion at lower temperatures (~773 K) which reduces the material and energy costs of the process. The sorption of carbon dioxide is also of interest as we are able to produce hydrogen with very low (or inexistent) carbon dioxide emissions. The sorbed carbon dioxide can be sent to storage facilities.

2. SERP proof of concept

In this work, the sorption enhanced reaction process (SERP) concept is tested for SMR using a Ni/Al_2O_3 catalyst and a hydrotalcite sorbent. The commercial hydrotalcite received from SASOL was impregnated with potassium and cesium (Nataraj, Carvill et al. 2000). The carbon dioxide sorption capacity was measured for different CO_2 partial pressures (Oliveira, Grande et al. 2008). The equilibrium sorption capacity is shown in Figure 1. The kinetics of steam methane reforming were measured in the commercial catalyst received from Degussa.

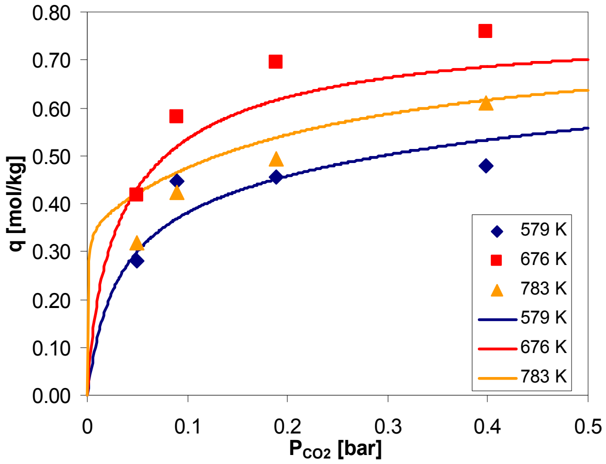

Figure 1. Carbon dioxide equilibrium sorption capacity in potassium modified hydrotalcites

The proof of concept was made by comparing the performance of the methane steam reforming reaction in the presence of just catalyst or in the presence of a 3:1 weight ratio of sorbent and catalyst. In Figure 2 the conversion is plotted as a function of time for the case of the traditional SMR and SERP.

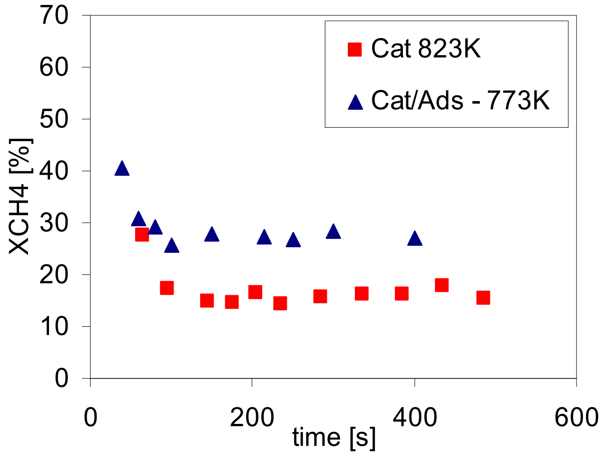

Figure 2. Methane conversion for traditional SMR and SERP.

Figure 2 shows that it is possible to achieve a higher conversion at a lower temperature in the presence of the carbon dioxide sorbent. Carbon oxides free hydrogen was produced for more than 80 s before the sorbent had to be regenerated.

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Adsorption of CO_2, CH_4 and N_2 on titanosilicate adsorbent

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Keywords: Adsorption, materials, synthesis, titanosilicate, ion-exchange

Introduction

In the last years the interest in new materials for adsorption has increased. Open-framework materials have numerous industrial uses as a result of their very regular pore openings on a molecular level. In 1989 a new class of framework materials containing both octahedral and tetrahedral atoms were reported by Kuznicki (1989) and designated as ETS (Engelhard Titanium Silicate). Chemical modifications, such as ion exchange or changing the framework composition can influence the pore size of these materials. Removal of nitrogen from methane-rich streams is one of the most energy intensive gas separations. Adsorption technologies may offer an interesting alternative for N_2 removal if this gas is selectively adsorbed in the adsorbent. In this work we have synthesized sodium titanosilicate (Na-ETS-4) and study the effect of exchanging Na with different alkali-earth cations in the structure, namely Sr and Ca. Adsorption properties of CH_4 and N_2 were determined as well as properties of CO_2 since normally is also found in these streams as contaminants and may be selectively adsorbed on the material reducing its potentiality.

Experimental Section

The ETS (titanosilicate) samples were statically grown from synthesis mixtures with molar composition 1.0 TiO_2; 5.5 SiO_2; 5.5 Na_2O; 1.5 KF; 350 H_2O, similar as reported by Kim et al. (2000). The crystallization was carried out in a 150 ml Teflon autoclave under autogeneous pressure and heated to 423 K for 7 days. The crystalline phase identification of the products was done by X-ray diffraction (XRD). The XRD patterns shows distinct peaks which constitute the characteristics signatures of ETS-4 framework, like reported by Marathe et al. (2004). The average crystal size of the sample was obtained by scanning electron microscopy (SEM), and images show the crystals with rectangular morphology and dimensions of ~2.0 X 6.0µm. To investigate the thermal stability and other advantages already reported about the ion-exchanged variants, the synthesized form of Na-ETS-4 was ion-exchanged with calcium and strontium (Sr-ETS-4 and Ca-ETS-4).

Adsorption of pure gases was performed in a magnetic suspension balance (Rubotherm, Germany) operated in closed system. The isotherms were measured at 323 K in the range of (0 to 650) kPa. The samples were activated at different dehydration temperatures (373, 448, 483 and 543 K) under vaccum. Kinetics of adsorption is analyzed following the uptake curve.

Results

Adsorption properties of CO_2, CH_4 and N_2 were determined and the effect of increasing activation temperature was studied in the modified samples. It was observed that activating the adsorbent at high temperatures prior to adsorption tests, the capacity of methane can be reduced. In the Ca-ETS-4 a shift in selectivity was observed: activating the adsorbent at higher temperatures result in an adsorbent with higher selectivity towards nitrogen (Figure 1). In all the samples tested, the loading of carbon dioxide is much higher than the loading of other gases indicating that this gas should be removed from the stream before nitrogen rejection.

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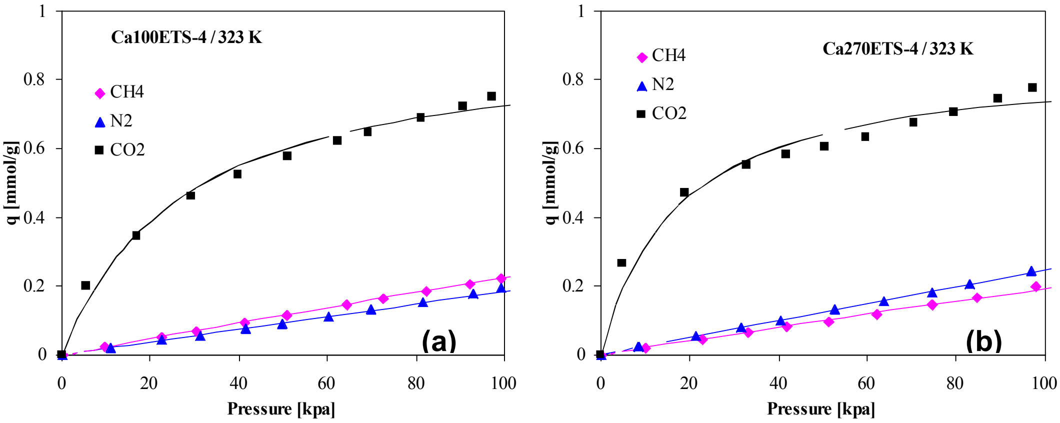

Figure 1. Adsorption Equilibrium on Ca-ETS-4 samples at 323 K and activation at 373 K (a) and 543 K (b): ■, Carbon Dioxide; ◆, Methane; ▲, Nitrogen.

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Modelling of light olefin transformation over ZSM-5 zeolites with different acid strengths

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Keywords: olefin transformation; acid catalysis; ZSM-5 zeolite; kinetic model

Data for the acid-catalysed transformation of propylene over four HNaZSM-5 zeolites with varying amounts of sodium (to ensure varying acid strength distributions), at various temperatures and partial pressures, was analysed. This transformation is characterized by the fact that, as the temperature increased, the propene consumption rate decreased and this has been interpreted as being generated by adsorption effects. In this work the rather complex network mechanism involved in the transformation of these light olefins was approximated by a simple quasi-homogeneous network kinetic model which only details the products up to the number of carbon atoms and does not explicitly include the steps corresponding to the adsorption of the reacting species. The kinetic constants for the various reaction steps in the network were quantitatively related to the acid strength of the sites over which the reactions takes place by Polanyi-type equations. The acidity of the sites was measured by the activation energy for the ammonia desorption and various ZSM-5 zeolites with different acid site distributions were used. The model is able to produce a good description of the observed product distribution.

Kinetic model

It is assumed that the transformation of small olefins is started by an oligomerization step that leads to heavier species which will then transform by complex reaction network. The model described in this paper couples the use of a reaction network model with the influence of the heterogeneity of the acid sites in the catalysts. Intervening species have only been detailed up to the number of carbon atoms (no distinction between olefins and paraffins is made at this stage). Under this approach a quasi-homogeneous model was developed. Thus, oligomerization/cracking reactions were described by the following general equation:

$$C_n + C_m \overset{\rightarrow}{\underset{\leftarrow}{}} C_{n+m} \qquad (1)$$

where *n* and *m* ≥ 2 and *n*+*m* ≤ 8. (no protolytic cracking was considered at this stage, and, thus, the formation of methane was excluded). Within this framework, the reaction network is described by just 9 different reactions. Assuming that reactions are of simple order in relation to the different species involved, this model requires a total of 18 kinetic rate constants, the forward (k_i corresponding to the oligomerization) and backward (k_{-i} corresponding to the cracking) rate constants.

$$r_i = k_i \cdot p(C_n) \cdot p(C_m) - k_{-i} \cdot p(C_{n+m}) \qquad (2)$$

These were assumed to obey an Arrhenius-type law and the activation energy was linked to the acidity of the site (as measured by the activation energy for the ammonia desorption) by a Polanyi-type equation. The global rate constant was taken as the summation over all acid sites present in the catalyst, as determined by NH_3-TPD.

$$k_i = k_{ref\ i} e^{-\frac{E_{a0i}}{R}\left(\frac{1}{T}-\frac{1}{T_{ref}}\right)} \sum_j q_j e^{-\frac{\delta_i E_{aj}^{NH3}}{RT}} \qquad (3)$$

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where $k_{ref\ i}$ and E_{a0i} are, respectively, the rate constant at the reference temperature and the activation energy for reaction *i* on a non-acidic site (one that does not bind NH_3), δ_i reflects the effect of the acid strength in the activation energy for a given reaction and q_j is a measure of the number of acid sites that bind NH_3 so that its activation energy for desorption is $E_{a_j}^{NH_3}$.

Results

The kinetic model was fitted to the experimental data for various catalysts, temperatures and partial pressures, described in detail elsewhere [1], using a non-linear least-squares regression technique implemented in commercial spreadsheet software (Excel). A single set of parameters was used, thus describing the results, regardless of catalyst, partial pressure or temperature.

All material balances were numerically integrated using the Euler method, to obtain a computed product distribution. The sum of the squares of the residuals was minimized (using Solver). In order to reduce correlation between the pre-exponential factors and the activation energies, in the Arrhenius kinetic term a reference temperature was used, as seen on equation 3; this temperature was the experimental temperature closest to the mean of the temperature range covered in the experiments.

The parity plot obtained and one example of the product distribution obtained using the model for a NaHZSM-5 catalyst that was used is depicted in Figure 1.

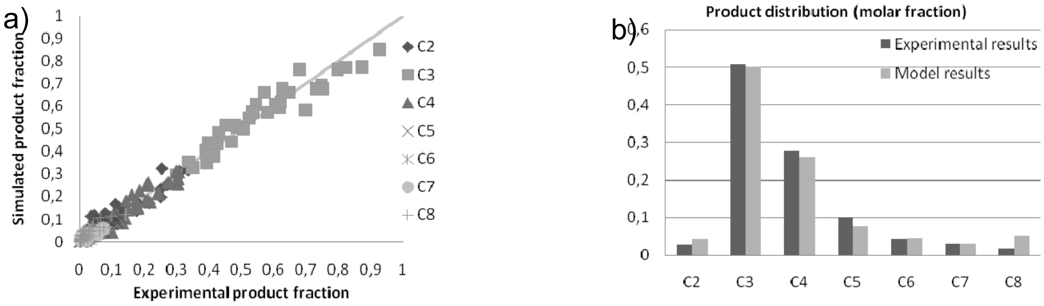

Figure 1 – a) Parity plot. b) Product distribution for the transformation of propylene at 30 kPa and 300ºC for a NaHZSM-5 catalyst and predicted by the Model.

Conclusions

Although the model used was relatively simple, it is able to provide a generally acceptable description of the activity and distribution of products for the experiments under analysis.

It is very important to note, that, although the number of parameters is relatively high, only one set of parameters is used to describe all the available experimental data, covering not only all the temperatures and partial pressures used in this study, but also all the catalyst tested. This result opens the way for the development of universal kinetic models that may be applied to the acid-catalysed transformation of hydrocarbons over zeolites.

Acknowledgments

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Carbon dioxide reforming of methane over Niγ-/Al_2O_3 in fluidized-bed ractor

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Keywords: Methane reforming, nickel catalysts, fluidized bed reactor

Abstract: To establish operational relations for the catalytic carbon dioxide reforming of methane into syngas, a one-dimensional Kunii-Levenspiel heterogeneous model for the fluidized bed reactor was developed. Effects of reaction parameters as temperature, and pressure on the dry reforming reaction were analyzed in terms of conversion, reagent and product concentrations. The fluidized-bed reactor presented the following dimensions: height of the reactor H_t = 1,180 mm, internal diameter D_{int} = 38 mm, particle diameter d_p = 70-85 μm, minimum fluidization velocity U_{mf} = 0.0011 m/s, superficial velocity U_o = 0.0191 m/s, mass of catalyst m_{cat} = 300 g, and the catalyst 4.98 wt% Ni/γ-Al_2O_3. The reactions were evaluate at 1023K, 1073K, and 1123K respectively, and under atmospheric pressure. Under these conditions and employing a Langmuir-Hinshelwood kinetic model, for 1073 K to 1123 K a methane conversion of 85,62 to 97.89% was obtained.

1. Introduction

Conventional fixed-bed steam methane reformers have some disadvantages, such as low heat transfer rates, diffusional resistance in catalyst pores and large temperature gradients. In addition, steam methane reforming, a highly endothermic process, is affected by the efficiency of the heat input into the reformer. The carbon dioxide reforming of methane may be presented as an alternative process, mainly when employed in a fluidized bed reactor. Improved heat transfer, catalyst uniformity, and the virtual elimination of diffusion limitations are the major advantages of when this reactor is employed (Chen, Honda and Zhang, 2004). The catalyst particles in a fluidized bed has to be in a proper range, and they have to withstand the mechanical environment. Catalyst particles with a mean diameter in the range of 70 to 85 μm were employed and the thermal uniformity of the fluidized-bed was obtained at a flow rate three times the minimum fluidization velocity.

2. Experimental

The column was built in stainless steel, it has a diameter of 38 mm and a height of 1.180 mm (Figure 1). The bed pressure drops were measured with a differential pressure transducer. The experimental fluidynamic data were obtained relating the pressure drop in the bed with the superficial velocity.

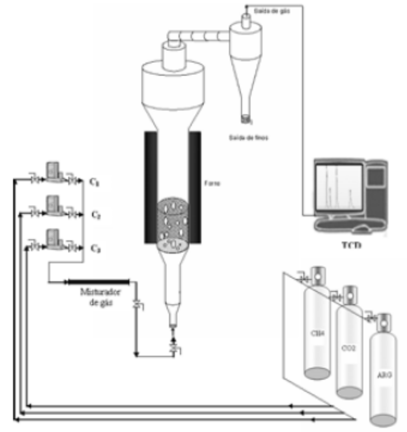

Figure 1. The Schematic representation

2. Results and Discussion

For evaluation of the process the main reaction considered were:

$$CO_2 + H_2 \leftrightarrow CO + H_2O \quad \Delta H_{298} = 41\,kJ/mol \tag{1}$$

$$2CO \leftrightarrow CO_2 + C \;\; \Delta H_{298} = -172\,kJ/mol \tag{2}$$

$$CH_4 \leftrightarrow C + 2H_2 \;\; \Delta H_{298} = 75\,kJ/mol \tag{3}$$

The solution of the mass balances, from the kinetic laws obtained from the experimental evaluation of steps 1, 2, and 3 of the reaction, and the temperature and the pressure employed, simulations were considered which produced the profiles presented in Figure 2.

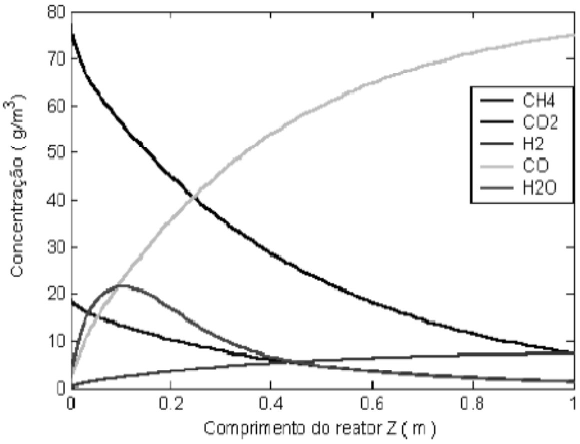

Figure 2. Schematic Profiles of reagents conversion and products.

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New polystyrene-based polymers as supports for ethylene polymerisation

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Keywords: Ethylene polymerisation, Polymeric supports, Spherical Polyethylene, Hydroxypolystyrene, polystyrene-b-polyisoprene

Abstract

The industrial use of metallocene and late transition metal catalysts /methylaluminoxane (MAO) catalytic systems requires the use of supports to improve the operational conditions (stability, prevention of reactor fouling). The use of supports also enables the control of the morphology of the obtained polymer, which is an extremely important benefit of heterogeneous supported catalysis versus homogenous non supported catalysis. However, the presence of residues of the inorganic supports at the end of the polymerisation reaction gives rise to some contamination problems. Therefore, important efforts are being nowadays employed in the replacement of inorganic supports by less contaminating organic supports, mainly based in polymers like polystyrene (PS).

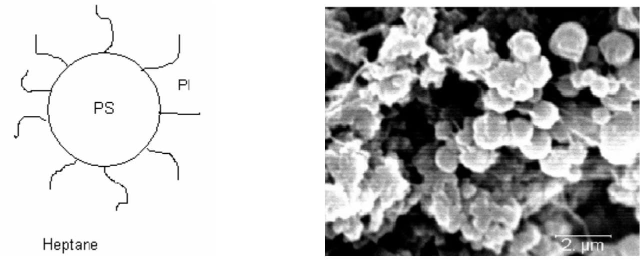
The introduction of polar groups in polystyrene-based supports has been described in literature as an efficient way to anchor MAO activators. Ethylene polymerisation takes place around the PS supports yielding polyethylene (PE) in the form of spherical particles. Klapper and co-workers reported the use of polystyrene supports with PEO (polyethylene oxide) and PPO (polypropylene oxide) functionalities [1]. To this purpose, Bouilhac *et al* also described a star-shaped polystyrene support having –O-CH_3 or –OH functionalities and new polystyrene functionalised microgels containing benzoic acid or benzophenone functionalities [2-3]. More recently, Bouilhac et al. have developed another strategy based on the self-assembly of end-capped PS and PS-based block copolymers that spontaneously form well defined micelles in a selective solvent [4] which is another important feature for the use of these polymers as organic supports.

On this basis, linear hydroxypolystyrene (PSOH), polystyrene-block-isoprene copolymer (PSPI) and plurihydroxy-functionalised PSPI (PSPI-OH_n) are tested as supports for ethylene polymerisation. First, the ability of these (co)polymers when dissolved in a selective solvent to self assemble and form spherical micelles is investigated by Light Scattering techniques [5]. Then, the capacity of these spherical polymer micelles to act as active supports in the presence of MAO towards MeDIP(2,6-$iPrPh_2FeCl_2$) are examined through ethylene polymerisation tests and morphology studies of the obtained PE.

Results show that the linear PS-OH polymer is able to form aggregates but only for concentrations of the polymer in toluene higher than those typically used in ethylene polymerisation. The synthesized PE using these supports shows however the formation of some spherical particles.

On the other hand well-defined micellar systems of PSPI with particle sizes around 200-250 nm are formed in heptane – Figure 1 a). After TMA addition (in an adequate PSPI/TMA ratio), the formation of bigger but yet stable aggregates is observed, enabling the synthesis of PE with spherical morphology – Figure 1 b). Polymerisation activities in the order of 900-1000 KgPE/(molFe.h.bar) are obtained at 20°C and 1bar ethylene pressure. A quite different behaviour is observed for the same PSPI block copolymers in toluene a good solvent of the two blocks. In this case, no stable micelles

are formed and, accordingly, the obtained morphology is much more irregular and polymerisation activities are low.



a) b)

Figure 1– a) Schematic representation of the formed micelles b) SEM microscopy image of polyethylene synthesized using the PSPI supports in heptane

Through the introduction of hydroxyles as side groups on the PI block of PSPI chains, it was aimed to anchor more efficiently MAO or TMA by covalent binding. However, light-scattering studies on PSPI-OH$_n$ in heptane showed a destabilisation of the micelles due to the presence of –OH groups which make the PI-OH block to become insoluble in heptane. In toluene, however, the presence of –OH groups was less sensitive, thanks to the solubility of the PS block, enabling the formation of micelles. However no benefits were found in polymerisation activities or in polyethylene morphology.

Light scattering results of PSPI and PSPI-OH$_n$ in heptane and toluene are resumed In Table 1.

Polymer	Heptane	Toluene
Non-Modified PSPI	PI block: soluble PS block: not soluble *Formation of micelles* *(PS Core; PI Shell)*	PI block: soluble PS block: soluble *Single PSPI Chains. No Micelles*
Modified PSPI (PSPI-OH$_n$)	PI-OH$_n$ block: not soluble PS block: not soluble *Destabilization of micelles, precipitation of the polymer*	PI-OH$_n$ block: not soluble PS block: soluble *Aggregation depending on the % of –OH groups inserted* *(PS Shell; PI-OH$_n$ core)*

In conclusion micelles of polyisoprene-polystyrene (PSPI) block copolymers gave promising results when used as support for ethylene polymerisation in heptane. They give rise to PE with spherical morphology. Moreover, the polymerisation activities in heptane (around 1000 KgPE/(molFe.h.bar) remain quite good if compared with the corresponding activities in homogeneous conditions (around 1600 KgPE/(molFe.h.bar).

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Cure of a fiberglass-vinyl ester I beam composite: a new heating strategy

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Keywords: pultrusion, polymeric composites, degree of cure.

Introduction

Pultrusion is a continuous process in which fibers are continuously pulled and impregnated in a resin bath before entering in a heated die. Normally the die is heated by electrical heaters coupled on its outer surface. In order to obtain parts of good quality, care must be taken through tooling and process design, to ensure that a uniform and sufficient degree of cure is achieved in all composite (Liu, X.L., 2001). A computer simulation model of the pultrusion process was developed by Kommu *et al.* (1998) using finite element/control volume (FE/CV). Coelho and Calado (2001) optimized the pultrusion cure cycle applying a Simulate Annealing Method. The pultruded I beam composite was also studied by Liu and Hillier, (1999). After the variation of pull velocity and the temperature of four rectangular heaters coupled on the die surface, these authors concluded that the fiberglass vinyl ester I beam should be pultruded maintaining all four heaters at the same temperature. The development of a new heating strategy to reduce the energy consumption in the pultrusion process is the main objective of this contribution. Simulation results were compared with results presented by Liu X.L. (2001).

Mathematical Model

The mathematical model of the steady state pultrusion process is composed by the energy balance and the kinetic cure reaction. These equations can be written, respectively, as:

$$\rho_c \; C_c \left(u_i \nabla T\right) = k_c \nabla^2 T + c_{in}(1-\phi)(-\Delta H)R_a \tag{1}$$

$$u_z \frac{d\alpha}{dt} = R_a = \left(A e^{\left(\frac{-Ea}{RT}\right)} \right)(1-\alpha)^n \tag{2}$$

where T is temperature, u velocity vector, C_c specific heat of the composite, k_c thermal conductivity of the composite, ($-\Delta H$) total heat generated, ρ_c the density of the composite, R_a resin reaction rate, Φ fiber volume fraction, c_{in} resin initial concentration, α degree of cure, A frequency factor, E activation energy, and $m + n$ reaction order.
In this work, the degree of cure was assumed to be zero ($\alpha = 0$) before the fiber-resin system entering into the die, and the inlet temperature was considered as 298 K. In order to compare our results with the results presented by Liu X.L. (2001), the same physical parameters were here considered. The mathematical model was solved by CFX™ computer software. After several computer simulations, the convergence of the profiles was obtained when a mesh with 34590 nodes and 37690 elements was employed. A heating configuration based on six internal electrical heaters inside an insulated die, as indicated in Figure 1, was here proposed and their simulated results were obtained.

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Results

Considering this heat configuration, simulated temperature and cure profiles along the beam are shown in Figures 2 and 3, and temperature profiles at three points of the beam are presented in Figure 4. It should be pointed out that the proposed configuration was able to cure the material in a more efficient way. This efficiency is characterized by approximately a 20 % reduction of the energy consumption in comparison with the heat strategy used by Liu X.L. (2001).

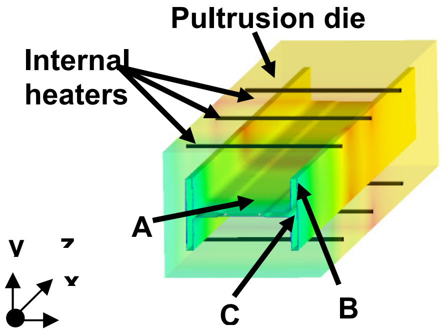

Figure 1 – Internal heating configuration.

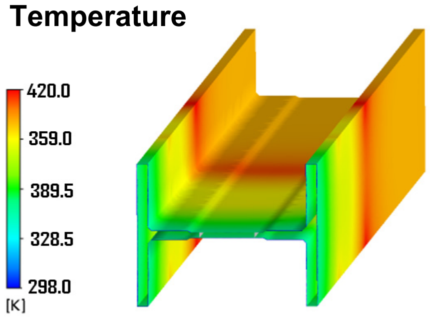

Figure 2 – Composite temperature.

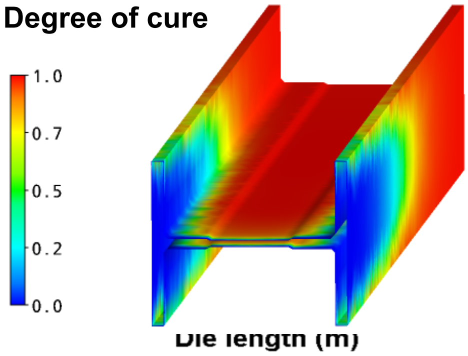

Figure 3 – Degree of cure of the composite.

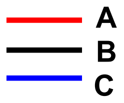

Figure 4 – Temperature profile.

Conclusions

Through intensive computer simulations, were verified that smaller energy consumption can be obtained with the suggested heat strategy in pultrusion process. This economy was verified by the reduction of the surface area of the heaters causing a significant decrease of the energy consumption. This internal heat configuration appears to be a good alternative to improve global thermal efficiency of the process.

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Preparation of polymeric hydrogel microparticles for textile applications

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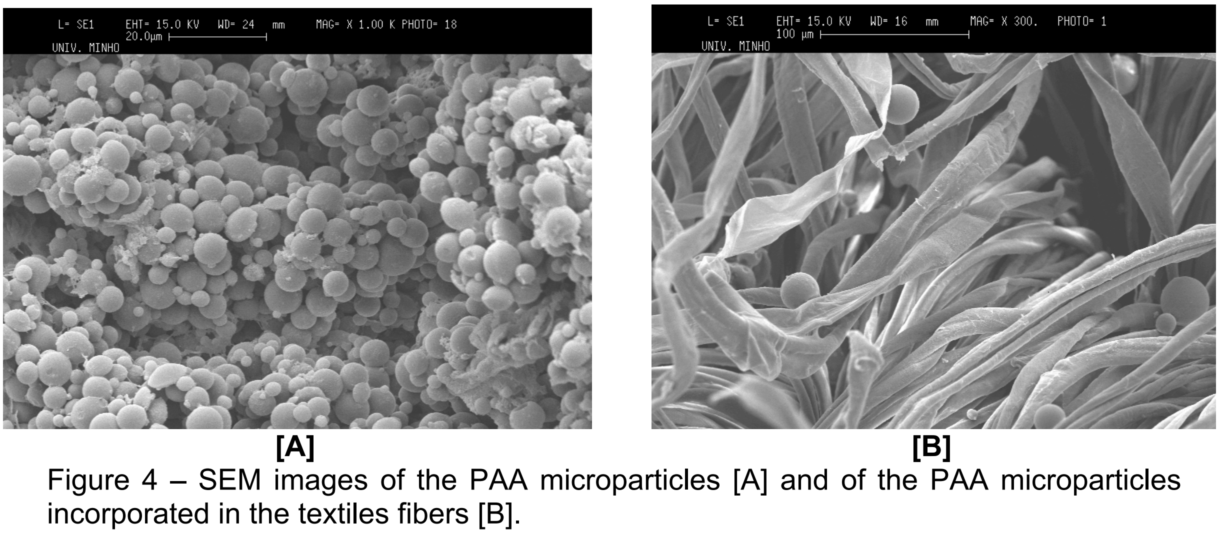
Keywords: Poli(Acrylic Acid), inverse-emulsion polymerization, hydrogel, textiles, swelling behavior.

Polymer hydrogels are three-dimensioned networks formed by chemical bonds and/or physical forces. They can absorb water to swell, but cannot dissolve in water. Recently the interest in the development of hydrogels has increased considerably. [1, 2]
In this work we wish to prepare polymeric microparticles based on a polymeric hydrogel - Poly(Acrylic Acid) (PAA), suitable for the textile industry.
The PAA microparticles were prepared by inverse-emulsion polymerization. [3]
The polymerization conditions and their effects on the morphology (analyzed by Scanning Electron Microscope (SEM)), structure (analysed by Attenuated Total Reflectance - Fourier Transform Infrared (ATR-FTIR)) and particle size (analyzed by Laser Diffraction Spectrometry (LDS) were evaluated.
The size and morphology of the polymeric microparticles has been optimized by the manipulation of proceeding factors such as the organic and aqueous solutions homogeneity, the time addition of the monomer and initiator, the cleaning time of the oxygen in the reactional atmosphere with N_2 and the isolation and cleaning of the solid polymer.
The swelling and humidity adsorption behavior of the PAA microparticles and after his incorporation in the textiles (cotton and polyester) were evaluated. The morphology of the polymeric microparticles after the incorporation in the textiles was also evaluated (Figure 1).



[A] **[B]**

Figure 4 – SEM images of the PAA microparticles [A] and of the PAA microparticles incorporated in the textiles fibers [B].

The polymeric microparticles were prepared with good yields. The scale up of the polymer production was possible from a 250mL reactor in the beginning to a 1L reactor. The SEM images (Figure 1) allow us to conclude that the incorporation procedure of the polymeric microparticles in the textiles does not affect the

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microparticles morphology. The swelling and water adsorption tests allow us to conclude to, that the incorporation of these microparticles in the textiles increases their swelling and capacity water adsorption.

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A shape selective MOF material for the adsorptive separation of linear and branched hexane isomers

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Keywords: Metal-Organic Framework, Adsorption, Hexane Isomers

Summary

Single and multicomponent fixed-bed adsorption of *n*-hexane (nHEX), 3-methylpentane (3MP) and 2,2-dimethylbutane (22MB) on the three-dimensional microporous metal-organic framework Zn(BDC)(Dabco)$_{0.5}$ (BDC=1,4-benzenedicarboxylate, Dabco=1,4-diazabicyclo [2,2,2]octane) has been studied in this work. The pores system consisting in one-dimensional large channels (7.5 Å) interconnected by 3.8 x 4.7 Å narrow pores can encapsulate linear hexane and block branched hexanes. The breakthrough experiments show a highly selective sorption with respect to nHEX.

Introduction

Porous metal-organic frameworks (MOFs) have been of significant interest because of their potential applications in gas storage, separation and heterogeneous catalysis. Unlike traditional porous zeolite materials, the pores within MOFs can be tuned varying their size and functionality for specific applications. This superior feature is significantly important to develop novel functional materials for gas separation which are mainly determined by shape selectivity effects.

Taking into account the Californian ban on MTBE and the fact that oxygenate content has to be reduced in 50% of the present value; the most sustainable way to boost octane ratings in gasoline is the development of novel materials and technologies for the separation of hexane isomers. As shown in Figure 1, the kinetic diameters of nHEX, 3MP and 22MB are slightly different; thus the micropores within MOFs need to be designed with suitable pore size for such kinetic separation. The 3.8 x 4.7 Å narrow channels in microporous MOF Zn(BDC)(Dabco)$_{0.5}$ (MOF **1**) (see Figure 1) can take up the linear nHEX but block the branched 3MP and 22MB, thus the hexane isomers might be kinetically separated by fixed-bed adsorption.

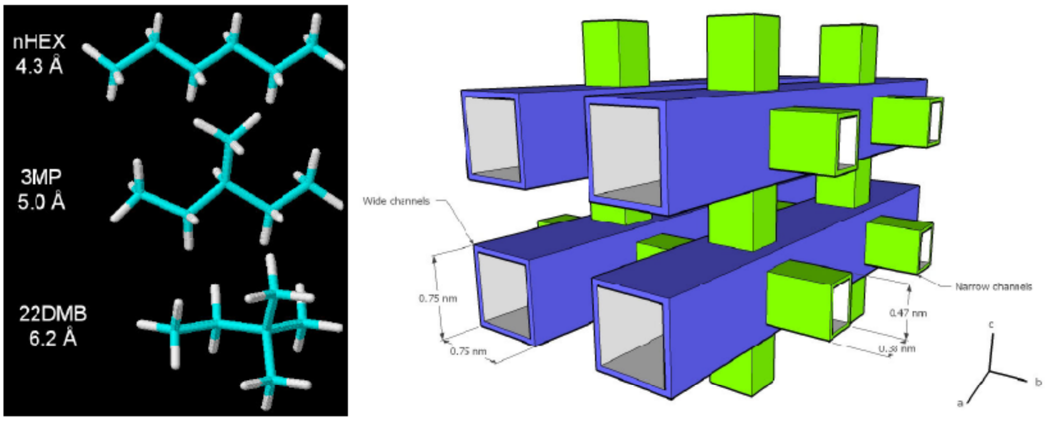

Figure 1. Three-dimensional optimized structure and kinetic diameters of hexane isomers (left); Perspective illustration of 3D intersecting channels (right).

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Experimental procedure

Adsorption equilibrium isotherms were obtained from breakthrough experiments. The adsorption column packed with crystals of MOF **1** was operated by introducing continuously a known composition of C_6 isomers in a helium stream. The procedure used for multicomponent breakthrough experiments consist in measuring the concentration history at the outlet of the packed bed in order to collect samples during the breakthrough curve. After the saturation is reached, the composition of each sample loop is evaluated by chromatography in a proper chromatographic column.

Results and discussion

In Figure 2a, we have plotted the adsorption capacities of MOF **1** for nHEX, 3MP and 22DMB. As both 22DMB and 3MP have only access to the larger channels their adsorption are significantly lower than those for nHEX. 3MP is longer than 22DMB, which leads to its slightly higher sorption capacities. The single breakthrough curve of nHEX (Figure 2b) exhibits an inflection indicating two types of interaction sites for nHEX in MOF **1**, which are probably attributed to the existence of two types of channels for the access of nHEX.

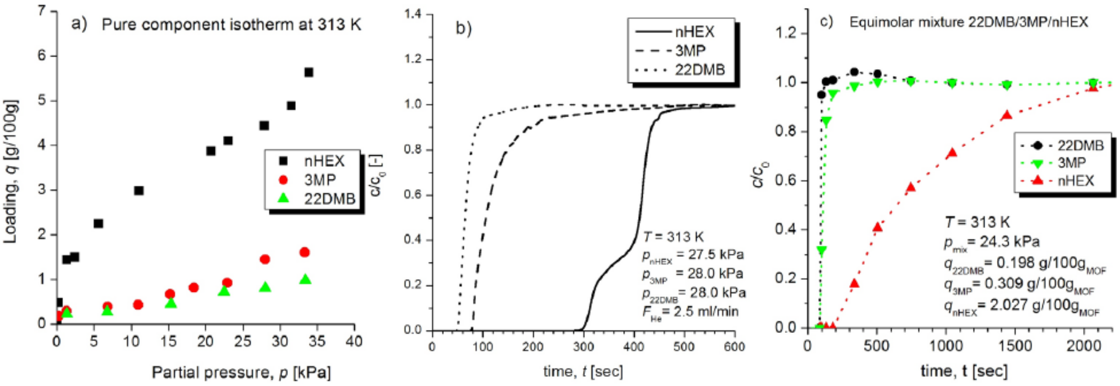

Figure 2. (a) Single adsorption isotherms at 313 K; (b) single breakthrough curve at 313 K and p = 28 kPa and (c) ternary breakthrough curve at 313 K and p_{mix}= 24.3 kPa.

The different breakthrough times following the order nHEX>>3MP>22DMB (Figure 2b) indicate that the adsorption strengths of the hexane isomers to MOF **1** decrease as the degree of branching increases simply because of their different van der Waals interactions with the microporous walls. The branched isomers can be readily separated from linear nHEX isomer, as shown in their ternary breakthrough curve (Figure 2c). This property is very important for its potential application in Total Isomerization Process (TIP), a process for separating branched hydrocarbons from linear components to enhance octane number. Future research will focus on the tuning of this material for the improvement of the separation degree.

Acknowledgements

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Photocatalytic oxidation of clofibric acid using nanocrystalline sol-gel TiO_2 under visible irradiation

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Keywords: Titanium dioxide, Sol-gel, Photocatalysis, Clofibric acid, Pharmaceuticals

Introduction

Treatment systems for water and air based in semiconductor heterogeneous photocatalysis represent an area of major technical importance in our days. Titanium dioxide (TiO_2) is the most popular semiconductor material in photocatalytic applications, mainly due to its strong oxidizing power, high chemical stability and relative inexpensiveness. TiO_2 exists in different crystalline forms: anatase, rutile and brookite. It has been recognized that TiO_2 photocatalytic efficiency is related to surface and structural properties of the semiconductor such as crystal structure, surface area, particle size distribution, porosity, band gap and surface hydroxyl density.

In the present work we produced TiO_2 nanocrystalline powders from a Ti(IV) alkoxide by an acid catalyzed sol-gel method. By changing the calcination temperature of the process we have obtained nanoparticles with different crystal structures, phase compositions, surface areas and particle sizes. These materials were tested in the photocatalytic oxidation of clofibric (2-(p-chlorophenoxy)-2-methylpropionic) acid (Fig. 1) under visible irradiation. Clofibric acid is the active metabolite of several fibrate drugs prescribed to reduce blood cholesterol levels – acts by lowering the serum lipids, thus reducing the low density lipoprotein fraction rich in triglycerides. Pharmaceuticals are a class of emerging environmental contaminants because are being used extensively and increasingly in human and veterinary medicine (Hernando et al., 2006). These chemicals are designed to be physiologically very stable, therefore when present in the environment they show refractory behavior, which may lead to bio-accumulation. Clofibric acid photocatalytic degradation kinetics was studied and reaction intermediates determined. A correlation between textural, crystalline and spectroscopic properties of the synthesized TiO_2 materials and its ability for the degradation of this organic pollutant is presented.

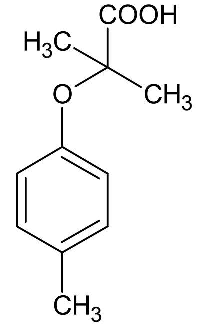

Fig. 1 - Clofibric acid.

Results and Discussion

Nanocrystalline TiO_2 was produced from the corresponding titanium isopropoxide by acid catalyzed hydrolysis. The powders were obtained after thermal treatment of the resulting xerogel at temperatures from 573 to 973K under nitrogen flow.

XRD measurements of titania obtained at different calcination temperatures revealed that below 723K only anatase crystalline phase is detected. Anatase to rutile transformation occurs between 723 and 773K. At temperatures above 723K there is a mixture between anatase and rutile phases. At 973K only the rutile phase is detected. The crystallite sizes of both anatase and rutile forms increased with calcination temperature. Thermal treatment of TiO_2 gels at higher temperature promotes phase transition from thermodynamically metastable anatase to more stable and condensed rutile phase. The gel possesses hydroxyl groups on the surface that are removed during heating treatment. As the dehydration process occurs, crystallites grow to dimensions larger than those of the original particles.

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The surface area of the TiO_2 samples was determined by nitrogen adsorption isotherms at 77K. Higher the temperature lower the surface area. Materials obtained at 573K exhibited a surface area of 134 m^2 g^{-1}. As the temperature increases the surface area gradually decreases, reaching 3 m^2 g^{-1} for temperatures higher than 873K. This results from the increase in the crystallite dimensions.

In order to explore the photo-efficiency of the prepared materials, TiO_2 nanocrystalline catalysts were tested in the photocatalytic oxidation of clofibric acid aqueous solutions under visible ($\lambda_{exc,max}$=366 nm) irradiation. The kinetics of photodegraration followed a pseudo-first order model. The higher apparent rate constant (k_{app}) of 1.11×10^{-1} min^{-1} was obtained with TiO_2 produced at 673K. For this catalyst, close to complete clofibric acid removal was achieved after only 30 minutes of irradiation. Catalysts obtained at higher temperatures resulted on progressively lower kinetic rate constants. Catalyst produced at 973K, constituted by pure rutile crystals, showed a k_{app} of 1.02×10^{-2} min^{-1}, close to ten times less than the pure anatase catalyst produced at 673K. In terms of total organic carbon (TOC) removal, a 79.4% of decrease in organic content was achieved for reaction in the presence of the catalyst produced at 673K, while a merely 20.0% TOC removal was achieved using pure rutile catalyst.

The main reaction intermediates detected by HPLC analysis were found to be 4-chlorophenol, isobutyric acid, 4-chlorocatechol, hydroquinone and benzoquinone (Fig. 2). Reaction using TiO_2 obtained at 673K produced an abrupt abatement on clofibric acid concentration. Its complete removal was achieved at very early reaction times. Clofibric acid removal appeared to be much slower when catalyst produced at 973K was used. In the later case, at the end of the irradiation run there was still a considerable amount of the initial compound and reaction intermediates in solution.

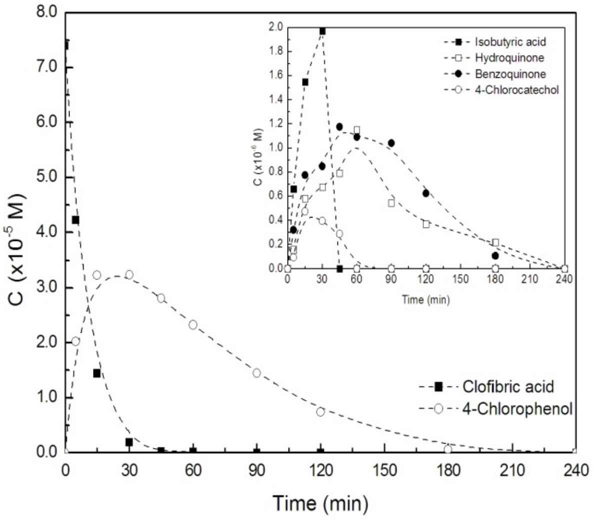

Fig. 2 - Concentration profiles of clofibric acid and reaction intermediates during the photocatalytic reaction using TiO_2 obtained at 673K.

Conclusions

The most effective catalyst is pure anatase, leading to complete conversion of the clofibric acid in less than one hour. The intermediates generated are more persistent, the most important being the 4-chlorophenol. However their presence is not likely to reduce catalyst activity. On the other hand, surface area seems determinant for the catalyst efficiency and therefore some adsorption effect cannot be ruled out.

Acknowledgments: The authors gratefully acknowledge FCT and FEDER for financial assistance (fellowship SFRH/BD/16966/2004 and project POCI/EQU/58252/2004).

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Catalytic cracking of n-decane as model molecule of Fisher-Tropsch synthesis

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Keywords: Catalytic cracking; N-decane; Acid zeolites; Activity; Selectivity; Deactivation

1. Introduction

The Fisher-Tropsch (FT) synthesis is an attractive route to produce high-quality liquid fuels from coal, natural gas and biomass via conversion of syngas (mixture of CO and H_2). The long chain paraffins (C_{10}-C_{60}) obtained from this process can then be fed in fluid catalytic cracking (FCC) units to produce transportation fuels (mainly gasoline). [1,2]

In this work, n-decane cracking, taken as model reaction, was carried out over HY and HZSM-5 zeolites, which form the active phase of FCC catalysts, but also over Hβ and HMCM-22 zeolites, which are used in other commercial processes.

2. Experimental

The catalytic tests were performed in a tubular Pyrex fixed-bed reactor, at 350°C and atmospheric pressure. The n-decane feed was diluted with dry nitrogen to achieve a partial pressure of 0.13 atm ($p_{nitrogen}/p_{n\text{-}decane}=7$). Different contact times were studied by varying the mass of catalyst (10 – 300 mg) and maintaining the n-decane flow constant (2.92 g/h). The reaction effluent was analysed using a VARIAN 3400 chromatograph with a 100 m PONA CP-Sil CB capillary column and a flame ionization detector.

3. Results and discussion

As shown in table 1, the following initial catalytic activity order can be proposed, when comparing zeolites with similar total Brönsted acidities: Hβ > HZSM-5 > HY > HMCM-22. Moreover, as expected, the activity increases with Brönsted acidity, for zeolites with a same pore structure.

Table 1 – Main obtained results for n-decane cracking over different zeolites

Zeolites (Si/Al)	**Brönsted Acidity[a] ($\mu mol.g^{-1}$)**		**Activity**		**% C[c]**
	Total	**Strong**	**Initial ($mmol.g^{-1}.h^{-1}$)**	**Residual[b] (%)**	
HY (5)	672	195	136	22	8.2
HY (15)	271	13	45	22	2.2
Hβ (15)	345	110	454	37	5.7
HMCM-22 (15)	651	55	81	59	3.5
HZSM-5 (15)	1161	459	307	88	1.0
HZSM-5 (25)	351	176	213	100	0.4
HZSM-5 (40)	297	46	66	92	0.1

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[a] Determined by pyridine adsorption/desorption followed by IR spectroscopy. Total acidity: 150°C. Strong acidity: 450°C

[b] Percentage of residual activity after 60 min of reaction

[c] Carbon content in coked catalyst for a n-decane conversion of 10%. Determined by total combustion at 1020°C

The very high initial activity of the Hβ sample (in comparison with HY(15) which possess the same Brönsted site concentration) could be related to the very small size of its crystallites: 0.02μm, compared to the other zeolites samples (0.5 – 1.0 μm).

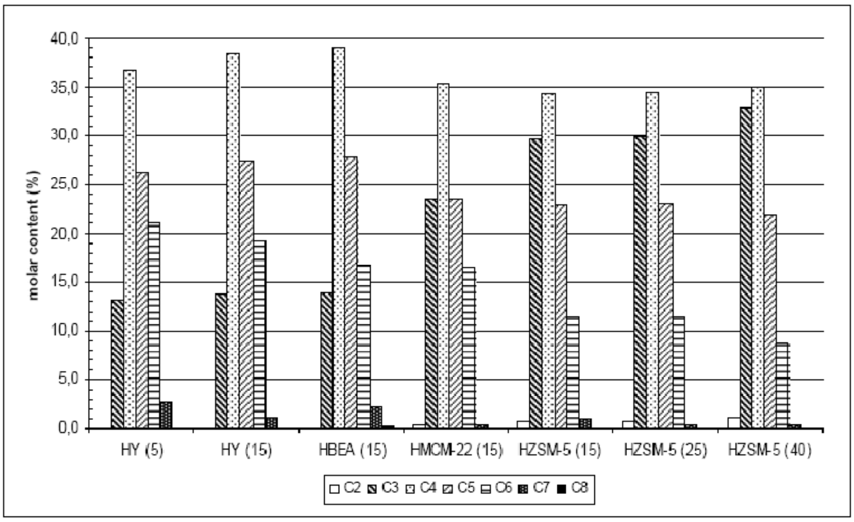

Figure 1 – Products distribution of n-decane catalytic cracking over the different zeolites, at 10% of conversion

Whatever the zeolite sample C_3, C_4, C_5 and C_6 (alkanes and akenes) are the main reaction products. As shown in Figure 1, the C_3/C_7 and C_4/C_6 ratios are higher than 1, and very high in the case of HZSM-5. This means that secondary cracking of hexenes and heptenes happens in a large scale, particularly over HZSM-5. This could be due to a slow diffusion of these compounds in the narrow channels of this zeolite, which would favour there cracking in smaller products. The intermediate behaviour of HMCM-22 could be related to the coexistence of large external cups on the outer surface of the zeolite crystallites and narrow 10-MR pores in its structure [3].

Moreover, the comparison of the product distributions obtained over HY (or HZSM-5) samples with different Si/Al ratios shows that zeolites acidity does not change the selectivity of the reactions. On the other hand, similar product distributions are obtained on HY and Hβ zeolites. Therefore, the product distribution, and in particular the extent of secondary cracking, seems to essentially depend on the pore size.

Finally, when time-on-stream is increased, a very fast and strong deactivation of the large pore zeolites (HY and Hβ) occurs, whereas only a very limited deactivation of HZSM-5 samples can be observed (see the residual activity values in Table 1). HMCM-22 presents, once again, an intermediate behaviour. This fact is explained by the formation of bulky polyaromatic compounds ("coke"), which remain trapped inside the zeolites porosity, as shown by the quantity of carbon deposed on the catalyst after 60 min reaction (%C, Table1). Table 1 suggests that coke content depends not only on the zeolite structure (coke formation is favoured over large pore zeolites), but also on the concentration of strong Brönsted sites.

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Optimization tools and economical issues in the design of a residential cogeneration system

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Keywords: Micro-cogeneration, Thermo-economic optimization, Heat Recuperating system design, multi-disciplinary project.

Cogeneration or Combined Heat and Power (CHP), is the simultaneous production of thermal and electric energies. It is a major option to achieve primary energy savings, avoid grid network losses and reduce GHGs emissions. It has been worldwide implemented in large scale installations (>1 MWe) but the use of microcogeneration units (<50 kWe), for domestic or small commercial buildings, is still at an incipient level. This scenario will change rapidly, triggered by rocketing fuel prices, climate change awareness and government support mechanisms (Onovwiona and Ugursal, 2006; DL n.º 363/2007; Directive 2004/8/EC).

The development of a new technology must take into account the socio-economic aspects, thus providing a realistic way to evaluate the production and operation costs of these systems. The economic aspects are important, namely for the final consumer.

Thus far, numerical methods of optimization have been used for the thermo and economic optimizations of large systems. The aim of these procedures is to minimize the total costs including those related with the thermodynamic inefficiencies (Silva et al., 2003). However, applications of these methodologies to small-scale installations, in particular, the use of numerical optimization algorithms in micro cogeneration systems, cannot be found in the literature.

In the present project, a multidisciplinary team has been adapting a thermoeconomic optimization method, which gave good results with multi-megawatt cogeneration systems, to small-scale (<1 MWe) and micro-scale cogeneration units (<50 kWe). Different technologies are available for cogeneration systems, and the choice depends on, among other factors, the size of the unit and the required power to heat ratio. The problem is then formulated as a non-linear optimisation problem, with both thermodynamic and physical constraints. The optimization problem was solved by using a direct method, the Box method implemented in FORTRAN language. This method solves nonlinear optimization problems with inequality constraints and is similar to the simplex method. In previous studies, this technique proved to be simple to use and to implement, by easily converging to the solution (Silva et al., 2003).

The thermo economic optimization model was tested on a small-scale cogeneration plant that delivers 100 kW of electricity and 0.05 kg/s of saturated steam. Based upon the optimal set point, the thermodynamic state over the plant can be determined, as shown in Figure 1 (Martins et al., 2008).

As part of this project, the design and construction of a small micro-cogeneration prototype for a single household (2≈5 kWe), based on a comercial IC engine-generator, fuelled by natural gas and complemented with a two-step exhaust heat recuperator, is also taking place. The main innovation will be this heat recuperating system (Figure 2).

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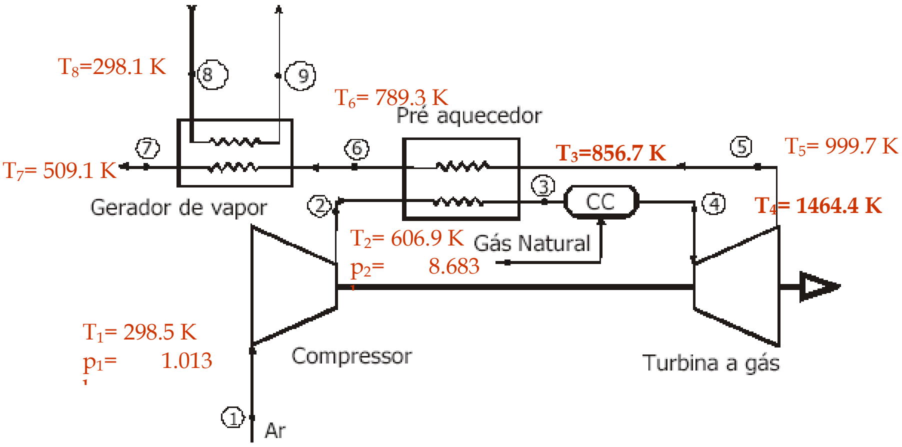
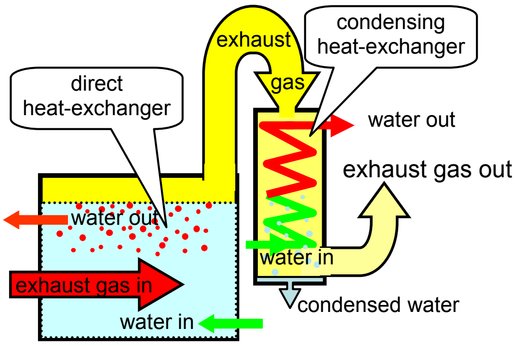


Figure 1. Temperature and pressure values for the streams in the optimal design of the micro-cogeneration plant.



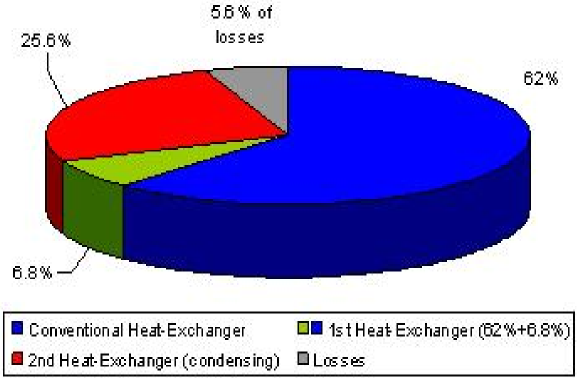


Figure 2. Heat Recuperating system.

Figure 3. Useful heat comparison, between a conventional heat-exchanger and the 2-step heat recuperating system.

This novel thermal system has been implemented in Excel in order to study its efficiency. It has been demonstrated that this layout leads to a lower exhaust gas temperature, i.e. to a higher global efficiency by comparison with traditional heat recuperators, Figure 3 (Teixeira, 2007).

At the moment, this optimization tool is being applied to the new microcogeneration system in order to improve design and to study the economical viability of the project.

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Environmental performance assessment in process design

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Keywords: environmental performance; life cycle assessment; environmental impacts

Abstract

An important issue in process design is which tools (indicators) should be used to include environmental concerns on the analysis and synthesis steps. According to Cano-Ruiz and McRae (1998) environmental indicators have been developed based on several concepts: minimization of the more concerning pollutants in the emissions, minimization of waste generated total mass, minimization of contribution for specific problems and minimization of an aggregated indicator. The environmental assessment based on the total mass of waste generated is not sufficiently enlightening since it does not consider the characteristics of the different substances emitted. The environmental assessment based on specific indicators is very restrictive and does not allow for the consideration of various aspects of the environmental impacts. The consideration of different environmental impacts is the best way to evaluate the environmental performance of a process.

This paper proposes a methodology to develop and determine a global (aggregated) potential environmental impact indicator based on the Guinée model (2002). In this model the impact categories were chosen considering a base list proposed by SETAC (*the Society of Environmental Toxicology and Chemistry*). To determine the global environmental indicator one should consider the baseline category impacts suggested by Guinée (2002) and select those that apply to the case in study. Since for the category human toxicity there aren't internationally accepted impact indicators it is suggested to use the ones that are more suitable for the case in study including the use of other models like the IChemE (2007). Then it follows characterization and normalization steps and finally, the establishment of weights to obtain an aggregated indicator. The normalization can be done using the mean value of the indicators values for all the compounds in each category as suggested by the WAR methodology (Cabezas et al., 1999). The application of this methodology implies the establishment of the scope (time and space). The environmental performance evaluation should be compatible with the information available since it can be interesting to perform a cradle to grave, a cradle to gate, a gate to grave or a gate to gate assessment. The energy environmental impacts should always be taken in consideration. This methodology was applied to the HDA process (Douglas, 1988) in a gate to gate approach considering energy environmental impacts, to select the best topologies by sorting.

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Environmental perfomance of the wine industry. opportunities for uptake cleaner production

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Keywords: winery, environment, water, energy, water vapour, wastewater, infrastructure.

Cleaner production and Eco-efficiency

The United Nations Environment Program defined cleaner production as “the continuous application of an integrated, preventative environmental strategy to processes, products and services to increase eco-efficiency and reduce risks to humans and the environment”.

The term eco-efficiency is used in different ways and other terms are used overlapping with these meanings, like environmental cost-effectiveness and environmental productivity. Starting point for the formal definition of eco-efficiency is the general definition of WBCSD (World Business Council for Sustainable Development, 1992): "To provide goods and services to a competitive price that satisfies the human necessities and the life quality, at time that reduces the environmental impact and the use intensity of resources progressively along the cycle of life, until a compatible level with the dear carrying capacity of the planet." Schaltegger and Sturm (1989), describing EE as a ratio between two elements: environmental impact, to be reduced, and value of production, to be increased.

The connection between CP and EE varies according different bibliographies. Howgrave-Graham and van Berkel (2007) say: “CP is most commonly understood as positive economic benefits arising from efficient use of materials and energy. EE is concerned with positive environmental benefits from economic efficiency”. For Pagan and Prasad (2007), CP and EE are similar concepts. However, for van Berkel (2007), the CP and EE are complimentary. EE focus on the strategic side of business (‘value creation’) and Cleaner Production on the operational side of business (‘production’).

The wine industry is one of the main industries in San Juan Province. This industry is not characterized by hazardous wastes, nevertheless during the vintage, considerable amounts of solid wastes and liquid effluents are produced. To make matters worse, many wineries are located near urban zones, where the gases generated by the fermentation processes and the unpleasant odours from the produced wastes, are nuisances for the surrounding neighbourhoods. In some stages of the vinification process it is necessary to maintain low temperatures. The cooling processes are characterized by high energy and water consumption and, by the toxicity of the refrigerants used. In the wineries, as in any other beverage or food industry, hygienic conditions must be maintained. The cleaning and sanitization of equipment and building requires great amounts of water. During the wine making process, some of the chemical substances manipulated may be dangerous for the employees. In addition to this, the gases generated during fermentation processes may also be harmful if the exposure time and the concentration levels are high enough.

The above comments justify the clean production analysis of the winery and the ecoefficiency evaluation. This work presents the opportunities for uptake of CP, using Cleaner Production Assessment and the results of EE evaluation applying the Schaltegger and Sturm´s definition.

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Environmental performance of wine industry

The eco-efficiency concept represents one of the most tangible paths to sustainability for economic players. Its objectives are to reduce the consumption of resources (e.g. energy, water and raw materials), as well as the impact on nature (e.g. air and waterborne emissions, waste disposal and dispersion of harmful substances), while maintaining or enhancing the value of the manufactured product (producing an equivalent amount, or even more). The eco-efficiency indicators (EEIs) form the eco-efficiency metrics. In order to describe the initial situation of the winery, different intensity indicators defined by Industry and Environment Council, (Murcia, Spain, 2002) were calculated. The data were gathered of eight wineries located in San Juan, Argentina. Table 1 shows each quantity used for the eco-efficiency indicators.

Title	Symbol	Units
Specific consumption of Electrical Energy	Es	Kwh/ product ton
Specific consumption of water	Ws	m_3/ product ton
Specific consumption of aditives	As	ton/ product ton
Specific quantity of generated solid wastes	Ss	ton/ product ton
Specific consumption of combustible	Cs	ton/ product ton
Specific quantity of generated wastewater	WWs	m_3/ product ton

Table 1: Quantities used for the eco-efficiency indicators.

Conclusion

The wine industry is facing serious challenges comprised of internally driven factors, such as rising costs of production due to carry out environmental practice. The majority of the large firms still consider the environmental issues as a cost driver. As a result of this consideration firms do not use more resources for environmental technologies. According to the indications, the firms use more resources for end-of-pipe technologies than the other environmental technologies. However, in this moment, a Program of Cleaner Production is implemented for this industry in San Juan, offering different economic opportunities.

In this study, an environmental diagnosis of winery industry was carried out, taking into account the main aspects and the collected data. A great results variation was observed. The winery 2 presented the higher indicators almost in all analyzed cases. Generally, the high indicators values result of bad management of process or effluents, inadequate technical equipment or planning.

The previously exposed diagnosis originates the necessity to propose the opportunities for uptake of Cleaner Production to wineries in San Juan. These opportunities are focused in each unit operation, stage process and services and utilities.

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Reducing the pollution of leather manufacturing process

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Keywords: tanning, wet white leather, free chrome leather

Chromium III salts are used extensively in the tanning process. Approximately 90% of the leather manufactured around the word is tanned using chromium III. It has been used in the leather industry for almost 100 years, primarily because it remains the most efficient and versatile tanning agent available; it is also relatively cheap. Due to toxicity of chromium III, which can convert to chromium VI with higher toxicity, tanners around the word are often place under pressure to reduce the chromium content of their effluent discharges and the resulting sluges. (1)

While much of the chromium used in the tannery may be recycled or reused or removed from waste streams on-site by precipitation as insoluble chromium III hydroxide, a small amount may be discharged to the sewage treatment works. This chromium will be disposed of with the waste sluge from the treatment works and this is one of the main disposal routes by which waste chromium leaves the tanning industry.

By the term „tannage" we understand an additional grind-like interlinking of the skin's collagen network, which results in stabilization against heat and micro-organisms and production of a material which remains supple after drying, retaining the flexibility of the fiber structure. Chrome tannage is quite obviously particularly suited to obtaining these desirable new properties in the hide material. These can be no other explanation for the 70-80% share held by this method in the main tannage of leather produced around the world and the resulting international Cr tanning agent consumption of approx. 400000 tons per annum (2). If we look at alternatives to chrome tannage, quite irrespectively of the question of actual ecological benefits to be drawn from using a different method, it is necessary to consider the extent to which a comparable tanning effect and the achievement of the required characteristics are possible at all on basis of the existing natural circumstances. To permit optimum stabilization of the hide material by the tannage process, it is necessary for the tanning agent to penetrate into the interior of the fibrils to cross-link the polypeptide chains. A stable bond is formed with the hide in the case of tanning with reactive organic substances as a result of reaction with the reactive groups in the collagen. The fact that the shrinkage temperature of leather tanned in this way hardly, in contrast to chrome tannage, exceeds the 80^0C mark is an indicative of stabilizing effect. The extent to which this type of tanning system can assert itself on a broad basis in the future in the field of pre-tannage or intermediate stabilization due to ecological and economic advantages remains to be seen. One class of syntans that has found application in high stability organic tanning (2) is resins (melamine resins, phenol resins, etc.). This resin can react with collagen under typical post tanning conditions, when their expected effect is to fill the fiber structure selectively, but they have little effect on raising the shrinkage temperature, either singly or as part of a combination tannage. A practical option is to use pre-polymer with defined particle size, which are incompletely cross linked and then to complete the polymerization reaction *in situ* using a cross linking agent. In general, this does not work, yielding only moderate shrinkage temperatures. The critical factor that controls the shrinkage temperature is the particle size of depending on the temperature of the solution. this is because the particles are aggregates of smaller particles; the distribution of particle sizes depends on exactly how the resin was polymerized.

Experimental

In the framework of this paper the tannage is performed in situ by making use of two pre-polymers, one based on a dialdehyde and the other on a diphenol, in the pickling bath. Having bound in hide the above two materials, the pH is risen up to 4.6 – 4.8 by sodium hydrogen carbonate or magnesium oxide. During this time a tanning resin is made within the leather that increases the hydrothermal resistance of leather, resulting in a shrinkage temperature of 70 – 72°C. The obtained leather is white and resistant to the mechanical operations. The wet white leather may be subsequently processed with chromium or only with vegetable and synthetic tanning agents according to the finished leather type to be obtained.

The process is capable of fitting into the current process flow and use existing equipment. The following flow chart, comparing „normal" practice with „wet-white" production indicates that this can be achieved.

The wet-white stock can be tanned with low chrome, vegetable tannins, aldehyde, acrylic/ zirconium, aldehyde / aluminum. The variety of leathers produced using „wet-white" stock has adequately demonstrated the versatility of the material.

This procedure was developed for shoe upper leather from bovine hides. This allows good quality, metal-free shoe for garment and upholstery leathers to be produced with no problem.

The quality of the crust leathers was directly comparable with those from standard procedures.

Taking the type of tannage into account there are no obvious problems shown in chemical analysis results. If any major differences are to show it is more likely to be highlighted by physical properties. But with appropriate process optimization, these differences would be minimized.

Conclusions

The wet-white leathers obtained have a white shade and a shrinkage temperature of 70-72°C. It can be mechanically processed in the same way as wet blue leather. By after treating this tannage with chromium, the shrinkage temperature can be increased over 100°C; with synthetic or vegetable replacement tannins, the shrinkage temperature can be increased to 90-95°C. This allows well –quality, metal-free shoe, garment and upholstery leathers to be produced with no problem.

This new „wet white" procedure does not claim to be a replacement for chrome, vegetable or other tannages, but an alternative. It is a pretannge which can subsequently be tanned with chromium, vegetable or synthetic tanning agents. The advantage of this is that the shavings arising are metal free and, therefore, a valuable by-product. Like untanned solid waste, they can be recycled and used as a starting material for gelatin, fertilizer, glue and other industrial products.

While it is true that from a manufacturing point "wet-white" production will fit into current tannery practice, it is also true that an „overnight" switch cannot be made. Machine handling, sorting and grading characteristics, although acceptable, are different and require that operators gain a familiarity with the material. At the same time the effort required to adjust tanning / retanning processes to produce acceptable leather should not be overlooked.

Despite these disadvantages the use of „wet white" as a raw material should not be dismissed. The reduction or elimination of chrome containing solid waste, greater control over chrome in the liquid effluent and the ability to produce so called „eco" leathers are factors that should be given serious

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Eco-friendly tanning agents to be used in leather manufacture

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Keywords: titanium wastes, waste valorization, tanning agents

Development of new tanning agents is required to get through the increasingly higher environmental pressure on the tanning materials and processes currently in use (tannage with chromium salts). Though some chromium-free processes (and products) were developed in the world, such an issue has not been solved wholly because of the features related to the output and profitability of the new processes and products in spite of the significant environmental drawbacks. This work is aimed at bringing an original contribution to solve such an issue by making use of wastes resulted from nonferrous metals industry, namely the solid titanium wastes (cuttings) unrecyclable in the titanium production.

Leather is perhaps the first material being processed chemically by the man (as yearly as 8000 year B.C. – leather was obtained by fat tannage).

Leather belongs to our life; daily we are coming into contact with leather objects making our life more comfortable. Today the traditions, culture, and science also are shared with the European societies through the leather.

Because of the specific tannery processes (uses raw materials of biological nature and a large range of chemicals known to be noxious, and not the last, large water consumption and energy expenditure), the tanneries are currently facing up major environmental problems.

Integrating the environment protection into the quality concept is a priority objective of the research in hide processing field because of the need of reducing water consumption and energy expenditure, and pollution. Under the general economic context (limited and varying resources, and a new regulation on chemicals - REACH - Registration, Evaluation and Authorisation of Chemicals) to be adopted by the EU) [1].

One of main ways of pollution prevention is the application of so-called 'clean' or 'environmentally friendly' processes developed onto three directions: [2]

- Reduced offer of chemical auxiliaries simultaneously with the application of hide support activation treatment;
- High bath exhaustion;
- Replacing the 'classic' chemical auxiliaries of high toxicity and adverse environmental impact with others ones of low or no toxicity (this paper's approach).

In this work a series of new tanning materials intended for partial or total substitution of chromium salts with the view of tanning operation optimization and rendering it profitable were obtained and tested.

To obtain such materials the use of wastes resulted from nonferrous material processing industry, namely the unrecyclable solid titanium wastes (cuttings) was suggested. Tannage is known to be a long term preservation of the 'labile collagen' by a complex physical-chemical process. Tanning compounds act to crosslink the collagen molecules in the hide, stabilizing thus the protein chains.

The crosslinking level resulted from tannage is revealed by the leather shrinkage temperature. The changes in leather shrinkage temperatures with the new tanning liquors are shown in Fig.1.

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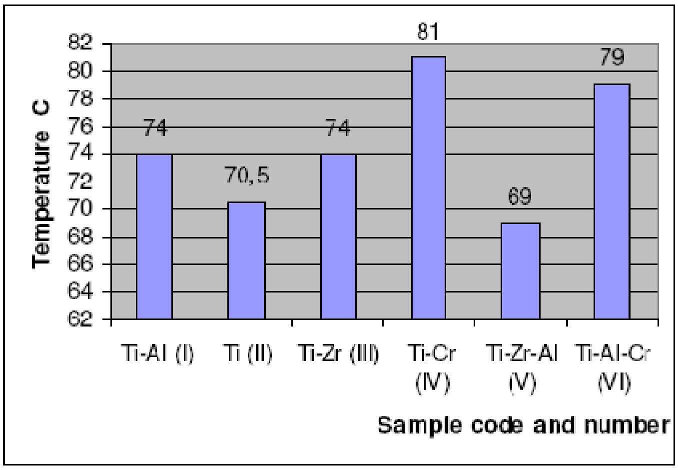

Figure 1 - Shrinkage temperature changes in leather tanned with the new tanning liquors

The hydrothermal resistance values obtained for all samples tanned with the new materials are not as high as for chromium tanned leather but provide for a very good behavior in the subsequent mechanical processing such as splitting and shaving. The doubtless tanning action of the new materials is revealed by the results of the thermal analysis [4] shown in Fig.2.

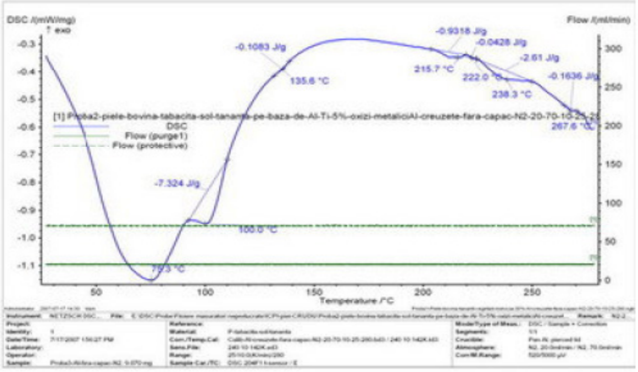

Figure 2 - DSC curve in nitrogen atmosphere for the leather tanned with Ti-Al tanning solutions

Obtaining new cost effective auxiliary materials easy to be applied, with low environmental impact, resulting in leather of high quality is surely the aim of the research in the leather sector.

This work is aimed at contributing to solve such problems by making a real use of unrecyclable wastes resulted from nonferrous metal (titanium) industry with the view of increasing the environmental efficiency in other sectors (leather manufacture).

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Valorization of tanning waste: production of an organic fertilizer

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Keywords: tanning industry, leather waste, organic fertilizer

Abstract

Leather products are used almost everywhere for many different purposes, from clothes to shoes, in transportation vehicles or in house goods. Leather is a product obtained by tanning hide from animals, an industry that is one of the most pollutants.

In fact, in spite of being able to process a waste (hide from slaughter houses), this industry is responsible for the consumption of many resources (water, energy, chromium, etc.) and for the production of a range of pollutants such as wastewater (containing high levels of chromium), tanned and non tanned solid wastes.

Generally, we can say that one ton of raw hide is responsible for 200 kg of leather, 50 m^3 of wastewater, 135 kg of tanned waste (leather containing chromium) as well as 270 kg of other non-tanned solid wastes, this means only 20% of the raw hide is converted into useful leather.

The huge amount of tanned waste represents a problem that needs a sustainable solution.

On the other hand, hide contains proteins and organic matter that could be used for land correction, instead of the use of synthetic fertilizers.

This was the main reason why the idea of waste leather valorization was studied in this project.

The aim of this work was to produce an organic fertilizer, through the alkaline hydrolysis of wet-blue scrapings. In the process, it was also necessary to reduce the amount of chromium contained in the initial wastes (3.3%) to such a level that the fertilizer could meat the legal regulations.

In the procedure, a certain amount of wet-blue was digested in a NaOH solution. The result was a chromium rich cake and a hydrolysate (the rich organic matter phase).

Chromium rich cake was used to produce chromium liquor that will be reused in the tanning industry.

The hydrolysate had a 3.2 ppm Chromium concentration. In order to reduce mineral salts and metal concentration, the hydrolysate was submitted to ion exchange in a column containing Amberlite IRA120 Na and Amberlite IRA402 Cl. pH could than be adjusted to 7, sometimes by adding NH_4^+ solution.

Depending on the characteristics of the fertilizer that we wanted to produce, the purified hydrolysate could be submitted to a drying operation, in order to obtain a concentrated fertilizer or even a powder fertilizer (very hygroscopic).

By applying this procedure, a 66% chromium recovery from the wet-blue scrapings was achieved. An organic fertilizer containing 17000 ppm of total nitrogen, from which 2900 ppm of organic nitrogen was obtained.

1090 cm^3 of raw hydrolysate were obtained from each 200g of wet-blue scrapings. The corresponding liquid fertilizer volume, obtained after ion exchange, was 1000 cm^3. The

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fertilizer had 77.2% of organic matter, 6.1% of solids, and a chromium concentration of less than 1.2 ppm.

Figure 1 – Leather waste **a)** Wet-blue scrapings and products obtained after applying the procedure: **b)** Chromium rich cake; **c)** Raw hydrolysate and **d)** Organic fertilizer

Comparing the organic fertilizer composition to the legal values in Decreto-Lei nº 118/2006 (from the 21st of June), we could conclude that it can be used for agricultural purposes.

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Biodiesel production from leather industry wastes

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Keywords: Biodiesel, Fleshings, Transesterification, Methyl esters, Beef tallow

Sustainable energy management is a main world concern, considering the fact that fossil fuels supplies are limited and energy demand continues to rise. Search for alternative renewable fuels has therefore gained fundamental importance. Among biofuels, biodiesel has very good properties in terms of utilization as well as environmental, and became a widely used alternative to fossil fuels, with ten years long commercial use in Europe and a rapidly growing world market. Biodiesel cost is mainly determined by feedstock prices. Presently, the cost of the cereals that are used to produce biodiesel is increasing due to the growing market demand. Also, this huge demand introduces a sustainability problem for the next coming years.

Significant research effort is being dedicated to the search for alternative feedstocks. Among these, animal fats from different sources, leather industry, butcher's and slaughterhouses, are potentially interesting. Furthermore, the re-use of these wastes is an environmental friendly disposal option.

Animal fats present usually a high acid value and its fatty acid composition shows a more saturated character than vegetable oils, as can be seen in table 1.

Table 1.Typical fatty acid compositions of vegetable oils and animal fats (Scrimgeour, 2005).

	Fatty acid (wt %)						
	Myristic (14:0)	Palmitic (16:0)	Palmitoleic (16:1)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Linolenic (18:3)
Soybean oil	0.1	11	0.1	4	23.4	53.2	7.8
Beef tallow	2,7-4,8	20,9-28,9	2,3-9,1	7,0-26,5	30,4-48,0	0,6-1,8	0,3-0,7

The purpose of this work is to investigate the possibility of using fleshings (bovine) from the leather industry in biodiesel production. These leather industry wastes contain 40 to 60 wt % of fat (dry basis). Beef tallow is also tested for comparison purposes, because it is a similar residue and previous works showed the feasibility of this feedstock for biodiesel production (Lebedevas, 2006).

To obtain the fat from beef tallow, the solid is melted at 140 ºC and the product is filtered to remove any solid particles that may be present.

To obtain fat from fleshings two steps are needed: first, the solids are mixed with water and heated in order to break the bonds between the fat and protein material. The optimal conditions for this step are solid:water mass ratio of 1:0.75 and 70 ºC.Three different phases are obtained, a fat rich phase, a water rich phase and a protein rich phase. In the second step, the fat rich phase is treated with n-hexane to extract the fat and leave the proteic residue. The operating conditions for extraction are: rich fat phase:solvent mass ratio of 1:10, ambient temperature and 1h. The product is filtered to remove the proteins and n-hexane is separated from the fat by distillation.

All these animal fats present high acid values ranging from 5 to 20 mg_{KOH}/g, depending on the source, and thus a pre-treatment step is needed. This pre-treatment consists of

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an esterification of the free fatty acids with methanol and sulfuric acid as catalyst, for 3h at 70ºC. After the phases are separated, the fat phase is transesterified with methanol using potassium hydroxide as catalyst. Initial experiments with a single step transesterification of the fleshings (fat:methanol:KOH mass ratio of 1:0.238:0.01 at 65ºC and 3h) resulted in a product with a low ester content (70%). Therefore a two-stage operation procedure was performed (Baptista, 2008). The first step consisted of a transesterification using 80% of the total volume of the methanol/KOH solution and 3h reaction time at 65 ºC. The glycerol phase was then removed and the ester rich phase was reacted again with the remaining methanol/KOH solution for 2 h. After the separation of the glycerol phase, the ester phase was washed with water and dried. The acid value, ester content, density and viscosity of the product were determined. These results, together with the mass yield (mass of product/mass of fat) are showed in table 2.

Table 2. Characterization and yield of methyl ester product.

Fat source	Acid value (mg $_{KOH}$/g)	Ester content (%)	Density, 15ºC (g/cm^3)	Viscosity, 40ºC (mm^2/s)	Yield (%)
Beef tallow	0.17	94.2	0.87	4.8	65
Fleshings	0.18	94.1	0.87	4.8	57

The values obtained are, except for methyl ester content, within the specifications for biodiesel (EN 14214 standard). The products from beef tallow and fleshings present methyl ester contents close to the standard value, so further optimization of operating and purifying conditions is needed.

This study shows the feasibility of using the fleshings from the leather industry to produce biodiesel but further adjustments are needed in the production process in order to improve the quality of the final product. Biodiesel produced from this kind of raw material, will present a high CFPP and will need to be blended (with biodiesel produced from vegetable sources or petroleum diesel) prior to final utilization.

Acknowledgements

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Influence of feedstocks and operation conditions in biodiesel production

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Keywords: Biodiesel, feedstocks, transesterification, oxidative stability

Extended Abstract

Due to the increasing cost and depletion of fossil fuels, there is a growing need for alternative fuels. Biodiesel, being a renewable, non toxic and biodegradable resource with positive influence in the environment, is the main alternative to fossil diesel. The production of biodiesel is usually achieved by an homogeneous transesterification reaction (catalysed by sodium hydroxide or methoxyde) between a lipid (vegetable oils or fats) and a short chain alcohol, such as methanol, to produce an ester and glycerol as a by-product (Felizardo et al., 2006) .

One of the main problems for biodiesel commercialization is its high cost. The feedstock, usually semi-refined vegetable oils, represents a wide part of the total cost of the biodiesel production is. Therefore, these costs may be considerably reduced if biodiesel is produced from low cost materials such as waste frying oils (WFO) or animal fats. However, the quality of biodiesel must be guaranteed because both factors influence its properties (Felizardo et al., 2006). So, the European Standard (EN) 14214 specifies the limits for 25 quality parameters. Moreover, WFO and fats often contain significant amounts of free fatty acids (FFA), which makes them inadequate for direct base catalyzed transesterification reaction, without the use of an extra amount of catalyst during the transesterification reaction to balance the oil acidity and/or a previous esterification reaction with an acid or with glicerol to convert the FFA into esters (Canakci, 2007). One of the main goals of this work was to study the effect of different transesterification reaction conditions as well as of the type of oils used on the final biodiesel properties. Another goal was to establish a relationship between the iodine value of the oils and the oxidative stability of biodiesel and the effect of the addition of several commonly used anti-oxidants.

Biodiesel samples were prepared by transesterification of soybean and WFO using the following procedure: 300 g of oil was transferred into a stirred tank reactor (1000 cm^3) immersed in a temperature-controlled water bath. For stirring, a single paddle round impeller (diameter = 6.5 cm) at 350 revolutions min^{-1} was used. The oil was heated until the desired temperature was reached (65°C). At this point, a mixture of methanol and sodium methoxide was added to the oil and the transesterification reaction began. The reactor was kept at around 65°C for 90 to 180 min. The majority of the reactions were performed using a molar ratio of methanol/oil of 6:1 and an amount of catalyst corresponding to 0.6% of the mass of oil sample. The acid value of the oils was obtained by titration with a KOH solution (prEN 14104), whereas the oils iodine value was determined by the Wijs method (prEN 14111). The oxidative stability of methyl esters, with and without antioxidant, was determined by the Rancimat method according to the European Standard EN 14112.

Several factors, including the reaction time and temperature, catalyst type and concentration (cat./oil) and alcohol/oil molar ratio (MeOH/oil), influence the efficiency of the transesterification reaction and the biodiesel purity. The results obtained show that the quantity of methanol influences significantly the purity of the final product, which decreases with the decreasing of the molar ratio methanol/oil. Concerning the amount of catalyst, it was possible to verify a decrease in the esters content of biodiesel with the decrease of the quantity of $NaOCH_3$ from 0.60 to 0.30%. Hence, for a methanol/oil molar ratio of 6:1 and a temperature of 65ºC, it was possible to produce soybean biodiesel with a methyl ester content above 97%, using $NaOCH_3$ as catalyst.

The iodine value of biodiesel is an important paramete which is a measure of the degree of insaturation of the methyl esters molecules and an indicator of how easily these molecules polymerize. This value influences several important properties of biodiesel such as its CFPP and oxidative stability. As expected, it was verified that the oxidative stability of biodiesel (EN14214 minimum induction time of 6h) decreases with the increase of the iodine value of the oil. Therefore, the biodiesel samples with higher induction times (above 13h) were the ones produced from palm oil, whereas soybean and rapeseed oils have induction times of 5.3 h and 5.8h, respectively. The samples produced from WFO presented the lowest values for the oxidative stability (induction times between 2 and 3h). However, one approach for improving the oxidative stability of biodiesel is to add an antioxidant. Therefore, in this work five antioxidants (identified as A to E) were added to biodiesel samples produced from soybean, rapseed and WF oils and the results are presented in Figure 1.

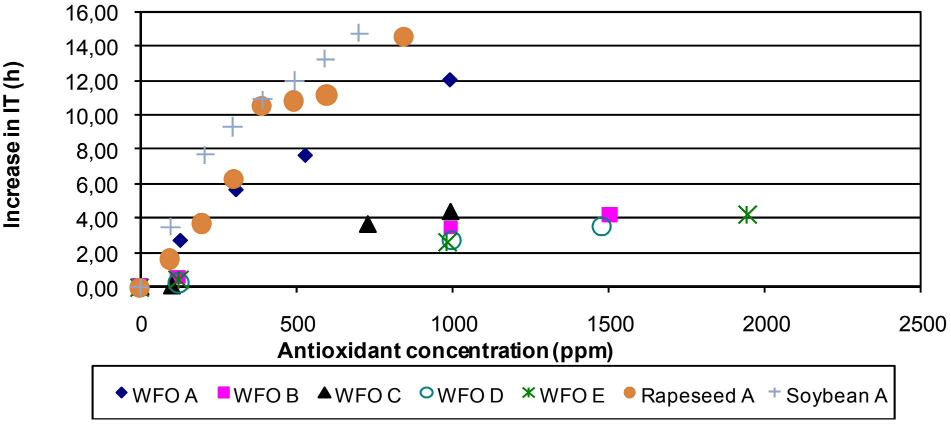

Figure 1 - Effect of the antioxidant concentration on the induction time.

As shown in Figure 1**,** the best results were obtained with the antioxidant A, even though all the antioxidants were able to increase the samples induction time. Furtheremore, the induction time increases directly with the concentration of antioxidant but, for higher values of antioxidant concentration, it stabilizes.

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High free fatty acids oils for biodiesel production

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Keywords: Biodiesel Feedstock, Methanolysis, Glycerolysis

Biodiesel (fatty acids methyl esters or FAME) is the main alternative to fossil diesel. The key advantages of its use are the fact that it is a non-toxic renewable resource, which leads to lower emissions of polluting gases. European governments are targeting the incorporation of 20% of biofuels in the general fuels until 2020.

FAME can be produced through a transesterification reaction between a lipid source (vegetable oils and fats) and an alcohol (mainly methanol) to produce an ester and a byproduct, glycerol. This reaction occurs stepwise, with mono and diglycerides as intermediate products. However, waste frying oils and fats often contain significant amounts of free fatty acids (FFA), which makes them inadequate for direct base catalyzed transesterification reaction, without the use of an extra amount of catalyst during the transesterification reaction to balance the oil acidity and/or a previous esterification reaction with an acid or with glicerol to convert the FFA into esters (Canakci, 2007).

This pre-treatment can consist in a reaction with an alcohol, such as methanol or glycerol in order to transform the free fatty acids into their corresponding esters (methyl esters or glycerides respectively). Good results concerning the reduction of the acid value were achieved even with high initial percentages of free fatty acids (Canakci and Gerpen, 2001, 2003; Pereda Marín et al., 2003). After this pre-treatment, glycerides in the oil/ester phase are transesterified with basic catalysis.

The goal of this work is to study the viability of biodiesel production from oil soap stocks with high acid value (~100 mg KOH/g of oil).

Methanolysis reactions were carried out using the following procedure: 200g of waste frying oil (75% m/m) and high free fatty acids oil (25% m/m) were transferred to a reaction vessel equipped with a condenser and a mechanical stirrer. The reaction was carried out at 350 rpm and 65º C using a methanol to oil mixture molar ratio of 9:1 and 3% of catalyst (mass of catalyst/mass of oil). Once the synthesis was completed, the product was submitted to distillation (at 90^{o} C under vacuum) in order to remove all methanol and water in the sample followed by a decantation step, and finally a washing step with water.

Glycerolysis reactions were carried out using the following procedure: The high free fatty acid oil and glycerol (Glycerol:FFA molar ratio = 1.10) were preheated (200º C) in the reaction vessel equipped with and a condenser and a mechanical stirrer. The reaction was carried out at 350 rpm for 2 hours. The sample was analyzed without further purification steps.

The acid value of all samples was determined (EN 14104), after sample purification, in order to determine the methanolysis and glycerolysis efficiency.

The preliminary results obtained from the two different processes show that both can reduce the acid value to values up to 10 times lower than the initial acid value (~100 mg KOH/g).

In fact both were able to reduce drastically the acid value of the original oil, resulting in feedstocks with acid values of 3 and 10 mg KOH/g for the methanolysis and glycerolysis, respectively. Figure 1 and 2 represent the kinetics of a methanolysis and a glycerolysis.

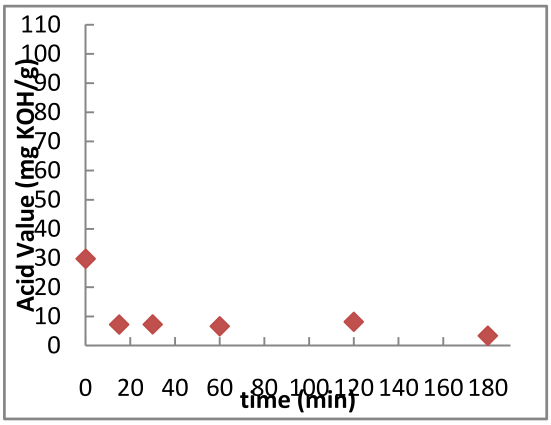

Figure 5 – High Free Fatty Acids Methanolysis (Reaction conditions: MeOH:FFA = 9; 3% Sulphonic Acid; T = 65° C; 350 rpm)

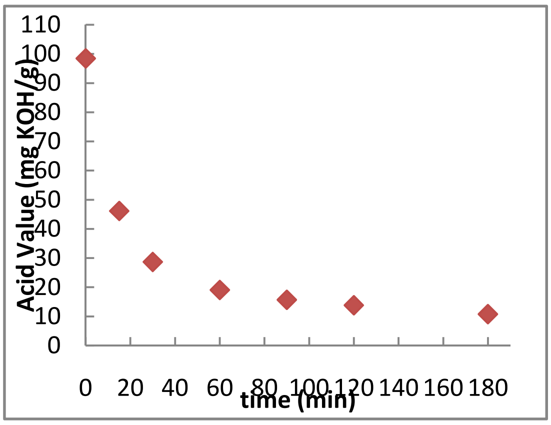

Figure 6 - High Free Fatty Acids Glycerolisis (Reaction conditions: Glycerol:FFA = 1.10; no catalyst; T = 200° C; 350 rpm)

It is also worth mentioning that both methods have advantages and disadvantages. For instance, methanolysis reaction produces water at the same time as an ester. This also occurs in glycerolysis but while in the first case the water remains in the reaction mixture, in the second process the water is removed by evaporation. Since water remains in the reaction mixture, high methanol to free fatty acids ratios is needed to try to reduce this limitation. Other aspect of methanolysis is that it requires an acid catalyst in order to achieve an ester. This is not the case of the glycerolysis, in fact, Figure 2 represent a glycerolysis reaction without catalyst, although high temperatures (200º C) are necessary for this reaction.

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Comparison between biodiesel produced from sunflower oil and waste frying oil

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Keywords: Biofuels, Biodiesel, Sunflower oil, Waste frying oil, Transesterification

Increased attention has been given to biodiesel, which is currently being used as an alternative fuel in compression-ignition engines. Biodiesel is an ester that can be produced by transesterification of vegetable oils or animal fats. During this reaction, the triglyceride is converted step by step into a diglyceride, monoglyceride and glycerol; at each step, one mol of ester is produced (Fig. 1). There are many parameters affecting the transesterification reaction. The ones known to greatly influence the reaction are: temperature, methanol/oil molar ratio, mixing rate, catalyst type and amount of catalyst (Meher, L. C., et al., 2006, Ma, F. and Hanna, M. A., 1999).

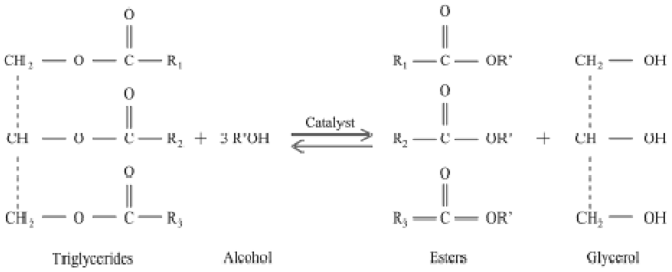

Fig.1 - Transesterification reaction of triglycerides (global reaction).

The increasing costs of refined oils are currently a great concern for biodiesel industry; therefore, alternative raw materials are being studied with more emphasis. The use of waste frying oils and other greasy wastes as raw materials for biodiesel production has two major advantages: i) decrease of biodiesel production costs (because it uses readily available raw-materials with low economic value); and ii) reduction of waste treatment costs (related with the waste management and wastewater treatment processes) (Wang, Y., et al., 2006, Encinar, J. M., et al., 2005, Tomasevic, A. V. and Siler-Marinkovic, S. S., 2003).

The objective of the present study was to evaluate the difference of using sunflower oil or waste cooking oil for the production of biodiesel through transesterification, using different concentrations of the currently more used catalyst in the industry (CH_3ONa). For that purpose, different experiments were conducted using a 6:1 methanol/oil molar ratio, temperature of 60 ºC, and varying the amount of catalyst used. The biodiesel yield was evaluated and so was the quality of the final product; the characterisation of the produced biodiesel was made according to European Biodiesel Standard EN 14214.

It was possible to obtain a product in agreement with EN 14214 using both oils. All samples fulfilled the specifications regarding the following properties: acid value, density, flash point, copper corrosion and linolenic acid methyl ester content. In terms of iodine value, sunflower oil showed a value close to limit. Dehydration step using an anhydrous salt was not effective; however evaporation at reduced pressure might be an efficient alternative.

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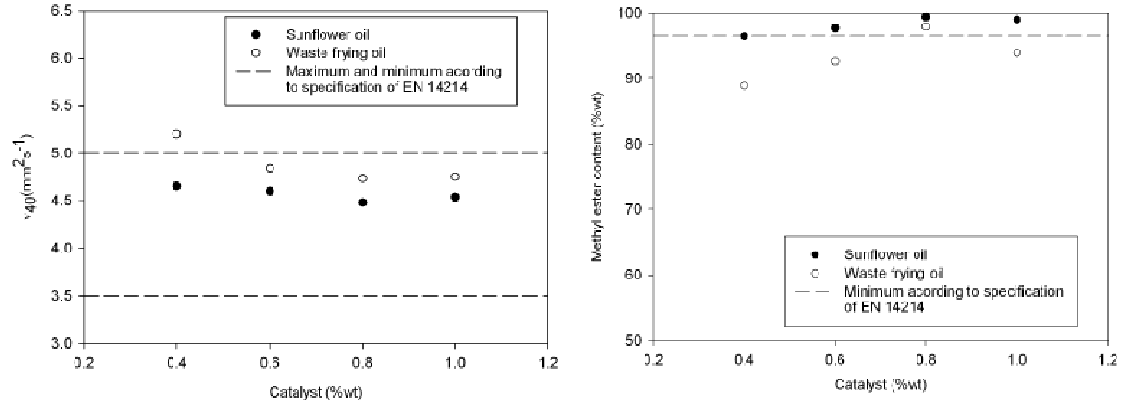
Viscosity and purity were the limiting parameters to select the best catalyst concentration; at 0.6%, both samples fulfilled the EN 14214 in terms of kinematic viscosity (Fig.2a); however, higher catalyst concentration (0.8%) was needed to achieve minimum purity using waste frying oil (Fig.2b).



(a) (b)

Fig. 2 - Biodiesel produced using CH_3ONa as catalyst: (a) kinematic viscosity (mm2s-1) and (b) methyl ester content (% wt).

The highest yield (93.4%) and purity (99.4%) was obtained using 0.8% catalyst concentration and sunflower oil as raw material. However, in order to optimize production costs, a smaller amount of catalyst might be used (0.6%).

Considering the present study, the optimum catalyst concentrations were: i) 0.6% for the production of biodiesel with a yield of 90% and presenting a purity of 99.4% using sunflower oil; and ii) 0.8% catalyst concentration for the production of biodiesel with a yield of 89% and presenting a purity of 97.9% using waste frying oil.

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Development of heterogeneous catalysts for transesterification of triglycerides in biodiesel

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Keywords: Biodiesel, heterogeneous catalysis, efficiency, transesterification, catalyst

Biofuels are all fuel substances produced from Biomass which includes Biodiesel (single alkyl ester of fatty acids), Bioethanol and Biogas (mixture of Methane and CO_2). The highway transports activity, in a global level, is 98% oil dependent. In the EU, this activity is responsible by more than 20% of total CO_2 emissions and, more than 50% of these emissions concerns to the individual transport, which increase 22% since 1999. The climate changes, the increase of crude prices and the supply energetic security leads to the growing interest about biofuels potential as substitutes of oil fuels, like gasoline's and diesel [1]. Nowadays, biodiesel is obtained essentially, from oleaginous plants by the transesterification process, which involves, as reactants, alcohol, essentially, methanol and a catalyst, preferably alkaline, liquid phase (NaOH, KOH, sodium methoxide). This process occurs with homogeneous catalysis. The main raw materials, which include the previously mentioned oils from oleaginous plants are, mainly Colza, Sunflower and SoyBean. It can be obtained, also, from other plants, such Palm. Chemically, biodiesel is described as a mixture of methyl ester's of fatty acids, where, through the transesterification process, are produced those ester's (single, double and triple triglycerides), and produces also, Glycerine as co-product reaction [1], [2].

The transesterification reaction is considered as the process with more technical effectiveness and with higher efficiency for biodiesel production in large scale, with minimization of by-products. Nevertheless, it is essential that, the raw materials (vegetable oils) mantain a minimum level of quality, which can be achieved in the presence of acids, alkalines or enzymatic catalysts, simples or supported. The use of acid catalysts, such as sulphuric acid, leads to a reaction kinetics much lower when compared with alkaline catalysts. Other disadvantage in the utilisation of this kind of catalysts is related with the necessity to perform biodiesel separation from the obtained reaction mixture, in order to prevent potential damages to the engine structures, caused by acid catalysts. Basic catalysis are faster and, in many cases, the steady-state is achieved at 15-30 minutes and leads to higher yields of biodiesel, normally higher than 50%. However, they have the inconvenient of being sensible to the presence of water and free fatty acids. These last ones consume the catalyst and conducts to the formation of gels and soaps. On the other hand, enzymatic catalysts offer some advantages when compared with acids and alkalines catalysts, like, for instance, less sensibility to water presence, more catalyst recovery and more efficiency in the Biodiesel separation steps. However, their utilisation implies higher costs [2].

This study concerns the use of heterogeneous catalysts for biodiesel production, in order to replace the conventional homogeneous alkalines or acids catalysts, to eliminate the difficulties found in the current biodiesel/catalyst separation.

These new catalysts are solid, and they consist of an alternative and innovative solution in biodiesel production, allowing, more economic rentability and competitiveness to the process bringing, more environmental compatibility, when compared with the conventional processes. In fact, the use of heterogeneous catalytic systems in the transesterification of triglycerides implies the elimination of several washing/recovery biodiesel/catalyst process steps, in order to ensure a higher efficiency and rentability of the process, lowering the production costs. There is, also, the strong possibility of being implemented in a continuous way, with the construction of a fixed bed catalytic reactor.

The utilisation of heterogeneous catalysts implies higher lifetimes, as there is no need to perform their recirculation to the initial step (mixture), since catalyst utilisation time is higher than the homogeneous catalytic processes. This means less catalyst replacements on the fixed bed catalytic reactor, which leads to a higher quality of the final product and, also, to the by-product, glycerine.

Figure 1 shows a possible *flow-sheet* for biodiesel production process using heterogeneous catalyst.

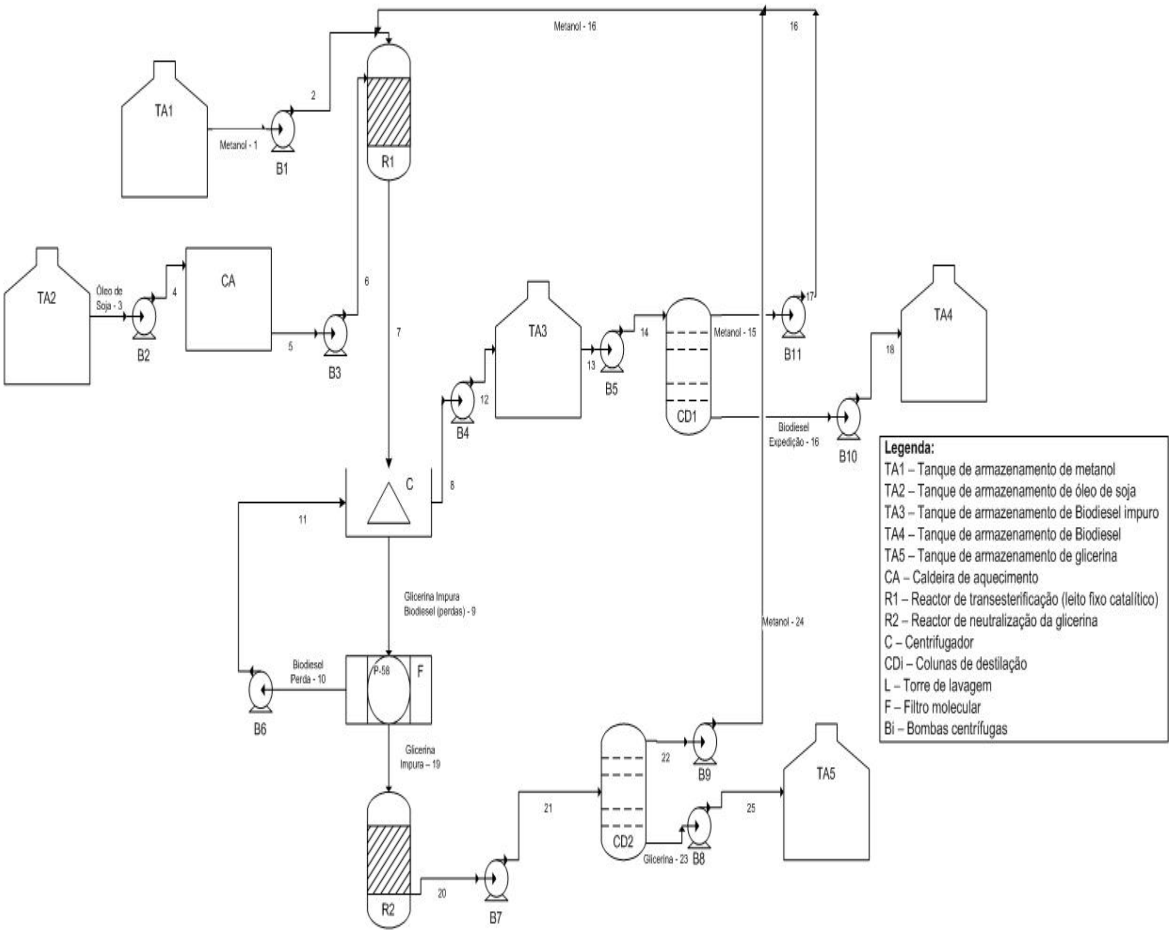

Figure 1 - *Flow-sheet* for biodiesel production using heterogeneous catalysis.

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Optimization of properties of biodiesel produced from vegetal oil mixtures by response surface methodology

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Keywords: Biodiesel, Vegetal oil mixtures, RSM

Abstract

With the increase in petroleum prices and environmental concerns associated with CO_2 emissions, there is a growing interest in vegetable oil and their derivatives as renewable fuels for diesel engines. Europe aims to replace 20% of fossil fuels for alternative fuels such as biofuels, natural gas or hydrogen until 2020. In Portugal the transport sector is the second highest producer of greenhouse gases, and the current goal is the replacement of 10% of fossil fuels by alternative fuels until 2010.

Besides renewability, the advantages of biodiesel include biodegradability, higher flash point, inherent lubricity, full miscibility with conventional diesel and reduction of emissions. In what concerns emissions, biodiesel, is advantageous when compared to fossil fuels, because it will allow an important reduction of COx, SOx, and particulated matter emissions.

Envisaging the necessity of more knowledge on the use of vegetal oil mixtures for the production of biodiesel as a means to minimize the product cost while still conforming to specifications, the transesterification of mixtures of soybean, colza and palm oil have been studied.

The goal of this work is to find the region of composition where the properties of biodiesel produced from mixtures of the three oils conform to the biodiesel national specifications. Response surface methodology (RSM) was used to evaluate methyl esters yield, iodine index, cloud point and properties such as density and viscosity. Design of experiments for three component mixtures were used to obtain the concentration region of interest, as showed in Table 1 (w/w).

Table 1- Design experiments for three components mixtures.

Colza	Soybean	Palm
1	0	0
0	1	0
0	0	1
½	½	0
0	½	½
½	0	½
⅓	⅓	⅓

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Modeling and simulation of process continuous production of biodiesel from soybean oil using immobilized Candida antarctica in fluidized bed bioreactor

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Keywords: Biodiesel, Candida Antarctica, Fluidized Bed

The objective this work was investigated a new route for biodiesel production using Immobilized Candida Antarctica in continuous Fluidized Bed **Bioreactor**. Conventionally, Biodiesel (fatty acid methyl esters) is produced by transesterification in which, oil or fat is reacted with a monohydric alcohol in presence of a catalyst. In recent years, the use of lipases as biocatalysts for biodiesel production has become of great interest due to its environment friendly. But some alcohols such as methanol inactivated the lipases to some extent and the enzymatic stability was poor. In order to enhance the stability of the lipase, three-step methanolysis was adopted, however, glycerol, as one of the products was easy to adsorb on the surface of lipase resulting in serious negative effect on the enzymatic activity. For to solve problems was used the interesterification kinetics of triglycerides and methyl acetate for biodiesel production was modeled. A heterogeneous model describing the interesterification process in an immobilized enzyme fluidized-bed bioreactor was developed. A simplified model based on Ping Pong Bi Bi with substrate competitive inhibition mechanism was proposed to describe the reaction kinetics of the interesterification.The model without any adjustable parameters was used to predict the interesterification process. The key parameters which measured the extent of external and internal mass-transport resistances, as well as the degree of back-mixing were quantified and discussed. The fluidized-bed bioreactor considered in this investigation is composed of two phases: a fluid phase comprised mainly of the triglycerides and methyl acetate and the product (Biodiesel); and a solid phase which is the immobilized enzyme. The effects of some operating and design parameters on the performance of the fluidized-bed bioreactor were also analyzed. The model was also tested for its sensitivity to changes in hydrodynamic parameters.

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Kinetic models for the homogeneous alkaline and acid catalysis in biodiesel production

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Keywords: Biofuels, Biodiesel, Reaction engineering, Kinetic models.

With ever increasing energy demand, global warming and oil prices, the world is now more concerned with sustainable energy management, saving of non-renewable resources and environment protection. In the EU context, the transport activities represent nowadays 30% of the total energy consumption and 67% of oil demand. To minor this scenery and comply with the Kyoto protocol, the European Directive 2003/30/EC, transposed to Portuguese legislation in 2006, established a series of measures to promote a more intense use of biofuels, in order to reach a 5.75% share of the energy used in the transport sector by 2010. The use of biodiesel (fatty acid methyl esters) is one possible and attractive alternative to fossil fuels. It's a direct substitute of diesel fuel, either in pure or mixed (ecodiesel) forms, with a more favourable energetic and air emission profiles in a life-cycle basis.

Many process alternatives are available for biodiesel production. One possible classification of them is according to the type of catalytic system used in the transesterification reactions: homogeneous – alkaline or acid; heterogeneous – alkaline, acid or enzymatic; non-catalytic – supercritical alcohol.

Numerous studies regarding the kinetics of transesterification and aspects of biodiesel production technology have been made, and critically reviewed by Vasudevan and Briggs (2008), Sharma et al. (2008) and van Gerpen (2005). Despite the considerable amount of work done in kinetic data evaluation, no systematic study that integrates experimental results from different authors is available at the moment in the open literature. Therefore, a direct comparison of the performance of different catalysts in distinct reaction conditions is not possible, and alternative design configurations and setup conditions cannot be evaluated based on existing systematic optimization methodologies.

The scope of this work is the derivation of kinetic models from experimental data in the literature of the transesterification reaction of vegetable oils, through homogeneous alkaline and acid catalysis (more used in industry). The alkaline catalyst studied was NaOH and data from Freedman et al. (1984), Noureddini and Zhu (1997), Jeong et al. (2004) and Leung and Guo (2006) was used. The acid catalyst studied was H_2SO_4, using data from Freedman et al. (1984), Canakci and van Gerpen (1999), Goff et al. (2004) and Zheng et al. (2006).

The kinetic models were obtained assuming: intense mixing rate; excess of methanol; oil is composed only by triglycerides; and negligible water and free fatty acid contents. Under these conditions, the reaction is practically irreversible, only limited chemically and pseudo first or second order relative to the triglyceride concentration in the reaction mixture (Zheng, 2006; Freedman, 1984; Noureddini, 1997). The independent variables under study for alkaline catalysis are the temperature, catalyst concentration and a catalytic activity factor. In the acid catalysis the variables are the same, plus the molar ratio of methanol/oil at the beginning of reaction.

Two approaches, designated as statistical and empirical, were used to fit experimental data into mathematical models. In the statistical approach, a multivariable linear regression was performed (using *STATISTICA*), considering as dependent variables the kinetic constant of reaction (K) or a transformation of it. Each predictor also represents an independent variable or a convenient transformation. In the empirical

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approach, *K* is viewed as a product of functions that individually describe the influence of each independent variable. Each function was obtained sequentially by plotting *K* vs. the independent variable under study, using *CurveExpert* to test and select the family of functions that have physical meaning and better fit the data, and *Mathematica* to refine the regression of the function's parameters. These procedures were performed for both the acid and alkaline catalysis, considering the reaction as pseudo 1st or 2nd order. At the end, the results of the kinetic models using both approaches were compared with the experimental data, for validation.

For the acid catalysis (H_2SO_4) the models better fitted assume a pseudo 1st-order reaction and were obtained, respectively, using a statistical and empirical approach, with mean errors of 7.2% and 5.9%. In the alkaline catalysis with NaOH, the models fitted better assuming a 2nd pseudo-order reaction, with a mean error of 4% and 4.5%, using also the empirical and the statistical approaches, respectively. This study is completed with the extension of these results to new data relative to the KOH, $NaOCH_3$ and $KOCH_3$ catalysts, and further data recently published relative to the kinetic models of NaOH (Rashid and Anwar, 2008).

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Selective extraction of organic compounds from transesterification reaction mixtures by using ionic liquids

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Keywords: liquid-liquid extraction, ionic liquid, transesterification reaction, selective separation

Ionic Liquids (ILs) are organic salts that are liquid close to room temperature. They normally consist of an organic cation, the most commonly used being dialkylimidazolium and tetraalkylammomiun salts, and a polyatomic inorganic anion (e.g. BF_4^-, PF_6^-). The most important advantage of ILs is their non-detectable vapour pressure, which makes them environmentally benign solvents compared with Volatile Organic Solvents (VOSs). They also show good chemical and thermal stability and can be used at high temperatures [1]. Additionally, the physical-chemical properties of ionic liquids, such as their hydrophobicity, density, viscosity, melting point, polarity and solvent properties, may be modified by altering the anion or the cation [2-3]. Indeed, this feature is a key factor for realizing successful extraction process since appropriate combinations of the cationic and anionic parts of the solvent can be made. In recent years, ionic liquids have been shown to be good solvents for use in many chemical [4] and biochemical [5] processes. At present, the amount of physico-chemical data available for ternary mixtures involving ionic liquids is insufficient to create a data base of knowledge relating to their applicability in separation processes. The almost limitless number of combinations of cations and anions that can be made to produce ionic liquids futhers complicate matters [8-9]. It is this correlation of the different combinations of cations and anions with the separation potential of the ionic liquid for specific mixtures of components that is of great potential interest to researchers.

The aim of this work was to evaluate the extraction of organic compounds from transesterification reaction mixtures by using ionic liquids, analysing the effect of the cation and anion composition in the efficiency of separation. The ionic liquids used in this work are based on 1-n-alkyl-3-methylimidazolium and 1-n-alkyl-3-methylpyridinium cations and diferent anions (hexafluorophophate, bis{(trifluoromethyl)sulfonyl}imide, tetrafluoroborate, methylsulfate, 2(2-methoxyethoxy)ethylsulfate, ethylsulfate, n-octylsulfate, dicyanamide, nitrate, tetrafluoroborate and chloride).

Initially, the partition coefficients of the compounds involved in a transesterification reaction (1-butanol, vinyl butyrate, butyl butyrate and butyric acid) between each ionic liquid and n-hexane (reaction medium) were evaluated. In all the ILs assayed the partition coefficients of the compounds follows the sequence: butyl butyrate < vinyl butyrate< 1-butanol < butyric acid. Furthermore, an important influence of the anion composition on the partition coefficients were observed. Regarding the cation composition of the IL, it was found that the partition coefficients of the compounds increase with the increase in the chain length of alkyl substituent of the cation.

The significant differences observed in the partition coeficients for the target compounds led us to extend the study to sixteen compounds involved in

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transesterification reactions (alcohols, vinyl esters, alkyl esters and carboxilic acids). The ionic liquids used for this study was based on two ILs: 1-butyl-3-methylimidazolium hexafluorophosphate, $[bmim^+][PF_6^-]$, and 1-octyl-3-methylimidazolium hexafluorophosphate, $[omim^+][PF_6^-]$. It is worthing to note that the partition coeficients between IL/hexane decrease with the increase in the alkyl chain length of the compounds for the same organic functional group. This parameter showed higher values for all the compounds when $[omim^+][PF_6^-]$ were used as extraction agent.

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Highly selective and efficient transport of transesterification reaction compounds across supported liquid membranes based on tetrafluoroborate ionic liquids

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Keywords: supported liquid membrane, ionic liquid, selective transport, transesterification reaction

Supported liquid membrane (SLM) is a novel and promising technique for the selective separation of organic compounds involved in pharmaceutical and fine chemical synthesis [1-3]. Their main advantages over conventional solvent extraction processes, such as simplicity of operation or economical utilization, are lessened by their well-known disadvantages relating to stability and lifetime [4,5], which are too low to enssure feasible commercial application. For this reason, the characteristics and application of ILs which can be used as solvents capable of stabilizing SLM have received growing attention during recent years [6]. Ionic liquids, which are liquid close to room temperature, normally consist of an organic cation, the most commonly used being dialkylimidazolium and tetraalkylammomiun salts, and a polyatomic inorganic anion (e.g. BF_4^-, PF_6^-) [6]. Due to their non-measurable vapour pressure, combined with the greater capillary force associated with their relatively high viscosity and the possibility of minimizing their solubility in the surrounding phases by the adequate selection of the cation and anion [2], it is possible to obtain very stable and environmentally friendly supported liquid membranes using ionic liquids as supported liquid phase.

This work analyses the permeability of different vinyl esters, alkyl esters, alcohols and acids through ILs based on the 1-n-alkyl-3-methylimidazolium cation (n-butyl, n-octyl) and tetrafluoroborate anions, immobilized in Nylon® (polyamide) membranes. The transport studies were performed using a glass diffusion cell with two independent compartments separated by the membrane. Hexane was used as feed and receiving phase.

It is worthing to note that the permeability values were decreased by the increase in alkyl chain length for the same organic functional group. In addition, significant permeability differences were found for the diferent organic functional groups. The increase in the permeability values for the different organic compounds was in agreement with the increase in the partitions coefficients of the organic molecules between IL and hexane. The obtained SLMs showed excellent operational and structural stability as was evidenced by SEM-EDX.

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Assessment of several commercial lipases for the catalysis of tranesterification reactions

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Keywords: Lipase, Transesterification, Biodiesel, Triglyceride, Stability

During the last decade, biodiesel production has been the focus of a large number of research works. The use of fatty acid alkyl esters as fuel in diesel engines presents a number of advantages, many of them related to environmental issues. Biodiesel is obtained by transesterification of triglycerides and alcohol, a reaction also known as alcoholysis. Methanol appears as the most commonly utilized alcohol, due to its relatively low cost and advantageous physical and chemical properties. However, other suitable alcohols include ethanol, propanol, butanol and amyl alcohol. In all cases, the presence of a catalyst is required, and an excess of alcohol is usually recommended to improve the reaction yield.

Current industrial production of biodiesel is mainly based on alkali- or acid- catalyzed processes, which lead to high conversion rates in short times. However, relatively high temperatures must be used, the residual glycerol is difficult to recover, a large amount of strongly acidic or alkaline wastewater is produced, and the reaction can be interfered by the presence of water and free fatty acids. Enzymatic catalysis has been proposed as a means to avoid some of these problems. Lipases can promote the transesterification of triglycerides, either in aqueous or non-aqueous media, at lower temperatures than those used in the chemical procedure. Also, recovery of glycerol is easier, and no acidic or alkaline wastewaters are generated. However, some important drawbacks have to be mentioned for this strategy, namely the high cost of the biocatalyst and the frequent deactivation of the enzyme due to the alcohols (Fukuda et al., 2001; Vicente et al., 2004; Marchetti et al., 2007). Therefore, a number of researchers have focused their activity in the optimization of the enzymatic process, either by protein engineering or manipulation of the reaction conditions (Kaieda et al., 2001; Du et al., 2004; Al-Zuhair, 2005; Nie et al., 2006).

In this work, the ability of three commercial lipases to catalyse the transesterification of sunflower oil and methanol has been assessed, and the relevance of some operating variables researched. Some important properties of the biocatalysts were determined, in order to identify potential restrictions in the reaction conditions. Lipolytic activity was determined spectrophotometrically, using 2.5 mM *p*-nitrophenyl laurate as substrate, at several pH (4.0-9.0) and temperature (30-60ºC) values, to ascertain optimum conditions for enzyme activity. The highest activity was found at pH 7.0 and 30ºC for two of the enzymes, and at the same pH and 55ºC for the other one, although it showed rather high activity for temperatures comprised between 35 and 55ºC. All the biocatalysts appeared to be more active at alkaline pH values.

Also, thermal stability and resistance to the presence of methanol and ethanol was studied, by measuring residual lipolytic activity after incubating aliquots of the enzymes at 40ºC during 3 hours, either in aqueous solution or mixed with the selected organic solvents. Two of the studied enzymes suffered significant loss of activity (around 70%) in aqueous solution, while the third one kept 80% of the initial activity in the assayed conditions. As for the effect of organic solvents, the activity loss was rather high in all cases, especially in the presence of ethanol.

The transesterification reaction was carried out in 10 mL test tubes, containing 4 mL reaction mixture, under magnetic stirring in a water bath at 40ºC. The operating variables investigated were enzyme concentration, oil:alcohol molar ratio, and water

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concentration, as well as reaction time. The progress of the reaction was preliminarily assessed by thin layer chromatography, using silica gel 60 F254 plates, and a mixture of petroleum ether, diethyl ether and acetic acid (85:15:1 v/v/v) as eluent. The plates were developed with iodine vapors.

The produced fatty acid methyl esters were determined by HPLC, using LC-18 precolumn and column. The injection volume was set at 20 μL and the isocratic eluent (90:10 acetonitrile/water) was pumped at a rate of 1 mL/min. Detection was performed using a diode array detector at 205 nm.

High conversion rates were obtained for the three commercial enzymes, with values above 80%. The influence of enzyme concentration on the reaction was assessed, and relatively high enzyme amounts were needed to attain significant conversion rates. Oil:methanol concentration was also confirmed to have significant influence on the reaction, and the convenience of employing excess methanol was confirmed. Also, the effect of water concentration in the reaction mixture was investigated, and the need for a minimum amount of water was found in all cases, indicating that the reaction might proceed through a hydrolysis-esterification route. Finally, the time-course of the reaction was assessed.

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Online monitoring of the transesterification reaction

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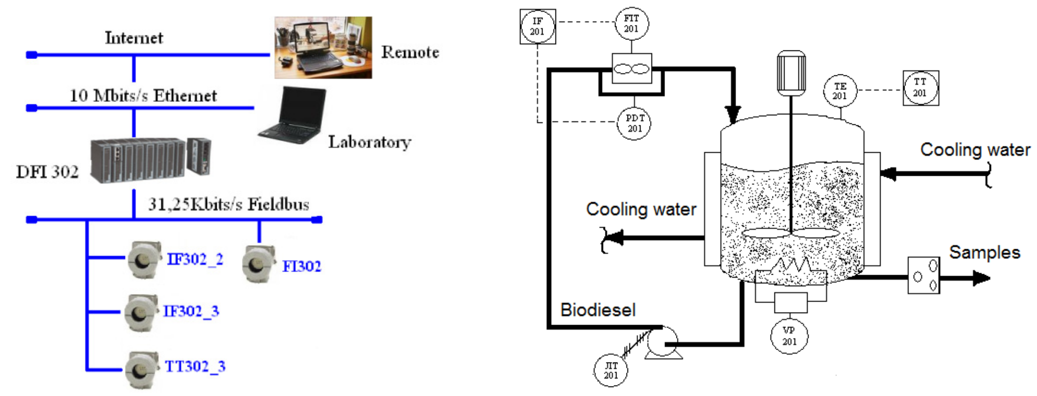
Keywords: Online monitoring, Bio diesel, Viscosity, Fieldbus

Bio diesel can be defined as the primary alkyl ester which is part of a long-chain of fatty acids. Bio diesel is normally produced from renewable sources such as, vegetable oil, animal fats and seaweed. Reduced viscosity is the major reason why vegetable oils or fats are transesterified to bio diesel; the high viscosity of neat vegetable oils or fats ultimately leads to operacional problems such as engine deposits (Knothe *et al*., 2006). One of the main problems in the study or industrial application of transesterification process for vegetable oils is how to measure the ethyl ester content (Fillipis *et al*., 1995). Analytical methods are based on chromatography and an online application in a transesterification plant is very difficult. Therefore the purpose of this work is to develop a method to be utilized as a controlled process. Many studies have shown that viscosity difference is sufficient to give an indication of the bio diesel content in a transesterification product. Kalotay (1999) proposed an online viscometer which can be assembled through a Coriolis mass flow meter and also a differential pressure transmitter; this system monitors the viscosity of the process fluid online.



a) **b)**

DFI = Fieldbus Universal Bridge
TT = TemperatureTransmiter/Fieldbus
IF = Electrical current/Fieldbus
FI = Fieldbus/Electrical current

TE = Sensor element
TT = Temperature transmitter
PDT = Differential pressure transmitter
FT = Mass flow transmitter

Figure 1. (a) Fieldbus network and (b) Batch stirred heater transesterification tank.

The process is carried out in a heated tank (1000mL) that has to be stirred constantly; a cooling jacket is maintained throughout the process in order to keep the heating temperature at a desired value. The heater has a power of 300W and the mechanical mixing process is driven by electrical motor rotating at 40 rpm both heat and the mechanical movement compose the energy input of the system. The set point of bulk temperature is 50ºC. Soybean oil, ethanol and an acidic catalyst are used to accelerate the reaction. The final result of this process is a batch of ethyl esters with

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conversion rate of about 95% of the mixture. The viscosity was calculated from mass flow value and differential pressure value. See Figure 1a. The whole process of ethyl ester production is managed by the digital control system from Fieldbus communication. Fieldbus network is connected to our laboratory network and provides us with the following variables: temperature, pressure and mass flow. These analog signals are converted into digital signals through analog to digital conversion (A/D). See Figure 1b. The transesterification product, a mixture of ethyl esters, unreacted oil, and mono-and diglycerides, was analyzed by gas chromatography (GC). The results are shown in the Table 1.

Table 1. Comparison between ethyl ester contents measured by GC and calculated from Viscosity of mixture data at 50°C ($\eta_{50°C}$)

Samples	Time (min)	ln ($\eta_{50°C}$)	$\eta_{50°C}$ (cSt)	Ethyl ester (wt %)		Error (%)
				by GC	from ($\eta_{50°C}$)[a]	
1[b]	0	-	16,1130	45,1	-	-
2	15	2,6588	14,2793	86,7	86,1	0,01
3	30	2,3921	10,9369	89,8	89,5	-0,27
4	45	2,1262	8,3828	92,2	92,9	0,81
5	60	2,0130	7,4855	94,9	94,4	-0,55

[a] EE=a ln ($\eta_{50°C}$) + b, x= $\eta_{50°C}$ (cSt), a= -12,905 ; b= 120,39; R^2= 0,9789

[b] If the ethyl ester content is higher than 85% the correlation allows the determination by a single viscosity measurement.

Conclusions

The viscosity measurements of the mixture can be used as a less expensive and practical online method used to evaluate the efficiency and conversion rate of biodiesel without the need for expensive GC. The Coriolis was used to replace tedious sampling and analytical expensive procedures.

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Exploitation of agroindustrial biomass by fractionation of its components. Characterization of hemicelluloses, celluloses and other fractionation products

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Keywords: lignocellulosic biomass, autohydrolysis, agroforestry residues.

The world is experiencing a growing shortage of raw materials which is especially severe in the energy sector and being worsened by the unfavourable environmental impact of a consumerist culture revolving around the exploitation of non-renewable resources. Accomplishing sustainable development and renewability entails finding and using new resources and chemical and consumer products where lignocellulosic biomass constitutes a "necessary" source of raw materials on account of its ubiquity, availability and low polluting power. In fact, lignocellulosic biomass has been widely acknowledged as the largest source of renewable energy available in the world to respond to the decline in fossil fuel sources (Ozcimen, D. et al., 2004; Jefferson, M., 2006; Semelsberger, T.A. et al, 2007). Processing the whole material rather than only its sugar and amylaceous fractions to obtain ethanol by fermentation, its fibre fractions to produce cellulose pulp and some residual fractions or all for burning is the only way of exploiting the whole potential of such an abundant resource (Kim, S. et al., 2004).

The alternative to integral exploitation of lignocellulosic biomass in a single process (usually of the thermal type) inevitably involves its fractionation; this is hindered by the inability to separate its main components without degrading the chemical structure of some. This was the origin of the "lignocellulosic material biorefinery" concept as the source of a wide range of products by analogy with the oil refinery concept. Although a number of refining schemes have been tested at the laboratory or pilot plant scale, none has to date been implemented on a commercial scale (Kamm, B. et al, 2004).

In this work, we characterized the raw material and the liquor resulting from the autohydrolysis of five different lignocellulosic materials (*viz. Eucalyptus globulus*, *Arundo donax*, sunflower stalks, *Chamaecytisus proliferus* (tagasaste) and *Pawlonia fortunei*) with a view to assessing their utility for the obtainment of energy and chemical products by integral fractionation based on autohydrolysis.

The final aim was to establish the viability of using effective crops in order to reduce the dependence of Spain on foreign countries as suppliers of energy materials and chemical products, and also to reduce agrifood surpluses by using a fraction of agricultural land to crop alternative plants.

Sunflower stalks and tagasaste exhibited higher holocellulose contents (72.2 and 80.3%, respectively) and lower lignin contents (19.9 and 19.8%, respectively) than did *Eucalyptus globulus*. All raw materials surpassed *Eucalyptus globulus* in xylan content (18.3 to 23.9%), but none equalled its glucan content (46.8%).

Hemicellulose solubility increased with increasing autohydrolysis temperature, the optimum extraction temperatures for *Arundo donax*, tagasaste and *Pawlonia fortunei* falling in the range 185ºC – 195ºC and being lower than the optimum value for *Eucalyptus globulus*. Also, the oligosaccharide contents of tagasaste, sunflower stalks and *Pawlonia fortunei* (13.9%, 21.5% and 11.7%, respectively) exceeded that of

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Eucalyptus globulus (10.3% at 196ºC). At temperatures in the region of 200ºC, Heitz and Garrote (Heitz, M., et al., 1991; Garrote, G. et al., 2002) observed substantial depolymerization of cellulose and a decreased amount of cellulose fibres in the autohydrolysis process.

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Influence of different chemical treatments in lipase immobilization in a lignocellulosic support

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Keywords: coconut fiber, lipase, immobilized enzyme, stability, chemical treatments

Lipases (triacylglycerol ester hydrolases, E.C. 3.1.1.3) are enzymes that catalyze both the hydrolysis and synthesis of esters from glycerol and long-chain fatty acids. These enzymes, under special conditions, can also catalyze interesterification, aminolysis and thiotransesterification reactions (Jaeger and Reetz, 1998). Thus, the increase on lipases applications in enzymatic synthesis and biotransformations impelled the study of immobilized biocatalysts preparation.

Considering the high cost of some available commercial support matrixes to enzyme immobilization, studies have been intensified in order to obtain cheaper supports. Some papers have reported the use of agroindustrial wastes as immobilization matrix for α-amylase, invertase and lipase. These studies showed that agroindustrial wastes are a suitable raw material source for immobilization matrix. In Brazil, an increase in the green coconut water market had a direct impact on the increase in coconut husk production, an agroindustrial waste (Brígida *et al.*, 2007). However, the presence of impurities on the surface of green coconut fibers requires treatment studies.

Therefore, the aim of this study was to investigate the effect of different chemical treatments on the immobilization of *Candida antarctica* lipase B (CALB) in green coconut fiber. For this purpose, two immobilization strategies were used: adsorption and covalent attachment. In order to evaluate the immobilization process in different chemical treated supports, the lipase loading and stability of the obtained derivates was evaluated.

Before being used as support, green coconut fibers were previously washed with water and then they were submitted to three different treatments: (i) coconut fibers were submitted to oxidation by H_2O_2, in basic medium, at 85ºC for 2 h; (ii) the fibers were soaked in 0.4% (v/v, in glacial acetic acid) NaOCl for about 2 h at 85ºC, and (iii) the last treatment was performed by soaking fibers in a solution of NaOCl 4-6% (v/v):H_2O (1:1) for 2 h at 30ºC and, after this time, the fibers were washed with water and soaked in 10% NaOH for 1 h at 30ºC.

Immobilized enzyme was obtained by adsorption or covalent attachment, at room temperature, by contacting enzyme and support. For each gram of dry support, 10 mL of lipase solution, in 25 mM sodium phosphate buffer pH 7, were used. For immobilization by covalent attachment, prior to enzyme immobilization, 3-glycidoxypropyltrimethoxysilane (GPTMS) was used for silanization of green coconut fiber. After immobilization, the biocatalyst was separated by filtration, rinsed with phosphate buffer (10 mL) and dried at vacuum for 10 min. The lipase activity was assayed by incubating, at 37ºC, 0.2 mL of free enzyme or 0.2 g of immobilized enzyme with 1.8 mL or 30 mL, respectively, of 560 μM p-nitrophenyl laurate dissolved in 50 mM potassium-phosphate buffer (pH 7.0). The reaction was followed in a spectrophotometer at λ = 410 nm. In this study, catalytic efficiency was calculated and defined as the ratio between the enzymatic activity of immobilized lipase and the difference between initial and equilibrium enzymatic activity in lipase solution.

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For each immobilized system, the catalytic efficiency and thermal stability of the immobilized lipases were studied. To evaluate the thermal stability, free enzyme and immobilized derivatives were incubated in a sodium phosphate buffer 0.1 M and pH 7.0 at 60 ºC. Periodically, samples were withdrawn and their residual activities were assayed. In this work, residual activity is given as percentage of lipase activity, being 100 % taken as the hydrolytic activity of the enzyme immobilized under standard conditions The thermal deactivation curves have been explained following the deactivation model proposed by Henley and Sadana (Arroyo *et al.*, 1999). By fitting this model to experimental data, all parameters (k_1, k_2, α_1, α_2), biocatalyst half-life ($t_{1/2}$) and the stabilization factor (F, considered as the ratio between soluble and derivatives half-lives) were calculated.

Preliminar results showed that lipases immobilized by adsorption had similar activity when immobilized in natural fiber and in treated fiber with H_2O_2 or NaOCl, 30.56 ± 2.0 U/g, 24.83 ± 5.3 U/g and 34.22 ± 1.6 U/g, respectively. On the other hand, low activity were achieved by lipase immobilized in NaOCl – NaOH treated fiber, 9.94 ± 1.3 U/g. All immobilizations carried out in the treated fiber showed a lower catalytic efficiency when compared to the enzyme immobilized in natural one. In addition to physical changes, observed by scanning electron microscopy, variations in the surface charge, indicated by pH change, may have influenced the immobilization process.

Finally, studies of lipases immobilized by covalent attachment in natural coconut fiber alowed to achieve an activity of 40.28 U/g, with catalytic efficiency of 20.51%, values that are near to those obtained by adsorption. However, lipase immobilized by adsorption was 1.4-fold more thermal stable than lipase immobilized by covalent attachment (F equal to 92.15 and 67.56, respectively). It was also observed that the GPTMS concentration during silanization step may influence the derivate stability. Thus, stability studies with lipases immobilized by covalent attachment in silanizated fibers under different concentrations of GPTMS should be made.

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Information CALS-model of the innovation technology of biologically active additives of a new generation

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Keywords: System analysis, CALS-technologies, Biologically active additives, Drying-agglomeration.

At the present time, as a promising information technology, methods of the Continuous Acquisition and Life Cycle Support (CALS) concept are widely implemented (2002). The CALS concept is based on a complex of unified information models and the standardization of methods for information access and correct information interpretation according to the international standard ISO 10303 (STEP). A series of CALS projects were developed for a number of technologies for producing special-purity substances (2006),(2007); however, in all the cases, CALS methods were used only to create project documentation.

In this work, using CALS methods, an information model was elaborated which includes all the steps (marketing, design, production, service and repair, and realization and sale) of the technology of producing biologically active additives of a new generation from an extract of medicinal herbs and gelatin starch complex (2003). In recent years, biologically active food additives therapeutic and prophylactic preparations produced from herbal raw material have successfully competed with synthetic drugs.

Let us sequentially consider elements of the information model that correspond to various steps of the technology of biologically active additives.

On the basis of marketing research results, at the marketing step, two information-analytical blocks are formed: consumer characteristics and risk factors. Each of the blocks includes lower-level parameters, whose analysis gives a complete pattern of the entire analytical block. For convenience of using the system, the parameters are inputted in text and table formats, as well as in graphic form. As basic consumer characteristics, the income, age, and sex distributions of consumers are analyzed. In this block, the reasons for buying biologically active additives and the main factors affecting the purchase decision are also studied. In the block of risk factors, an analysis is undertaken regarding the possibility of a change in tax laws and concerning inflation and competition, market capacity, degree of import substitution, and prices for similar products. At the marketing step, a conclusion is drawn about the expedience and prospects of developing technology and equipment for producing biologically active additives of new generation.

At the design step, the initial data for design are analyzed, which contain information on six basic stages: grinding of medicinal raw material, extraction, concentration, mixing, drying agglomeration, and packing. Particular attention was given to the limiting stage drying agglomeration.

The main methods for drying biologically active additives were analyzed (fig); these are spray drying, fluidized-bed drying, infrared drying, and drying in a vacuum dryer equipped with a rake agitator. It was shown that the equipment considered is inefficient if the product contains astringent substances (fructose, lactose, gelatin starch complex, and others), which, during drying agglomeration, form clots, stick to moving parts and the housing of the apparatus, and can render the apparatus inoperative. Therefore, a continuous convective agitator dryer was proposed, which gives a product of required quality and given particle size distribution.

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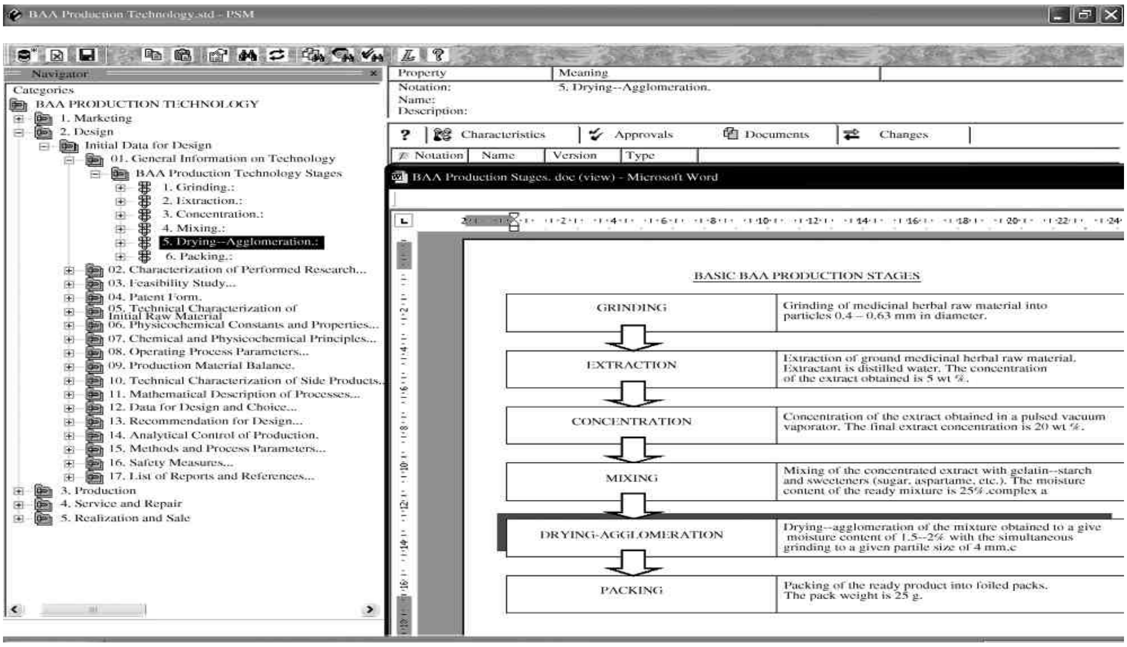

Property	Meaning
Notation:	5. Drying--Agglomeration.
Name:	
Description:	

BASIC BAA PRODUCTION STAGES

GRINDING	Grinding of medicinal herbal raw material into particles 0,4 – 0,63 mm in diameter.
EXTRACTION	Extraction of ground medicinal herbal raw material. Extractant is distilled water. The concentration of the extract obtained is 5 wt %.
CONCENTRATION	Concentration of the extract obtained in a pulsed vacuum vaporator. The final extract concentration is 20 wt %.
MIXING	Mixing of the concentrated extract with gelatin--starch and sweeteners (sugar, aspartame, etc.). The moisture content of the ready mixture is 25% .complex a
DRYING-AGGLOMERATION	Drying--agglomeration of the mixture obtained to a give moisture content of 1.5--2% with the simultaneous grinding to a given partile size of 4 mm.c
PACKING	Packing of the ready product into foiled packs. The pack weight is 25 g.

Fig. CALS Project element at the design step (basic stages of the technology of biologically active additives)

At the design step, four variants of agitator design are considered. The ultimately chosen variant was the design that ensured required product quality at minimal energy consumption, with the agitator of this design being constructed at minimal material and labor expenditures. Three methods for introducing a heat-transfer medium were analyzed, and the optimal variant ensuring the maximal uniformity and efficiency of heat transfer was chosen. Intermediate and final variants described in text and graphic files were included into the CALS project.

At the production step, technical documentation of extractors, dryers--agglomerators, and other equipment is analyzed by industrial equipment manufacturing plants.

At the service step, technical information on the main stages (switching on, operation, switching off, washing) of service of extractors, dryers--agglomerators, and other equipment is introduced.

Information at the repair step is structured at three levels: trouble – cause - troubleshooting. The developed module enables one to promptly, in dialog mode, select the optimal variant of troubleshooting.

The step of realization and sale includes product promotion to market (advertising in printed and electronic mass media; participation in exhibitions, contests, tenders, etc.) and also analysis of information on the use of the developed equipment not only in the technology of biologically active additives but also in the chemical and pharmaceutical industries.

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Hexane extraction process using ionic liquids as solvents

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Keywords: Azeotropic mixtures, Green technology, Extraction, Ionic Liquid, 1-alkyl-3-methylimidazolium hexafluorophosphate

An important problem for the industrial processes is the presence of azeotropic or close-boiling mixtures in their production, since the separation is impossible by ordinary distillation. Extractive distillation is the most widely used process to remove the components from the azeotropic system, but this process needs energy to get a fluid phase system. With the reduction of lead in gasoline, a growing number of processes in which alkanols and alkanes co-exist to produce oxygenated additives for gasolines are under development or have already reached the industrial production stage. In this work, the azeotropic mixture hexane with ethanol is chosen due to it is hard to separate it into each component by conventional distillation. The liquid – liquid separation leads to an environmentally friendly extraction process for azeotropic mixtures as alternative to the azeotropic distillation where volatile organic compounds, high pressures and energy to evaporate the mixture are necessary to separate it into the desired pure components.

Ionic liquids (ILs) have become one of the growing “green” media for engineers due to their superb physicochemical properties. The application of these remarkable salts in extraction processes has been extensively investigated and reviewed. Moreover, ILs have had increasing attention as possible replacement solvents for volatile organic compounds in the development of a sustainable green chemistry. In this paper, we suggest the use of two ILs formed by 1-alkyl-3-methylimidazolium (CnMMIM) as cation and hexafluorophosphate (PF_6^-) as anion.

The knowledge of the alkane/alkanol LLE is essential for the design of the separation technique applied. In our case, two ILs (1-hexyl-3-methylimidazolium hexafluorophosphate HMIM PF_6 and 1-methyl-3-octylimidazolium hexafluorophosphate OMIM PF_6) are used as solvents and the research is focused on liquid – liquid extraction involving the azeotropic mixture hexane with ethanol. The suitability of these ILs was assessed by means the experimental determination of the liquid–liquid equilibria (LLE) for the ternary systems hexane + ethanol + HMIM PF_6 or OMIM PF6 at 298.15 K. The solute distribution ratio and the selectivity are determined from the tie–line data. As design of a separation process requires knowledge of the phase equilibria, correlation of LLE data are made using the NRTL equation. Extraction process with the best solvent are simulated using conventional software and the separation sequence is determined.

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Designing highly stable supported ionic liquid membranes by the adequate selection of the ionic liquid composition and surrounding phase nature

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Keywords: supported liquid membranes, ionic liquid, stability, SEM-EDX

The stability of the SLMs, porous supports whose pores are filled with a liquid, is one of the major limitations of this separation technique [1]. Therefore, the main challenge in the use of SLMs at industrial scale is the design of highly stable SLMs. Unlike others solvents previously used in SLMs, ILs possess unique properties that are interesting in the context of liquid membranes. Ionic liquids are organic salts that are liquid at room temperature consisting in an organic cation (e.g. dialkylimidazolium, tetraalkylammomium) and a polyatomic inorganic anion (e.g. tetrafluoroborate, hexafluorophosphate). Among the most remarkable properties of the ionic liquids it can be highlighted their negligible vapour pressure and great surface energy combined with high viscosity. These properties are very suitable to prevent the liquid flow that is consistently retained within the porous support even under pressure. Another interesting aspect of ILs to be accounted for is the possibility of tailoring their physicochemical properties through the selection of the proper constituent ions [2]. Therefore, an adequate design of the IL could reduce their solubility in the surrounding phases, increasing the stability of the SLM. All these mentioned properties have made ILs to be considered as “green designer solvents”, finding potential use in numerous applications [3-5].

This work evaluates the operational stability of supported liquid membranes (SLMs) based on ionic liquids (ILs) into different feed/receiving phases. $[\mathrm{bmim}^+][\mathrm{PF}_6^-]$, $[\mathrm{bmim}^+][\mathrm{BF}_4^-]$ $[\mathrm{bmim}^+][\mathrm{NTf}_2^-]$ and $[\mathrm{bmim}^+][\mathrm{Cl}^-]$ were used as supporting phase in Nylon® membranes. Scanning electron-microscopy (SEM) combined with Energy Dispersive X-ray (EDX) analysis was used to characterize the membrane surface morphologically and examine the global chemical composition of the membranes and the distribution of the ILs within them. The effect of the ionic liquid composition and the surrounding phases nature on the SLM stability was analysed.

Study of the freshly impregnated membranes showed that, in all cases, the ILs were homogeneously distributed, mostly filling the pores of the membranes although, in some cases, a small amount of excess of IL was located on the external membrane surface. Stability tests were performed by keeping the respective impregnated membranes immersed for a week in a diffusion cell including two independent compartments and using hexane/hexane, hexane/TBME hexane/acetone, hexanea/DMSO, hexane/water as the respective feed and receiving phases. When hexane/hexane was used as feed/receiving phases, the SEM-EDX study of membranes after continuous operation showed that the ILs were still retained within the membrane pores, showing an homogeneous distribution of the ILs and only small losses of the IL initially located on the external surface were observed. However, when

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more polar solvents were used (hexane<TBME<acetone<dimethylsulfoxide< water) as surrounding phases, less amount of ionic liquid was detected in the polymeric membrane. It was also observed that the stability of the supportted ionic liquid membranes increased with the increase of the ionic liquid hydrofilicity ([bmim$^+$][Cl$^-$] > [bmim$^+$][BF$_4^-$] > [bmim$^+$][PF$_6^-$] > [bmim$^+$][NTf$_2^-$]) for all the surrounding phases studied. These observations are in complete agreement with the ionic losses determined by mass balance.

These observations underlines the importance of the hydrophobic/hydrophilic character of the ionic liquid and the polarity of the surrounding phases in the design of supporting liquid membranes based on ionic liquids.

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Improving the selectivity in the simultaneous separation of organic compounds through supported liquid membranes by the use of water-miscible ionic liquid

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Keywords: supported liquid membranes, ionic liquid, selective transport, green chemistry

The selective separation of organic compounds is a critical issue in the chemical industry. Most of these methods are technically demanding, involve considerable energy cost and/or result in large amounts of waste solvents. Membrane-based separation processes have been recognized as a promising alternative [1] since they do not require high energy consumption for their operation and because they can be conducted under moderate operating conditions and sequencial operation. Among membrane-based separation processes, the use of supported liquid membranes (SLMs) has received growing attention during recent years [2]. SLMs are porous supports whose pores are filled with a liquid; among their numerous advantages is the fact that the amount of solvent needed in the SLM process is minimal and that the processes of extraction and stripping are combined into one single stage. Nevertheless, their industrial application is still limited, mainly due to concerns about SLM stability and long-term performance [3]. Ionic liquids (ILs) have recently been revealed as interesting clean alternatives to classical organic solvents [4]. They are organic salts that are liquid close to room temperature and they normally consist of an organic cation, being the most commonly used dialkylimidazolium and tetraalkylammomiun salts, and a polyatomic inorganic anion (e.g. BF_4^-, PF_6^-). The use of these new solvents as a liquid membrane phase results in the stabilization of the SLMs due to their negligible vapour pressure, the possibility of minimizing their solubility in the surrounding phases by adequate selection of the cation and anion [5], and the greater capillary force associated with their high viscosity, which could reduce displacement of the liquids from the micron pores under pressure.

Recently, we reported the selective simultaneous separation of the substrates and products of a transesterification reaction (vinyl butyrate, 1-butanol, butyl butyrate and butyric acid) using SLMs based on miscible and immiscible ionic liquids [6]. Better selectivity results were obtained by the use of water miscible ionic liquids, which led us to further investigate the effect of new water miscible ionic liquid used as liquid phase on the selectivity of the resulting SLM.

In this work, a wide range of new water-miscible ionic liquid membranes based on 1-n-alkyl-3-methylimidazolium and 1-n-alkyl-3-methylpyridinium cations and diferent anions (methylsulfate, 2(2-methoxyethoxy)ethylsulfate, ethylsulfate, n-octylsulfate, dicyanamide, nitrate, tetrafluoroborate and chloride) have been developed and tested for the selective simultaneous separation of the target compounds, evaluating the influence of the nature of the ionic liquids on the behaviour of the resulting supported liquid membranes.

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The transport studies were performed at 30 ºC using a glass diffusion cell with two independent compartments, with 30 mL each, separated by the SLM. O-rings were inserted on each side of the SLM. In each experiment, the initial solute concentrations in the feed phase were 100 mM in n-hexane. n-Hexane was used as a receiving solution in all cases. The solute concentrations were monitored by GC.

The permeability of vinyl butyrate, 1-butanol, butyl butyrate and butyric acid through SLM based on water miscible ionic liquids was determinated. Significant permeability differences between the organic compounds were obtained in all cases. It is worthing to point out that the permeoselectivity values were higher when water-miscible ionic liquids were used respect to water-immiscible ionic liquids. Furthermore, in order to understand the molecular mechanism involved in the transport phenomena, the partition coefficients of the targets compounds between each ionic liquid and the feed/receiving phase were evaluated. An important influence of this parameter on the permeability values of the compounds was observed, since an increase in the partition coefficients involves an increase in the permeability values. This relationship allows the easy prediction of the capability of a given SLM based on miscible IL to separate two compounds by simply evaluating the partition coefficient of each compound between the ionic liquid and the feed/receiving phase.

Furthermore, the influence of the nature of the ionic liquids on the behaviour of the resulting supported liquid membranes was evaluated. The anion composition of the ionic liquid has been found to be a key parameter for the selective transport of the target compounds, obtaining the following sequence of selectivity: $[MeSO_4^-]$ > $[MDEGSO_4^-]$ > $[EtSO_4^-]$ > $[Cl^-]$ > $[dca^-]$ > $[BF_4^-]$ > $[NO_3^-]$ > $[OcSO_4^-]$. Regarding the cation composition, higher separation efficiences were obtained with 1-n-alkyl-3-methylimidazolium respect to 1-n-alkyl-3-methylpyridinium cations. By comparing the octanol/water partitions coefficients (K_{ow}) of the ionic liquids assayed, it can be concluded that an increase in the hydrophilic character of the ionic liquids results in an increase in the selectivity for the separation of the assayed organic compounds. This underlines the clear relationship between the nature of the ionic liquid and the permselectivity of the supported liquid membranes.

The operational stability of the supported liquid membranes were also studied SEM-EDX, showing a remarkably stable behaviour when tested over seven days operation.

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Improving the stability of supported ionic liquid membranes by chemical immobilization of the ionic liquid

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Keywords: supported liquid membranes, ionic liquid, stability, SEM-EDX

Unfortunately, the industrial application of supported liquid membranes, porous supports whose pores are filled with a liquid, on a larger scale is still very limited due to insufficient membrane stability. These effects have been attributed to loss of solvent from the supporting membrane, either by evaporation or dissolution/dispersion into the adjacent phases. In this sense, new changes have to be done in the design of supported liquid membranes in order to increase their stability. Unlike the solvents previously used in SLMs, ILs possess unique properties that are interesting in the context of liquid membranes. Ionic liquids are organic salts which are liquid close to room temperature and which normally consist of an organic cation (e.g. dialkylimidazolium, tetraalkylammomium) and a polyatomic inorganic anion (e.g. tetrafluoroborate, hexafluorophosphate). Among the numerous advantages of ionic liquids as regards SLM stability can be highlighted their negligible vapour pressure and their high capillary force associated with their high viscosity, which could reduce the displacement of the liquids from the micron pores under pressure [1].

In a previous work, supported liquid membranes were developed by physical immobilization of the ionic liquid in a porous membrane [2] and its stability was studied by SEM-EDX after continuous operation, showed that the ILs were still retained within the membrane pores when n-hexane was used as feed and receiving phase, which demonstrates at microscopic level their high operational stability [3]. However, some losses of IL were observed when the surrounding phases consist of high polar solvents (e.g. water, DMSO, etc).

New supported ionic liquid membranes have been developed by chemical immobilization of ionic liquids in Nylon organic membranes using a grafting method. The ionic liquids chosen for this study were based on 1-n-alkyl-3-methylimidazolium cations and bistriflimide, hexafluoroborate, tetrafluoroborate and chloride anions. The stability studies were performed at 30ºC using a glass diffusion cell with two independent compartments, of 30 mL each, separated by the SLM. Hexane/hexane, hexane/TBME hexane/acetone, hexane/DMSO, hexane/water were used as feed and receiving solutions, and both compartments were mechanically stirred. The membranes thus obtained were characterized immediately after preparation and after seven days´ immersion in the aforementioned diffusion cell by FT-IR and Scanning electron-microscopy (SEM) combined with Energy Dispersive X-ray (EDX) analysis in order to examine the global chemical composition of the membranes, characterize the membrane surface morphologically and examine the distribution of the ILs within them. The new immobilization methods showed improved stability in high polar solvents.

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Stability of hydrolase enzymes in ionic liquids

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Keywords: stability, hydrolase enzyme, lipase, penicillin acylase, ionic liquid.

In nature, enzymes act as catalysts of living systems and are designed to function in aqueous solutions. However, anhydrous conditions are needed for synthetic transformations using enzymes. The use of organic solvents as non-aqueous environments offers the possibility of carrying out synthetic reactions by hydrolytic enzymes, increasing the solubility of organic substrates [1,2]. However, organic solvents are usually volatile liquids that may evaporate into the atmosphere with a detrimental impact on the environment and human health. The use of ionic liquids in biocatalytic processes has recently gained much attention as an environmentally attractive alternative to classical organic solvents [3], and they have been seen to be good solvents in a wide variety of biochemical processes [4]. Ionic liquids are organic salts that are liquid close to room temperature. From an environmental point of view, the most important properties of ionic liquids are their negligible vapour pressure, which makes them environmentally benign solvents compared with volatile organic solvents (VOSs) [5].

Here we attempted to evaluate the stability of penicillin G acylase (PGA) from *Escherichia coli* in their native form and free *Candida antarctica* lipase B (CaLB) at low water content. The hydrolysis of penicillin G to 6-aminopenicillanic acid (6-APA) and phenyl acetic acid (PAA) catalysed by PGA and the synthesis of butyl butyrate from vinyl butyrate and 1-butanol catalyzed by CaLB were chosen as activity test. The influence of these new solvents on enzyme stability was studied by incubating the enzyme (PGA or CaLB) in several ILs: $[\text{bmim}^+][\text{BF}_4^-]$, $[\text{bmim}^+][\text{PF}_6^-]$, $[\text{omim}^+][\text{PF}_6^-]$, $[\text{emim}^+][\text{NTf}_2^-]$, $[\text{bmim}^+][\text{NTf}_2^-]$ at 40°C and 30 °C, respectively.

To compare the effect of ILs on enzyme deactivation, the half-life times of the enzymes in ionic liquids were calculated. It is worthing to note how the presence of ILs enhance the stability of PGA and, in the case of $[\text{emim}^+][\text{NTf}_2^-]$, an over-stabilization phenomenon is produced, increasing the half-life time by about 2000-fold with respect to 2-propanol, used as reference organic solvent. The most important effect on enzyme (PGA and CaLB) stability was observed by changing the nature of IL anions. The lower half-life time of enzymes was observed in $[\text{bmim}^+][\text{BF}_4^-]$ for PGA and in $[\text{bmim}^+][\text{dca}^-]$ for CaLB. This could be explained by the fact that these ILs are fully miscible with water, so they could interact with the absolute amount of water indispensable for acquisition and maintenance of the catalytic conformation of the enzymes.

Accordingly, the potential advantages of these new solvents, together with the unique stabilization effect on enzymes, may open up the possibility of development of new biocatalytic processes based on ionic liquids

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Fructooligosaccharides production using immobilized cells of *Aspergillus japonicus*

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Keywords: Sucrose, Fermentation, *Aspergillus japonicus,* Fructooligosaccharides, Immobilization.

Fructooligosaccharides (FOS) are naturally occurring sugars that have beneficial effects as food ingredients, because of its low calorie, noncariogenic nature and ability to promote the growth of beneficial bifidobacteria-rich intestinal flora and immune system modulation. Although FOS is present in several plants, the concentration of them in these sources is low and choice of plants as a source of FOS is limited by seasonal conditions (Wang & Zhou, 2006). Hence, microbial FOS production by the action of fungal fructosyl transferase on sucrose is more feasible at industrial level (Sangeetha et al., 2005).

In the process of FOS production with fructosyltransferase, the main problem is that the activity of the enzyme is severely inhibited by glucose, which is generated as a byproduct. As a result, maximum conversion yield of sucrose to FOS ranges from 55 to 60% (w/w) due to unreacted sucrose and glucose. Since the produced FOS mixture contains considerable amounts of sucrose and glucose, its use has been limited (Yun, 1996). Therefore, the development of processes that permit the FOS production in industrial scale with higher yields, and lower necessity of purification of the final product, is necessary.

The use of immobilized enzymes or cells can be useful for the development of effective and economic methods for large-scale production of FOS. Nevertheless, the selection of the immobilization material is essential to design an effective system for this purpose. The aim of the present work was to evaluate the FOS production using immobilized cells of the fungus *Aspergillus japonicus* ATCC 20236. Polyurethane foam, stainless steel sponge, vegetal fiber sponge, pumice stones, zeolites and cork were tested as microorganism carrier during the production of FOS under submerged fermentation conditions.

Batch experiments were carried out in 500 mL Erlenmeyer flasks containing 1 g of carrier and 100 mL of sucrose medium enriched with nutrient sources. The flasks were agitated in an orbital shaker at 160 rpm, 28°C, for 48 h. Samples were withdrawn during the fermentation to determine the consumption of sucrose, liberation of glucose and fructose to the medium, and production of FOS (kestose (GF_2), nystose (GF_3) and fructofuranosylnystoses (GF_4)). At the end of the fermentation, the cell mass adhered to the carrier was quantified.

According to the results, the microorganism adhesion to the carrier varied to each tested material. Consequently, the FOS production also varied to each medium, due to the differences in the amount of free and immobilized cells present. Among the evaluated carriers, the highest amount of immobilized cells were achieved by using vegetal fiber sponge and stainless steel sponge, while the zeolites and cork gave the worst immobilization results.

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Development of a biosensor based on ion-selective electrode for urea in milk by using immobilized amidase from *Pseudomonas aeruginosa*

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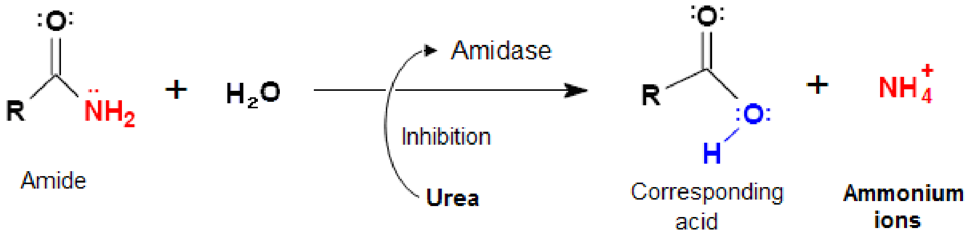
Keywords: Urea in milk; Amidase from *Pseudomonas aeruginosa*; Biosensor based on ISE; Membranes; Active-site inhibitor

The food processing industry requires suitable analytical methods for quality control of food which are fast, reliable, specific and cost-effective since current wet chemistry tests are time-consuming and some may require highly skilled labour as well as expensive equipment (Verma *et al.*, 2003). This urgent need is due to increased regulatory action and consumer concern about food composition and safety (Luong *et al.*, 1997).

Milk has been considered as a human's most nearly perfect food as far as nutritional properties are concerned and its consumption depends on strict sanitary control employed by the dairy industry (Potter and Hotchkiss, 1995). Urea is present as an adulterant in milk since it is not a natural constituent of milk which has originated increasing concern from the public as the consumer. The presence of urea in milk may be due to excessive nitrogen uptake whose concentration is in the range of 1.66 – 4.16 mM and high urea concentrations are responsible for reduced fertility rates in dairy cattle (Butler *et al.*, 1996)

The present work involves the development of a biosensor based on ion-selective electrode (ISE) for assay of urea in milk by using a novel enzymatic method. Urea is a powerful time-dependent active-site directed inhibitor for aliphatic amidase (acylamide amidohydrolase EC 3.5.1.4) from *Pseudomonas aeruginosa* which catalyses the hydrolysis of a small number of aliphatic amides producing the corresponding acid and ammonia (Martins *et al*. 2006; Tata *et al*, 1993) according to the following reaction:



Increasing concentrations of urea were found to inhibit proportionally amidase activity which was detected by the hydrolase reaction. Therefore, urea concentration in samples was inversely proportional to amidase activity which was measured with acetamide as substrate by using ISE for ammonium ions.

Cell-free extracts containing amidase activity were immobilized on nylon and polyethersulfone membranes in the presence of gelatin and glutaraldheyde as the bi-functional reagent. Membranes containing immobilized amidase activity were used to set up a biosensor based on ISE for ammonium ions by using acetamide as the substrate and the enzyme reaction was followed by measurement of ammonium ions due to hydrolysis of the aliphatic amide (Fig.1)

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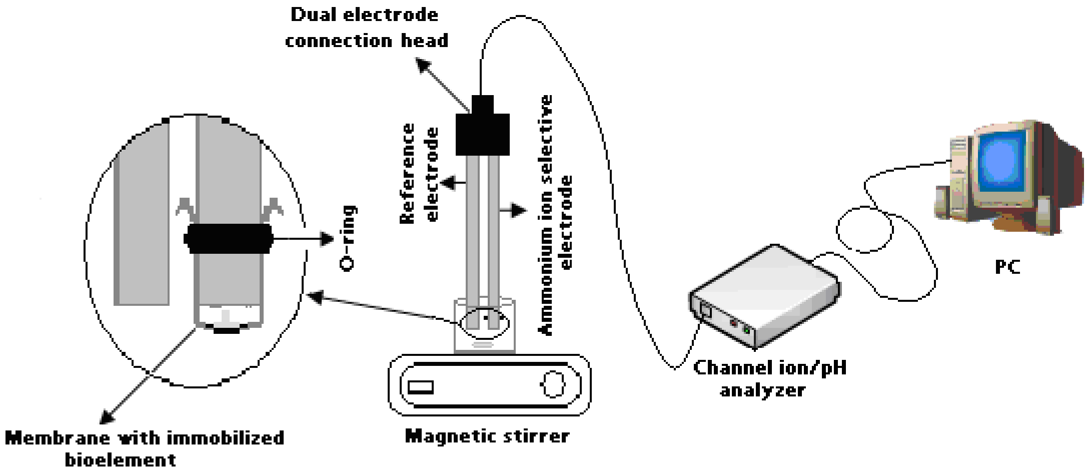

Fig.1 - Experimental set-up for potentiometric biosensor: ammonium ion selective electrode containing polyethersulfone membrane with immobilized enzyme and reference electrode, electrochemical cell, magnetic stirrer, ISE analyser and PC.

Subsequently, this biosensor was washed and incubated with milk containing increasing concentrations of urea in the range of 0 - 10 μM, for 1 h. After the incubation period, the biosensor containing amidase activity was assayed again by using acetamide as the substrate. There was a linear relationship between a decrease of the biosensor response in mV and urea concentration in milk. This biosensor exhibited a linear response in the range of 2.0 – 10.0 x 10^{-6} M of urea either in a buffer solution or in milk and a response time of 60 s. The biosensor containing amidase activity could be re-used again by reactivating enzyme activity with hydroxylamine for 2h. This biosensor was stable for at least 1 month since it did not lose enzyme activity and it is cheap because cell-free extracts containing amidase activity can be used for quantification of urea in milk.

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Characterization of the fungi tissue of *Agaricus bisporus* for its future use as a component of a biosensor for phenolic compounds determination

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Keywords: tyrosinase, *Agaricus bisporus*, fungi, phenol

Phenolic compounds are extremely diverse. Although they may have hazardous adverse effects, they are the major pollutant present in the wastewaters from several industrial activities: coalmining, petrol refining, pharmaceutical production, steel and iron manufacturing, and the tanning and finishing of leather (Atlow et al., 1984).
Several procedures have been developed for the determination of phenolic compounds, such as chromatographic and fluorimetric techniques, and spectrophotometric methods. However, these techniques do not easily allow continuous monitoring in actual samples. They are expensive, time-consuming, need skilful operators, and sometimes require pre-concentration and extraction steps that increase the risk of sample loss.
Biosensors are generally defined as an analytical device, incorporating a biological or biologically derived sensing element, like enzymes, intimately associated with or integrated within a physicochemical transducer. They represent promising tools to supplement already existing techniques, due to their intrinsic characteristics such as selectivity, low cost, potential for the construction of simple flow devices for fast continuous monitoring. Among biosensors for phenols there is the enzyme tyrosinase as a bio-component, which is isolated from mushrooms due to economic reasons.
Tyrosinases (monophenol, o-diphenol:oxygen oxidoreductase, EC 1.14.18.1) belong to a larger group of proteins named type-3 copper proteins. Tyrosinase has been found widely distributed throughout the phylogenetic scale, which catalyses the *o*-hydroxylation of monophenols and the subsequent oxidation of the resulting *o*-diphenols into reactive *o*-quinones, both reactions using molecular oxygen. Subsequently, the *o*-quinones undergo non-enzymatic reactions with various nucleophiles, producing intermediates, which associate spontaneously in dark brown pigments (Topçu et al., 2004; Marin - Zamora et al., 2006).
This work aimed to characterize the fungi tissue for its future use as bio-componte of a biosensor for the determination of phenolic compounds. The tissue of *Agaricus bisporus* was chosen to be a cheap and rich source of the enzyme tyrosinase. Currently, some works were found in literature using tyrosinase of this mushroom as biological component of a biosensor (Topçu et al., 2004; Marin-Zamora et al., 2006), however they do not use it in the natural tissue (immobilization *in natura*).
For the development of work, fruit bodies of mushrooms (*Agaricus bisporus*) were obtained from a local market. The extraction enzymatic based on Kameda et al., 2006 and tyrosinase activity was measured spectrophotometrically according to Campos et al. (1996), using a diluted sample (ratio 1:10) of enzyme. The resulted mixture was homogenized and the changes of the absorbance were monitored at 280 nm in a spectrophotometer at a regular interval of 30 seconds during 999 seconds. One unit of enzymatic activity (U) was defined as the amount of enzyme that increased absorbance 0.001 (λ=280 nm) per minute.
Phenol analysis was done according to the direct colorimetric method per Standard Methods (Apha, 1996), where the absorbance was read at 500 nm. It is Important to

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clarify that each sample was previously filtered in a C18 PR-COLA cartridge for the interference removal.

Enzymatic reactions due to the presence of phenol in the mushroom tissue had been carried out following the conditions: 5g of mushroom tissue; erlenmeyer with 250 ml of distilled water free of phenol and chlorine; the agitation was rated at 200 rpm in a shaker, and the temperature was kept constant in 30°C.

The existence of phenol adsorption in the mushroom tissue was verified through enzymatic reactions by comparing the results of phenol remotion. The raw or the preheated (woven boiled previously at 100 °C during five minutes) tissues are used. The accomplished tests had the same conditions above, except the controlled temperature of 45ºC.

Obtained results showed that a significant amount of the enzyme remained in the paste of mushrooms even though extracting the first one. Therefore, a larger activity in the second (or third) enzymatic extract in relation to the first one may be due to the largest cellular breaking during the re-extraction process. That result is corroborated by the literature; see Papa et al. (1994) and Kameda et al. (2006). The activities were obtained, they varied from 20.2 U/ml to 980.36 U/ml. Furthermore, this work identified the degree of maturation of the mushrooms as the weight factor to interfere with the activity of the tyrosinase in the extract. That result is corroborated by Bevilaqua (2000).

Finally, phenol is present in the composition of mushrooms, and the average concentration is 0.307 ppm and the hypothesis of adsorption of the phenol in the pieces of tissue of *Agaricus bisporus* can be eliminated based on the satisfactory results of experiments that were performed during this work.

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Automated nanosensor for monitoring aromatic compounds in industrial environments

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Keywords: BTX monitoring, Optical fibre sensing, Automated nanosensor, Confined industrial atmospheres, Remote sensing

Volatile organic compounds (VOCs) are an important class of indoor air pollutants, with a high potential hazard to human and environmental health. The monitoring of these compounds *in situ* and in real-time is of great interest for several technological areas such as, industrial process control, environmental monitoring and food safety. The exposure level to VOCs, including benzene, tolune and xylene (BTX) in an industrial environment is considerably higher than in non-industrial environments. Human exposure to air pollution is a function of both time and concentration, and symptoms can include fatigue, headaches, nausea, dizziness, skin and eye irritation and central nervous system damage. Poor air quality in work places could negatively affect worker's efficiency and productivity beside increasing absenteeism. Therefore obtaining reliable data, *in situ* and real-time of VOCs concentration in indoor industrial atmospheres to prevent exceeding safety levels and assuring an acceptable air quality becomes an important aim in environmental science as well as in sensor technology applied to environmental protection. Optical fibre (OF) sensors show very appropriate analytical characteristics and attractive advantages, especially when compared to the more traditional chemical sensors and classical analytical methods, for pollutants monitoring. They show electromagnetic interferences immunity, high durability, small size, low maintenance cost, fast response and simple design (M. Campbell, 1997). Furthermore, OF sensors provide the possibility for accurate and safe detection of toxic compounds besides showing high potential for remote measurements in inaccessible and harsh environments, in a continuous mode operation. In recent years, many research groups (Bariáin et al., 2001; Elosúa et al., 2006; Abdelmalek et al., 1999; Abdelghani and Jaffrezic-Renault, 2001) focused their attention on OF sensors as a tool for monitoring the concentration of VOCs, including BTX. Some miniaturized devices for field VOCs detection have been reported, including for example a microfluidic device mesoporous silicate adsorbent (Ueno et al., 2003), but they show a low analytical selectivity and/or sensitivity.

This work aimed at the development of a new and portable OF sensor for remote BTX monitoring in confined athmospheres of industrial environments. The optical component of the developed sensor consists on a monomode OF pigtail, core and cladding diameters of 9/125 µm, respectively, integrated in a 50:50 Y optical coupler. A small length of the fibre was uncladded and cleaved on a length of 20 mm with a precision fibre cleaver and sensitized with a nanometric film of Poly[methyl(3,3,3-trifluoropropyl)siloxane] (PMTFPS) deposited by spray technique. The polymeric film thickness was estimated as about 2 nm by Rutherford backscattering spectrometry (RBS). The polymeric film exposure to organic vapours leads to a variation in its refractive index and consequently changes in the reflected optical power. The complete OF sensor set-up included a portable laser diode at 1550 nm and a photodetector (both from Oz Optics) to measure the modulated optical signal. The air sample from the industrial environment is obtained by a vacuum pump by continuously sampling of air

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at a flow rate of 0.2 Lmin-1 controlled with a mass flowmeter. The sensor system is also constituted by a glass tube (GT) with an incorporated tape heater containing a fused silica fibre coated with polydimethylsiloxane (PDMS) and an analytical tube (AT) containing the sensitized OF fibre sensor. Data acquisition was performed by wireless between the laptop and the photodiode with a software home made. The OF sensor was previously calibrated with a standard mix of aromatic volatiles compounds: benzene, toluene and o-xylene, 100 µg/mL of each compound in methanol. Changes of up to 17 dB in the reflected optical power were detected after injection of 0.03 µg of BTX which highlights the high potential of the sensor for aromatic compounds detection, allowing their determination at very low levels. The estimated detection limits of the aromatic hydrocarbons analysed, based on 3 times the residual standard deviation were, 1.4 ng for benzene, 1.3 ng for toluene and 1.2 ng for o-xylene. In order to test and compare the performance of the developed sensor with gas chromatography-flame ionization detector (GC-FID), ten different concentrations of a standard mixture of BTX were determined with both methods and no statistically significant difference ($\alpha = 0.05$) were found between the two methods. After some laboratorial tests the sensor was applied to air monitoring in a confined environment at a Portuguese solvent industry. Air samples were also collected for GC-FID analysis according NIOSH method 1501 (NIOSH, 2003).The obtained results at the confined atmosphere are much lower than the occupational exposure limits recommended for benzene (3.19 mgm^{-3}), toluene (3.77 mgm^{-3}) and o-xylene (4.34 mgm^{-3}) by NIOSH (NIOSH, 2003). It is important to higlight that the analytical signal monitoring was performed at 60 m from the sampling local. From the obtained results it was possible to conclude that the developed sensor allows both qualitative and quantitative BTX monitoring *in situ*, in a continuous way and in real-time. The easiness of operation, short analyical time, lower safety requirements, compact design and no expensive technology were also figures of merit checked for the developed remote sensor.

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Influence of the concentration of locust bean gum on the gelling ability of whey peptic hydrolysates

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Keywords: Whey Protein, Locust Bean Gum, Gelation, Hydrolysis, Rheology

The functionality of whey proteins can be changed by the presence of other components. Synergistic effects have been found between whey proteins and several polysaccharides such as galactomannans, xanthan or carrageenan (Croguennoc et al., 2001; Turgeon and Beaulieu, 2001; and many others). The effect of limited proteolysis in the interaction with polysaccharides is hardly ever mentioned. Locust bean gum (LBG) is a galactomannan (non gelling neutral polysaccharides found in the endosperm of *Leguminosae*) widely used in the food industry as a thickening agent.

In this work the effect of LBG on the gelation of aqueous whey protein hydrolysates (10 % w/w) from pepsin was assessed at pH 7.0. Whey protein concentrate (WPC) and hydrolysates with a degree of hydrolysis (DH) of 1.5 (P1.5), 2.5 (P2.5) and 4.9 % (P4.9) were used. Different LBG concentrations were tested: 0, 0.1, 0.3 and 0.55 % (w/w). Dynamic oscillatory tests were performed in a controlled stress rheometer AR2000 (TA Instruments, Delaware, USA) fitted with a parallel plate geometry (40 mm diameter, gap 800 μm). Each sample was equilibrated during 5 min; this step was followed by a frequency sweep ("mechanical spectrum") from 100 to 0.1 Hz at a strain of 5 %. Then a temperature ramp from 20 to 80 ºC was applied, at a rate of 2 ° C.min-1, after which the temperature was maintained at 80 ºC for 3 h. At the end of this time sweep the sample was cooled back to 20 ºC, at the same constant rate (2 ºC.min-1). The mechanical properties of the resulting gel were monitored at 20 ºC for 1 h. A full factorial design was used considering two factors (LBG concentration and degree of hydrolysis) and three levels for each factor and a quadratic model was adjusted with Design Expert 6.0.6 (Stat-Ease, Inc. Minneapolis). Two replicates of the experiments with WPC (DH = 0) were used to estimate errors and determine if the lack of fit of the chosen model was significant. Further refinement of the empirical model was made by excluding the factors that were found to be insignificant, one at a time, as the exclusion of one factor may influence the other.

The evolution of the parameters follows the general behaviour reported for many biopolymer heat-set gelation processes including whey proteins gelation (see for instance Paulsson et al., 1990; Gosal and Ross-Murphy, 2000). Initially *G"* is slightly higher than *G'* because of the liquid nature of the sample and the absence of pre-aggregated protein molecules. As the temperature rises both moduli decrease until the gelation treshold is achieved (either before the end of the temperature ramp or during the time sweep step). As this point approaches, a sudden increase in the values of *G'* and *G"* is observable. However *G'* rises much faster and the crossover *G'*-*G"* point was considered the gelling point. By the same time the values of the loss angle decrease even more markedly, sign of the increase of the elastic behaviour. The increase in the storage modulus and the reduced phase angle indicate the formation of viscoelastic gels. *G'* continues to increase after the gel point as more and more protein reinforces the weak tridimensional network initially formed, enhancing its elasticity.

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Although the overall gelation patterns were similar for all tested samples the corresponding gelling parameters (*G'*, *G"*, δ, *Tg*, *tg*) were quite different.

In the case of hydrolysates alone, *G'* was higher for P1.5 (657 Pa) followed by P2.5 (138 Pa). These two were stronger than WPC at this concentration (*G'* = 204 Pa) indicating that they were stiffer. They were also more elastic as the loss angle was smaller. Apparently pepsin is effective in improving the gelling ability of whey protein gels for low degree of hydrolysis, possibly because β-Lg (the main gelling protein) is resistant to pepsin. In fact, P1.5 still has all the β-Lg intact and P2.5 still has 96 % of intact β-Lg. This improvement might be due either to the presence of low molecular weight hydrophilic peptides which can reduce electrostatic repulsions between intact β-Lg molecules enhancing protein-protein interaction or to the partial unfolding of α-La and BSA exposing their hydrophobic residues, therefore improving their individual gelling ability and/or allowing for a better interaction with the intact β-Lg.

LBG alters the microstructure of whey protein gels by modifying the equilibrium between aggregation and segregation. The gelation time was also decreased. The behaviour of gels from whey proteins or whey protein hydrolystates towards the presence of LBG was very similar. The increase in the LBG concentration generally led to a decrease in the gel strength. However, for whey proteins a small amount of LBG (0.1 %) in the presence of salt leads to a big enhancement in the gel strength probably due to an increase in the protein concentration of the protein enriched phase. Further increases in the LBG concentration led to a decrease in the gel strength.

When analysing the influence of the concentration of LBG on gelling properties (*G'*, *G"*, tan δ and temperature of gelation), the differences between the hydrolysates and WPC with no polysaccharide and the hydrolysates with 0.1 % of LBG were usually sharp; this difficults modelling with a simple quadratic function. Therefore the statistical analyses and empirical modelling were performed only with the data from mixed systems for the LBG range of 0.1 – 0.55 %. By means of an analysis of variance it was concluded that all the adjusted models are significant. The effect of LGB concentration and of the degree of hydrolysis on peptic hydrolysates gelation was negative for *G'* and *G"*, though an interaction factor has also to be considered. For tan δ and the temperature of gelation a minimum value exists in the studied range of LBG concentration, while a maximum exists in the studied range of *DH* for the temperature of gelation.

The gelation process is very sensible to environmental conditions and to processing and often leads to rather coarse data. The factorial planning used allowed validating conclusions using fewer experiments than those needed if no planning had been used, while still getting statistical significance out of the results. However, as many factors are involved, the modelling of the process was not straightforward. A simple quadratic function was generally not enough to accurately describe the system behaviour.

As a general conclusion, it can be stated that it is possible to make all kinds of different gels by manipulating the protein concentration, the degree of hydrolysis, the amount of LBG and the salt content. It is important though to master the mechanism of phase separation in order to be able to design the adequate conditions for the desired texture.

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Enzymatic agent from red king crab hepatopancreas and its applications

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Keywords: King crab hepatopancreas, enzyme, hydrolysis, enzymatic hydrolysates.

Currently, biochemists in different countries are aimed at the development and improvement of enzymatic processes that are used in the production of enzymatic protein hydrolysates and modification of natural polysaccharides.

Special attention to this direction is determined by the high specificity of enzymatic reactions, which makes it possible to obtain unique compounds of a certain structure, and environmental friendliness of biochemical manufacture, in contrast to purely chemical manufacture, which is expressed in renunciation of large volumes of aggressive chemicals and absence of contaminated chemical discharges. Of special interest are peptides and chitooligosaccharides, whose biological activity is higher than that of natural protein and chitin.

The invertebrates of the Barents Sea have the great importance for development of a fishery of Northwest region of Russia. Except for food industry these objects have interest as sources of enzymes with various substrate activities.

The proteolytic activity of enzyme preparations from different taxons of the Barents Sea invertebrates, such as mollusks (*Serripes groenlandicus*, *Biccinum undatum*), crustaceans (*Paralithodes camtschaticus*, *Pandalus borealis*) and echinoderms (*Cucumaria frondosa*, *Asterias rubens*), was studied. The choice of them was conditioned by essential stocks of these animal species in the Barents Sea and insufficient development of rational technologies to process them.

The research of enzyme properties is actual for an (evaluation of a physiological state of trade objects, for extraction high-activity enzymes, and also to prepare with the its help the enzymatic protein hydrolysates.

The waste of Red King crab processing includes hepatopancreas, which is of interest as a source of various enzymes, including proteolytic ones (Kupina et al., 1999). Total proteolytic and collagenolytic activity of enzyme system of crab is greatly higher that activities in digestive organs of Vertebrates (Chen et al., 1991).

We studied the temperature, pH, and time dependence of proteolytic activity of enzymatic agent obtained from Red King crab hepatopancreas (Mukhin et al, 2001a). It was discovered also a chitin-degrading activity of this preparation (Novikov et al., 2003).

Enzymatic preparation from Red King crab hepatopancreas were shown to be capable of producing protein hydrolysates. Hydrolysis of protein-containing waste of Northern shrimp, King crab and Iceland scallop occurs most successfully at pH about 8 and 50-55 °C for 5-6 h in presence of 3-6 g enzyme per kg substrate (Mukhin et al, 2001; 2001a).

It is demonstrated that the hydrolysates can be used for growing test cultures (Mukhin et al., 2001b; 2005), and feed supplement for chickens and fishes (Mukhin et al, 2001a).

Our study confirms the presence of chitinolytic and chitosanolytic activities in the hepatopancreas of the red king crab, related to the specific diet of this species (Novikov et al., 2003; 2005). The maximum rate of chitin/chitosan hydrolysis by an enzyme preparation from crab hepatopancreas occurs at 36.5–37.0°C. Two pH optimums have been found for the enzymatic reaction under mildly alkaline and acidic conditions for both exo- and endochitinase activities. The enzyme preparation is most affine to partly deacetylated chitin with an acetylation degree within 40–50% (Novikov et al., 2007).

We have confirmed the presence and quantified the exochitinase and endochitinase activity of the enzymatic agent as well as demonstrated the absence of exochitosanase, deacetylase and N-acetylglucosamine deacetylase activities. We assumed that the chitinase and protease activities of the enzyme preparation are apparently exhibited by different enzymes.

The practical significance of our results is that they demonstrated that crab hepatopancreas can be used as a source of enzymes exhibiting not only proteolytic and collagenolitic activities but also chitinolytic and chitosanolytic activities for industrial modification of chitin and chitosan.

This enables maximum utilization of all components of commercial aquatic organisms and increases the efficiency of sea fishery owing to utilization of its processing waste. The study of the chitinolytic activity of the enzymatic agent from the red king crab hepatopancreas is of practical interest in terms of designing chitinase drugs for fighting against pests and treating fungal infections.

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Induction of lignolytic and hydrolytic enzymes production in *Pycnoporus sanguineus* by industrial wasterwater

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Keywords: xylanase, laccase, textile, cotton, bioscouring, dyeing

Several studies have shown that these fungus can degrade various xenobiotics, so they have the ability to produce lignolytics enzymes (laccases and peroxidases) and hydrolytic (cellulases and xylanases). Several studies in literature have demonstrated a possible application for the treatment of textile effluents and degradation of dyes of the following genera of fungi: *Phanerochaete, Trametes, Pleurotus, Pycnoporus* and others (Eichlerová, et al., 2007; Yang et al., 2005; Yesilada et al., 2003; Kunz et al., 2002), because they can synthesize a variety of metabolites, such as pigments and enzymes lignolíticas. However, despite these enzymes are related to processes of degradation also by other genera of Basidiomicetos, no report was found in literature reporting the ability of bioremediation of Pycnoporus sanguineos. Considering this, the present study has as main goal, to illustrate the ability of induction of laccase, peroxidase, cellulase, xylanase by *Pycnoporus sanguineos*, a white-rot fungi native from Florianopolis. The tests were performed by incubation with the same effluent individually and combined, from the process of scouring and dyeing, knitting 100% cotton. It was used to dye C.I. Reactive Black 5. This dye was selected for this study because it is widely used as a model for obtaining laccase of *Pleurotus sajor-caju* (Murugesan et al., 2007) and also to be quite resistant to natural degradation and proven mutagenic and carcinogenic character. (Zollinger, 1991). The production of enzymes was examined every 24 hours for 7 days and the efficiency of bioremediation was verified by reducing BOD, TOC and color. The results showed that there was a significant reduction of color and BOD effluent from the process of dyeing after the fourth day of incubation, corresponding to 78 and 55%, respectively. There were also high induction of all enzymes mentioned, especially the laccases.

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Alkaline hydrolysis of chitin and chitosan

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Keywords: chitin, chitosan, deacetylation, crystallinity

Chitin is unique natural polysaccharide occupying the second place after cellulose. In the initial state it does not find wide application because of power solubility and small reactivity. Usually chitin is exposed to modification by various ways for reception of derivatives, in particular chitosan.

Basic hydrolysis of chitin and chitosan results in splitting mainly acetamido linkages with formation of free amino groups and preservation of polysaccharide chain. In result well soluble high molecular weight polysaccharide is formed. Chitosan finds wide application in medicine, cosmetics, and agriculture (Muzzarelli, 1977; Goosen, 1997).

In this work we have studied process of basic hydrolysis of chitin and chitosan. The main purpose has consisted in an establishment of the mechanisms responsible for splitting of amide links in an alkaline condition.

Many researchers mark, that at alkaline deacetylation of chitin it is not possible to receive degree of deacetylation (DD) equal 100 %. The rate of deacetylation falls practically up to zero at continuous conducting process in 60-90 minutes. Only retreatment by alkali allows to increase DD. After that the further deacetylation is again retarded. Features of deacetylation kinetics are considered in works (Kurita et al., 1977; Mima et al., 1983; Tsaih and Chen, 2003). Authors of work (Kurita et al., 1977) explain specificity of deacetylation process, first of all, by crystalline structure of natural chitin. The assumption that speed of deacetylation of chitin in crystalline regions proceeds more slowly than in amorphous has been stated.

On the other hand, authors of work (Ottoy et al., 1996) have assumed, that the diffusive mechanism of reaction of deacetylation prevails at basic hydrolysis. Diffusion rate of alkali inside of solids plays the general role.

We investigated influence of change of chitin and chitosan crystallinity on deacetylation kinetics (Chebotok et al., 2007).

We have confirmed with a method of X-ray crystal analysis, that at reprecipitation from alkaline and acid solutions crystalline structure of the dried up samples of chitin and chitosan practically is completely reduced. Only reprecipitated wet samples had diffraction patterns of completely amorphous solids.

It is known, that crystallinity of chitin decreases at increase of DD, and then (after 90-95 %) new crystalline structure of chitosan is formed (Kurita et al., 1977; Focher et al., 1992). If this fact takes place, the rate and depth of deacetylation should grow with increase of chitin DD, because of increase in amount of amorphous regions, and then in samples of chitosan again to decrease.

In works (Novikov et al., 1990; Novikov, 2003; Novikov, 2005) it has been shown, that the kinetics of chitin and chitosan deacetylation does not depend on an initial DD of polysaccharide. The form of kinetic curves appeared identical to chitin and chitosan with various starting DD up to 95 %. In our opinion, formation of solvated structures as a result of interaction of polysaccharide macromolecules and low molecular weight electrolyte with solvent affects on reaction rate of deacetylation.

So, rate of deacetylation and limiting DD at basic hydrolysis of wet starting chitin appeared below, than at dry one.

Further we have found completely unexpected kinetics at deacetylation of reprecipitated chitin. In the dry sample having reduced crystalline structure similar on structure of initial chitin the rate of deacetylation on the first part appeared less though limiting DD has reached those some of initial dry chitin. But at the deacetylation of wet amorphous chitin the rate of deacetylation on the first part and limiting DD were the lowest.

Obtained results allow to approve, that degree of crystallinity of the investigated samples renders considerably smaller influence on the rate of deacetylation, than an opportunity of formation of a hydrated environment around of polysaccharide macromolecules. Last interferes with nucleophilic attack of hydroxyl ions on glycosidic linkages and retards the reaction rate. The passing of two simultaneous reactions - deacetylations and formations low-activity intermediate which collapses at washing is possible. It is shown, that speeds of these two reactions are proportional to a degree of acetylation. Constants of reaction rates of deacetylation and formation of low-activity intermediate are estimated.

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Adsorption isotherms of galactomannan films

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Keywords: adsorption isotherms, galactomannan, water activity, edible films

The aim of this work was to study the behavior of water activity in different samples of edible films made of blend of galactomannan-corn starch, and glycerol used as a plasticizer, in order to characterize these films.

Seeds from *Adenanthera pavonina* L. were collected in Fortaleza-Ceará, Brazil. One hundred grams of seeds were boiled for 20 minutes. After that, the seeds stayed 24 hours in distilled water for imbibition. Testa and cotyledons were removed manually and only endosperms were used. About 500 mL of distilled water were used to homogenize endosperms to obtain a viscous liquid. This liquid was added to ethanol 95º (1:2, v/v, respectively) to precipitate soluble polysaccharides. The precipitate was washed with acetone and dry in desiccators.

A dispersion of corn starch was prepared according to Bertuzzi et al. 2007. This dispersion was mixed with different parts of a dispersion of galactomannan 1.0% according to a designed experimental matrix. Glycerol (30% w/w) was used like a plasticizer. Different films encoded by different parts of polysaccharide dispersions were prepared. The mixes of galactomannan and corn starch were dried at 35ºC for 15 hours. When the films have been formed, they were removed from moulds. The masses of the films were determined and the films were put on desiccators for about 15 to 20 days to remove water until their weight were kept constant.

An equipment LabMaster-aw (Novasina, Switzerland) was used to perform all measurements of water activity. The standard salts used were 0.11, 0.33, 0.53, 0.75, 0.90 and 0.97 aw (Greenspan, 1977). All data acquired were done at constant temperature of 25 ºC. The weight of samples was registered in a precision balance Sartorius model 2462 (0.0001 g). Adsorption curves were built with Microsoft Excel software. All adsorption curves showed distinct plots. The water absorption in lowest values of aw was influenced by corn starch content. With the increasing of the parts of corn starch dispersion into galactomannan dispersion, it was possible to see the films had absorbed water in the lower values of aw (0.11, 0.33 and 0.53). An opposite behavior was seen with decreasing of parts of corn starch dispersion. The behavior of water activity in the different samples of films made of blend of galactomannan-corn starch is an accurate parameter to evaluate the stability and characterize these films.

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Application of surfactants to membrane separation processes

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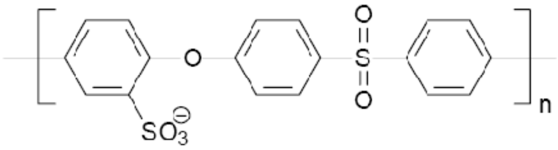
Keywords: modified polyethersulfone, ultrafiltration, bovine serum albumin, cadmium

Membrane separation processes being more environmental friendly than conventional separation techniques are receiving considerable interest in biotechnology and metallurgical industry. Among membrane processes ultrafiltration has deserved some special interest. However wider application of ultrafiltration has been hindered so far due to the detrimental effect of fouling, i.e. an irreversible process caused by solute sorption. In biotechnology, ultrafiltration membranes are commonly used for concentration and purification of protein solutions. Very often protein molecules block membrane pores that result in a decline of membrane flux. Most thermo-resistant polymers have adequate chemical and biological stability suitable for membrane formations but their strongly hydrophobic character accelerates sorption of proteins on the membrane surface. Polyethersulfone (PES) popular membrane material belong to such polymers. There are several methods for the membrane protection from fouling: physical modification by surfactants [Jönsson and Jönsson, 1991] and introduction of ionic groups to the membrane materials by plasma [Poźniak et al., 2002] or chemical treatment [Poźniak et al., 1995].

Ultrafiltration (UF) has proved to be an efficient way to remove from water high molar substances (polymers, proteins) - particles of a size ranging from 2 to 100 nm and M larger than 500 Da. As a consequence, the pore size of UF membranes is too large to reject small molecules, like metal ions. Micellar enhanced ultrafiltration (MEUF) is a surfactant-based separation process that has been used to remove heavy metal ions from dilute streams [Scamehorn et al., 1994]. In this process, an anionic surfactant at a concentration higher than its critical micelle concentration (cmc) is added to the aqueous stream containing the dissolved solutes. The negatively charged micelles force the cations to be bond to the micelle interface. Micelles (size above 10 nm) with the bonding cations are then separated by UF using membrane of suitable porosity, capable of retaining micelles.

The two systems were used to obtain more permeable membranes from polyethersulfone (PES):
(i) adsorption of surfactants: anionic - sodium dodecyl sulfate and nonionic - monoether dodecylpentaoxyethylene glycol
(ii) chemical introduction of sulfonate groups to the polymeric chains.



The polyethersulfone (ULTRASONE E-2020P from BASF) was sulfonated using a mixture of chlorosulfonic acid (CSA) and 1,2-dichloroethane. Porous asymmetric membranes were formed by a phase-inversion method from: 13.5%-wt. solution of PES, and 30%-wt. solution of sulfonated PES in N,N-dimethylformamid. Water was a coagulation medium.
Effect of the membrane hydrophilization by surfactants adsorption was monitored by evaluation of permeate fluxes and resistance of membrane to fouled by bovine serum albumin. Hydrophilization was carried out according to different protocols: in one cycle

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by immersion in a surfactant solution and in two cycles by a subsequent sorption of two surfactants - anionic and nonionic one. From the flux values before and after protein filtration the transport parameters: fouling index (FI), reduction of the flux in filtration (RF) and solute rejection (R), were calculated according to our procedure [Poźniak et al., 2006]:

$$FI = 1 - (J_f/J_o) \quad (1)$$

$$RF = 1 - (J_p/J_o) \quad (2)$$

$$R = 1 - (c_p/c_o) \quad (3)$$

J_o - flux of buffer (pH 8) through the freshly mounted membrane, J_p - flux of buffered BSA through the same membrane, J_f – flux of buffer through the membrane after BSA filtration, c_p and c_o concentration of protein in the permeate and in the feed solution, respectively.

In comparison with an unmodified PES membrane, the modified species show a significant improvement of their performances in protein filtration. Fouling (FI) is less intensive and reduction of the flux in BSA filtration (RF) shows lower values. It is well known that the BSA molecules have a negative charge at pH=8. The negative charge appeared also on the membrane modified with SDS, hence some repulsive effect should be expected. With a nonionic surfactant (EDPEG), improvement in the filtration indices are ascribed to the thick hydrophilic layer on the membrane surface which keeps the protein molecules over the pore entrance. [Chen et al., 1992]. It is worthy to note that the best results were obtained for the BSA filtration through the membrane modified with a consecutive sorption of SDS and EDPEG.

The second reason of surfactants use in the filtration processes is a phenomenon of enhanced removal of harmful species conducted by the micelle that are too large to penetrate membrane pores. To check the effect of surfactant that is needed to remove Cd(II) ions, the MEUF and the ligand-modified micellar enhanced ultrafiltration (LM-MEUF) processes were investigated. 8-hydroxiquinoline was used as the ligand. The latter has a high degree of solubilization in the micelles and an increased tendency to selectively complex the target metal ion [Paulenovà et al., 1996]. The highest rejection coefficient was observed for SDS concentration equal to 81 mmol/dm^3 in MEUF (10 cmc) and 40.5 mmol/dm^3 (5 cmc) in the LM-MEUF process. The sulfonated polyethersulfone membrane worked more effectively that its off-charge analogue.

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Comparison of the antimicrobial efficacy of commercial surfactants with the respective pure components

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Keywords: Surfactants; antimicrobial action; sanitation, environmental impact, mixed bacterial growth; bacterial resistance

Surfactants constitute the most important group of detergent products. Generally, these are water-soluble surface-active agents comprised of a hydrophobic portion, usually a long alkyl chain, attached to hydrophilic or water solubility enhancing functional groups (McDonnell and Russell, 1999) . They are generally characterized by properties such as the critical micelle concentration (CMC), the hydrophile-lipophile balance (HLB), chemical structure and charge, as well as by source (chemical or biological) (Pereira, 2007; Van Hamme *et al.*, 2006).

In real world, microorganisms are often found associated in complex communities (usually comprising more than one microbial species) and exposed to stress factors that can lead to the development of bacterial resistance (Versalovic and Relman, 2006) and thus to the failure of the traditional sanitation procedures.

The development of surfactants with antimicrobials properties aims to reply to a bigger consumption and effectiveness. Nowadays, there is a huge diversity of new products that give rise to new concerns. We assist to an increasing efficiency of the detergent products mainly due to the inclusion of aggressive chemical products to guarantee the inhibition of microorganism growth (Gilbert and Mcbain, 2003;McDonnell and Russell, 1999;Simoes *et al.*, 2006). In this scenario, environment questions emerge aiming to safeguard the Nature and thus the conditions where we live and enlightening to the negative impact that the residues of those products detergents can have in the Environment.

With this study it was aimed to investigate the antibacterial effectiveness of commercial surfactant formulations (Dettol and Cutasept), used in the domestic cleanliness and disinfection of surfaces and equipment, as well as, in food industries and medical area. It was also aimed to compare their sanitation performances with the respective pure surfactants products: cetyl trimethylammonium bromide - CTAB and benzalkonium chloride - BAC (cationic surfactants) and dodecylbenzene sulfonic acid - DBSA (anionic surfactant) using Gram positive (*Staphylococcus aureus*) and Gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria from collection. In order to mimic conditions similar to actual situations, simple and mixed growth (achieved through the development of the bacteria, respectively, separately or in mixture) were implemented, as well as, *P.aeruginosa* strains previously adapted to BAC and CTAB.

The susceptibility of the several bacterial strains to the antimicrobial action of all the surfactants was characterized through the determination of the Minimum Inhibitory Concentration (MIC) defined as the minimum concentration where no suspended microbial growth was detected. To obtain the MIC of each product, single and mixed bacteria growth curves were developed in microtiter plates, during 48 h, in the presence of increasing concentrations of each one of the surfactant products.

The analysis of all results gathered with this work allowed to realize that, for equal concentration of the same product, the growth of Gram positive bacteria was more easily inhibited than the growth of the Gram negative bacteria. In the set of the surfactants tested, the commercial formulations Dettol and Cutasept showed to be the most efficient, since they inhibited single and mixed bacterial growth even when diluted

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4-fold or even 8-fold, in the case of Dettol. These results seem to point out that the concentrations of the commercial products are very elevated and higher than the really needed. It is important to take this fact in account since residues resulting from the use of those products can have tremendous negative impact in Environment and be responsible for bacterial resistance development. The MIC of the products when applied to the bacteria in mixed growth are comparatively higher than the ones determined with simple growth. This evidence indicates that the bacteria establish favourable microbial relations when developed together. In mixed growth, *P.aeruginosa* appeared to dominate over the others since the trend of the mixed growth curves was similar to the one obtained with that bacteria solely. The experiments carried out with the adapted bacteria revealed that the products concentration required to inhibit the single or mixed growth of the adapted *P.aeruginosa* was higher than the one needed in the experiments with the non-adapted bacteria. These results seem to be a sign of development of bacterial resistance to the products tested. Moreover, some cross-resistance seems also to occur since *P.aeruginosa* adapted to CTAB become less susceptible to the action of BAC. The increase of the MIC to values close to the CMC, in the case of the pure active agents, represents an additional concern as the use of those doses can be responsible for generating toxicities problems.

With the present study it was possible to conclude that: i) the commercial products appear to be more aggressive than the pure components even when used diluted; ii) bacteria developed in mixed cultures are less sensitive to the chemical products tested; iii) the previously contact of *P.aeruginosa* with sub-MIC concentrations of BAC and CTAB give rise to bacterial resistance.

The data collected with this study highlighted for the rational and conscious use of chemical commercial products to control bacterial growth in actual environments.

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Functionalized nano-microstructures to combat biofilms

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Keywords: micro- and nanoparticles, biofilms, biocides

Biofilm formation causes serious problems in the food industry, cooling water systems, medical equipment, etc. In order to combat the growth and persistence of these undesirable biological matrices, chemical products with antimicrobial properties like biocides and surfactants are used, but these compounds are obviously deleterious to the environment.

Water treatments with antimicrobial purposes should therefore be carried out with a minimum consumption of biocides to reduce the environmental load downstream. The present work suggests a new technological approach to minimize the use of antimicrobial agents, based on the principle of drug-delivery systems where the antimicrobial compounds are transported on nano-microparticles or in nano-micro-capsules. In this preliminary study, the efficacy of the method against biofilm of *Pseudomonas fluorescens* was assessed, using benzyldimethyldodecylammonium chloride (BDMDAC), which is a surfactant belonging to the family of benzalkonium chloride, as a biocide carried on microparticles (diameter: 4 µm). The latter were prepared using the layer-by-layer self-assembly (LBL) technique (Cordeiro *et all*, 2004). The oppositely charged polyethyleneimine (PEI), sodium polystyrene sulfonate (PSS) and BDMDAC were assembled on polystyrene (PS) cores (Figure 1).

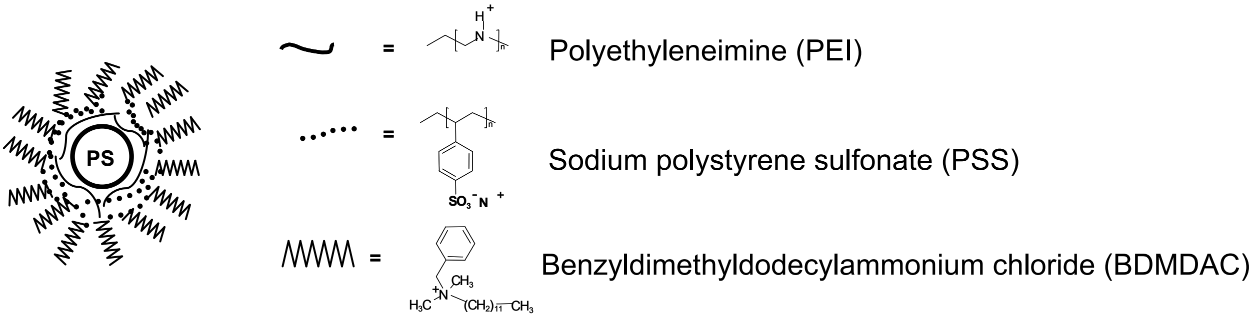

Figure 1: Schematic sequence of the formation process of the microparticles.

The BDMDAC coated particles were observed by CryoSEM and their composition by X-ray microanalysis (Figure 2). Their size distribution (Coulter Particle Size Analyzer) and zeta potential (Nano Zetameter) were also determined.

A pure culture of *Pseudomonas fluorescens* was grown in a 4 L tank for 24 h, under optimal growth conditions (T = 27 ºC, pH = 7 and glucose as carbon source) (Vieira and Melo, 1999). After the incubation period, the suspension was diluted in 5 L of distilled and sterilized water that was put to recirculate between the fermenter and a flow cell (where biofilm development on PVC coupons was monitored). After nine days, the coupons were collected and incubated for 30 minutes under two different conditions: i) saline solution; ii) solution of BDMDAC coated particles. In sterile saline solution (0.85% NaCl), the biofilm contained 1.6×10^7 CFU/ml. On the other hand, the biofilm exposed to the microparticles with biocide for 30 min contained no cultivable cells (determined by removing the biofilm from the coupon surface, diluting and spreading it on Plate Count Agar, followed by incubation at 30ºC during 24 h) – see Figure 3.

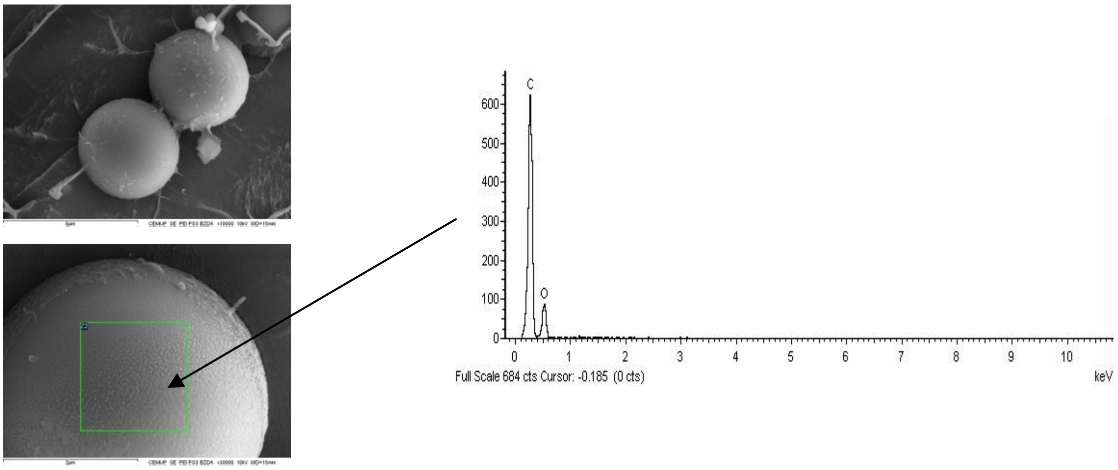

Figure 2: CryoSEM image of the coated particles and X-ray Microanalysis of the coating layer.

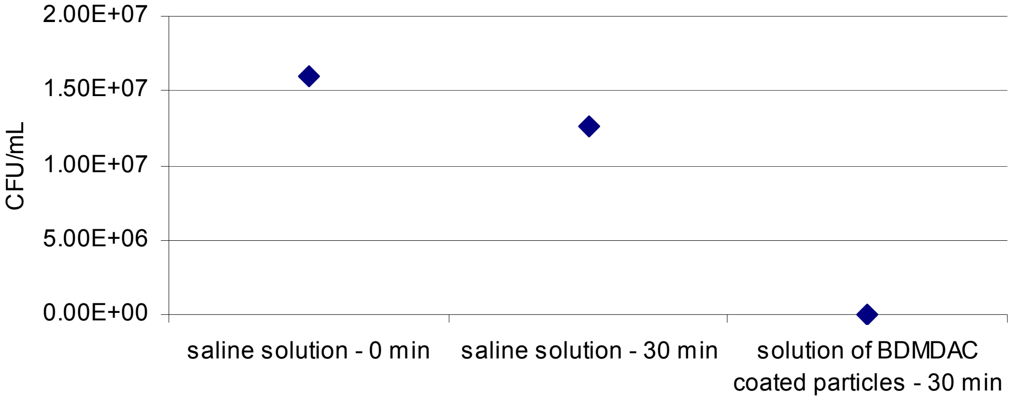

Figure 3: Number of bacteria colonies (CFU/mL) in biofilms subjected to different conditions.

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Synthesis and characterization of organic phase gold nanoparticles obtained through chloroaurate ions reduction with sodium citrate

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Keywords: gold, nanoparticles, synthesis, sodium citrate

The reproductible making of gold nanoparticles of well-defined size and good monodispersion is still a challenge for researchers.

Nanomaterials have a great importance in industrial applications due to their electronic, magnetic, optical [Wyatt et al.] and catalyst [Králik and Biffis] properties. Another important feature of nanoparticles is their large spectrum (range), an important characteristic in catalytic processes. Nanoparticle applications are found in communications, data storage [Schmid and Ditlbacher], solar energy conversion [Graetzel, M.]. It is well known that certain physical and mechanical properties of materials modify with the reduction of their particle size.

The synthesis of powders and their subsequent processing have a great influence over the final properties of the resulted materials. The selection of suitable processing methods determines, to a great extent, the results obtained for the final products.

At present, the obtaining of nanopowders for different practical applications uses nonconventional processing methods, capable to offer better homogenity, increased purity, low sintherizing temperatures and a fine microstructure, with as small as possible grains.

Specialized literature presents a series of synthesis methods of different shapes [Hayat] and size [Jana] gold nanoparticles which, by large, can be classified into two sections, function of their growth environment, i.e. polar (aqueous) or non-polar (organic) environment.

This paper presents a method to produce stable gold nanoparticles by the reduction of hydrogen tetrachloroaurate ions in the organic phase, with sodium citrate. The characteristics of colloidal gold solutions reduced with sodium citrate were determined by UV-visible mass spectrometry, by using a CECIL spectrophotometer within a wave length between 400 and 700 nm.

Figure 1 presents the UV-Vis spectra of gold colloidal solutions in the first experiment (10^{-2}M) and in the second experiment ($5x10^{-3}$M) respectively. Curve 3 corresponds to the $Oct_4N^+AuCl_4^-$ complex resulted from the mixture of the tetracloroauric acid with the tetraoctil ammonium bromide solution in toluene.

A strong absorption can be noticed in case of curve 3, around the 347nm value of the $Oct_4N^+AuCl_4^-$ complex resulted from the mixture of the tetrachloroauric acid with the tetraoctil ammonium bromide solution in toluene. The absorption band is characteristic for the $AuCl_4$ $^{6-}$transfer band between the metal and the ligand. One can also notice that the absorption spectrum for curve 1 is flatter, which indicates a certain degree of aggregation of the obtained nanoparticles, in contrast to the sharper absorption spectrum registered for curve 2.The reduction of the $Oct_4N^+AuCl_4^-$ complex with sodium citrate leads to the formation of gold nanoparticles covered with the citrate ions, as illustrated by spectra 1 and 2 which reached their maximum around 530 nm.

The particle size and size distribution were determined by electronic transmission microscopy (TEM) and the obtained results exhibit their nanometric dimensions.

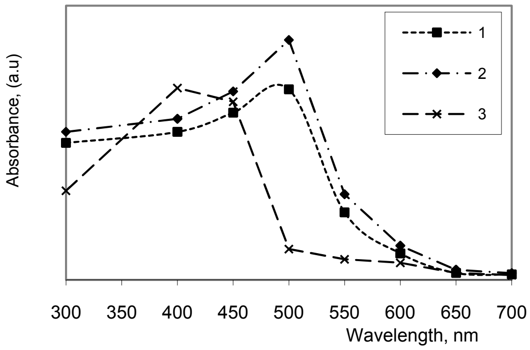

Figure 1. UV-Vis Spectrum of 10^{-2}M (curve 1), $5x10^{-3}$M (curve 2) gold colloidal solutions and of $Oct_4N^+AuCl_4^-$ complex (curve 3)

Figure 2 A presents the image obtained by electronic transmission microscopy of gold nano-particles obtained by the reduction of 0.011M tetrachloroauric acid with 10^{-2} M sodium citrate (**1**). A uniform gold nanoparticle distribution may be noticed. Figure B presents the distribution curve of nanoparticles dimensions. The continuous line overlapping the histogram is a Gaussian curve which gives information on the mean particle size, found to be in the range of 5-7 nm.

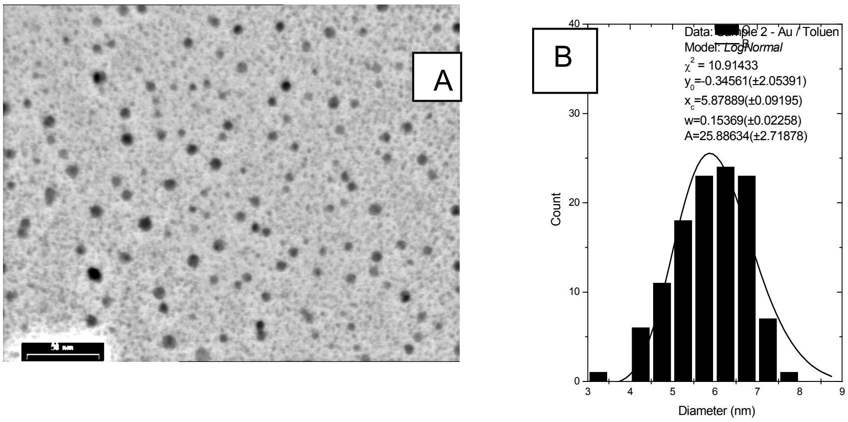

Figure 2. TEM image for A nano-particles obtained in the organic phase through 10^{-2} M sodium citrate reduction; B- Histogram of gold nanoparticle size distribution for the same sample.

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Flexible silica based xerogels and aerogels for spatial applications

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Keywords: sol-gel synthesis, silica based aerogel/xerogel, flexible nanomaterial.

Silica aerogels synthesized with $Si(OR)_4$ precursors have a nanostructured threedimensional network and exhibit low density (~ 40 Kg/m^3), high surface area (~ 1000 m^2g), low thermal conductivity (0,02 W/mK) and high transparency (~ 90 %); these unique properties allow their use in several applications, namely as thermal or acoustic insulators, dielectric or optical materials, filters and catalysts (Pierre and Pajonk, 2002, Rao et al., 2006). However, these aerogels are very brittle, absorb moisture and deteriorate with time.

Flexible and hydrophobic aerogels would allow achieving improved performance of these materials in particular applications. Using methyltrimethoxysilane (MTMS) as precursor, the obtained silica based aerogels can fulfill this need. In MTMS, one OR group of $Si(OR)_4$ is replaced by R, with $R=CH_3$. This group is not attacked during the hydrolysis step of the synthesis and remains in the aerogel structure, providing the hydrophobicity and flexibility required (Rao et al., 2006, Baghat et al. 2007). The density and the surface area are also controlled by the drying technique, being the ambient pressure dried gels – xerogels – more dense and less porous than the gels dried in supercritical conditions – aerogels.

Materials and methods

MTMS (98 %), methanol (99,8 %), oxalic acid (99 %) and ammonium hydroxide (25 %) were used as precursor, solvent, acid and basic catalysts, respectively. The silica based gels were synthesized by a two-step acid-base catalyzed sol-gel process, as described by Rao, Baghat et al. (2006, 2007). At first, MTMS was diluted in methanol and then water was added, in the form of an oxalic acid solution (0.1 M), to promote the hydrolysis of MTMS – acid step. After 24 h, an ammonium hydroxide solution (10 M) was added, drop by drop, and the condensation of monomers began, forming a sol – basic step. The molar ratio of MTMS:methanol:acidic water:basic water was 1:35:4:[1.8-9.4]. The sol has gone through a gelation and an alcogel was formed. The alcogel was aged for two days at 27 ºC and then dried. In the beginning of this study, the drying was done at ambient pressure. To form the xerogel, the alcogel was transferred to a ventilated oven, at atmospheric pressure, and submitted to a temperature cycle: 24 h at 60 ºC, followed by three stages, of one hour each, at 100ºC, 150 ºC and 200 ºC. The autoclave to dry the gel at supercritical conditions is being set and the results will be published in the final paper. The elemental composition (C,H,N,O), chemical structure, surface area, density and contact angle of the xerogels were accessed by elemental analysis, Fourier transform infrared spectroscopy, nitrogen gas adsorption, weight and volume measurements and a contact angle technique, respectively.

Results and Discussion

During the basic step of the synthesis, a sharp pH increase from ~ 2 to ~ 9 was observed with the addition of the first 2-3 drops. After, the pH increases slowly towards its final value (10-11). The condensation started with the beginning of the catalyst addition and the solution became milky, proving that the main effect was achieved with a tiny amount of catalyst. In a fully condensated material, the repetition units of the inorganic polymer have Si:O:C:H molar ratio of 1:~1.5:1:~3, neglecting the H atoms on the terminal groups. This corresponds to a theoretical C:O:H mass percent of 18.1:36.3:4.5. Comparing these values with the elemental analysis results – Table 1, it can be concluded that all the samples have only one CH_3 group per Si in its structural formula, since a good agreement for the % C is obtained. This means that the hydrolysis was complete. Also, the hypothesis of a fully condensed material is validated by this agreement, especially for samples with B ≤ 6. This was expected, because the pH rise is accomplished by a small volume of the catalyst, as a consequence of the high concentration of its solution. The measured values for the % H are higher than 4.5 %, what can be explained by the contribution of H in OH terminal groups. The N content obtained in the experimental results proves the existence of impurity traces in the dried material, since only the basic catalyst had N atoms. The theoretical and experimental values for the % O are very different, because the temperature used in the elemental analysis (1060 ºC) was not enough to break the Si-O bonds.

Table 1 Elemental compositions, surface areas and pore sizes, and contact angles of the obtained xerogels.

Molar ratios	Elemental analysis				Surface areas and pore size		Contact angle
B [a]	% C	% H	% O	% N	BET surface area (m2/g)	BJH pore size [b] (Å)	
1,8	19,8	5,8	1,5	0, 17	-	-	-
3,0	17,4	5,6	1,6	0,32	315,26 ± 4,53	32,5	152º
4,0	19,1	6,0	1,5	≤ 100 ppm	385,77 ± 6,61	32,3	141º
6,0	19,5	5,8	1,5	0,14	388,46 ± 7,08	31,6	-
8,0	16,5	5,6	1,6	0,14	-	-	126º
9,4	15,4	5,4	1,5	≤ 100 ppm	-	-	-

a) B = $(H_2O)_{Basic}$/MTMS; b) Based on desorption isotherms.

A decrease in the density of the xerogels with the increase of B is observed – Fig. 1. This decrease is more pronounced up to the sample synthesized with B = 4. For higher B values, the variation is almost within the experimental uncertainty. As the xerogel became more powdery and weak with the increase of B (with B = 9.4, the xerogel was a powder), 4 was considered the optimum B value to the desired properties.

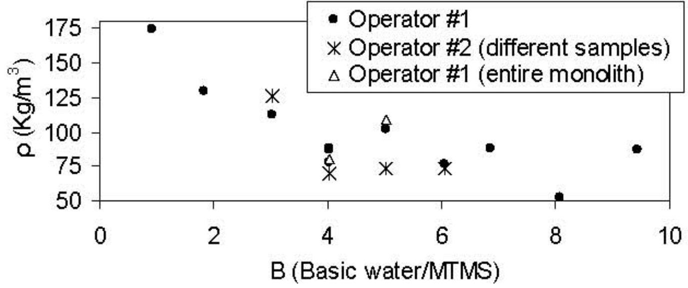

Figure 1 Density for several molar ratios of {basic water/MTMS} tested.

Figure 2 Xerogel monolith.

The sample with B = 3 has lower surface area than the samples with B = 4 and 6. This was expected, because the xerogels with B < 4 are more dense and rigid. The surface areas are comparable in the other two samples. The pose sizes obtained for the tested xerogels are consistent with a mesoporous structure, as they are in the interval 20-500 Å. The contact angle values – Table 1 – confirm the high hydrophobicity of the obtained xerogels and Fig. 2 shows the flexibility of these materials.

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Effect of operating conditions on the extraction of β-glucans from barley

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Keywords: β-glucan, barley, extraction

The aim of this work is to analyze the effect of operating parameters on the extraction of β-glucans from different varieties of barley. Several experiences have been carried out in batch extractors with a reference experiment in order to check the influence of temperature, pH, time, solvent and flour:solvent ratio. Time and temperature showed to have a strongly marked influence: the higher temperature, the higher extraction rate. Higher temperatures led to improve the extraction yield, increasing the amount of starch co-extracted. Extraction time was a critical factor up to 3 hours. Longer times did not increase the amount extracted. The influence of pH was not very remarkable, just generating a slight increase in extraction yield up to 8; beginning to decrease from that value. The use of alcohols (methanol and ethanol) as solvents, did not improve the extraction rate. On the other hand, it was observed an important decrease on the β-glucan solubility, greater the higher concentration of alcohol employed and the longer carbon chain. Finally, a extraction yield form 40-50% was obtained when operating at solvent:flour ratio form 7 to 12, at 55ºC, in almost all range of pH, when lasting the extraction process more than 2,5 hours..

Introduction

β-glucans are a kind of non-starchy polysaccharides that can be found in several kinds of cereals such as barley, oat or rye in concentrations from 2 to 12% in dry basis. They form part of the dietetic fibre and contribute to improve the human health by means of controlling of cholesterol and glucose concentration in blood (Brennan, 2005). Extraction of β-glucans form cereals has to deal with several difficulties. A typical extraction process involves, at least, three stages (Laroche, 2007): inactivation of endogenous enzymes, extraction of β-glucans with a suitable solvent, and finally a purification-isolation step, because other chemicals present in cereals (proteins, saccharides, or starch) can be co-extracted. All these steps increase the complexity of the overall process, increasing the economical costs, therefore. This is the most limiting factor for the extraction process.

In this work a simple solid-liquid discontinuous extraction process from different waxytype barley (H13-06 (hull-less) and D24-06) with hot water was proposed. The effect of most important operating parameters such as temperature, pH, extraction time and polarity of the solvent was checked in order to optimaze the continuos extraction process. Moreover, a reference experience based on literature revision carried out at 55ºC, 3 hours, water as solvent, solid:liquid ratio 1:10 was chosen as a comparative tool (Papageorgiou et al., 2005).

Results

β-glucan extraction yield, expressed as the ratio of β-glucan in the extract generated to the total amount of β-glucans in the barley flour, was 41,5±1,0% for D24 and 47,2±2,4% for H13 for the reference operating conditions.

<u>Temperature effect</u> (35-75ºC): It was observed a costant increase in the extraction yield when increasing the temperature. However when temperature is higher than 55ºC, operational problems connected with the higher suspension viscosity and the

coextraction of starch in the extract are observed. For these reasons, the optimal extraction temperature was fixed in 55ºC.

Extraction time effect (0.5-5 h): Times under 2,5 hours were found to be too short (yield fell down under 40%), but an extraction time of 5 hours did not entail a dramatic increase in the amount of β-glucan extracted, rather it kept constant. After three hours of extraction, equilibria was reached.

pH effect (6-11): An slight increase in extraction yield was produced when pH increases in the operating range 6-9. However, a further increase (pH 11), decreases the amount of β-glucans extracted. This decrease with pH was more marked in the case of H13 type. This could be due to the depolymerisation of our targed compounds.

Solid (flour):liquid ratio (5-15) was also tested for H13 variety. An important change could be observed when this ratio was shift from 5 to 10, when extraction yield was increased by 50%. The increase of this ratio to 15, did not produce a positive effect in the extraction of β-glucans. When using ratios of 8, 10 or even 12, it was observed a constant yield, around 47%. This result showed that it is possible to operate at lower solid:liquid rate (at 8), which is important taking into account a further purification step. Using less amount of solvent means simplifying the concentration of β-glucans.

Polarity of the solvent can be classified as a critical parameter. This part of the study was developed using the D24 variety. The better results were obtained with water as solvent (yield was 41,5%). When an alcohol was used as solvent (5% in volume), the extraction yield decrease slightly to 41,4% in the case of the use of methanol and to 34,1% when ethanol was used. When the concentration of alcohol was increased (50% or 100%), it was not possible to extract β-glucans. The amount of polymer extracted in those cases was under the detection limit of the analysis method. According to this result, alcohols can not be used as co-solvents. This property would allow to the alcohols to be used to purify β-glucans, using the alcohols as anti-solvent.

Conclusions

Initial conditions fixed for discontinuous extraction of β-glucans from barley with water (55ºC, 3h and flour:solvent ratio of 8) are suitable to get extraction yields in the range 40-50% for both varieties of barley tested. Time and temperature showed to have a strongly marked influence: the higher temperature, the higher extraction rate. Longer times than 3 hours didn't increase the amount of β-glucans extracted. The influence of pH was not very remarkable, just generating a slight increase in extraction yield up to 8; beginning to decrease from that value. The use of alcohols (methanol and ethanol) as solvents, did not improve the extraction rate. The operation in 3 steps of one hour each one, allowed to increase the maximum amount of β-glucan up to 60%. This result shows that β-glucan extraction can be enhanced when increasing the number of extraction steps, which justifies the need to develop a continuos extraction process.

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Alternative route to poly(lactic acid) synthesis

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Keywords: Poly(Lactic Acid), Biodegradable, Polycondensation, Molecular weight, Sustainability

An increasing effort in the development of sustainable alternatives to petrochemical origin materials has been recently registered, mainly due to environmental reasons. Poly(lactic acid), PLA, is an aliphatic polyester, biodegradable/compostable, biocompatible, and bioreabsorbable, obtained from a 100 % renewable raw material, the lactic acid. Therefore, for some aplications, PLA is a promising substitute of conventional polymers. Due to its hydrolytical instability and good biocompatibility, poly(lactic acid) can be used in biomedical, pharmaceutical and cosmetic industries, and is approved by the US Food and Drug Administration (FDA). On the other hand, to be used as a commodity, lactic acid polymers need to have a high molecular weight and good mechanical properties. PLA, with high molecular weight, has mechanical properties similar to the conventional thermoplastics, like PET, and can find applications in important technological fields, as films for agriculture, fibers for the textile industry, packaging and composites. However, high molecular weights are difficult to obtain, mainly due to the amount of water that is formed during the polymerization and its influence upon reactions´ equilibrium. High molecular weight PLA can be synthesized by two routes: ring opening polymerization of the lactide, ROP, or step-growth polymerization. The first mechanism is an effective and the most used method, but quite complex and an expensive process [1,2]. The second one, developed by Ajioka et al. 1995, has disadvantages for the environmental, since it uses organic solvents with high boiling point as reaction medium, however it has proved to enable achieving high molecular weights [3].

The limitations of the methods mentioned previously justify the study of new ways to obtain biodegradable lactic acid based polymers. In the first stage of this project, the polymerization of lactic acid by a step-growth mechanism has been experimented. Although this technique is less developed, it is considered to be less complex, and there is room for improving the current reactions´ procedure, in order to reduce some of its disadvantages. On the other hand, with this method it is expected to be possible to act upon the properties of final polymer by controlling the molecular weight, crystallinity, isomer ratio.

In this work, the discussion is centered in the main results obtained in the first stage of this project. The procedure for PLA synthesis begins with the distillation of the lactic acid solution. After the distillation, the catalyst is introduced and the reaction is performed at high temperature, under inert atmosphere, for two/three days[4]. The experiments were carried out with an aromatic compound as solvent, the initial monomer concentration was 20% V/V, a tin compound was used as catalyst (0,1 % mol of catalyst), and the temperature was in the range 130 - 145ºC. Different strategies were tested to remove the water from the reaction medium. Figure 1 shows the ^{1}H NMR spectra for one of the samples of PLA obtained (PLA1). The bands for the CH_3 and CH groups appear at 1,6 and 5,2 ppm, therefore confirming the synthesis of PLA. The band at 7,3 ppm refers to the solvent used in the analysis, $CDCl_3$. Size Exclusion Chromatography (SEC) was used to obtain the molecular wheight distribution of three PLA samples produced in this study and the spectra are shown in Figure 2. The main

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differences in between these experiments refer to the procedures tested to control water content in the reacting mixture.

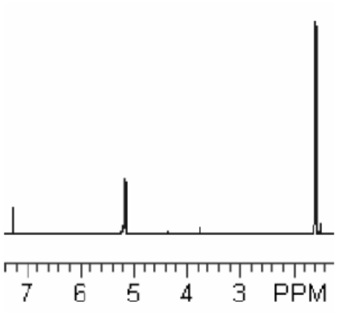

Figure 1: ^{1}H NMR spectra for PLA1 in $CDCl_3$ at 500 MHz.

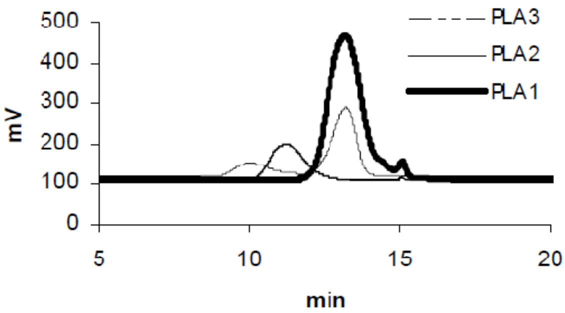

Figure 2: SEC curves of the PLA obtained under different reaction conditions.

PLA1 was obtained at atmospheric pressure, without removing water during the reaction. The SEC analysis shows a low average molecular weight, MW=3 163 g/mol, as expected. PLA2 has a higher average molecular weight, MW=23 185 g/mol. In this experiment, prior to condensation, the vapour passed through a packed tube filled with molecular sieves, in order to absorb water, a side product of the polymerization reaction. In the third experiment, in order to facilitate water absorption in the drying agent, the pressure was slowly decreased through the course of the experiment. The SEC results point out the presence of two predominant molecular weights, the curve of low exclusion times exhibits the highest average molecular weight, MW=75 448 g/mol.

The use of a drying agent combined with reduced pressure seems to be an efficient method to remove water and to achieve high molecular weights. This polymerization method allowed to obtain high molecular weight PLA in one single step, instead of the three steps required in the ROP method. From an the economic point of view, it is the best interest to develop an efficient, effective and less expensive method to produce a promising polymer, Poly(lactic acid).

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Biodegradability of mixed plastics containing PVAL/Gellan and PVAL/Xanthan in soil environment

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Keywords: biodegradation, PVAL, Gellan, Xanthan, gas-chromatography

Plastics represent one of the most important contemporary materials. Their features, easy workability and price far exceed classicality of metal, wood, glass etc. However, the long decomposition period of some polymeric materials as well as growing scores of plastic waste make a considerable problem. One of the possible solutions is development and production of biodegradable polymers which are more rapidly decomposed in biotic environment and thereby eliminated. Good examples of biodegradable materials are wraps, compostable biowaste bags, agricultural mulch foils and protective painting and others. [1]

Poly (vinyl)alkohol (PVAL) is considered to be relatively well biodegradable polymer. Hydrophile feature of PVAL makes its decomposition easier by using microorganisms but limits its range for practice application and that is why the PVAL is modified. Polymeric materials of biologic origin (biopolymers), for example proteins (colagen, protein hydrolyzate), polysacharides (starch), polyesters (e.g. poly-3-hydroxybutyrate, PHB) are often used for the purpose of modification. It is possible to use additives of extracellular polysacharides type such as Dextran, Xanthan and Gellan which are the most remarkable agents. These polysacharides can be added to the mixed polymers for the purpose of the increasing and accelerating of their biodegradability. Required feature low price can be limited factors.

The aim of the study was to review the impact of different Xanthan and Gellan proportion to biodegradability in soil environment of their mixtures with polyvinylalcohol (PVAL) and glycerol, with regard to mechanical properties of the mixtures (tensile stengh, fracture strain). Tested mixed granulates containing 10,5 %, 21 % and 42 % of Xanthan or Gellan were prepared on two - screw thread extruder LABtech.

The tensile strengh of the mixtures was decreasing with increasing polysacharides content, in the tested range within cca 20%, whereas the Gellan mixtures constantly showed lower values than Xanthan ones. The strain decreasing was more evident - between first two samples at cca 30%, by the last mixtures (containing 42% of polysacharides) the values approximated to zero. Obviously, the reaching of aplicable physicomechanical features will be compromising but eventually hardly achieved.

Mixed granulates biodegradation in soil environment was tested under aerobic conditions in reaction flasks [2,3]. The CO2 production via gas – chromatography together with O2 usage for aerobic conditions keeping control was monitored during all tests.

According to DCO2max level of biodegradation, all the polymeric mixtures appeared as hardly degradable in the soil environment. The mixed granulate N/68/107 containing 10.5% of Gellan degraded the worst of all the set, respectively only of 17,9 %. Mixed granulates N/68/108 (21 % of Gellan) and N/68/109 (42 % of Gellan) degraded of 35,9 % and 39,6 %.

Granulates N/68/110 (10,5 % of Xanthan), N/68/111 (21 % of Xanthan) and N/68/112 (42 % of Xanthan) degraded of 21,2%, 45,2% and 47,3%. These results showed that the polymeric mixtures containing Xanthan were better degradable than mixtures containing Gellan. It means that the kind and amount of used polysacharide had a significant effect over the biodegradation of polymeric mixtures but the biodegradability of PVAL itself was not influenced. The level of biodegradation was approximately in relation with content of degradable components (PVAL and glycerol).

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Development of semi-transparent wood polymer composites

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Keywords: Wood polymer composites; acrylic polymers; design of experiments; free radical bulk polymerization

Introduction

We have developed a new material consisting of pieces of wood within a matrix of an acrylic polymer, resulting in a transparent or semi-transparent product. This material present quite appealing aesthetics features, opening new possibilities for new decorative products.

Acrylic and methacrylic monomers are in the liquid state for temperature ranges including room temperature. Therefore, it is possible to introduce wood (in our case, walnut wood) in a mixture of acrylic (HPA – hydroxypropyl acrylate) and/or methacrylic monomers (MMA – methyl methacrylate and HEMA – 2-hydroxyethyl methacrylate) in the presence of a chemical initiator (benzoil peroxide). Introducing this reaction mixture along with pieces of wood in a mould, we obtained a transparent polymeric matrix with wood dispersed, through bulk free radical polymerization, i.e., a wood polymer composite (Kumar et al., 2006).

Experimental Design

In order to develop the new material, a 2^{4-1} fractional factorial design was conducted, where the impact of four factors in the relevant physical and chemical properties was systematically assessed. These factors were the presence of HEMA, HPA, dioctyl phthalate (DOP) and walnut wood. Two levels were adopted for each factor (Table 1): 0 and 10 w/w % for the first three factors and presence/absence for the fourth factor, the walnut. This experimental design as resolution IV, meaning that no main effect is aliased with any other main effect or with any two-factor interactions, but two-factor interactions are aliased with each other (Montgomery, 2001). The composition of the samples was completed by adding MMA.

Table 1 – Experimental design followed in this work

Test	**Design Factors**			
	***A* – HEMA (w/w %)**	***B* – HPA (w/w %)**	***C* – DOP (w/w %)**	***D* – Walnut**
1	0	0	0	present
2	10	0	5	present
3	0	10	5	present
4	10	10	0	present
5	0	0	5	absent
6	10	0	0	absent
7	0	10	0	absent
8	10	10	5	absent

Materials Characterization

All samples produced were characterized by DMTA (determination of T_g), TGA (study of the thermal stability), tensile testing (determination of tensile mechanical properties), HDT (heat-deflection temperature) along with a study of the mass (%) and volume (%) variation through a cycle of water absorption/desorption (7 days in water at ambient temperature; 7 days at a temperature of 50 ºC). All results obtained were analyzed through appropriate statistical methods.

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Results and Discussion

A resume table of the regression models obtained from the analysis of the experimental design runs, regarding the glass transition temperature (T_g), ultimate strength (σ_M), elongation at the ultimate strength (ε_M), modulus of elasticity (E_t) and heat-deflection temperature (HDT) can be seen in Table 2.

Table 2 – Regression Model, R_2 and P-value obtained for Tg, σ_M, ε_M, E_t and HDT

Propriety	Regression Model	R^2	P-Value
Glass Transition Temperature (frequency 1 Hz), T_g	T_g (ºC) = 103,81 – 7,44 x **B** -7,61 x **C**	0,8195	0,0138
Ultimate Strength, σ_M	σ_M (MPa) = 40,22 + 18,46 x **D**	0,9497	0,0001
Elongation at the Ultimate Strength, ε_M	ε_M (%) = 2,74 + 1,77 x **D**	0,9447	0,0001
Modulus of Elasticity, E_t	E_t (MPa) = 1862,20 + 456,72 x **D** -324,38 x **BD**	0,8428	0,0098
Heat-Deflection Temperature, HDT	HDT (ºC) = 70,83 - 8,68 x **B**	0,7861	0,0033

The TGA analysis determined that the materials produced in this work present a good thermal stability up to temperatures close to 200 ° C.

The introduction of monomers with OH groups in the reaction mixture didn´t cause any significant increase in the ultimate strength (σ_M), which means that greater adhesion between the polymeric and the wood phase was not achieved. Thus, the monomers to be used (even in the case of producing flexible materials, by the addition of acrylic monomers) can be chosen only on the basis of cost and not in terms of polarity.

The presence of HPA in the reaction mixture (internal plasticization) is the most important factor for determining the flexibility of new materials when subjected to load. Thus, if the intention is to develop materials showing flexibility when subjected to loads, attention should be focused on internal plasticization and not in external plasticization.

The presence of wood was not important in the mass (%) variation of the material immersed in water. The wood is highly hygroscopic, so we can conclude that the wood is fully impregnated by the polymer and, as such, does not absorb water (Kumar et al., 2006).

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Solubility of acetamide and acrylamide in supercritical carbon dioxide

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Keywords: Supercritical solubility, acetamide, acrylamide, Soave-Redlich-Kwong equation of state

Abstract

The solubilities of acetamide and acrylamide in supercritical carbon dioxide (SC-CO2) were measured at T = (308.2, 318.2, and 333.2) K over the pressure range from (9.0 to 40.0) MPa by a flow type apparatus. The solubility of acetamide (2.3×10^{-4} to 31.3×10^{-4}) in the overall region of measurements is approximately twice that of the acrylamide (1.2×10^{-4} to 16.3×10^{-4}). The increase of pressure increases the solubility of the components and the relative location of the crossover region is observed at 12.0 MPa. Furthermore, the experimental data were correlated by using the Soave-Redlich-Kwong cubic equation of state (SRK EoS) with the one-fluid van der Waals mixing rules.

Introduction

Both acetamide and acrylamide, in pure form, are white crystalline solids. The particular importance of those components is that they have an amide bond, similar to the essential bond between amino acids in proteins and are used as starting material in the synthesis of many other organic compounds. Acetamide has been detected recently as a compound in the center of the *Via Lactea* galaxy (Hollis et al., 2006). Acrylamide was found in foods such as potato chips and bread in (Tareke et al., 2002). To the best of our knowledge, solubility data for acetamide and acrylamide in SC CO2 have not been reported in the literature till present. The experimental solubility data was correlated by the SRK cubic EoS with the one-fluid van der Waals mixing rule.

Materials and methods

The supercritical fluid extraction experiments were performed in a flow apparatus (Coelho et al, 2005). This equipment allows carrying out studies at a temperature up to 393.2 K and a pressure up to 60.0 MPa. The conditions of the solubility studies were: CO_2 flow rates of 9.65x10-3 $g \times s^{-1}$ (linear velocity of 8.74×10^{-3} $cm \times s^{-1}$), pressures up to 40.0 MPa and temperatures up to 323.2 K. The extracts were collected in a system of glass U tubes, at atmospheric pressure and a temperature controlled with an ice bath. The CO_2 (99.995 % purity) was supplied by Air Liquide (Portugal). The compounds are supplied from Sigma Aldrich with a maximum purity of 98 %.

Three replicates were performed at each experimental condition and the solubility was obtained as an average of these results. The uncertainty of the solubility measurements was 2 - 4 %.

Results and discussion

Experimental data

The experimental solubility of acetamide and acrylamide in SC CO_2 and the results from the modelling are shown in Figures 1 and 2, respectively. With the increase of pressure at constant temperature, the solid solubility increases due to an increase of the fluid density. However, solubility curves intersect each other around 12.0 MPa.

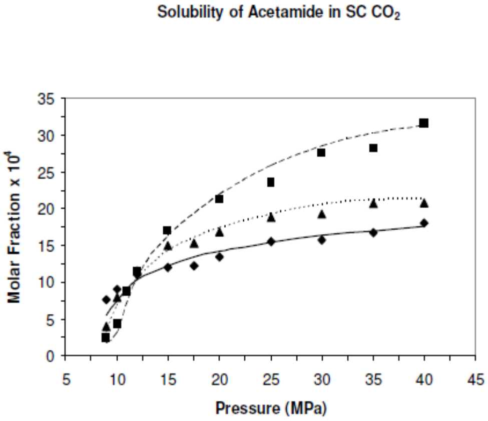

Figure 1 – Lines represents the solubility correlated by the SRK EoS at temperatures: ♦-308.2 K, ▲-313.2 K, ■-323.2 K.

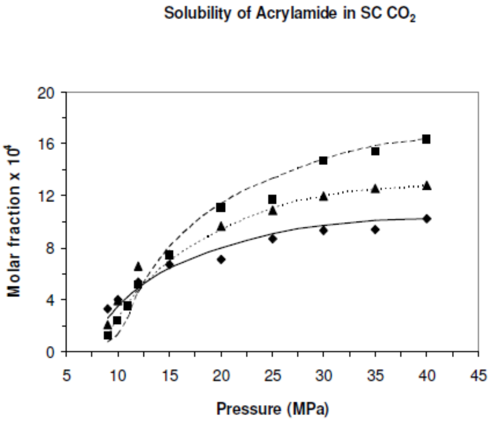

Figure 2 – Lines represents the solubility correlated by the SRK EoS at temperatures: ♦-308.2 K, ▲-313.2 K, ■-323.2 K.

Thermodynamic Framework

For the fugacity coefficient of a solid solute the following expression is applied (Prausnitz et al., 1999).

$$\ln\varphi_s = \ln\varphi^{SCL} + \frac{\Delta H_m}{R}\left(\frac{1}{T_m} - \frac{1}{T}\right) + \frac{(V_s - V^{SCL})}{RT}\left(P - P_s^{subl}\right) \qquad (1)$$

Two types of data are required for calculating the fugacity coefficient of a pure solid solute: its fusion properties and its critical parameters. For the acetamide and acrylamide some of the properties required are experimentally available, while for those which are either not available or hypothetical the algorithm discussed in detail in (Fornari et all. 2005) is employed.

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Solubility of some sulphates in supercritical water

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Keywords: catalysts regeneration, sulphates solubility, sub and supercritical water.

Abstract

Despite the extended use of three-way catalysts (TWCs) in automobile industry for so many years, only a few attempts have yet been made aiming at expanding the durability of gasoline-driven aged TWCs and in situ regenerating their catalytic performance after applying economically attractive and environmentally friendly techniques. Among these attempts are extraction of contaminants by using liquid solvents such as weak organic acids (e.g., acetic, oxalic, and citric acids) and employment of different combinations of thermal and chemical treatments by oxygen, hydrogen, and chlorine-containing gas mixtures or other chlorine-containing reagents (such us carbon tetrachloride, hydrochloric acid, etc.) to reverse thermal aging by redispersion of the large particles of catalysts noble metals.

However, all of these technologies present some objections, i. e., the efficiency of the process is not complete because of one or several reasons: only partial elimination of the impurities is achieved, there are exclusive treatments for specific pollutants, the recoveries of the catalytic activity and the oxygen storage capacity are limited and wastes streams are generated that need to be treated in order to yield a process environmentally benign. It is for this reason that we propose the use of sub and supercritical water to recycle the TWCs by extracting selectively the pollutants poisoning the catalysts surface. Data recently published (1, 2) suggest that the treatment could work.

Thus, the present study has been undertaken to study for the first time the possibility of using sub and supercritical water to improve the catalytic activity and oxygen storage capacity of aged TWCs. More specifically, the solubility in sub and supercritical water of TWCs pollutants such as $Al_2(SO_4)_3$ and SO_4Na_2 is investigated in order to optimize the experimental conditions to perform the extractions that would improve the catalytic and oxygen storage properties of the catalysts.

The solubility experiments have been performed using a continuous flow apparatus. The system pressure was maintained with a syringe pump. The equilibrium cell consisted of a 250 cm^3 cylinder. The saturated sub or supercritical water was depressurized through a metering valve. The dissolution of the inorganic salt in water at ambient pressure was collected in a bottle and the dissolved solute quantified by classical chemical SO_4^{2-} analysis.

As regards to the experimental results obtained, it should be noticed that they cover a wide range of temperatures and fill the gap in the information available in the literature since no previous measurements in the water critical region were found for $Al_2(SO_4)_3$ and very few for SO_4Na_2. The experiments have been performed at a fluid flow of 1 cm^3/min at pressures and temperatures ranging between 200 - 300 MPa and 300 - 400 ºC. For both salts it has been found a rapid decrease in solubility around the critical temperature.

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Generation of pharmaceutical cocrystals using supercritical fluid technology

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Keywords: crystal engineering; supercritical fluids; solubility; theophylline; pharmaceutical cocrystals

Bioavailability of an active pharmaceutical ingredient (API), the amount of the intact drug that reaches systemic blood circulation, relates to its aqueous solubility. Some fundamental strategies in improving the aqueous solubility or manipulation of technical properties of drugs include manipulation of solid state structure (polymorph changes or amorphous form), formation of a salt, for liquid drugs adjustment of pH of the solution (Gourinchas et al, 1996). Drug delivery technologies, modifying chemical or mechanical environment surrounding the drug molecule, or physically altering the macromolecular characteristics of aggregated particles, have traditionally been considered for improving the solubility of drug substances (Hakuta et al, 1997).

Pharmaceutical cocrystallization is a relatively recent technology and offers an alternative platform in solving the above mentioned problems of APIs through the development of new class of crystalline solids, called pharmaceutical cocrystals. The choice of pharmaceutical crystalline form can be used to optimize drug properties, like solubility and dissolution rate, and so cocrystals are emerging as new alternatives (Basavoju et al, 2007).

Specifically, in addition to the traditional means of preparing cocrystals by cocrystallization from solutions, the use of SCF`s (Supercritical Fluids) offers new flaform that may enhance the rate of cocrystal formation and offers a solvent free product, allowing the single-step generation of particles that are difficult or even impossible to obtain by traditional techniques, particularly for substances with thermal sensitivity or structure instability. However, there is no report on using environmentally friendly SCF methods in the generation of pharmaceutical cocrystals.

In this work, theophylline cocrystals, using saccharin as a cocrystal former, were produced by using high pressure CO_2 or N_2 as supercritical media. The potential cocrystalline phase was characterized by several techniques, namely SEM, DSC (see Figure 1) and PXRD. The theophylline-saccharin cocrystal structure was previously determined from single crystal X-ray difraction data and its pharmaceutical relevant properties, such as dissolution rate, were evaluated. We are currently studying several other cocrystal systems for in depth understanding of cocrystal formation behaviours in supercritical fluids.

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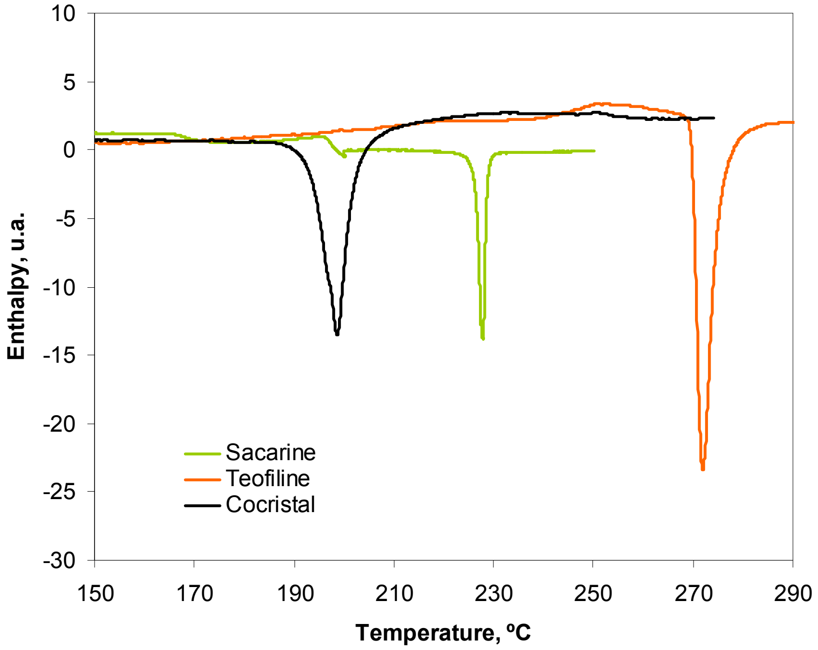

Figure 1 - DSC thermograms for saccharin, theophylline, and theophylline-saccharin cocrystals.

CO_2 capture with CaO / MCM-41 materials

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Keywords: CO_2 capture, CaO, MCM-41,TPD

In the short term, fossil fuels will continue to be the main energy supply, and technologies associated with CO_2 capture and storage are increasingly considered to achieve these emission reductions (IPPC Special Report, 2005). Developed post-combustion technology consists in the flue gas absorption in an aqueous solutions with amines. Adsorption at low temperatures is considered as one of the potential alternatives to absorption processes, with added advantages, and the development of adsorbents with a high CO_2 selectivity and adsorption capacity is needed. Modified carbons, zeolites, mesoporous materials, metal oxides and amines supported have been tested as adsorbents, but no work has been done by using calcium bases supported on MCM-41 materials.

The aim of this work was to study the carbon dioxide adsorption of sorbents prepared by aqueous mixture at room temperature of CaO and MCM-41 support. Temperature-programmed desorption (TPD) analysis of these materials previously treated with CO_2 was used. The influence of time of contact, percentage of CO_2 in the flow and temperature of contact in the adsorption was studied. The behaviour in consecutive adsorption-desorption cycles was also tested in order to check the stability of the adsorbents, in comparison to the use of a commercial CaO adsorbent.

MCM-41 was prepared with two silicon sources, heated under reflux and calcined at 550ºC. The molar composition of the gel was: 1.00 SiO_2: 0.25 Na_2SiO_3: 0.25 CTMABr:0.10 TMAOH:100 H_2O. The CaO /MCM-41 sorbents were prepared at the same conditions that CaO/smectite sorbents (Renedo et al., 2006) by mixing at room temperature CaO with distillate water, adding the support and stirring for 30 minutes, filtering and drying at 110ºC to constant weight. The weight percentage of CaO /support varied from 2.5%, 5% and 10% and the water to solid ratio was 10.

The prepared sorbents were characterized by determining their BET specific surface area and their pore size distribution by N_2 adsorption in a Micromerics ASAP-2000 apparatus.

TPD experiments were performed by using an AutoChem II 2920 equip. Before each TPD experiment, the sample was outgassed in He flow by thermal treatment from room temperature to 550ºC. After being cooled to the temperature at which adsorption was carried out (50 to 200ºC) the adsorbent material was contacted to a mixture of CO_2 in He (10% normally, but 50% was also checked) for times varying from 30 minutes to 24 hours. The reversible adsorbed CO_2 was removed by treatment of the sample in He flow for 30 minutes at the adsorption temperature. The TPD tests were carried out by heating the sample with a ramp of 10ºC/min until 550ºC in a constant He flow. The signals obtained were integrated in order to obtain amounts of CO_2 desorbed, previous calibration of the device. To study the regenerability of the adsorbents, after holding the sample temperature at 550ºC during 30 minutes, the temperature was decreased to the initial value and the adsorption-desorption cycle repeated.

Results of the BET specific surface area of the sorbents showed a drastic reduction with respect to the support when $Ca(OH)_2$ was introduced, decreasing gradually with the $Ca(OH)_2$ increase (from 1085 m^2/g of the MCM-41 support to 204, 196 and 159 m^2/g in sorbents with 2.5%, 5% and 10% $Ca(OH)_2$ /support respectively) The pore size distribution also evidence that the $Ca(OH)_2$ produces the closure of the pores.

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In order to maintain the structure of the support, prepared by calcination at 550ºC, sorbents were treated in flow with Helium up to 550ºC. It was proved previously that CO_2 is not retained in these sorbents as $CaCO_3$, which decomposes at 625ºC.

Results of TPD obtained with 2.5% CaO/MCM-41 showed three peaks, with maximums at 100ºC, 200ºC and 550ºC, being the most important peak centered at 200ºC. Similar profiles were observed by using the 5% or 10% CaO / MCM-41 sorbents, indicating that CO_2 is desorbed mainly near 200ºC.

To study the influence of the CO_2 concentration and the time of contact with CO_2 several experiments were performed. An experiment with a flow of 50% CO_2 in He during 30 minutes at 50ºC was carried out during five cycles and compared with data obtained with 10% of CO_2 in He for 10% CaO / MCM-41 sorbent. The same value, 3.2 mg CO_2 captured / g of sorbent, was obtained in the first cycle fot both conditions, being the differences in the values in the rest of the cycles less than 2%. Therefore, no influence of the CO_2 concentration was found.

After the exposition to 10% CO_2 at 50ºC in consecutive cycles during times varying from 30 to 1440 minutes (24 hours) of 10% CaO / MCM-41 sorbent, TPD experiments were performed with reversible desorption times of 30 minutes. The time of reversible desorption was also checked, varying from 30 to 1440 minutes, in the same experiment. No influence was observed, being 30 minutes enough to eliminate the reversible CO_2 adsorbed. Nevertheless the retention of CO_2 in the sorbent increases as the contact time does, reaching the equilibrium near 18 hours, which indicates the slow retention rate of CO_2 in the basic sorbent.

New experiments at 18 hours of contact time were carried out at 50ºC, to determine the maximum CO_2 adsorption capacity of these sorbents (measured by the desorption data obtained in the TPD experiments). An important increase in the CO_2 captured took place, being the sorbents with less CaO content (2.5%) that obtain the highest capacity of capture (9.8 mg CO_2/g). This is an important result in order to optimize the preparation of these sorbents with the minimum cost.

To study the influence of the temperature in the CO_2 captured, a second consecutive cycle was performed in each sorbent (after the first exposition to 10% of CO_2 at 50ºC during 18 hours and desorption) during 18 hours at 200ºC. Results of the TPD indicate, according to the desorption profile of these sorbents, that an enormous reduction in the CO_2 retained takes place, varying the values obtained for the different sorbents within 1.3 mg / g for 2.5% CaO / MCM-41 and 2.1 mg / g for 10% CaO / MCM-41 sorbent.

Results obtained during five consecutive cycles of 30 minutes at 50ºC in all the CaO / MCM-41sorbents showed that the reproducibility in the capture was higher than 85%.

Commercial $Ca(OH)_2$ used as sorbent, without support, showed higher CO_2 retention values than the prepared sorbents (22 mg CO_2 / g sorbent was captured in 30 minutes at 50ºC and 29 mg CO_2 / g was found in 18 hours). But the regeneration must be done at temperatures higher than 700ºC, in order to decompose the $CaCO_3$ formed, and the loss-in-activity found was 42% in the fifth cycle of 30 minutes and 27% after the third cycle of 18 hours.

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Enhancing CO_2 selectivity in activated carbons for H_2 production applications

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Keywords: Modification of activated carbon; Activation; Carbon dioxide; Adsorption

Nowadays, the steam methane reforming (SMR) is the most economical route for hydrogen production (Sircar, 1999). The hydrogen produced by SMR is frequently purified in a Pressure Swing Adsorption (PSA) unit. To have a sustainable production of hydrogen employing natural gas (fossil fuel), a capture process would be necessary to remove the undesired by-product (CO_2) for permanent sequestration. This is the main objective of the European Research Project HY2SEPS (Hybrid Hydrogen Carbon Dioxide Separation Processes) where the PSA technology is combined with a membrane process to produce high purity H_2 (>99.99%) integrating a CO_2 capture technology.

In this work, a commercial sample of activated carbon (AC) was used as the starting point for the preparation of a new material with enhanced selectivity towards carbon dioxide in order to attain better performance within the PSA unit. The modified activated carbons (ACs) were prepared from activated carbon extrudates by activation with carbon dioxide using different conditions: flowrates of activating agent, activation temperatures, pressures and activation times. The changes in micro and macro porosity of the new samples were evaluated by adsorption of N_2 at 77 K and by mercury intrusion. The maximum surface area was obtained at 1173 K and 1 bar, using a carbon dioxide flowrate of 5.0×10^{-10} m^3/s during 2400 s. A scale-up of this procedure was carried out to prepare 400 g of modified AC.

The kinetics and the adsorption properties of H_2 and CO_2 as well as of the minor species CH_4, CO and N_2 were studied for the original AC and for the final scale-up sample of modified AC. The transport mechanism and diffusivity parameters of the pure gases in these adsorbents were obtained from breakthrough or pulse experiments of pure gases. Figure 1 shows the breakthrough curves of CO_2 on the modified AC and on the original AC at 303 K and 1 bar of total pressure. The adsorption equilibrium of the pure gases on the two ACs were measured gravimetrically in a magnetic suspension microbalance (Rubotherm, Germany) operated in closed system with a Lucas Schaevitz 0-7 bar pressure transducer for measurements inside the adsorption chamber. As an example, Figure 2 shows the adsorption equilibrium of CO_2 and H_2 on both ACs at 303 K. The kinetics and the adsorption properties must be represented by reliable models in order to do the necessary theoretical design, control and optimization of the process. The adsorption equilibrium of all gases (H_2, CO_2, CH_4, CO and N_2) on the two ACs was fitted with the virial isotherm equation (Kiselev, 1971; Barrer, 1981) that allows direct prediction of multicomponent adsorption (see Figure 2).

The validation of the multicomponent adsorption equilibrium was accomplished performing breakthrough curves in the fixed-bed setup of multicomponent mixtures of CO_2-H_2-CH_4 at different experimental conditions for both activated carbon materials. The multicomponent extension of the virial isotherm model was used to predict the adsorption equilibrium of the mixtures for which the multicomponent behaviour is purely based on the information obtained from pure component adsorption equilibrium data. The simulations were performed with gPROMS (PSE Enterprise, UK) using centered finite differences as the numerical method. Figure 3 shows the experimental results

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obtained for measurements at 303 K and 1.2 bar of the ternary CO_2-H_2-CH_4 breakthrough experiment on the modified AC together with the simulation results.

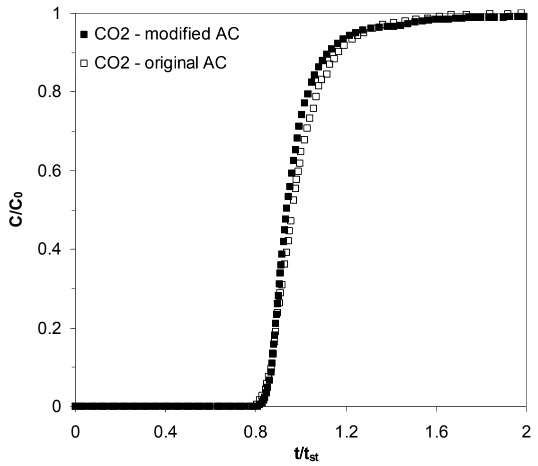

Figure 1. Breakthrough curves of CO_2 on the modified AC (■) and on the original AC (□) at 303 K; 1 bar of total pressure.

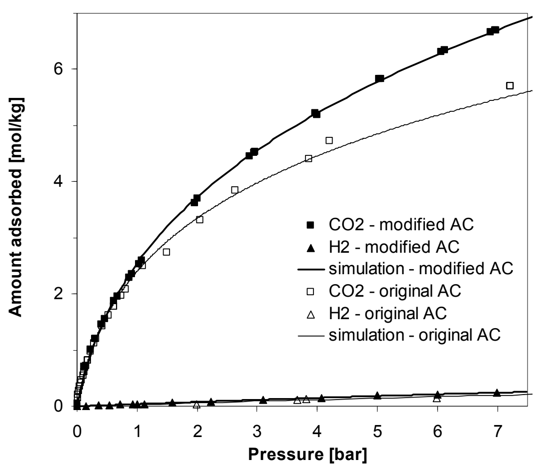

Figure 2. Amount of CO_2 and H_2 adsorbed on the modified AC (■,▲) and on the original AC (□,△) at 303 K; —— virial isotherm fitting.

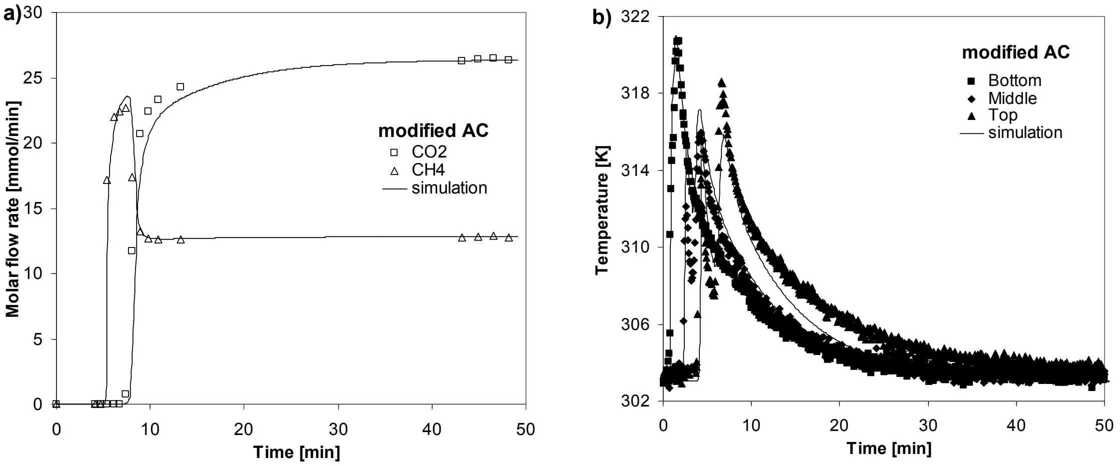

Figure 3. CO_2 (□) and CH_4 (△) molar flowrate histories at the column outlet (a) and temperature histories (b) obtained for the CO_2-H_2-CH_4 breakthrough curve at 303K and 1.2 bar on modified AC; Mixture with 58.0% of CO_2 and 28.2% of CH_4 balanced with H_2; Temperatures measured at 0.175 m (Bottom (■)), 0.424 m (Middle (◆)) and 0.677 m (Top (▲)) from feed inlet.

Acknowledgments

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Preparation of mesoporous carbons using a porous clay heterostructured as template

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Keywords: Mesoporous carbons, Template synthesis, Porous Clay Heterostructure, Furfuryl alcohol.

The extensive use of porous carbons is the natural consequence of their properties, such as high specific surface areas, large pore volumes, chemical inertness and good mechanical stability. Most carbons are primarily microporous, however there are numerous potential applications for which materials with wider pores, preferably in mesopore range, would be advantageous (Lee, 2006). In this sense, since the pioneer work of R. Ryoo el al. (1999), much effort has been made concerning the preparation of Ordered Mesoporous Carbons (OMCs). Such materials are important for applications in emergent areas such as energy storage in double-layer supercapacitors, in addition to the common applications as adsorbents and catalyst supports (Lee, 2006).

The synthesis of OMCs is generally made by the templating approach. The main steps are: i) infiltration of the porous structure of a mesostructured inorganic material (template) by the carbon precursor (generally a polymer or pre-polymer); ii) polymerization of the infiltrated substance; iii) carbonization of the nano-composites formed and iv) elimination of the template. Depending on the type of mesostructured silica used as template, carbons with different structures and pore sizes can be prepared (Fuertes, 2004).

Although silicas of MCM and SBA families have been extensively employed as templates, the use of other inorganic matrixes have also been envisaged. In fact, in the literature there are some studies where clay based solids or zeolite frameworks were used, as is the case of the work developed by D. Nguyen-Thanh and T.J. Bandosz (2006) that, to our knowledge, is the only study were the potentialities as template of a porous clay heterostructured (PCH) for mesoporous carbons preparation was evaluated.

In the present study a new approach of templated synthesis of mesoporous carbons was tried, using a PCH as inorganic matrix and furfuryl alcohol as carbon precursor. The materials were characterized by nitrogen adsorption at – 196 ºC, X-ray powder diffraction and scanning electron microscopy. The ash content of the carbons was evaluated according to the procedure described in A.S. Mestre et al. (2007).

The PCH used was prepared from a natural Portuguese montmorillonite from Benavila (Alentejo, Portugal). The preparation procedure consisted in the intercalation of cetyltrimethylammonium bromide and decylamine to direct the interlamelar hydrolysis of tetraethylortosilicate (that will form the pillars) within the galleries of modified clay. Two carbon samples were prepared, CF and CF-TS, differing only in the fact that in the preparation of CF-TS a previous impregnation of the PCH with p-toluenesulfonic acid was made. The purpose of this impregnation was to increase the surface acidity of the solid which will catalyse the polymerization process of the carbon precursor used, furfuryl alcohol. The infiltration of this organic compound onto the PCH porosity was

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made by incipient wetness at room temperature, using a volume of alcohol equal to the total pore volume of the silica. The materials were then placed in a covered recipient and heated overnight at 95 ºC, after what they were carbonized in a horizontal tubular oven at 700 ºC for 1 h, under nitrogen flow (6.7 cm^3 s^{-1}). The composite samples so obtained are designated as CF/PCH and CF-TS/PCH. After removing the inorganic template using HF, the samples were washed with distilled water until pH 7.

The results displayed in Fig. 1 show that the methodology followed permitted the preparation of carbons presenting a by-modal mesoporous distribution, the smallest mesopores being slightly narrow than those of the PCH. So, the layered structured of the matrix seems to be inherited in some extension, what was not revealed in the X-ray diffraction analysis. The textural characteristics of the carbons prepared with and without surface acidification (see samples CF and CF-TS in Table 1) allow us to conclude that the surface acidity of the PCH is sufficient to catalyze the furfuryl alcohol polymerization.

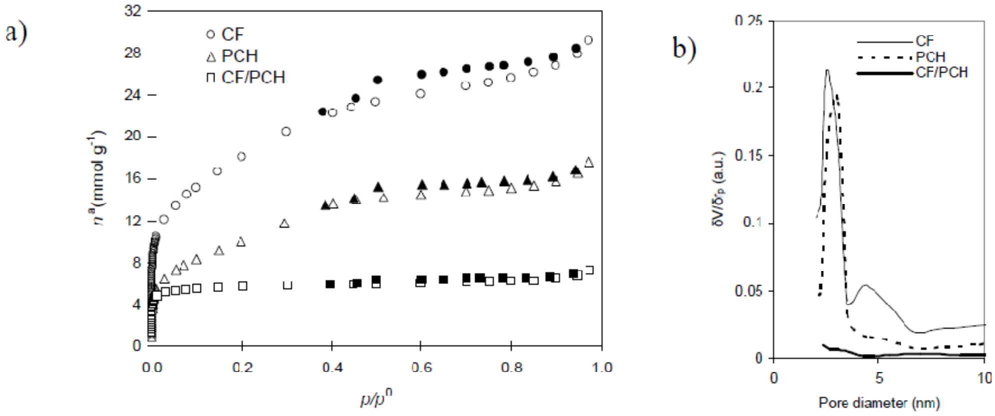

Figure 1 - Nitrogen adsorption-desorption isotherm at – 196 ºC for the samples indicated (a) and respective mesopore size distribution (b).

Table 1 - Textural characteristics of the samples.

Sample	S_{BET} m^2 g^{-1}	V_{micro} (α method) cm^3 g^{-1}	V_{meso} cm^3 g^{-1}	V_{total} (p/p^0 = 0.95) cm^3 g^{-1}
PCH	818	0.46	0.12	0.58
CF/PCH	452	-	-	0.24
CF	1469	0.59	0.42	0.98
CF-TS/PCH	428	-	-	0.24
CF-TS	1433	0.59	0.43	0.99

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Influence of the preparation methodologies in the performance of Pd-Cu/AC and Pt-Cu/AC catalysts for nitrate reduction with hydrogen

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Keywords: nitrate reduction, activated carbon, bimetallic catalysts, water treatment.

Introduction

Nitrate concentrations in surface water and especially in groundwater have increased in many locations in the world, including in Europe. The catalytic reduction with hydrogen has been suggested in the literature as a promising method for nitrate removal from water without the drawbacks of the conventional methods (Pintar, 2003; Prusse and Vorlop, 2001). This process was reported for the first time by Vorlop and Tacke (1989) and consists in the reduction of nitrate towards nitrogen over bimetallic catalysts in the presence of a reducing agent. Nitrite as intermediate and ammonium as by-product are considered the main disadvantage of this process (Pintar, 2003). The activity and selectivity of the bimetallic catalyst is highly dependent on the preparation method, on the way the noble metal is promoted, on the metal/promoter ratio and on the operation conditions (Matatov-Meytal and Sheintuch, 2005). In the present work, Pd-Cu and Pt-Cu bimetallic catalysts supported on activated carbon were prepared according to different methodologies and the respective activities and selectivities for the reduction of nitrate in water with hydrogen were assessed.

Experimental

The active metals were supported on a commercial activated carbon NORIT GAC 1240 PLUS (AC). The catalysts were prepared by incipient wetness co-impregnation, with aqueous solutions of the corresponding metal salts (H_2PtCl_6, $PdCl_2$, $Cu(NO_3)_2$). Then, the samples were dried at 100 ºC for 24 h. Different temperatures were selected for calcination (T_{Cal}) under a nitrogen flow for 1h and reduction (T_{Red}) in hydrogen flow for 3 h. No calcined and no reduced catalysts (N_{Cal} N_{Red}) were also studied. The contents of noble and promoter metals were maintained constant at 1%wt and 0.3%wt, respectively. The catalysts were characterized by hydrogen chemisorption, N_2 adsorption isotherms, XRD, TEM and XPS.

The catalytic tests were carried out in a semi-batch reactor, working at room temperature and pressure, with an initial nitrate solution of 100 ppm and using hydrogen as reducing agent. CO_2 was used as a buffer. Nitrate and nitrite concentrations were simultaneously determined by HPLC. The concentrations of ammonium ions were quantified using a convenient selective electrode. pH values were also measured.

Results and Discussion

The effects of calcination and reduction temperatures on the catalysts activity and selectivity during the reduction of nitrates were studied. The activities of 1%Pd-0.3%Cu/AC and 1%Pt-0.3%Cu/AC catalysts are show in Figure 1. The reduction of nitrate is quite different depending on the supported metal and the preparation conditions. For all the catalysts the activity decreases with the increase of calcination and reduction temperatures. Since these catalysts have the same amount of metals, the different performances should be related to the metal dispersion. In addition, a

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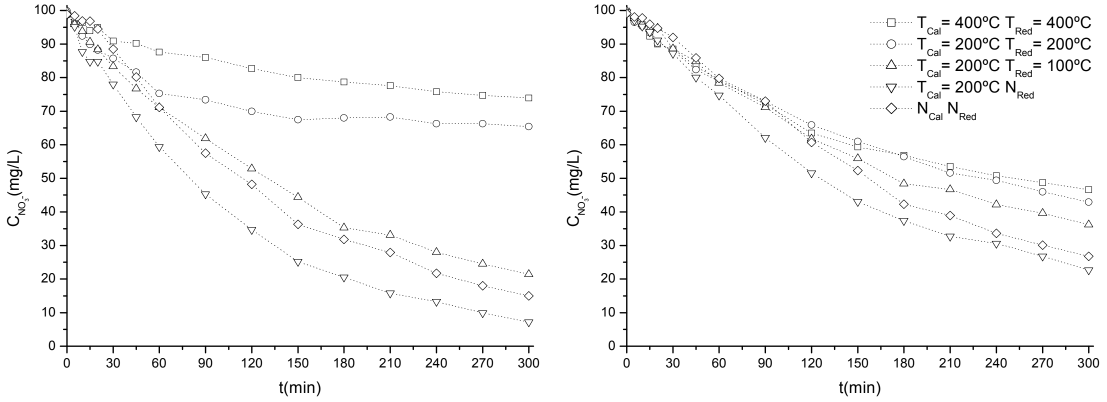
support promoting effect on the metals may also be considered. Higher temperatures used during the preparation of the catalysts will result in larger metal particles with an overall loss of catalytic activity.



a) b)

Figure 1. Nitrate concentration as a function of time during nitrate reduction in the presence of a) 1%Pd-0.3%Cu/AC and b) 1%Pt-0.3%Cu/AC catalysts ($C_{NO3^- i}$ = 100 mg/L, catalyst = 0.5 g/L, pH = 5.5, Q_{H2} = 100 Ncm^3/min, Q_{CO2} = 100 Ncm^3/min, T = 25 ºC).

For high calcination and reduction temperatures the Pt-Cu catalysts were more active than the Pd-Cu catalysts. However, using a low reduction temperature or not carrying out the reduction, the Pd-Cu catalysts were the most active. The order of activity for both catalysts is similar: T_{Cal} = 200ºC N_{Red} > N_{Cal} N_{Red} > T_{Cal} = 200ºC T_{Red} = 100ºC > T_{Cal} = 200ºC T_{Red} = 200ºC > T_{Cal} = 400ºC T_{Red} = 400ºC, with conversion values after 5h of 93, 85, 79, 35 and 25%, and 77, 73, 64, 57 and 53%, respectively for the Pd-Cu and the Pt-Cu catalysts.

For all the catalysts, the nitrite concentration goes through a maximum and the ammonium concentration increases with nitrate conversion, indicating the occurrence of consecutive/competitive reactions. For both pairs, the reduced catalysts give rise to similar nitrite concentrations, which are much lower than the concentrations obtained with the non-reduced catalysts. This situation is more evident for the Pt-Cu catalysts, which cause higher nitrite concentrations. A similar trend is observed for ammonia, but in this case the highest concentrations were measured for the Pd-Cu catalysts.

The performance of the catalysts is maximised for low calcination and reduction temperatures. For every preparation conditions tested the Pd-Cu pair seems to be more selective in the transformation of nitrate into nitrogen.

Acknowledgments

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Modification of zeolite porosity by alkaline treatments

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Keywords: Zeolites, Alkaline Treatment, Desilication, Mesoporosity, Acidity.

Zeolites are crystalline aluminosilicates with a unique combination of properties, such as, high surface area, well-defined microporosity, high thermal stability and intrinsic acidity.These materials have been widely used in catalysis as well as in separation and purification fields (Yousheng, 2006).

The modification of the purely microporous character of zeolites is an important aspect that can be explored. In fact, a more efficient performance of zeolites as catalysts can be envisioned upon enhanced accessibility to the active sites and shortening of the diffusion path length in the micropores (Groen, 2006).

The traditional dealumination treatments, in steam or acidic medium, generally induce limited mesoporosity in zeolites and directly impact on the acidic properties due to depletion of Brönsted acidity (Groen 2006). Recently, the controlled extraction of framework silicon in alkaline medium, referred to as desilication treatment, was proposed by Groen *et al.*(2004). This treatment has resulted in a promising methodology to produce extended mesoporosity in zeolites crystals, without impacting on the acidity (Groen, 2006). Several publications have emerged, especially using MFI type zeolite.

In this work, MOR, BEA, MFI and FER zeolites, supplied by Zeolyst, with a Si/Al ratio of 10, 12.5, 25 and 27.5, respectively, were desilicated using the experimental conditions described by Groen *et al.* (2004). For example, approximately 2.64 g of MFI zeolite sample was vigorously stirred in 80 mL of 0.2 M NaOH solution at 65 ºC for 30 min. The suspension was then centrifuged and washed with distilled water. The alkaline-treated samples were converted into the H-form by three consecutive exchanges with 1 M NH_4NO_3 solution for 3 h. Subsequently the suspension was filtered and the solid was dried overnight. Final calcinations of the zeolites was done in air flow at 500 ºC for 3 h. After desilication the samples were characterized by X-ray powder diffraction (XRD) in a Philips PW 1710 difractometer and scanning electron microscopy (SEM) in a Jeol Model JSM – 6301F microscope. The porosity modifications were studied by N_2 adsorption at low temperature, performed in an automatic apparatus ASAP2010. The possible changes in the acidity were evaluated by the model reaction of n-heptane cracking, performed at 350 ºC. The experiments were performed for 90 min using a flow of 3 mL h^{-1} of n-heptane (molar ratio N_2/n-C_7=9) and WHSV=20.5 h^{-1}, after a pre-treatment of 10 h at 500 ºC in situ, under N_2 flow of 60 mL min^{-1}. The products were analysed by an on-line gas chromatograph (HP689) equipped with a FID.

The XRD patterns (not shown) do not evidence significant losses of cristalinity during the desilication treatment in all samples. Figure 1 show, as an example, the nitrogen adsorption/desorption isotherms of the alkaline treated MOR (HMOR-des) and the parent HMOR zeolites. Table 1 presents, for the same samples, the values for micropore (V_{micro}), mesopore (V_{meso}) and total porous volumes (V_T), as well as the external surface area, A_{ext}.

The isotherm for HMOR zeolite shows a sharp rise in uptake at lower pressure, that confirms the predominant microporous character of the parent sample. The isotherm of HMOR-des sample shows an increase in nitrogen uptake for high relative pressures, revealing an increase in V_{meso} upon desilication treatment. The hysteresis loop

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observed is H4 type. The porous size distribution, from BJH method, is very broad showing that the treatment created porous with openings covering all the mesopores range.

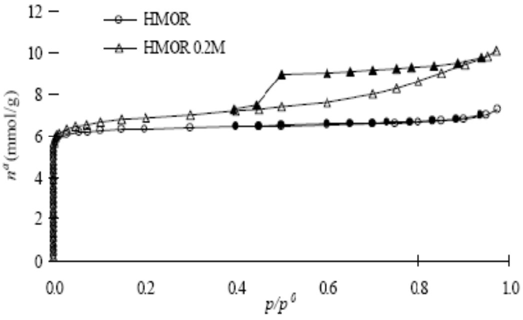
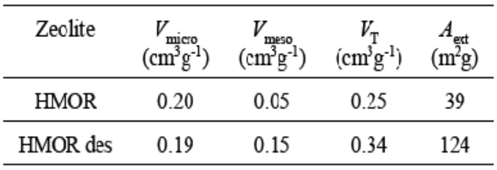


Figure 1 – N_2 adsorption/desorption isotherms at -196 ºC for parent and alkaline treated HMOR zeolites. Open and closed symbols represent adsorption and desorption points, respectively.

Table 1 – Textural properties of the parent and alkaline treated HMOR zeolites; micropore (V_{micro}) by the α-method, mesopore (V_{meso}) by subtracting the microporous volumes from the total pore volume at $p/p^0 \sim 0.95$ (V_T) and external surface area (A_{ext}).



Zeolite	V_{micro} (cm^3g^{-1})	V_{meso} (cm^3g^{-1})	V_T (cm^3g^{-1})	A_{ext} (m^2g)
HMOR	0.20	0.05	0.25	39
HMOR des	0.19	0.15	0.34	124

n-Heptane cracking was used as a model reaction to characterise the acidity of the samples and, in this sense, evaluate possible changes in acidity after desilication treatments. The main reaction products are hydrocarbons (olefins and paraffins) with three and four carbon atoms. After desilication all samples show identical catalytic behaviour as the parent ones. Table 2 shows the main parameters related to n-heptane cracking reaction for HMOR and HMOR-des samples.

Table 2 – Main parameters for n-heptane cracking at 350 ºC after 5 min. of time-on-stream

Sample	Conversion (molar %)	O/P (C4)	B/L (C4)	Deactivation (%)
HMOR	31.4	0.2	2.3	85.3
HMOR-des	24.3	0.2	2.7	83.2

The alkaline treated sample shows a slightly lower initial activity but a similar deactivation. The olefin/paraffin (O/P) ratio remains constant after desilication, which indicates the maintenance of the acidic properties. The branched/linear products (B/L) show a slight increase for HMOR-des, indicating that the space available within the pores became larger due to the presence of mesopores created during the alkaline treatment.

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Application of stochastic methods for modeling of the large reaction network of oligomerization

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The kinetic modeling of complex reaction networks, which involve thousands of species, intermediates and elementary steps, is of great industrial importance. Such complex processes, oligomerization for example, may contain an excessive number of kinetic equations and rate parameters so that there is a common need to identify techniques for simulation of these gigantic networks. The classical modeling approach applies the deterministic solution for a reduced network consisting of a small number of reactions between pseudo-components or lumped species.

Nowadays, the chemistry of acid catalysed reactions is well defined through introduction of carbo-cation intermediates and number of reaction types for a specie is limited to several elementary reactions. These chemistry rules enable us to construct entire reaction network in terms of elementary steps of cation chemistry using computer algorithms. The single event concept explains the effect of structure on the entropy contribution of rate coefficient and reduces the rate parameters to tractable numbers [1]. Generation of such reaction network for oligomerization is difficult because of the number of species expands exponentially [2]. Moreover, no real thermodynamic equilibrium between species can be considered in case of oligomerization and thus, no lumping based on thermochemical considerations can be done [2]. It follows that no deterministic simulation of the complete network and full detail on species can be done.

In recent years, stochastic simulation of chemical reactions is becoming important. The stochastic kinetics is a probabilistic approach for the time evolution of spatially homogenous systems [3]. In this work, the stochastic simulation approach is applied for simulation of oligomerization reaction to predict the performance of the stochastic method. The interest of this approach for our application is to generate an appropriate network of possible reactions and identify the population of species and evolution of the reaction network at each moment. Using the stochastic simulation algorithm allows us to have the most probable reaction paths. It is shown that the stochastic simulation could be an advisable approach for simulation of the oligomerization network. On the other hand, a certain number of parameters of simulation are difficult to estimate, duration of simulations is relatively high and adequate simulation runs are required to obtain a stable average because of stochastic nature of proposed method.

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The kinetics of ampicillin release from hydroxyapatite porous for bones regeneration

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Keywords: Antibiotic release, Hydroxyapatite, Ampicillin, Kinect study.

Semi-synthetic beta-lactam antibiotics are the most important class of antibacterial agents. Their use in veterinary and human medicine is in continuous expansion. Some examples of semi-synthetic penicillins and cephalosporins are: amoxicillin, ampicillin, cephalexin, cefadroxil, cefazolin, among many others. They have in common the presence of the beta-lactam ring, responsible for their anti-microbial activity. They irreversibly inhibit the last step of the bacterial cell wall biosynthesis. The beta-lactam antibiotics can be described in terms of a beta-lactam nucleus with a side-chain (see Figure). Many different nuclei and side-chains are found in the antibiotics that are in use today. Different combinations of side-chains and nuclei form antibiotics with distinctive properties; for example, replacing the phenylacetic acid side-chain of penicillin G with D-phenylglycine (PG) results in the beta-lactam antibiotic ampicillin, which in contrast to penicillin G, is orally stable (Cole, 1969; Bruggink et al., 1998; Wegman et al., 2001; Ribeiro et al., 2005). Ampicillin (6-[2-amino-2-phenylacetamide] penicillanic acid) is in the penicillin group of which penicillin proper was the first antibiotic to be used in therapy (Bruggink et al., 2001; Pessina et al., 1988).

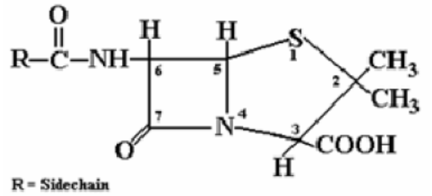

Figure: General structure of beta-lactam antibiotics.

There is a growing need for developing bioactive implants, due biomaterials are biocompatible, resorbable, and present osteoconductive properties. It is known that the use of bone substances has many inherent disadvantages in practical applications, and it is linked to many surgical problems (Suchanek and Yoshimura, 1998).

Advantages of implantable drug delivery tools can include high release efficiency, precise dose control, low toxicity, and allow to overcome disadvantages connected with conventional methods (Uhrich et al., 1999). In this respect, hydroxyapatite (HA) is an elective material. It enables to produce architectures similar to those of real bones. Here we studied a kinetic model to describe ampicillin release from HA.

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HA analogous to the mineral component of bones, its properties make it desirable as implant materials and delivery agents of drugs. HA was synthesized by the aqueous precipitation method from CaO and H_3PO_4 as the reagents, and used as a carrier for charging ampicillin. HA grafts were impregnated with 25 mM of ampicillin buffer solutions at 37 ºC for 48 h until dried. Morphological investigation was performed using a scanning electron microscope. Antibiotic adsorbed in HA has been quantified by comparing sample weight before and after loading. In the course of the release experiment, ampicillin was dissolved in phosphate buffer to make a stock solution, maintained at 37 ºC and shaken at 60 strokes/minute. Kinetic experiments to determine the amount of ampicillin adsorbed as a function of contact time were conducted by stirring. Samples were withdrawn periodically for analysis and then returned to the mixture. Adsorbed amounts were measured by the difference of the concentration of the antibiotics before and after adsorption using UV adsorption at 215 nm.

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Solvent effect on the antioxidant activity of extracts of *Myrtus communis* L. leaves

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Keywords: Myrtle, Myrtus communis, antioxidant activity, extraction, Rancimat

Extracts obtained from herbs and spices have been found to present antioxidant activity, and their use instead of synthetic antioxidants is becoming increasingly popular. Several works show that the majority of antioxidant active substances occur in extracts, but some essential oils were also studied to analyse their antioxidant activity, namely essential oil containing thymol and carvacrol, and other phenolic compounds, which exhibit high antioxidative activity. Esquível *et al.* (1999) evaluated the antioxidant activity of savoury extracts. Ribeiro *et al.* (2001) studied the antioxidant activity in supercritical fluid extracts residues. Reddy *et al.* (2005) determined the antioxidant activity of amla, drumstick leaves, and raisins. Pellegrini *et al.* (2007) studied the efficiency of extraction of a sequence of solvents of some fruit and vegetable foods, in order to evaluate their antioxidant capacity. Ali *et al.* (2007) studied several Indian medicinal herbs as sources of antioxidants. Yanishlieva *et al.* (2006) presented a review with results on the stabilization of lipids and lipid-containing foods with different herbs and spices (ground materials and extracts). Although many plants has been investigated, only rosemary and sage are commercially available as flavourless, odourless, and colourless antioxidant extracts.

Myrtle (*Myrtus communis* L.) leaves, from plants originated from the Centre region of Portugal, were subjected to different treatments to obtain extracts with antioxidant activity.

The sample leaves were submitted to hydrodistillation (Clevenger distillation) and the deodorised solid residues were solvent extracted. The solvents tested were *n*-hexane, diethyl ether, isobutyl acetate, ethyl acetate, acetone, isopropyl alcohol, *n*-butanol, ethanol, and methanol. The largest extract yields were obtained with methanol, followed by ethanol.

There are several methods for the evaluation of antioxidant action on fats and oils (Frankel and Meyer, 2000; Becker *et al.*, 2004). The Rancimat method has some advantages such as a quick answer, and allowing the stability evaluation at high temperatures, which are important factors for the use of the antioxidants in food processing.

The Rancimat method is based on the increase of electrical conductivity due to the formation of volatile dicarboxylic acids as a result of lipid oxidation. Metrohm Rancimat 679 was used. Samples of extract were dissolved in 7.0 g of sunflower oil, at various concentrations of extracts, and were heated at 110 ºC. A continuous air stream (20 L/h) was passed through the heated samples, and the volatile compounds were absorbed in a conductivity cell. Conductivities were continuously monitored until a sudden rise signified the end of induction period. The protection factor (PF) is the ratio between the induction time found for the sunflower oil with a certain quantity of extract and the induction time obtained for sunflower oil with no addition of extract.

Two extraction methods were used: solid-liquid extraction (SLE) and Soxhlet (Sox). Protection factors of extracts obtained from solid residues of Clevenger distillation, at 2h of extraction time, with different solvents, are presented in Figure 1. The extraction method did not affect significantly the antioxidant activity of the extracts. The higher values of protection factors were obtained with solvents of intermediate polarity, such as ethyl acetate (PF=2.58) and diethyl ether (PF=2.23). However, alcoholic extracts also presented high antioxidant activity.

When both the yields and the protection factor were compared, the isopropyl alcohol extract presented the best values (PF = 1.8; Yield = 25 g extract / 100 g deodorised solid residue).

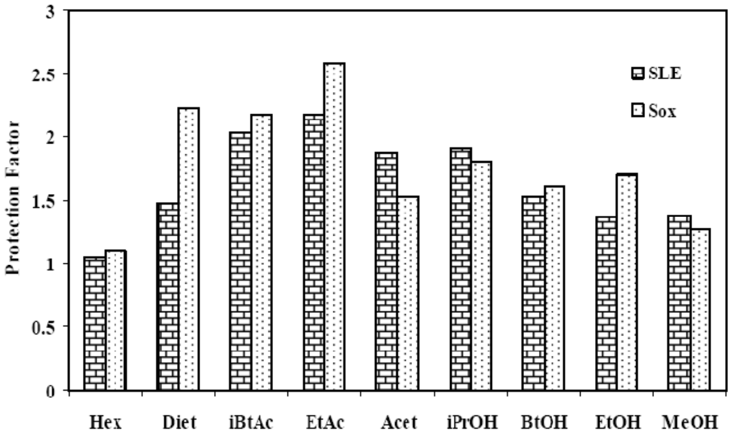

Fig. 1. Protection Factors of extracts obtained from solid residues of hydro-distillation, with different solvents, after 2h of extraction time. SLE – solid-liquid extraction at solvent boiling temperature with the solid immersed in solvent; Sox – Soxhlet method.

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Selection of a suitable adsorbent for decoloring sugar solutions from beet industry with ion exchange resins

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Keywords: Sugar technology; Color removal; Melanoidins; Resins; Size exclusion chromatography

Introduction

Juices and syrups formed during sugar beet processing contain impurities that impart yellow or brown color to white sugar. These compounds are mainly formed through beet processing as a result of sugar degradation reactions, pH changes, thermal effects and reactions between amino compounds and carbohydrates (Maillard reaction). High molecular weight colored impurities may be occluded within sugar crystals, causing a negative effect on the quantity and quality of white sugar. The colorants in beet sugar juices are mainly anionic compounds that may be removed by adsorption using ionic exchange resins. Acrylic resins are commonly used to decolorize refined sugar solutions. These resins show low adsorption selectivity but can be easily regenerated. Polystyrenic resins show high decolorization efficiencies but its regeneration is more difficult than acrylic resins. The aim this work was to enhance the sugar beet juice quality by removing colored compounds present in thin juice. Styrenic and acrylic resins were studied in order to select a suitable decolorization scheme based on a single adsorbent or combinations of adsorbents for a sound factory scale design. Analysis by size exclusion chromatography coupled with diode array detection was chosen to yield information about the evolution of high molecular weight colorants.

Material and Methods

- *Adsorbent*: Three resins have been tested: the strong styrenic resins Lewatit S 6368 and Lewatit S 6328 A (Bayer) and the acrylic resin Lewatit VP OC 1074. Prior to being used, resins were washed with distilled water.
- *Adsorbate*: Sugar beet solutions containing 17-18% dry matter, pH 8.5-9.0, purity 96-97% and 3000-3300 IU were used in all experiments. Sugar solutions were prepared by diluting thick juice from a Northern beet sugar factory.
- *Experimental set up*: Experiments were carried out in duplicate in jacketed columns operating in parallel. Each column held 207 mL of resin (1 Bed Volume). Decolorization experiments were performed at 60 ºC feeding a flow of 11 BV/h in down flow direction. The cycle length was prolonged up to reduce about 90% of color. After decolorization, resins were washed with 9 BV/h of distilled water in up flow mode. Resin regeneration was achieved with an alkaline calcium saccharate (Bento, 1999) solution containing $CaCl_2$ (25 g/L), CaO (5 g/L) and sucrose (120 g/L). Regenerant was fed through the resin in down flow at 2.3 BV/h and 60 ºC. Finally, the resin was washed with distilled water at 9 BV/h in up flow mode. All the experiments were carried out in duplicate.
- *Analytical procedures*: Color, dry matter content, pH and purity were determined according to ICUMSA methods (ICUMSA, 1994). The molecular masses of colored compounds were estimated by size exclusion chromatography (SEC). Separation was achieved in an Ultrahydrogel™ 250 column. Identification was achieved by using a Waters 996 Photodiode Array Detector.

Results and Discussion

Decolorization experiments were carried out feeding 5.5 BV of juice. The volume of regenerant was 6.9 BV. Table 1 compares the mean decolorization percentages. Color load was calculated according to references (Bento, 1997).

Table 1: Decolorization efficiencies of thin juices with ion exchange resins.

Resin	Decolorization, %	Adsorbed color load, %
S6328A	84.4	84.7
S6368	87.1	88.6
VP OC 1074	87.0	87.4
VP OC 1074 + S6368	91.8	95.3
S6368 + VP OC 1074	91.5	95.5

Purity, pH and dry matter content values remained almost constant during the experimental runs. From the experimental results showed in Table 1, it can be concluded that there were no considerably differences among the resins studied. The styrenic resin S6368 provided slightly higher color removal percentages, about 87.1%, which corresponded with an adsorbed color load about 88.6%. The decolorization percentages slightly increased using two beds in series. The acrylic resin followed by the styrenic resin provided the higher decolorization percentages, about 91.8%, which an adsorbed color load of 95.3%. The combination styrenic-acrylic resins provided a similar color reduction percentage, 91.5%.

Size exclusion chromatograms (Figure 1) showed that the colorants of higher molecular masses, 100-50 kDa, were more efficiently removed than the colorants of lower molecular weigh (about 2-3 kDa). Colorants of higher molecular mass are likely to be related to melanoidins (Coca et al., 2004). Both styrenic resins were more efficient in removing lower molecular weight colorants than the acrylic resin VP OC 1074.

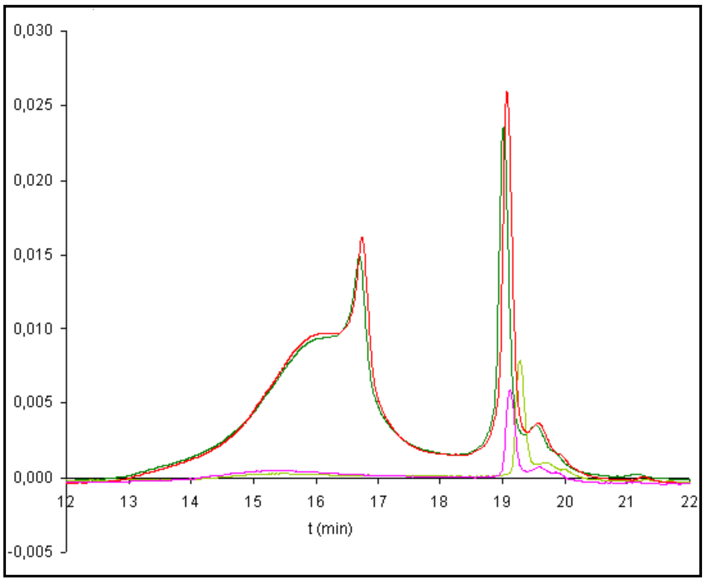

Figure 1: Decolorization of thin juices. Size exclusion chromatograms

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Assessment of the efficiency of N,N´-ethylenedi-L-cysteine as a potential green chelator agent

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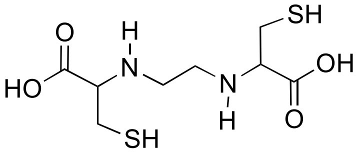
Keywords: N,N´-ethylenedi-L-cysteine, synthesis, chelator agent, potential biodegradability

Chelants sequester metals and prevent unwanted side reactions. Chelants are used in domestic products such as laundry detergents, houshold cleaners, personal care products and industrial applications such as pulp bleaching and metal cleaning. More recently, they have been used as environmental metal remediation agents [Vandevivere *et al*., 2001].

Chelating agents in widespread use include polyphosphates (e.g. pentasodium tripolyphosphate), hydroxycarboxylates (e.g. citric acid), aminocarboxylates (e.g. NTA and EDTA) and phosphonates (e.g. aminotrimethylenephosphonate).

The chemical industry produces 166,000 tonnes EDTA + NTA per annum. The majority of these chelators are discharged into sewers where they are not readily biodegradable. Environmental concerns, which have recently resulted in the banning of EDTA, in some states/countries, have led to efforts to find readily biodegradable replacement ligands which have parity in terms of chelating power.

The N,N´-ethylenedi-L-cysteine [2,2'-(ethane-1,2-diylbis(azanediyl))bis(3-mercaptopropanoic acid)] was synthetized using units of enantiopure aminoacid, L-cysteine, the isomeric form more abundant in living organisms [O´Dowd and Hopkins, 1998]. In addition, N,N´-ethylenedi-L-cysteine is a ethylenediamine disubstituted derivative with two secondary nitrogen atoms in the molecule, which are potentially biodegradable [Pitter and Sykora, 2001].



These facts together point to N,N´-ethylenedi-L-cysteine as a potential green chelating agent that should combine several desired properties: biodegradability, low toxicity and high affinity for most heavy metals.

The N,N´-ethylenedi-L-cysteine was prepared in a two-step synthesis process [Blondeau et al., 1967], starting from cysteine hydrochloride. From reaction with formaldehyde the precursor L-thiazolidine-4-carboxylic acid was obtained and under the action of sodium metal in liquid ammonia the final product was formed.

Metal formation constants with Ca, Cd, Co, Cu, Mg, Ni, Pb and Zn are presented, some being determined as part of this study. These constants were used for computer simulation modelling of N,N´-ethylenedi-L-cysteine equilibria and compared with EDTA simulations in order to assess the efficiency of N,N´-ethylenedi-L-cysteine as a potential green chelator agent for substituting EDTA applications.

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Delignification kinetics of kraft pulps by laccase-violuric acid system

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Keywords: Laccase, Mediator, Kraft pulp, Delignification, Kinetics

After the cooking process the pulp has a significative amount of residual lignin, which should be removed in order to obtain bleached pulp. This process involves consecutive stages, usually using chlorine dioxide, sodium hydroxide and eventually oxygen, ozone and hydrogen peroxide. Some of these compounds generate effluents with organic chloride compounds which negatively affect the environment. Laccases have been receiving increasing research efforts due to its potential as oxidizing agents. Because of the laccase dimensions and the pore size of the cell wall, it seems likely enzyme can not penetrate into the cell wall to degrade directly the residual lignin and a mediator should be used (Bourbonnais et al, 1997). Several mediators have been tested in the recent years. A comparative study with 2-2´azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 1-hydroxybenzotriazole (HBT) and violuric acid (VA) has demonstrated the advantage of the violuric acid (Oudia, 2007). The potential of laccase-violuric acid system (LMS) on *E. globulus* kraft pulp has been recently investigated (Oudia et al, 2007) and delignification extent up to 70% was achieved using two consecutive LMS stages. The present paper reports on the delignification kinetics of laccase-violuric acid system on *E. globulus* and *Pinus pinaster* kraft pulps. The residual lignin content and their chemical structure are very different, enabling to extend the kinetic studies. The kinetic studies were carried out at very low solid content (0.62%), and under relatively high mixing intensity in order to minimize the mass transfer resistance outside the pulp fibres. The pH and the temperature of the reaction medium were adjusted to 4.5 and 45ºC, respectively. The oxygen pressure was fixed at 6 bar. Laccase doses between 20 IU/g and 200 IU/g of pulp were investigated, while the mediator charge has varied from 0.5% to 11% on pulp. The reaction was followed by monitoring the pulp residual lignin content with time, after submitting the oxidized pulp to an alkaline extraction to remove the degraded lignin. The oxygen consumption was also investigated for some experiments.

Figure 1-a shows the evolution of the residual lignin content, measured in terms of kappa number, along the reaction time. The experimental results show a very fast decrease of the residual lignin followed by a very slow phase, probably limited by diffusion. Several hypotheses can be pointed out to explain the data, but lignin inaccessibility after a given oxidation extent is the most plausible. In fact, practically no additional lignin degradation occurs when the oxidized pulp was submitted to a new LMS stage without previous alkaline extraction. On the contrary, substantial delignification occurs when the pulp is previously submitted to an alkaline extraction stage.

In order to study the kinetic of the reaction, series of experiments were designed with much lower reaction times: 1, 5, 10, 20 minutes. Figure 1-b shows the kappa number profile for different mediator charges.

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The new experimental data reveal that the major delignification takes place during the first minute of reaction. The slight but consistent increase of the lignin content for 5 minutes reaction time could suggest competition between lignin degradation and polymerization of the dissolved lignin fragments (Rittstieg et al, 2003).

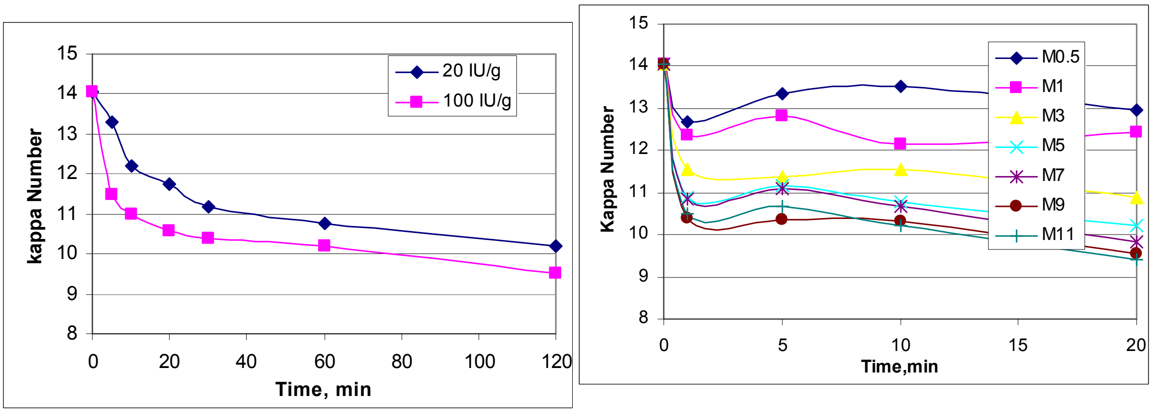

Figure 1: Kappa number vs time at 4% of mediator (a-left) and at a laccase dose of 100 IU/g of pulp (b-right).

Figure 1-b also shows the very positive effect of the mediator charge on the initial reaction rate in the range 0.5%—9%. Considering –Δkappa number/Δt, for t=1 min, as a good estimation for the initial reaction rate, Figure 2 shows the evolution of the reaction rate with the mediator charge. It resembles the Michaelis-Menten equation, but other mechanisms are under investigation. The key role of the mediator on the reaction rate and on the reaction extent, however, is clearly demonstrated in Figure 1-b. Similar experiments were carried out with *P. pinaster* kraft pulp, with much higher residual lignin content, which shows similar trends but other reaction rates, as expected.

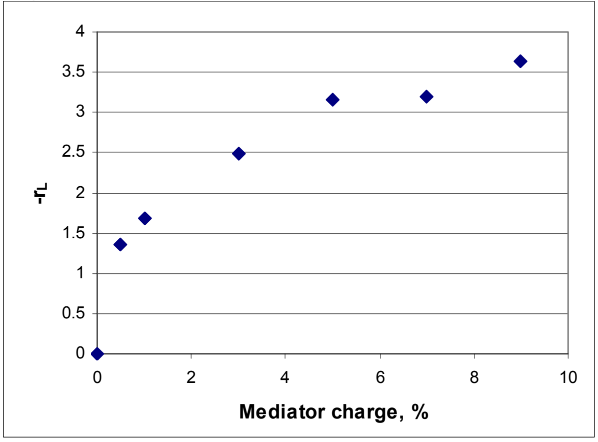

Figure 2: Initial (average of 1 min) reaction rate of delignification vs mediator charge.

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Kinetics of Kraft pulp brightening with hydrogen peroxide in a final ECF stage

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Keywords: Hydrogen Peroxide, Kinetics, Final bleaching stage, Brightness, Eucalyptus

Elemental chlorine free (ECF) sequences are presently the most used technology in wood pulp bleaching. The consolidation of this multistage pulp bleaching technique still needs better coordination between bleaching stages and target brightness, enhanced flexibility and higher washing efficiency, within the so-called concept of minimum impact mill. Regarding eucalyptus bleached pulps, there is an ever-increasing market demand, mainly for printing and writing paper grades. These specific products demand high strength pulps bleached to high brightness levels and exhibiting low brightness reversion. In this context, the final bleaching stage plays a key role in determining the quality of a market end-product.

The final stage in a chemical pulp bleaching sequence is a truly brightening stage to gain the difficult brightness points with the destruction of the chromophores remaining in pulp. The origin of many of these chromophores has been recently assigned to quinone type structures (Rosenau et al., 2007), thus justifying the final bleaching stage with hydrogen peroxide, which degrades these kinds of structures. Under alkaline conditions the brightening agent is considered to be the hydrogen peroxide anion (HOO^-), a strong nucleophile that reacts preferentially with carbonyl and conjugated carbonyl groups (Lachenal, 1996). However, besides chromophores destruction, the waste of hydrogen peroxide in decomposition reactions should be also considered. Some of these competitive reactions are catalysed by transition metals, producing unselective radical species that attack polysaccharides thus reducing pulp quality and/or yield and wasting oxidation power.

In previous studies (Carvalho et al., 2008; Loureiro et al., 2008), it was demonstrated that the substitution of the conventional final chlorine dioxide stage (D) by a hydrogen peroxide stage (P) results in better beatability and papermaking properties of the bleached pulp with a notable improvement of brightness stability. In addition, the ISO brightness of DED prebleached pulp should be at least 87% before the final P stage in order to attain a final ISO brightness of 90%. In order to implement this final P stage in a mill situation, a previous kinetic study is required to ensure the best results regarding both process control and optimization. A kinetic study is also important to clarify both the adequate conditions for the final P stage and to provide some insights on brightening and decomposition reaction rates, which is the aim of this work. The *Eucalyptus globulus* kraft pulp, used in this study, was collected after the D1 stage in the industrial ECF D0ED1ED2 bleaching plant. The D_0ED_1 pre-bleached pulp had the brightness of 87.5% ISO and an intrinsic viscosity of 1066 dm^3/kg. This pulp was submitted to a final P stage with the following range of bleaching conditions: 0.25-2.0% odp (*o*ven *d*ried *p*ulp) of H_2O_2, 0.6-0.8% odp of NaOH, 60-90 ºC, 0.25% odp of DTPA (Diethylene triamine pentaacetic acid).

As can be seen in Figures 1 and 2, there is a fast brightness evolution during the first minutes of reaction which slows down afterwards. In some cases, asymptotic levels are

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attained and in others a negative evolution is even observed. These results suggest that a kinetic model to be developed may comprise two phases with different kinetic parameters due to the presence of chromophoric structures with dissimilar reaction rates. The brightness evolution is faster as hydrogen peroxide charge and/or the temperature increases. The moderate temperature of 70ºC renders a continuous evolution of brightness values for several hydrogen peroxide charges. In fact, even with the lowest charge, a brightness ceiling is not attained (Figure 1). Although an initial higher development of pulp brightness as temperature raises is observed (Figure 2), for temperatures greater than 70ºC, all hydrogen peroxide is consumed after nearly 60 min which leads to a slight pulp darkening for longer times. Therefore, for the pulp used, residence times greater than 120 min are not advisable for temperatures greater than 70ºC. On the other hand, the higher the temperature, the lower the pulp intrinsic viscosity and the pulp yield (results not shown). Therefore, 70ºC seems to be the most advisable temperature for the final P stage of *E. globulus* pulp bleaching. This value is quite lower than those reported in related literature, e.g. 90ºC (Anderson and Amini, 1996).

To assess the hydrogen peroxide consumed in the parallel reactions, the same industrial pulp was used but bleached to very high brightness (with negligible chromophores content). The total hydrogen peroxide consumed in the final P stage and the fraction consumed in decomposition reactions were both evaluated and a strong dependence on the temperature was perceived. In fact, the peroxide decomposition reached half of its total consumption at 90 ºC.

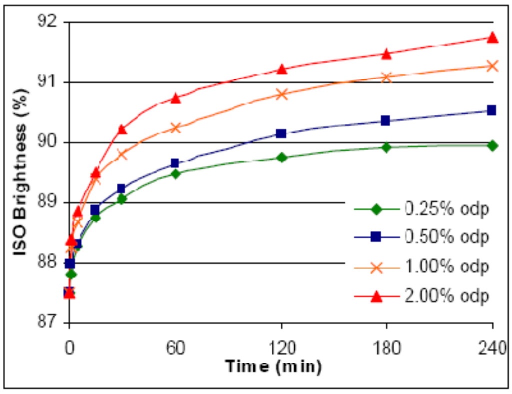

Figure 1. Brightening kinetics at 70 ºC with different H_2O_2 charges.

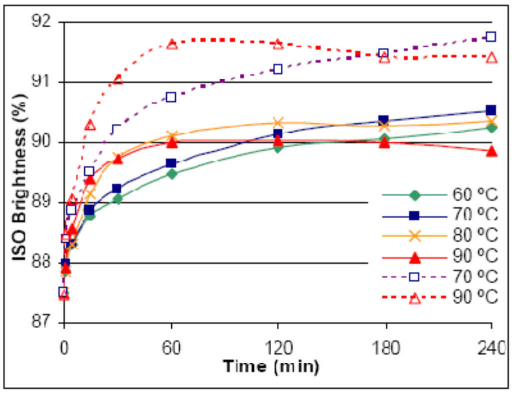

Figure 2. Effect of temperature on the brightening kinetics. H_2O_2 charges of 0.5 (filled symbols) and 2.0 % odp (empty symbols).

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A case study of an absorption process for flue gas treatment

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Keywords: Flue gas, Absorption, CO_2, SO_x, NO_x

The emissions produced by fossil fuels combustion have been the object of a lot of research work and many developments were observed within last decades. Meanwhile these fuels continue to be very attractive and energy demand keeps growing, environmental concerns increase and legislation is more and more exigent. All measures taken to improve the efficiency of combustion units are of prime importance for emissions reduction. Good results were obtained with those strategies in many power generation units. Even in these cases the removal of pollutants in flue gases is normally necessary to meet legal standards.

Special attention has been devoted to the removal of SO_x, NO_x and recently CO_2, due to its effect on global warming. Wet scrubbing, semidry and dry processes for SO_x removal, selective non-catalytic or catalytic reduction of NO_x are used for emissions treatment. Costs associated with these technologies are significant, mainly for the budget of small and medium size industrial units. Absorption processes used to eliminate acid gases from flue gases are widely described in the literature as wet scrubbing. They are considered the most efficient for SO_x removal, but usually more expensive. The equipment includes spray towers, plate columns or packed towers. Bubble columns are not fully explored. Typical absorbents are aqueous solutions of lime, limestone and sodium carbonate, the production of CO_2 being now unsuitable. Simultaneous removal of NO_x is less efficient but recent developments brought new results.

The selection of integrated procedures that take the industrial process as a whole is a quite recent concept. The replacement of common absorbents by alkaline effluents produced in *situ* is an attractive solution, as the neutralisation of both currents may be accomplished in a single step. In many industries (Portuguese industrial plants included) both kind of effluents are effectively produced. This study was designed under this perspective, bearing in mind the optimisation of absorption processes. This is considered in the literature an important aspect to be developed.

In the frame of this project and with the objective of studying real cases, a privileged cooperation protocol was established with an industrial Portuguese company: PORTUCEL (Cacia, Aveiro).

A laboratory apparatus was designed and constructed, including a bubble column (inside diameter of 0.14 m, total height of 0.70 m) as the absorption system. Two different types of gas injectors were used: perforated plate and perforated tubes.

In all absorption experiments, the column was operated in counter current flow at atmospheric pressure and ambient temperature, with partial liquid recirculation. The flue gas composition was simulated using synthetic gas mixtures. The experiments were carried out varying: total gas flowrate (200 to 360 cm^3/s, 1bar, 20ºC), liquid to gas flowrate ratio and gas composition ($8\%<CO_2<12\%$, $470ppmv<SO_2<980ppmv$, $340ppmv<NO<720ppmv$).

In a first set of experiments, an industrial effluent from Cacia plant ($10.2<pH<11.7$) was used as the liquid phase. In order to simplify the experimental planning and to allow a better comparison of results from different essays, a synthetic effluent was selected. For this, several solutions of Na_2CO_3:NaOH (in different proportions) were tested in the

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same experimental conditions as the industrial effluent. A proportion 6:4 (Na_2CO_3:NaOH), in meq/L, with a total concentration of 1.5 g $CaCO_3$/L, was found to have a similar absorption performance. This synthetic effluent was used in further absorption essays.

Gas and liquid flowrates were regulated and measured inline. The pH of the liquid outlet was monitored during each experiment. Except for the initial set of experiments, the gas composition at the inlet and outlet of the column has been continuously monitored by means of a gas analyser (O_2, SO_2, NO_x, CO_2), specially acquired for this purpose.

The main conclusions of this study were:

- both the industrial and the synthetic alkaline effluents were effectively neutralised to 7< final pH <9.5;
- the removal values of CO_2 from the gaseous current were up to 25% and the SO_2 was completely removed, for every experiment.
- the absorption of NOx still claims for further work, that will be performed analysing other important parameters.

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Influence of nitrogen oxides on ozone concentrations at urban and remote areas

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Keywords: ozone, nitrogen oxides, urban area, remote area

Introduction

High concentrations of superficial ozone have negative impacts on human health, vegetation, materials, climate and atmosphere composition (WHO, 2000). Considering the harmful effects of this pollutant, standards that should not be exceeded were legislated by the European Union: i) information threshold (IT) - 180 μg m^{-3} (1-hour average); ii) alert threshold (AT) - 240 μg m^{-3} (1-hour average); and iii) target value for the protection of human health (TVPHH) - 120 μg m^{-3} (8-hour running average) (Directive 2002/3/EC). Photochemical ozone is formed by reactions involving solar radiation and anthropogenic pollutants (methane, non-methane volatile organic compounds, carbon monoxide) in the presence of nitrogen oxides. There is a competition between VOC and NO_x for the OH radical. When [VOC]/[NO_x] is low, the reaction of OH with NO_x can predominate (VOC limited), removing OH from the VOC oxidation cycle and retarding the further production of O_3. Under these conditions, typical of polluted areas, an increase in NO_x concentration leads to O_3 decrease. When [VOC]/[NO_x] is high, OH will react mainly with VOC (NO_x limited), generating new radicals and accelerating O_3 production. Under these conditions, typical of rural areas, an increase in NO_x concentration accelerates O_3 formation. Therefore, while increasing of VOC concentration always increases ozone formation, increasing of NO_x leads to more or less ozone, depending on the prevailing ratio between [VOC] and [NO_x] (Seinfeld and Pandis, 1998).

The purposes of this work were to compare ozone concentrations at remote areas with urban ones influenced by traffic and to evaluate how nitrogen dioxide influences ozone concentrations in both, urban and remote areas.

Methodology

The study here reported was based on data measured at one urban site influenced by traffic in Oporto (PO) and at six remote sites: five at Bragança district (BG_1, BG_2, BG_3, BG_4 and BG_5) and one at Vila Real district, Lamas d'Olo (LO). Pollutant concentrations were measured with passive samplers in two periods during the summer of 2006. To test their performance, the sampling tubes were co-located with continuous monitors, at sites PO and LO.

The pollutants measured were ozone and its precursors, recommended by the Directive 2002/3/EC: nitrogen dioxide and some volatile organic compounds, namely, benzene, 1,2,4-trimethylbenzene, 1,3,5-trimethylbenzene, m/p-xylene, o-xylene and toluene.

Ozone and nitrogen dioxide concentrations were also continuously monitored using, respectively, UV-absorption photometry and chemiluminescence methods.

Results and Discussion

The lowest concentrations of O_3 were observed at the PO site being 51.0 μg m^{-3} and 40.9 μg m^{-3} for the 1st and 2nd periods, respectively. From all remote sites analysed,

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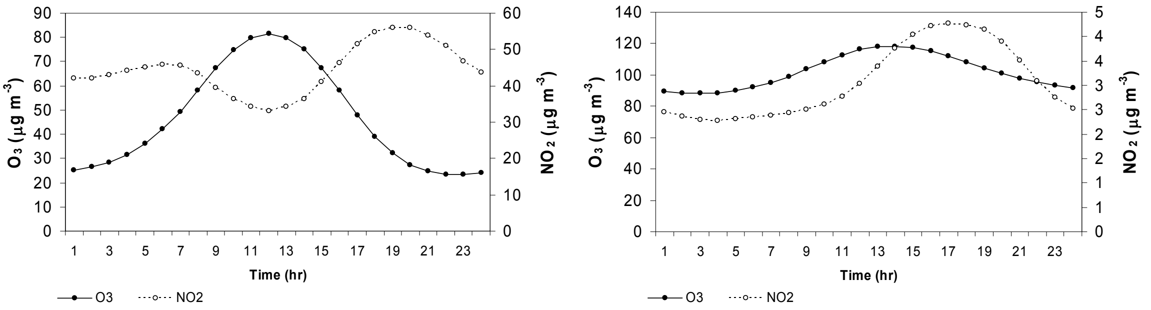
BG_4 was the one with the highest concentrations during both periods (97.9 μg m^{-3} and 122.0 μg m^{-3}, respectively). As expected, during the period of passive sampling the exceedances of IT, AT, and TVPHH were greater at the LO site than at the PO site, being, approximately, 5, 8, and 2 times greater, respectively. It could be observed for PO site a typical profile of an urban area where O_3 formation was controlled by VOC concentrations, leading to a decrease on O_3 concentrations when NO_2 concentrations increased.

NO_2 concentrations at the PO site were from 12 to 20 times greater than those at remote sites, due to the influence of traffic emissions. The VOCs concentrations were also considerably high at the PO site, being from 2 to 8 times greater than at remote sites, again confirming the traffic influence.

It could be observed for LO site a typical profile of a rural area where O_3 formation was controlled by NO_2 concentrations. Accordingly, a decrease on O_3 concentrations was observed when NO_2 concentrations decreased. These results showed that the ratios of VOC-to-NO_2 concentrations where relatively high at LO site because of the relatively quicker removal of NO_x from distant sources compared to that of VOC, coupled with the absence of NO_x local sources (Figure 1 (a) and (b)).



(a)

(b)

Figure 1. Daily profile of 8-hour running averages for the average for all the period of passive sampling: (a) at PO site and at (b) LO site.

Conclusions

At the urban site, ozone formation was controlled by volatile organic compound concentrations, leading to a decrease in ozone concentrations when nitrogen dioxide concentrations increased. At the remote sites, ozone formation was controlled by nitrogen dioxide concentrations, due to its very low level. Accordingly, a decrease on ozone concentrations was observed when nitrogen dioxide concentrations decreased.

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Influence of tobacco smoke on carcinogenic content of indoor particulate matter

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Keywords: PM_{10}, $PM_{2.5}$, PIXE, tobacco smoke, carcinogenic elements

Introduction

In the past years, many epidemiological studies showed a strong association between the exposure to PM_{10} (particles with aerodynamic diameter smaller than 10 μm) and especially to $PM_{2.5}$ (particles with aerodynamic diameter smaller than 2.5 μm) and the increase of morbidity and mortality rates, caused by pulmonary and cardiovascular diseases. One of the most important parameters of particles is their size because it determines the place of particle deposition in the respiratory system; the smaller the particles, the deeper the penetration. Thus the recent attention has been focused on particles of smaller sizes - $PM_{2.5}$ and PM_1 (particles with aerodynamic diameter smaller than 1 μm). Chemical composition of PM is also an important parameter, as it is common belief that it may significantly enhance the negative health effects.

Tobacco smoking is certainly one of the greatest sources of the indoor inhalable particles, not only for the smokers but also for all those who are somehow exposed to it. In 1993, the US Environmental Protection Agency classified passive smoking, i.e., exposure to secondhand tobacco smoke, also called environmental tobacco smoke (ETS), as a “Class 1” human carcinogen (EPA, 1993); accordingly, the International Agency for Research on Cancer unanimously stated that ETS is carcinogenic to humans. Over 4000 different chemicals have been indentified in tobacco smoke that is a complex mixture of gaseous components and of particles of different sizes, mainly constituted by carbonaceous compounds, aromatic hydrocarbons with high molecular number, nicotine, and heavy metals; as most of particle components have known impacts on health, the exposure to particles represents serious risk to human health.

To further understand the impact of tobacco related particulate matter on public health, this study evaluated the influence of tobacco smoke on the characteristics of PM_{10}, $PM_{2.5}$ and PM_1, considering concentration and elemental composition of carcinogenic elements.

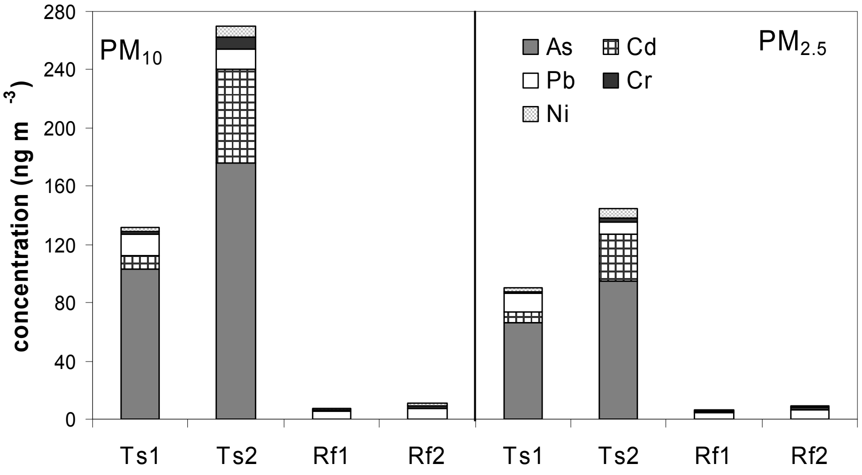
Methods

The monitoring of PM_{10}, $PM_{2.5}$ and PM_1 was conducted during summer-autumn 2006 at four sites situated in Paranhos district of Oporto city in Portugal: two directly influenced by tobacco smoke, one office (Ts_1) and one cafeteria (Ts_2), and two homes not directly influenced by tobacco smoke, reference sites Rf_1 and Rf_2. At each site the sampling was conducted for 28 consecutive days. Samples of indoor PM were daily collected for 12-h in order not to over-load filters for consequent chemical analysis. Sampling was done using TCR TECORA Bravo H2 constant flow samplers, combined with PM EN LVS sampling heads in compliance with the norm EN12341, using an air flow rate of 38.3 L min^{-1}. Inlets were placed 1.5 m above the floor and about 1 m from the walls, without obstructing the normal usage of the rooms. PM masses were determined gravimetrically by subtracting the initial average mass of the blank filter from the final average mass of the sampled filter; the difference was then divided by the total volume of air that passed through filter (at 25°C and 101.3 kPa). The elemental compositions of PM_{10} and $PM_{2.5}$ were determined by Proton Induced X-ray Emission (PIXE).

Results and discussions

At sites influenced by tobacco smoke, PM_{10} concentrations ranged from 21.3 to 220 $\mu g\ m^{-3}$ (mean of 107 $\mu g\ m^{-3}$) and from 111 to 242 $\mu g\ m^{-3}$ (mean of 156 $\mu g\ m^{-3}$), the corresponding $PM_{2.5}$ concentrations ranged from 17.6 to 202 $\mu g\ m^{-3}$ (mean of 103 $\mu g\ m^{-3}$) and from 95.0 to 213 $\mu g\ m^{-3}$ (mean of 132 $\mu g\ m^{-3}$). The reference sites exhibited lower values of PM, ranging from 8.4 to 58.8 $\mu g\ m^{-3}$ (mean of 29.3 $\mu g\ m^{-3}$) and 6.8 to 44.8 $\mu g\ m^{-3}$ (mean of 23.6 $\mu g\ m^{-3}$) for PM_{10} concentrations; the corresponding $PM_{2.5}$ concentrations ranged from 6.9 to 58.2 $\mu g\ m^{-3}$ (mean of 24.6 $\mu g\ m^{-3}$) and from 6.1 to 40.5 $\mu g\ m^{-3}$ (mean of 16.9 $\mu g\ m^{-3}$). PM_1 concentrations ranged from 17.1 to 153 $\mu g\ m^{-3}$ (mean of 84.2 $\mu g\ m^{-3}$) and from 69.3 to 181 $\mu g\ m^{-3}$ (mean of 110 $\mu g\ m^{-3}$) at the sites influenced by tobacco smoke; at the references sites PM_1 ranges were 5.5 to 44.7 $\mu g\ m^{-3}$ (mean of 17.3 $\mu g\ m^{-3}$) and 3.6 to 30.2 $\mu g\ m^{-3}$ (mean of 13.4 $\mu g\ m^{-3}$). In general, the results showed that tobacco smoke increased concentrations 270-560% for PM_{10}, 320-680% for $PM_{2.5}$ and 390-720% for PM_1. These results allowed concluding that tobacco smoke strongly increased PM concentrations, this increase being clearly higher for smaller particles.

In total, 25 elements were determined using the PIXE technique to characterize PM_{10} and $PM_{2.5}$ sampled at the two sites influenced by tobacco smoke and at the two reference sites. Five elements were carcinogenic ones: Cr, Ni, As, Cd and Pb. The mean concentrations of Cr, Ni, As, Cd and Pb in PM_{10} and $PM_{2.5}$ at smoking (Ts_1, Ts_2) and non-smoking sites (Rf_1, Rf_2) are shown in the following figure.



From this figure it is clear that at sites influenced by tobacco smoke, As was the most abundant element in both PM (ranging from 103 to 176 $\mu g\ m^{-3}$ and from 66 to 95 $\mu g\ m^{-3}$ in PM_{10} and $PM_{2.5}$, respectively). As and Cd occurred only in PM of sites with tobacco smoking, showing its strong influence on the presence of carcinogenic elements in indoor air. Pb concentrations at these sites were 110-180% and 30-190% higher than at the reference sites for PM_{10} and $PM_{2.5}$, respectively. Cr and Ni exhibited lower concentrations than As, Cd and Pb, mainly for the reference sites. Still, at the sites influenced by tobacco smoke, Cr and Ni concentrations in PM_{10} were 15-680% and 15-480% higher, respectively; the concentrations in $PM_{2.5}$ were 20-250% and 10-490% higher for Cr and Ni, respectively. The obtained results showed that by tobacco smoke increased 1100-2400% and 840-2200% the total concentrations of five carcinogenic elements (Cr, Ni, As, Cd and Pb) in PM_{10} and $PM_{2.5}$, respectively.

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Study of NO oxidation before absorption in bubble and packed columns

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Keywords: NO_x, Absorption, Flue gas, NO_x chemistry

The control of nitrogen oxides (NO_x) is still nowadays one of the more pertinent targets regarding environmental protection, in energy production plants, from fossil fuels combustion. Moreover, in some emission sources such as co-generation plants, NOx represent the main concern in terms of legal restrictions.

Using adequate combustion equipment and fuels, and optimising the operating conditions (regarding temperature control, air staging, etc) are some preventive measures that largely contribute to the reduction of NO_x emissions in combustion processes.

However, this is often insufficient to meet the more and more exigent legal limits, and so it is necessary to adopt simultaneously end of line technologies in order to eliminate the exceeding NO_x.

The different nitrogen oxides (NO, NO_2, N_2O_3, N_2O_4, HNO_2) are produced in unequal proportions, depending on the fuel characteristics and combustion conditions. The predominant in flue gases being NO and NO2, all nitrogen oxides are easily involved in several reversible chemical reactions that may cause a wide variability of NO_x composition in a gaseous stream. The various nitrogen oxides have different values of solubility in water (as in other liquids) and this is a very important aspect when absorption processes are considered for the reduction of nitrogen oxides.

The lowest solubility corresponds to the predominant species (NO) in flue gas typical compositions. So it is of the uttermost importance to find adequate means of oxidizing NO (and even NO_2) to produce the most soluble gases of the group.

This has been achieved by typical oxidising agents as ozone and hydrogen peroxide, but at considerably high operating costs.

The present work intends to: i) evaluate the influence of dissolved oxygen and other oxidising agents in the production of more soluble nitrogen oxides and ii) study the absorption of those compounds in a laboratory apparatus.

The main objectives underneath this experimental study are to explore low cost alternatives concerning the oxidising agents as well as the absorbent liquids. Ideally, in a real application, the liquids to be used might be rejected effluents of the same industrial plant where the NO_x are being emitted.

The absorption experiments are performed in two different laboratory units: bubble and packed columns.

The gaseous stream is water saturated before entering the absorption unit. It contains NO, SO_2, CO_2, N_2 and O_2, and is obtained by the appropriate combination of two calibrated synthetic gas mixtures to simulate typical compositions of flue gases.

To improve the oxidation of nitrogen oxides, the liquid is previously aerated or mixed with other oxidising agents. Different tests are performed to optimize this first step, varying: system pressure, total gas flowrate, liquid to gas flowrate ratio and bubbling conditions. The main absorption unit is operated in counter-current flow with no

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recirculation of the liquid phase. The same parameters (system pressure, total gas flowrate, liquid to gas flowrate ratio and bubbling conditions) are studied in order to evaluate the better NO_x removal conditions. As absorbents, different liquids are tested.

The characterisation of inlet and outlet gaseous streams is accomplished by a gas analyser (Horiba PGA250). The effluent liquid is also analysed in order to evaluate the need of a specific treatment before rejection.

A cooperation protocol was established with an industrial company nearby Porto, in which a cogeneration unit is being operated, with continuous NO_x emissions control. In the frame of this study, it is expected to perform some tests in that unit, to validate the results obtained in laboratory experiments.

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Mechanized separation of plastics from municipal solid waste: evaluation of the separation efficiency by selective dissolution and infrared spectroscopy

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Keywords: Polymer recycling, Polymer separation, Polymer analysis, Selective Dissolution, Fourier Transform Infrared Spectroscopy

Abstract: The recycling of plastics from Municipal Solid Waste (MSW) is a difficult problem to solve due to the level of contamination and the presence of a large variety of plastics. The recycling of heterogeneous mixed plastics must be preceded by their separation, due to the difficulty in obtaining recycled materials with good mechanical properties in only one operation, without separation. The present work is part of a project aiming at MSW plastics separation using mechanized methods adapted from mineral separation methods. The contribution reported concerns the chemical analysis and quantification of the plastic mixtures before and after separation using different methods.

There are two main types of plastics separation, the first is based in the separation by one physical-chemical property (Agante et al., 2004), such as differences in density, tribological separation and use of infrared spectroscopy, and the second is the separation based on differences in solubility. The processes based on solubility are an alternative path for secondary recycling by type of plastic, and implicates the dissolution of the waste plastic with solvents and their subsequent recovery.

The separation processes based on solubility include steps of dissolution of a number of incompatible polymers in a common solvent, at different temperatures, or in different solvents, separating each polymer at a time. The polymer can be recovered either by fast evaporation of the solvent, using selective dissolution, or by the addition of an appropriate non-solvent (NS), that precipitates the polymer. The application of these methods to the separation of plastic waste brings environmental problems due to the use of large amounts of solvents (Pappa et al, 2001).

The present work was developed in the aim of a project under development in Portugal for the design of a pilot plant that will be used in the mechanical separation of granulated plastics from packaging waste. The project SEMEC, financed by SPV, intends to develop a low-cost, environmentally friendly methodology for the separation of the different plastics occurring in drop-off-points. The partners of the project are CERENA, a research center of Instituto Superior Técnico, PIEP, a research center of Minho University, Tratolixo, a solid waste management system, responsible for the collection of solid wastes in an area nearby Lisbon (corresponding to almost 1 million people), and Selenis-Ambiente, a Portuguese PET recycling company. The partners from CERENA are involved in the development of separation technologies, while the PIEP/DEP partners are involved in the development of analytical methods for the characterization of the composition of the separated plastics.

The analysis of the plastic mixtures from MSW before and after separation was based on a selective dissolution method (Nauman and Lynch, 1994). The method used involved the following steps: (a) addition of a solvent to selectively dissolve only one of

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the polymers, under certain conditions; (b) filtration to remove the non dissolved polymers; (c) solvent evaporation to recover the dissolved polymer and for reuse of the distilled solvent (d) application of the same procedure for each polymer in the mixture.

In practice, the plastics from MSW were washed and the polyolefin fraction separated by density difference, in a water tank. The remaining plastics were constituted mostly by polystyrene (PS), polyvinyl chloride (PVC) and polyethylene terephthalate (PET). The dissolution process consisted on dissolving polystyrene, in the mixture of polymers, with toluene at room temperature (toluene dissolves PS at room temperature, but doesn't dissolve PET or PVC). The solvent was evaporated from the solution on a rotary evaporator and the PS was obtained as a film. The remaining mixture of PET/PVC was treated with tetrahydrofuran (THF) to dissolve PVC, at room temperature and to recover PET as the undissolved polymer. THF was evaporated from the solution with the dissolved PVC, the PVC remaining as a film. PET was washed and quantified as the final solid fraction. The separated plastics were then dried in a vacuum oven for 6 hours at 120 ºC for PS and PVC, and at 160 º C for PET. After drying, all the samples were identified by Fourier-Transform infrared spectroscopy (FTIR).

This analytical method allowed the systematic characterization of the fractions of MSW plastics separated by different mechanized methods, and the assessment of possible applications of the separated plastics according to the degree of purity or type of contaminations present.

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PAHs soil decontamination by an innovative combined method: desorption and electrochemical treatment

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Keywords: desorption, electrochemical treatment, desorption, PAHs, soil remediation, surfactants

Polycyclic aromatic hydrocarbons (PAHs) are widely distributed in the environment. PAHs emitted into the atmosphere by natural processes (e.g. forest fires) or industrial sources are largely adsorbed on particulate matter in the atmosphere. The adsorbed PAHs then can be deposited into aquatic and soil environments by fallout, subsequently leading to water and soil pollution. Toxic PAHs are persistent and these molecules are strongly adsorbed on soils, sludges or sediments because of their strong hydrophobicity which makes them less bioavailable, while simultaneously limiting conventional remediation measures (Bamforth and Singleton, 2005). PAHs removal from soils and aquifers by natural attenuation mechanisms or traditional remediation efforts such as pump-treatment is slow due to the low solubility of these compounds in water (Zhou and Zhu, 2005). Therefore, the addition of different water miscible solvents, especially surfactants, is a natural first step to enhance PAH solubility. The PAHs are desorbed with surfactants through solid-liquid equilibrium, and PAHs present in the solution collected could be degraded in a second stage by an adequate treatment.

The aim of this study is to evaluate the potential of a combined treatment in two stages: PAHs removal from kaolin and electrochemical treatment once in solution (desorption with nonionic surfactants followed by detoxification steps to generate innocuous products by electrochemical treatment). The selection of the most appropriate surfactants from a list of five (Brij 35, Tergitol, Tyloxapol, Tween 20, and Tween 80) was carried out based on the extraction capacity for each PAH. Secondly, the electrochemical degradation process was conducted with neat solutions of PAHs with the best surfactants, according to the results of previous experiments.

In soil preparation kaolin was spiked with different PAHs (Anthracene, Benzo[a]pyrene, Phenanthrene, Benzanthracene, Fluoranthene and Pyrene). 0.1 grams of PAH was added to 200 grams of soil to yield 500 mg PAH per kg soil (typical PAH concentrations at contaminated sites; USEPA, 2000). The desorption was performed in 250 mL Erlenmeyer flasks, containing 2.5 g of polluted kaolin and 50 mL of the surfactant solution with a concentration of 10 g/L for all the experiments tested. Based on previous papers (Sartoros et al., 2005; Alcántara et al., 2008) the surfactants used were: Brij 35, Tergitol, Tween 20, Tween 80 and Tyloxapol. PAHs were determined by HPLC using an Agilent 1100 HPLC equipped with a XDB-C8 reverse-phase column (150×4.6 mm i.d., 5 µm). The injection volume was set at 5 µL and the isocratic eluent (70:30 acetonitrile/water) was pumped at a rate of 1 mL/min. A diode array detector, operating from 200 to 400 nm was used to monitor the eluate. The concentration of PAHs in the spiked soil was determined through the extraction process from kaolin using a Soxhlet apparatus (Alcantara et al., 2008). The electrochemical degradation of PAHs solutions was carried out in a Cubic Plexiglass electrochemical cell with a working volume of 0.4 L and graphite electrodes with an immersed area of 52 cm^2, and an electrode gap of 8 cm (Cameselle et al., 2005). For every experiment neat mixtures of contaminant (100 µM), surfactant (1%) and electrolyte (0.1 M Na_2SO_4) were prepared. A constant potential difference (5 V) was applied with a power supply (HP model 3662) and the process was monitored with a multimeter (Fluke 175).

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There are significant differences in the levels of PAH removal depending on the surfactants and PAHs studied. Thus, the surfactants selected for each PAHs were Tyloxapol and Brij 35 for Anthracene and Benzo[a]pyrene, respectively and Tween 80 for the rest of PAHs tested. These results are in accordance with those obtained by Cheng and Wong (2006a, 2006b). They found that Tween 80 would be the most suitable candidate, among the four surfactants (Tween 80, Brij 35, Triton X-100, and sodium dodecyl sulfate) for improving solubilization and extraction of PAHs in a soil-water system.

Once in the water phase, engineered treatment steps may be used to enhance the PAHs remediation. An environmentally friendly approach for PAH degradation in aqueous solution is based on the use of electrochemical treatment, which is known for degrading a great variety of other polluted compounds such as dyes (Sanroman et al., 2004; Cameselle et al., 2005). Liquid electrochemical oxidation is an alternative that has the potential to replace already existing processes (Torres et al., 2003). Nowadays, electrochemical technology is receiving increasing attention, due to its convenience and simplicity. In this study, the electrochemical degradation of PAHs was investigated, with the surfactant that gave the best results. Near complete degradation was attained for all the experiments, showing a direct relationship between the ionization potential and the degradation rate.

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Photo-degradation studies of polypropylene geotextiles

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Keywords: Geotextiles, Durability, Photo-degradation, Chimassorb 944, FTIR, HPLC.

Geotextiles are polymeric materials widely applied in construction of infrastructures (such as, landfills, roads, railways, tunnels, dams and reservoirs) due to economical, technical and environmental advantages. In those applications, the geotextiles can be exposed to several agents (ultraviolet radiation and other weathering agents, atmospheric oxygen, high temperatures, chemicals and microorganisms), that may decrease their durability. The degradation suffered by the generality of the polymeric materials is often retarded and/or inhibited by the incorporation of chemical additives, such as UV stabilizers and antioxidants, in their composition.

In this work, non-woven geotextiles, made from polypropylene fibres stabilised with different amounts of the additive Chimassorb 944 (0%, 0.2% and 0.4%, *w/w*), were exposed to ultraviolet radiation (different total UV radiant exposures) in a laboratory weatherometer (the QUV). The photo-degradation of the geotextiles was evaluated by physical and chemical tests.

The degradation suffered by the polypropylene fibres was studied by infrared spectroscopy (FTIR) and by scanning electron microscopy (SEM). FTIR analysis showed the appearance of a new band (centred at 1720 cm^{-1}) on the infrared spectra of the exposed geotextiles. This band, which indicates the presence of carbonyl groups resulting from the photo-degradation process of polypropylene, was used to evaluate the degradation suffered by the geotextiles. SEM analysis was used to characterise the morphological changes on the polymeric structure of the geotextiles, showing that materials exposed to higher ultraviolet irradiances were more damaged.

Simultaneously, the level of Chimassorb 944 on the geotextiles was monitored by liquid chromatography with spectrophotometric detection. The methodology developed for the determination of Chimassorb 944 can be divided in two main steps: (1) ultrasonic extraction with chloroform at 60 ºC for removing Chimassorb 944 from the geotextile sample; (2) separation and determination of the additive by liquid chromatography with detection at 244 nm. The obtained results showed that the level of Chimassorb 944 in the stabilised geotextiles decreased as the ultraviolet exposure increased. This way, it was possible to correlate the degradation suffered by the stabilised geotextiles with the decrease of the level of Chimassorb 944 in these materials.

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Biosorption kinetic of Orange II dye with non living leaves of *Posidonia oceanica*

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Keywords: Biosorption, Orange II, dyes, *Posidonia oceanica*

Synthetic dyes are extensively used in many industries such as textile, paper, printing, leather tanning, food technology, hair colorings, etc. There are over 100000 commercially available dyes and two per cent of dyes produced are discharged directly in aqueous effluent. The presence of very small amounts of dyes in wastewater is highly visible, and many of these dyes are toxic, carcinogenic and present a serious hazard to aquatic living organisms (Aksu, 2005).

Several physical, chemical and biological methods for dye removal have been reported: coagulation-flocculation, membrane separations, oxidation process, ion-exchange, adsorption, etc. Activated carbon adsorption is an extended procedure, but this sorbent is expensive. In last years many non-conventional low cost adsorbents, including natural materials, have been used for dyes removal (Crini, 2006).

In this work the adsorption potential of dead biomass of *Posidonia oceanica* (inexpensive material abundant in the beaches of the Mediterranean Sea) for Orange II removal is evaluated.

EXPERIMENTAL, RESULTS AND DISCUSSION

Biosorbent. Non living leaves of *Posidonia oceanica* were collected from the beaches of Mediterranean Sea at the Region of Murcia, washed repeatedly with tap water to remove sand and salt and dried in an oven at 50-60ºC. This material was ground and sieved to obtain various size fractions.

Dye*.* An azo dye, Orange II (Acid Orange 7) provided by Sigma, was used without further purification and stock solutions were made in distilled water. The dye concentration in the solutions was determined spectrophotometrically measuring the absorbance at λ= 485 nm. Calibration curve was obtained with standard solutions and the amount adsorbed was found by mass-balance procedure.

Effect of particle size. In Figure 1 can be observed that no significant differences in adsorption capacity (q_e) for Orange II exits with particle size from <0.15 to 2.5-3.2 mm (q_e varies between 9.8 – 11.7 mg/g adsorbent).

Effect of biosorbent dose. As can be seen in Figure 2, an increase in the dose of adsorbent until 4 g/L increased appreciably the removal of Orange II. A greater dose did not increase the removal of dye to any great extent.

Effect of pH. The effect of pH values (from 1 to 12) on the sorption of Orange II by *Posidonia oceanica* was studied using an initial concentration of 100 mg Orange II /L. The pH was kept constant by using HNO_3 or NaOH solutions. The removal of dye was the greatest at pH 1 (q_e=16 mg/g) and decreased significantly when pH increased from pH 1 to 12. At pH>6 the q_e value was small (< 5 mg/g), and was near 0 at pH 12 (Figure 3). At acidic pHs the sorbent surface acquire positive charge due to the excess of protons in solution. Thus, the electrostatic attraction between the negatively charged adsorbate species and the positively charged adsorbent particles increases.

Kinetic of Orange II removal. Kinetics experiments were carried out at pH 2 and at different temperatures (10ºC, 25ºC and 40ºC) in continously stirred beakers containing

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0.5 liter of solution (100 mg Orange II /L) and 2 g of biomass. The pH was adjusted with HNO_3 or NaOH solutions. Samples were collected from the mixture at pre-determined time intervals for analysis. The results are presented in Figure 4. A two-staged kinetic behavior is evident: a rapid initial adsorption occurred during the first hours, followed by a long period of much slower uptake. Experimental data have been adjusted to pseudo-first order, pseudo-second order and Elovich kinetic models (Ho and McKay, 1998) by non linear regression. The pseudo-second order equation provides the best correlation (r ≥ 0.99) for all of the experimental data.

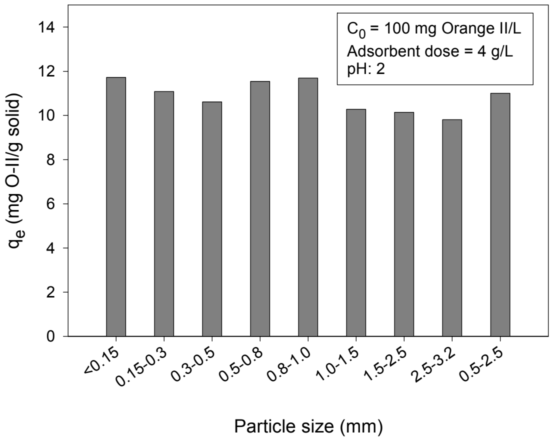

Figure 1.- Effect of particle size

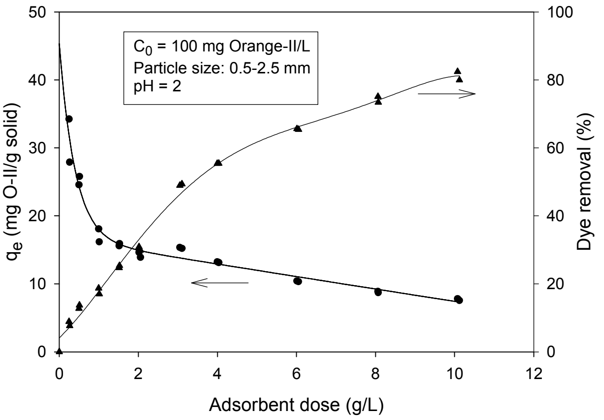

Figure 2.- Effect of adsorbent dose

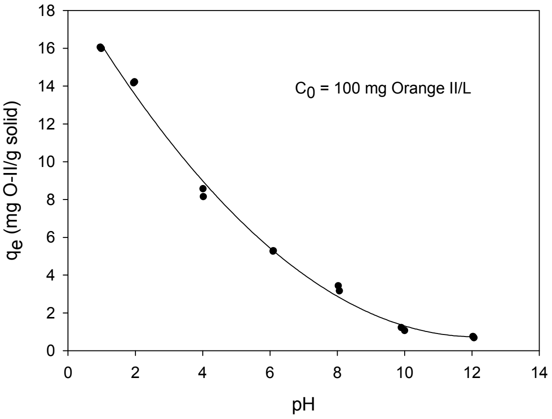

Figure 3- Effect of pH

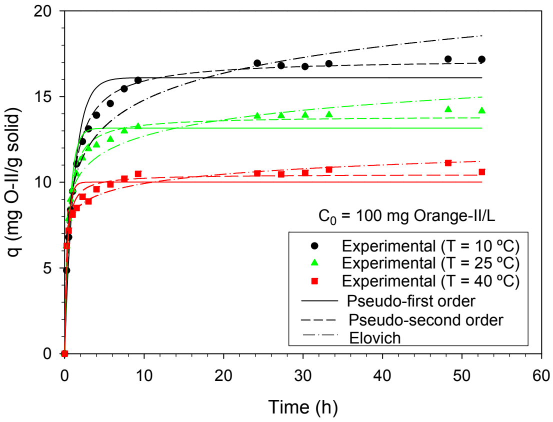

Figure 4.- Kinetics of O-II adsorption

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Continuous biosorption of methylene blue in packed bed of *Posidonia oceanica* leaves

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Keywords: Biosorption, methylene blue, packed bed, *Posidonia oceanica*

Methylene blue (MB) is a cationic dye widely used in the textile industry. Various low-cost alternative adsorbents, from industrial waste to renewable agricultural or natural products or by-products, have been proposed for the removal of dyes (Aksu, 2005). In this work, the continuous adsorption of MB in fixed bed of *Posidonia oceanica* leaves, Mediterranean Sea phanerogam, is studied.

The continuous packed bed experiments were performed with different bed heights (6.64 cm, 11.6 cm and 18 cm), and different solution flow rates (11.5 mL·min^{-1}, 16.5 mL·min^{-1} and 21.5 mL·min^{-1}) in order to obtain experimental breakthrough curves. The column was a methacrylate tube with 2.1 cm inner diameter. The biosorbent particle size was between 2.5 mm-0.6 mm and the compacity of the packed bed was 160 g·L^{-1}. In all experiments, the pH and concentration of the solution feed were 6.0 mg·L^{-1} and 20 mg·L^{-1}, respectively.

A known quantity of *Posidonia oceanica* leaves was placed in the column to yield the desired bed height of the sorbent. MB solution was pumped upward through the column at the desired flow rate by a peristaltic pump. Samples of the effluent were collected from the exit of the column at different time intervals. The pH of the effluent samples was recorded and its MB concentration was determined spectrophotometrically measuring the absorbance of solution (λ= 664 nm).

Four models (Table 1) were applied to experimental data to predict the breakthrouh curves and to determine the characteristic parameters of the column, useful for process design (Aksu and Gonëm, 2004).

Table 1. Mathematical models

Mathematical models	Equation	Parameters
BDST	$\frac{C}{C_0}=\frac{1}{\left(e^{\left(K_a N_0 H / v\right)}-1\right)\cdot e^{-K_a C_0 t}+1}$	K_a, kinetic constant (L·mg^{-1}·h^{-1}). N_0, volumetric sorption capacity of bed (mg·L^{-1})
Thomas	$\frac{C}{C_0}=\frac{1}{1+\exp\left(\frac{K_{Th}}{Q}\left(q_0\cdot X-C_0\cdot V_{eff}\right)\right)}$	K_{Th}, kinetic constant (L·mg^{-1}·h^{-1}) q_0, equilibrium sorption capacity, (mg·g^{-1}) X, sorbent amount in the column
Clark	$\frac{C}{C_0}=\left(\frac{1}{1+A\cdot e^{-r\cdot t}}\right)^{\frac{1}{n-1}}$	A, constant of the Clark model r, adsorption rate (h^{-1}) n, Freundlich adsorption constant
Yoon-Nelson	$\ln\left(\frac{C}{C_0-C}\right)=K_{YN}\cdot t-\tau\cdot K_{YN}$	K_{YN}, kinetic constant (h^{-1}) τ, time when C = 0.5·C_0 (h)

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Experimental breakthrough curves are shown in Figure 1. Breakthrough time (t_b) and sorption capacity of fixed bed (q_b) were evaluated and they are included in Table 2.
As can be expected, breakthrough time decreases when flow rate increases and when the bed height decreases. The sorption capacity varies between 288 mg·g^{-1} and 387 mg·g^{-1}. This value is similar to that obtained from the adsorption isotherm.
The pH of the efluent increases initially until 8, and then decreases until pH near 6.
Experimental data were fitted, by non-linear regression, to the mentioned mathematical models. Values of the models parameters were determined by non-linear regression analysis and they are shown in table 2.

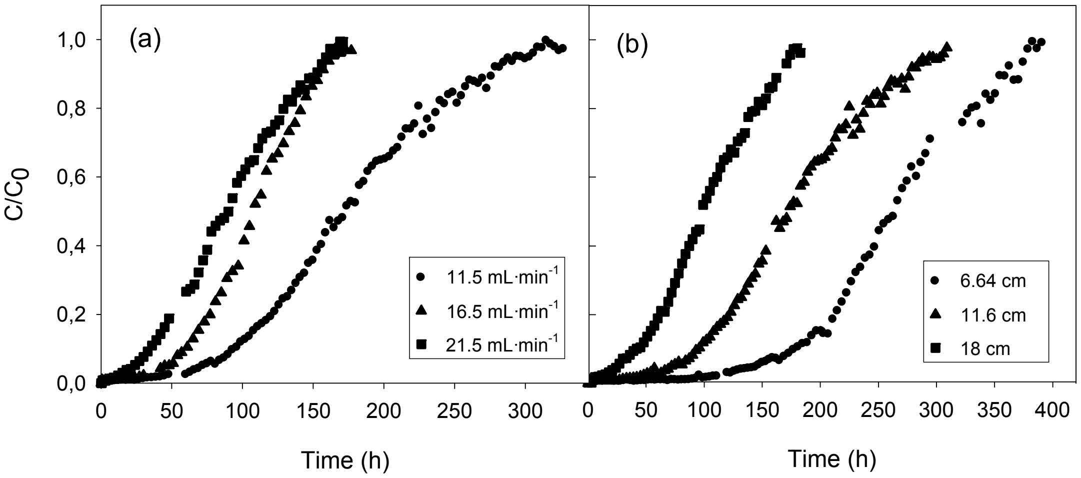

FIGURE 1. **BREAKTHROUGH CURVES. INFLUENCE OF (A) SOLUTION FLOW RATE (C_0 = 20 MG·L^{-1},**
PH = 6.0, H = 11.6 CM), (B) BED HEIGHT (C_0 = 20 MG·L^{-1}, PH = 6.0, Q = 11.5 ML·MIN^{-1})

Table 2. Experimental and models parameters. (Units: Q: mL·min^{-1}; H: cm; t_b and τ: h; q_b and q_0: mg·g^{-1}; K_a and K_{Th}: L·mg^{-1}·h^{-1}; N_0: mg·L^{-1}; r and K_{YN}: h^{-1}).

		Exp.		BDST		Thomas		Clark		Yoon-Nelson	
Q	**H**	**t_b**	**q_b**	**K_a·10^3**	**N_0·10^{-3}**	**K_{Th}**	**q_0**	**r**	**A**	**K_{YN}**	**τ**
	6.64	25	337	1.87	54.1	1.87	345	0.022	0.226	0.037	100.0
11.5	11.6	72	341	1.38	53.1	1.36	341	0.016	0.367	0.027	172.9
	18	146	387	1.24	52.8	1.24	368	0.014	0.950	0.025	266.5
11.5		72	341	1.38	53.1	1.36	341	0.016	0.367	0.027	172.9
16.5	11.6	45	288	2.26	47.94	2.26	307	0.025	0.368	0.045	108.5
21.5		23	314	1.94	51.67	1.94	328	0.024	0.182	0.039	89.1

Taking into account the goodness of the fit for the different models investigated, we can assume that they all describe properly the column performance.

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Using a flocculent brewer's yeast strain of *Saccharomyces cerevisiae* in the removal of heavy metals

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Keywords: Bioremediation; copper; flocculation; nickel; zinc.

Heavy metals pollution is a growing problem mainly caused by industrialization, being of particular concern in developing countries. Metal-bearing effluents required a pre-treatment step before being discharged in a water body or directed to a municipal sewage treatment plant. Conventional technologies (precipitation-filtration, ion exchange and membrane technologies) are not adequate (the metal removal is incomplete) or often economically prohibitive and/or impracticable for the treatment of large volumes of wastewater containing relative low metal concentrations.

An effective and economic alternative to traditional treatments in detoxification of metal-bearing wastewaters is the use of biological processes. Among the different kinds of biomass, yeast cells of *Saccharomyces cerevisiae* can constitute a good alternative to wastewater treatment since has the ability to accumulate a broad range of heavy metals under a wide range of external conditions (Blackwell *et al.* 1995; Ferraz and Teixeira 1999; Wang and Chen 2006). A common problem of biomass application is usually associated to its difficulty of separation from the reaction mixture. To solve this problem, immobilized microbial biomass has been used (Tsezos 1990). Nevertheless, immobilization techniques are very expensive when used in large scale. A novel approach may involve the use of flocculent yeasts. Recently, it was shown that a flocculent strain of *S. cerevisiae* accumulated more Cu^{2+} than the isogenic (except for the marker genes and the gene *FLO1*) non-flocculent strain (Soares *et al.* 2002).

In this work, the effect of cell concentration, the presence of different heavy metals and the killing temperature on the yeast settling profile were studied. Our results shown that yeast strain used was able to sediment in the presence of Cu^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+} and Cr^{3+}, which evidences that the flocculation can be used as a cheap and natural separation process for an enlarged range of industrial effluents. For a biomass concentration higher than 0.5 g/l, more than 95 % of the cells were settled after 5 minutes; this fact shows that the auto-aggregation of yeast biomass is a rapid and efficient separation process. Cells inactivated at 45 ºC maintain the sedimentation characteristics.

Additionally, a detailed comparison (using kinetic and equilibrium uptakes studies) of the ability of live and dead (killed at 45ºC) flocculent cells of *S. cerevisiae* to accumulate nickel, copper and zinc was carried out. Equilibrium studies have shown that inactivated biomass displayed a greater Zn^{2+} and Ni^{2+} accumulation (163 Zn^{2+} and 134 Ni^{2+} μmol g^{-1} dry weight biomass), than live yeasts (12 Zn^{2+} and 8 Ni^{2+} μmol g^{-1} dry weight biomass). For Cu^{2+}, live and dead cells displayed similar accumulation: 122 and 156 μmol g^{-1} dry weight biomass, respectively.

Fluorescence and scanning electron microscopy and infrared spectroscopy studies have shown that no appreciable structural or molecular changes occurred in the cells during the killing process. The increased metal uptake capacities observed in dead

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cells can be most likely explained by the loss of cell membrane integrity, which allows the exposition of further metal-binding sites inside the cells. Infrared analyses suggest the involvement of carboxyl, amino, hydroxyl and amides groups in yeast metals uptake.

In conclusion, flocculent cells, inactivated at 45 ºC, retain the ability to sediment rapidly in the presence of different heavy metals. This type of biomass shows a higher heavy metal removal ability, being more suitable for further bioremediation works. Together, the results evidences that the use of this type of biomass to remove heavy metals from industrial effluents is particularly promissory as it is a residue from brewing industry available in large quantities at low cost. Furthermore, the costs associated with cell separation after effluent treatment are minimized due to the natural aggregation characteristics of the strain. In addition, it is possible the use of different configurations of suspended biomass reactors without the risk of biomass washout.

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Optimization of chromium adsorption upon chitosan by surface response analysis

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Keywords: Chromium adsorption, experimental design, optimization, chitosan

The continuously increasing demand for the commodities produced by chemical industries has triggered heavy metals accumulation in the ecosystem. Being one of the heavy metals, chromium- which is mostly discharged to the environment as a result of metals processing, galvanization, leather tanning and electrolytic coating- exists in the aquatic media as trivalent chromium (Cr^{+3}) and/or hexavalent chromium (Cr^{6+}) of which the latter is more toxic and carcinogenic (Ramnani and Sabharwal, 2006).

The conventional methods applied for Cr^{6+} removal are electrochemical precipitation, ion exchange, adsorption and reverse osmosis. Chemical precipitation produces great amounts of mud, whilst ion exchangers and reverse osmosis are relatively of very high cost (Nomanbhay and Palanisamy, 2005). Therefore, adsorption is the most frequently applied technique owing to its advantages such as variety of adsorbent materials, high efficiency at a relatively lower cost (Babel and Kurniawan, 2003).

Chitosan, the major derivative of chitin and second abundant biopolymer in nature after cellulose, is a good scavenger for metal ions owing to the amine and hydroxyl functional groups in its structure (Zhao *et. al*, 2007). The formerly conducted studies considering the adsorption of Cr^{6+} have proved that the process parameters, namely, pH, initial concentration, temperature, adsorbent dose and particle diameter, influence the removal efficiency immensely (Rojas *et. al*, 2005). This influence can be realized by inspection of the reported values for Cr^{6+} adsorption capacity of chitosan which varies from 27.3 mg Cr^{6+}/g chitosan to 273 mg Cr^{6+}/ g chitosan (Bailey *et. al*, 1998). The confusion arising from the dependency of adsorption capacity could be overcome by optimization of the parameters involved. Therefore, this study aimed to investigate the effects of process parameters on the Cr^{6+} adsorption capacity of chitosan and to determine the set of parameters leading to maximum Cr^{6+} removal. The effects of pH, initial concentration and adsorbent dose were studied however, the efffects of temperature and particle diameter were excluded intentionally.

Surface responce methodology was utilized for optimization studies. Classical methods of optimization involve the change of one variable at a time which is quite time consuming especially when a large number of variables are considered. Alternatively, response surface methodology aims to optimize the response surface shaped under the influence of the process parameters.

Materials and Methods

Stock Cr^{6+} solution of 500 ppm (mg/L) was prepared using $K_2Cr_2O_7$ (Merck) with deionized water. Solutions of 50 mL volume were prepared by dilution of this stock to the predetermined concentration. The effects of process parameters pH, ion concentration and adsorbent dosage were investigated at five levels within the ranges summarized in Table 1. The pH of the solutions were regulated by micro additions of 0.2 N H_2SO_4 and 0.1N NaOH. Cr^{6+} concentration was analyzed by diphenyl carbazide method. Chitosan flakes (Sigma) were washed with double distilled water, dried at 50°C overnight and stored in desiccator until use. All experiments were conducted batchwise in 100 mL stoppered flasks in a water bath kept at 298K. Agitation rate was held constant at 120 rpm.

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Table 1. Lower and upper bounds of the parameters

Parameters	**Coded Value**				
	-1.6	**-1**	**0**	**1**	**1.6**
pH	1.5	3	5.5	8	9.5
Initial concentration (mg/L)	15	30	55	80	95
Adsorbent Dose (g/L)	1.8	6	13	20	24.2

The total number and sequence of experimental runs were determined using the MINITAB software. All experiments were done in triplicates to ensure the accuracy of the results. Surface plots were constructed by using MINITAB and main effects and interactions of the factors were determined by ANOVA method. The significance level was chosen as 0.05. Optimum factor levels were tested to check accuracy.

Results and Discussion

The highest adsorption capacity was calculated as 22.09 mg Cr^{6+}/g chitosan. Maximum Cr^{6+} removal was attained from a solution as concentrated as 30 ppm at pH 3 with an adsorbent dosage of 13 g/L. pH was found to be the most effective parameter for removal percentage which was followed by initial concentration and adsorbent dosage. The order of effects was the exact opposite for adsorption capacity. In both cases, effects implied by interactions were determined to be poor. However, the square effect implied by pH was quite significant. Regression coefficients of the predictive models were calculted above 0.9 for both adsorption capacity and removal percentage. Adsorption data was found to be consistent with Freundlich isotherm model with model parameters k and n being calculated as 2.79 and 1.7, respectively.

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Influence of different cations of N3 dyes on their photovoltaic performance and stability

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Keywords: dye sensitized solar cells, solar energy, photovoltaics

Nowadays, a particular interest in the development of alternative energy sources arises, especially motivated not only by the necessity of reducing the dependency on fossil fuel resources, but also for providing the reduction of the CO_2 emissions. An attractive strategy in order to turn over the actual energy problem is by using a renewable energy source with great potential as solar energy. In this sense, the direct conversion of sunlight into electricity by means of photovoltaic systems could make important contributions to this energy contend in an environmentally friendly way (Grätzel, 2001; Zweibel et al., 2008; PV TRAC, 2007). In conventional solar cells, the charge separation occurs at the interface of two materials of different conduction mechanisms, exploiting the photovoltaic effect (Grätzel, 2003). More recently, a new generation of cells emerged, the Dye Sensitized Solar Cells (DSC). DSCs are considered very promising since they use low cost, abundant and environmentally safe raw materials and they show relatively high-energy efficiency (Grätzel, 2001). DSCs apply the dye-sensitization concept by taking advantage of a broader region of the solar spectrum. In fact, by using a« nanoparticulated semiconductor coated with a light absorbing dye it is possible to extend the light absorption range of the semiconductor, which otherwise would only absorb UV-light (O'Regan et al., 1991).

A DSC is made of a nanoparticulated titania film coated with an adsorbed dye monolayer. This thin film is applied on a glass substrate coated with a transparent conducting oxide (TCO) that collects the generated electrical current. A back electrode consists of the same conducting glass and a platinum layer. This serves as a catalyst of the redox reaction occurring in the electrolyte placed in between the two electrodes (figure 1).

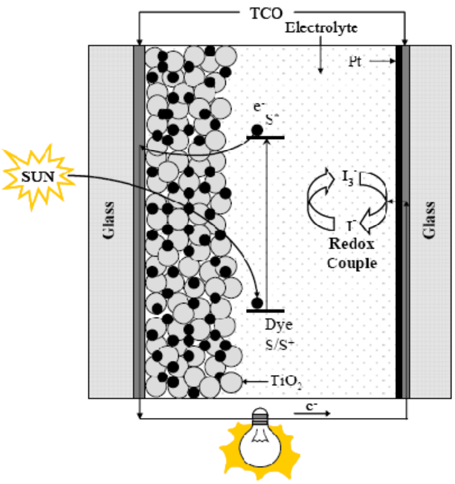

Figure 1 – Scheme of a DSC.

Since the development of the N3 dye in 1993 (Nazeeruddin et al., 1993), its tetrabutylammonium salt N719 has been used as the standard red dye because of its unmatched performances. Similarly to the N719 dye, all the dyes from this family were developed bearing in mind that the number of protons in the dye influences the open-circuit potential and the short circuit current of the DSC [Nazeeruddin et al., 2003]. In this work we modified N3 dye protons by substituting with different cations and forming new dyes in order to evaluate their performance and stability of the DSC devices during thermal and light soaking ageing. The devices prepared with these new dyes were subjected to full sunlight irradiation at 50ºC and monitored the variations in the photovoltaic parameters at regular time intervals for 1000 h. It was concluded that the ones with the sodium and potassium cations were the most stable. These two systems were further characterized for incident photon to current conversion efficiency (IPCE), impedance spectrum and electron lifetime. Electrochemical impedance spectroscopy (EIS) was used to investigate the charge transfer phenomena occurring in the cells. The charge-transfer resistance at the TCO layer, the charge-transfer resistance at the counter-electrode/electrolyte interface and the chargetransfer resistance related to the recombination of electrons at the TiO_2/dye/electrolyte interface were obtained by means of the appropriate equivalent circuit [Kern et al., 2002].

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Membrane charge effects on the recovery of Fe-EDTA species in aqueous solution by nanofiltration

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Keywords: Nanofiltration, iron, EDTA, speciation, membrane charge

Membrane processes are being widely studied as useful technologies to recover contaminants from polluted waters. Its success is mostly due to the easiness of membrane processes to be scaled-up and operated. Nanofiltration is a promising membrane technology that allows the recovery of small size organic compounds as well as of multivalent metal ions from aqueous streams. Nanofiltration mechanisms allowing the retention of such compounds are based on electrostatic interactions between the charged molecules (or ions) and sieving effects due to the molecular weight cut-off of the membranes and the molecular weights of the targeted compounds (Van der Bruggen et al., 1999). Thus, it is of high importance to control both the chemistry of the solutions to be filtered as well as the membrane properties for predicting the retention mechanisms involved in the separation process.

Nanofiltration membranes have been used for the recovery of a wide variety of organic compounds such as dyes (Van der Bruggen et al., 2001), herbicides (Plakas et al., 2006), aromatic compounds (Zhang et al., 2006), etc. In addition, nanofiltration has been used for the recovery of metal ions such as copper (Tanninen et al., 2006), chromium (Frenzel et al., 2006), or cobalt (Choo et al., 2006) among many others.

Advanced Oxidation Processes (AOP's) are usually applied for the partial mineralisation of biorefractory organic compounds so that their subsequent abatement in biological wastewater treatment plants could be satisfactorily completed. Belonging to AOP's, Fenton and Fenton-like processes are based on the formation of hydroxyl radicals by the decomposition of hydrogen peroxide aided by the addition of homogeneous iron (II) or iron (III) salts (Pignatello et al., 2006). Dissolved iron (II) and iron (III) ions are involved in oxidation-reduction cycles and they are present in the partially-treated effluents from the oxidation process causing both environmental and economical problems. Thus, it is strongly recommended the installation of a catalyst recovery unit for preventing undesired iron discharges.

Nowadays, chelating agents, such as ethylenediaminetetraacetic acid (EDTA), are broadly used in fertilizers, herbicides, detergents, as effective agents to remove metals from contaminated industrial containers, and even as promoters of the oxidation of biorefractory organic compounds when iron is also present in the reactive media. For the latter, as in the Fenton and Fenton-like processes, the homogeneous catalytic pair (in this case, Fe-EDTA) also leaves the oxidation reactor together with the partially-treated effluent, increasing the costs associated to the treatment and also adversely affecting the environment. Thus, the recovery of the complex Fe-EDTA is also an absolute necessity for solving the abovementioned problems.

In this study, nanofiltration is presented as a successful process for recovering Fe-EDTA chelates from synthetic wastewater. In addition, as Fe-EDTA complexes change in nature when the pH of the solutions is modified, the sieving and electrostatic effects involved in their retention have been investigated in connection with pH.

Three commercial nanofiltration membranes (NF, NF90 and NF270, manufactured by Filmtec, Dow) have been selected for performing the filtration experiments by means of

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a stirred batch filtration cell. The transmembrane pressure was fixed at 6 bar and Fe-EDTA chelates were formed by the addition of equimolar amounts of EDTA disodium salt and iron (III) nitrate into deionised water. The pH of the solutions was adjusted by either the addition of HCl or NaOH, when necessary. Samples of permeate were periodically withdrawn so that the iron retention evolution along the time could be monitored. The filtration was conducted in discontinuous operation, i.e., the permeate was not recycled back to the feed. The test was run until the concentrate volume passed from 240 mL (initial volume) to 40 mL, i.e. a volume reduction factor (VRF) of 6 was used. In this way, the evolution of the iron retention at different inlet concentrations could also be explored.

The different membrane properties (isoelectric point, charge density, membrane material and pore size) and the Fe-EDTA speciation diagrams of the tested solutions allow the understanding of the phenomena occurring during the filtration process and highlight the relevance of the possible rejection mechanisms. The results show that iron retention is strongly affected by the pH, which control the structure of the chelates as well as that of the membrane surface. High iron rejection can be achieved, up to 98%, if the solution properties are properly selected and adjusted. From the results, it is obvious that both electrostatic phenomena and sieving effects allow the effective concentration of iron chelates by nanofiltration. However, it is found that pH is a crucial variable in the nanofiltration of Fe-EDTA chelates as it influences the charge of both the membrane surface and that of the Fe-EDTA chelates. For instance, at VRF of 6 and pH around 2.5, iron retentions were 90%, 95% and 53% for NF, NF270 and NF90, respectively. However, for NF90 at a VRF of 6, iron retentions were as high as 98% at pH 4.3 and 99% at pH 8.4, which clearly substantiate the key role of pH in the separation performance.

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Sorption Isotherms of organic-inorganic hybrids

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Keywords: Cellulose/Silica hybrids, O/I hybrids, Water Sorption, Isotherms

Organic–inorganic hybrids (OIHs) are promising composite materials compiling in a synergetic way the properties of the starting components depending on the synthesis mode. Cellulose/Silica hybrids (CSHs) are an example of such materials (Sequeira *et al*. 2007) fitting well in the concept of green chemistry.

Cellulose is the most abundant biodegradable, recyclable and renewable natural polymer. The incorporation of silica to cellulose at the nanometer scale was accomplished by a mild sol–gel process, involving hydrolysis and polycondensation of tetraethoxysilane (TEOS) used as silica precursor. The reactions were performed at room temperature, using solid acid catalysts and benign solvents such as water and alcohol (co-solvent). The advantage of this sol–gel process is that silica particles are generated *in situ* and evenly dispersed in the polymeric host matrix (i.e. cellulose), bounding to the polymer through hydrogen or covalent bonds thus forming a threedimensional network with -O-Si-O-Si- linkages on the cellulose surface (Sequeira *et* al. 2007).

The combination of nanoscale inorganic moieties with organic polymers, such as cellulose, has high potential for future applications. When compared to the starting materials these new OIHs exhibit improved thermal and mechanical stability, excellent heat and acoustic insulation properties, and improved dimensional stability to humidification (Sequeira *et al.* 2008). Altogether these properties render these materials suitable for insulation or packaging purposes.

In this paper the water sorption properties of cellulose/silica hybrid materials, prepared by sol–gel process from *Eucalyptus globulus* kraft pulp and TEOS, were evaluated and related to suitable adsorption models.

Sorption isotherms were determined by a static-gravimetric method (Gibert *et al*. 2006, Arlabosse *et al*. 2003) at different temperatures, in the range of 10 - 45 ºC, using an experimental setup similar to that presented in Figure 1. Saturated aqueous salt solutions of KOH, CH_3COOK, $MgCl_2.6H_2O$, K_2CO_3, $Mg(NO_3)_2$, KI, $(NH_4)_2SO_4$ and KNO_3 were used to control the atmosphere's moisture content (RH, %) in the range 7% to 95% (Greenspan, 1977). Prior to sorption testing samples were oven-dried overnight at 105 ºC and then placed in closed flasks containing the saturated salt solutions. The flasks were placed in a shaking water bath at controlled temperature (± 1 ºC) and weighted regularly until reaching constant weight (≈ 10 days).

Preliminary results, presented in Figure 2, have been modeled using several isotherm equations, such as BET (Brunauer, Emmet, Teller), GAB (Guggenheim, Anderson, De Boer) among others. The correspondence of fit of each equation to sorption data was quantified and compared. The effect of temperature on the model parameters was discussed and the heat of sorption calculated from the experimental data.

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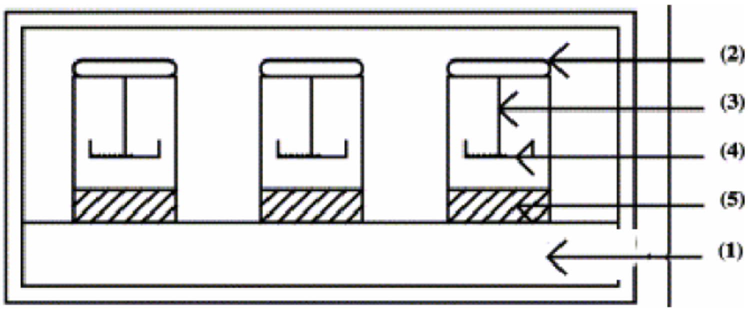

Figure 1: Experimental setup for measurement of sorption isotherms: 1. Thermostated bath, 2. Erlenmeyer containing salt solution, 3. sample holder, 4. cellulose/silica hybrid, and 5. saturated salt solution.

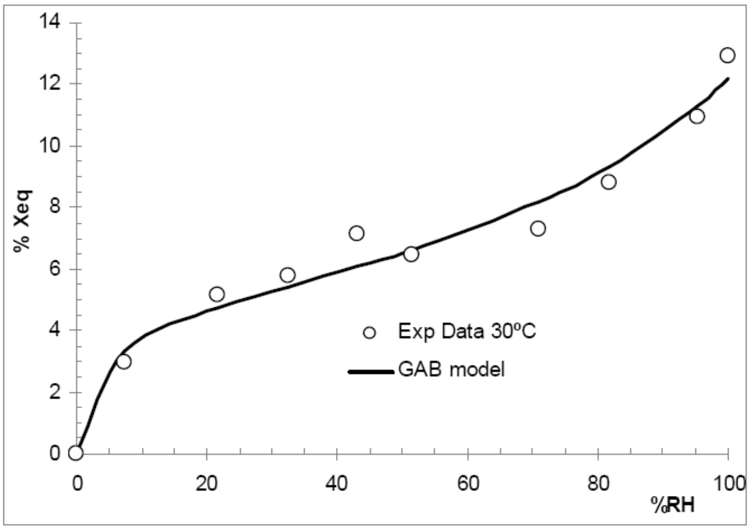

Figure 2: Experimental data (equilibrium water content –Xeq, *versus* Realtive Humidity – %RH) for celulose/silica hibrids at 30ºC, and GAB sorption isotherm fitting.

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Evaporation solar produced water and fresh water in trays of Teflon

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Keywords: Solar Evaporarion, Produced Water, Vapour Pression, Salting Effect, Boiling Point

The water, considered the universal solvent, it is one of the substances most important for the maintenance of life on Earth. Its presence for survival of living beings, as well as for implementation of human activities is essential.

In industrial processes, water, solvent and serves well as raw materials, as input for heating or cooling systems and appears to like byproduct of some processes as is the production of oil. Regardless of your use, the legislation, which is increasingly restrictive, requires that the waste generated is treated and that they receive appropriate final disposal.

Understanding and predicting for the behaviour of aqueous systems is useful and necessary in all senses whether to optimize its use in industry (minimizing the waste), whether to provide improvements in the systems of treatment of effluent and its final disposal or reuse (Bezerra, 2004).

Among the operations with the water in the industry, it is here the process of evaporation. In many industrial processes is producing an effluent of high salinity in such cases if need separate salts from water and solar evaporation can be a viable alternative to perform this operation, either by allowing the use of salts, as a way to return the water to the environment.

The pure water enter into boiling at 100 ° C at atmospheric pressure and although the water is present on the planet is not pure, mainly due its power to solvency, that temperature can change in real systems, as they are the elements composing this mixture.

Evaporation of water, however, starts at 0 ºC. In the triple point coexist called the three states of matter (van Ness, 1980). With the elevation of temperature, for a system isobaric, increases to the rate of evaporation coming to the boil at approximately 100 °C.

It is known that the saline solutions increase of Point Boiling (Pb) of water to reduce its pressure vapour. Meanwhile, volatile organic increase the vapour pressure of the water and consequently its rate of evaporation.

The produced water is a complex mixture of water, salt, oil, microorganisms and traces of heavy metals (Azevedo, 1998). Because of this complexity, the Pb this water, may change to one side or the other and that displacement is defined by their individual characteristics that are fairly inconstantes, ranging from one field to another and even producer of treatment employed for obtaining oil.

This paper aims to study the variation of solar evaporation of samples of fresh water (groundwater) and treated produced water (low concentration of organic), the same pressure on aluminum trays impregnated of comercial Teflon.

The work was developed in the city of Natal RN, Northeast Brazil (5°47'42"S and 35°12'32" W), whose average solar radiation is approximately 5000 W/m^{2}d. It used Teflon tray, with capacity 2.8 L. The experiment is to put 1L sample of each type in the tray and leave them in exposure to the sun for equal time period: from 10 to 14 hours. Monitors up its temperature through mercury thermometer, and there is evaporated on the end of each tray. It measured the pH and conductivity of the samples that showed

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the following average values: 357 μS/cm for fresh water and 3500 μS/cm for produced water and pH of 8.8 and 8.7 for the samples of fresh and produced water, respectively.

The results show that had not significant variations in temperature between the trays, which keeping between 39 and 40 °C for the experiment. The average volumes evaporated in that period of time were 400 mL for fresh water and 403 mL of produced water, which is not set as significant. These results indicate that for samples with these characteristics, the effects tend to undo in opposition to the expected by tests of conventional distillation, which showed a Tb greater than 100 ° C, showing that case, the effect of the salt.

The work highlights the importance of understanding more deeply the process of solar evaporation of produced water, because the needs of the industry, sometimes returning the water to the hydrological cycle with the least possible environmental damage and collecting the most appropriate provision for salt .

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An innovative biological system to treat wastewater: A year of operation of the first wastewater treatment plant in real scale

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Keywords: wastewater, symbiotic treatment, wetland, nitrification/denitrification

A wastewater treatment plant was constructed at the University of Murcia in order to achieve a treated wastewater with adequate quality for reuse as garden irrigation waters. Supported by the successful experience of an innovative biological treatment, symbiotic depuration (Golftrat, 2007), in assays carried out in pilot scale (Llorens et al., 2004), this technology has been transferred to the construction of a real plant to treat the 500 m^3 of water daily generated at the Campus of Espinardo (University of Murcia).

The symbiotic treatment has as its main characteristic the inclussion of two well-distinguished areas, the depuration area and the cultivation area. The water is applied underground to the percolation beds for increasing efficiency of the treatment (less evaporation losses) and the green areas can be used even during irrigation and direct contact of users of such areas with residual waters is prevented. The depuration area consists of a gravel bed, 120 cm thick, which is isolated from the ground by a waterproof layer. A network of subterranean drippers, placed directly on the gravel, is used to apply the wastewater. The cultivation area, composed by approximately 30 cm of sandy substrate, is situated over the depuration area. The cited substrate permits air entry into the bed and the depuration takes place in aerobic conditions.The whole depuration process consists of four stages of treatment in series with a filtering surface of 205 m^2/stage.

The wastewater is pretreated before entering the symbiotic plant. The pretreatment consists of two subsequent rotary sieves with 0.5 and 0.25 mm sieve openings, respectively, a clarifier (recentely installed in order to reduce the inlet solid content to filters) and a series of twelve 50 µm mesh ring filters. Pretreated wastewater enters the first stage of the symbiotic plant through the drippers falling by gravity through the gravel bed until it reaches the bottom waterproof layer. The effluent is collected in a deposit and then filtered through a series of three 75 µm size mesh filters before being pumped to the next stage. The effluent from the fourth stage falls by gravity into an artificial wetland or a storage pond. Treated water is reused as irrigation water.

Physico-chemical analysis was made of samples collected from: influent and effluent of the rotatory sieves, influent and effluent of the clarifier, influent and effluent of each filter battery and inlet and outlet of each one of the four treatment stages. Samples were also collected in the wetland to asses the improvement in the water quality after this final treatment. The range and the mean value of the parameters measured at the inlet and outlet of the the fourth symbiotic stages are shown in Table 1, as well as the percentage removal. As can be seen, there is a great variation in the influent composition. The raw wastewater is comparable to a domestic wastewater typical of a town with high strength (Metcalf&Eddy, 1995).

The results obtained during more than a year of experimentation show high overall removal rates (Table 1). Taking into account the contribution of the different elements to the final depuration, rotatory sieves provide a redution of total suspended solids (TSS) and chemical oxigen demand (COD) of almost 10 %; the clarifier 30% in TSS

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and 18 % in COD and each symbiotic treatment eliminate more than 50 % of the total suspended solids that entry in each stage. It is neccesary to point up that, after the pretreatment, symbiotic beds rather than filters accounts for the main contribution to the contaminant parameter removals. The small amounts of sludge generated by sloughed off biofilm from the surface of the gravel beds, settle down rapidly in the deposits of treated wastewater of each stage and they were carried to the clarifier without pass to the filters. A sludge purge in the clarifier was done daily.

Table 1.- Characteristics of wastewater at the inlet and outlet of the plant and percentage removal.

	Inlet	Outlet	Removal (%)
pH	7.90 (7.13-10.3)	7.24 (6.8-8)	-
Conductivity (mS/cm)	2.22 (1.64-3.55)	2.31(1.62-3.02)	-
DO (mg O_2/L)	3.03 (0.2-6.52)	4.20 (1-8.02)	-
T (ºC)	20.32 (15.9-28.2)	19.98 (14-29.3)	-
SST (mg/L)	490 (123-1140)	13.2 (2-31)	96.0 (80.8-99.5)
COD (mg O_2/L)	944 (253-2320)	32.5 (16-63)	95.6(85.6-98.7)
BOD_5 (mg O_2/L)	504 (150-1100)	6.24 (0-22)	98.7(94.4-100)
TKN (mg N/L)	44.4 (10.6-98.8)	7.68 (0.2-45.8)	85.6 (35.8-99.7)
N-NH_4^+ (mg N/L)	35.7 (6-60.9)	6.86 (0.1-40)	83.8 (27.7-99.8)
Nitrates (mg NO_3^-/L)	0.85 (0-5.6)	80.8 (19.6-192)	-
TP (mg P/L)	11.0 (5.8-21.7)	4.02 (1.1-7.7)	60.9 (6.1-85.5)
P-PO_4^{3-} (mg P/L)	4.4 (0.5-16.7)	3.27(0.5-6.57)	-

In the first stages of the treatment solid content and organic removal takes place, and in the third and forth stage final organic matter removal and nitrification. Along the stages, where an increasing in dissolved oxygen is obtained, the decomposition by microorganism of organic nitrogen to ammonia and then the complete oxidation to nitrates takes place. Furthemore, with the final treatment in the wetland an important reduction of nitrogen and phosphorous content is achieved.

Before more than a year of operation of the cited wastewater treatment plant it can be concluded that:
- A high quality of effluent is achieved with the treatment. The effluent complies with the Directive 91/271/ECC effluent requeriments of $\leq$ 35mg/l TSS, $\leq$ 125mg/l COD and $\leq$ 25mg/l BOD_5.
- The plant presents a high stability to changes in flow and load.
- A quick stars-up of the process.
- Simplicity of operation and maintenance.
- The sludge generated with this biological system is considerabily lower than obtained with other wastewater technologies.
- No unpleasant odours were observed.

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Denitrification in an anoxic rotating biological contactor under two carbon/nitrogen ratios

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Keywords: anoxic rotating biological contactor, C/N ratio, denitrification

Biological treatments are most promising, versatile and preferred technologies to remove nitrate from wastewaters than physicochemical methods, which are expensive and may generate toxic residuals (Reyes-Avila et al., 2004). Microorganisms first reduce nitrate (NO_3^-) to nitrite (NO_2^-) and then produce nitric oxide (NO), nitrous oxide (N_2O), and, finally, nitrogen gas (N_2), in the absence of dissolved oxygen (DO) or under limited DO concentrations (Tchobanoglous and Burton, 1991). A rotating biological contactor (RBC) is an attached growth bioreactor, which is used for both municipal and industrial wastewater treatment. Due to its advantages: simplicity of operation, short hydraulic retention time (HRT), low land area requirement, low operating and maintenance cost and high biomass concentration per reactor volume, RBCs constitute a very unique and superior alternative technology for carbon oxidation, nitrification, denitrification and phosphorus removal. Although in the last decade RBCs tightly closed to avoid air entrance have started to be used for denitrification, few studies have still been conducted with anoxic RBCs. The aim of the present work was to compare the performance of an anoxic bench-scale RBC under two carbon/nitrogen (C/N) molar ratios (2 and 3).

The anoxic RBC single-stage system consisted of 8 polymethylmethacrylate (PMMA) discs (diameter = 130 mm, thickness = 3 mm, 20 mm interspace) mounted on a horizontal shaft, having a working volume of 2.5 L. The reactor was operated at a rotational speed of 4 rpm and the temperature was maintained at 28 ºC by means of a heating jacket. The submergence of the discs was 93.5%. The reactor was inoculated with 2.5 L of acclimatized activated sludge and microbial attachment onto the discs was allowed to occur in batch mode. A visible attachment of biomass on the discs was noticed after 4 days of inoculation. On day 6, the anoxic RBC mixed liquor was removed, the reactor was re-filled and started to be fed continuously with synthetic wastewater. The synthetic influent contained acetate as carbon source and a phosphorus concentration of 10 mg P/L. According to the experiment, acetate and nitrate concentrations were adjusted to give a ratio of C/N=2 or C/N=3. To evaluate the reactor efficiency and to allow biofilm development the carbon and nitrate loads were doubled on the 8th day of operation and the hydraulic retention time was reduced from 10.00 h to 5.68, keeping the ratio C/N constant, in both experimental conditions (C/N=2 and C/N=3). The study was conducted for a period of 28 days (for each C/N ratio). During the course of continuous operation, samples of the RBC influent and effluent were collected routinely and analysed for various parameters such as nitrate, acetate and nitrite. In order to remove interfering suspended particles samples were filtered over a 0.2 µm membrane filter and used for nitrite, acetate and nitrate quantification according to standard methods (APHA, 1989). Periodically, gas samples were analysed by gas chromatography.

Variations in the removal efficiencies of nitrate-nitrogen (NO_3^--N) and acetate-carbon (CH_3COO^--C) as a function of operating time are shown in Figure 1 (a) and (b), respectively.

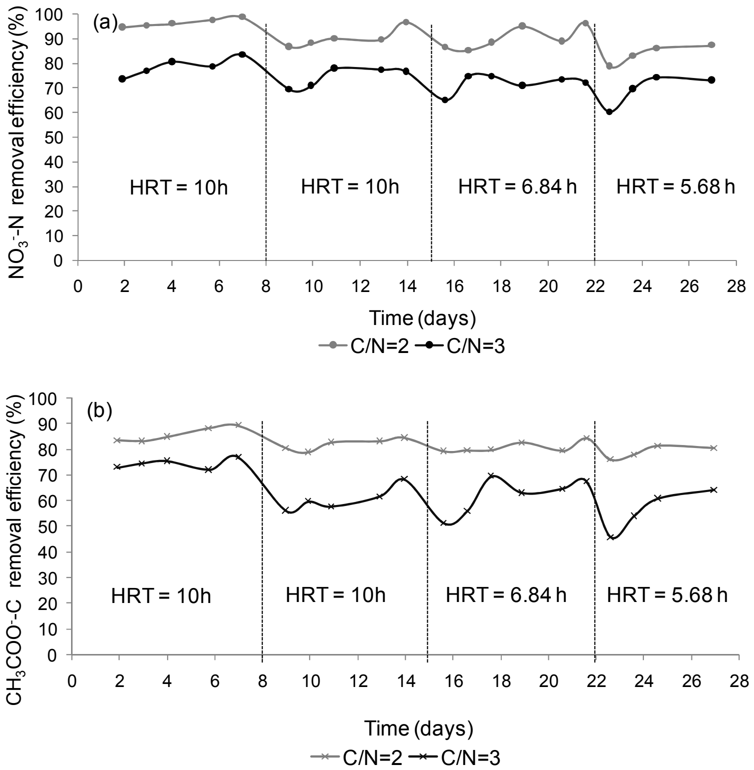

Figure 1. Nitrate-nitrogen (a) and acetate-carbon (b) removal efficiency along the time at two C/N ratios: 2 and 3.

These results show that, as the C/N ratio increased from 2 to 3, the substrate removal efficiencies decreased. The bench-scale RBC achieved a NO_3^--N overall efficiency of about 90.4% at a C/N=2 lowering to 73.7% at a C/N=3 (Figure 1 (a)). Considering acetate-carbon removal, an overall efficiency of 82.0% and 63.6% was attained at a C/N ratio of 2 and 3, respectively (Figure 1 (b)). Consequently, in the experiment at C/N=3 the biomass present in the RBC did not metabolize all the substrates and as a result they were accumulated along the time. For economical and environmental reasons the use of C/N=2 is more appropriate. The maximum substrate removal efficiencies were obtained in the first period of operation, with an influent nitrate concentration of 50 mg NO_3^--N/L and a hydraulic retention time of 10 h. The removal efficiencies were adversely affected by a twofold increase in nitrate and acetate loading rate and a decrease in HRT. The accumulation of nitrite-nitrogen (NO_2^--N) was verified and it can be speculated that the influent phosphate concentration limited the conversion of nitrite to nitrogen gas. Alternatively, the accumulation of nitrite can be the result of the microbial population present inside the RBC rich in nitrate reducing bacteria. Relatively to gas composition, the results indicate that N_2 was the most abundant gaseous product. The RBC had a very good performance at C/N=2, using acetate as carbon source, attaining a nitrate removal of about 90%, that could probably be enhanced with an increase in phosphorus concentration. As a general conclusion, the RBC proved to be very robust in coping with high nitrate loads and shock loads.

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Integration of advanced and biological oxidation processes: Enhancing biodegradability of phenol solutions

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Keywords: wastewater treatment, phenol, process integration, activated sludge, advanced oxidation process

Abstract – Due to the toxic nature of some phenolic compounds the Environmental Protection Agency (EPA-USA) has set a water purification standard of less than 1 part per billion (ppb) of phenol in surface waters (Melo *et al*., 2005). They are often found in industrial effluents such as from pulp and paper, timber, plastics and synthetic polymer, pharmaceutical, pesticide, oil and petrochemical industries. Conventional oxidative methods for the wastewater treatment utilize the combination of biological, physical and chemical treatments. Biotreatment processes tend to be very large due to the slow rate of the biological reactions; physical methods generally transfer waste components from one phase to another; chemical treatment, such as chlorination, can result in the formation of chlorinated phenols and their byproducts which are as toxic and non-biodegradable as the original medium (Kusic and Koprivanac, 2006). Advanced Oxidation Processes (AOP´s) have been used as one of the most effective processes to remove organic pollutants from aqueous solutions containing soluble organic compounds that are either toxic or non-biodegradable. AOP´s involve the generation of the hydroxyl radical (•OH), which has a very high oxidation potential and is able to oxidize almost all organic pollutants (Esplugas *et al*., 2002; Kusic and Koprivanac, 2006). Although these processes have shown high efficiency for water and wastewater treatment, they have high costs related to investment (complex installations) and operation (high consumption of energy and/or reagents) (Esplugas *et al*., 2002). A promising alternative to complete oxidation of biorecalcitrant wastewater is the use of an AOP as pre-treatment to convert the highly toxic organic compounds to readily biodegradable intermediates (Devlin and Harris, 1984), followed by biological oxidation of these intermediates to biomass and water (Hsu et al, 2004; De Morais and Zamora, 2005; Al Momani, 2006). Fenton's reaction may be recommended as a pre-treatment process to enhance later microbial transformation, lowering the operational costs, by increasing its biodegradability, generally through the cleavage of large organic compounds into smaller ones (Walling, 1975; Chamarro *et al*., 2001; Zazo *et al*, 2005). This work aims to treat a phenol solution via Fenton reaction to enhance medium biodegradability and using it as an effluent in a continuous activated sludge (biological) process. The Fenton´s condition used was 1000mg.L^{-1} of TOC, 1mg.L^{-1} of $FeSO_4$ and 1000mmol of H_2O_2 added with a peristaltic pump during 2 hours of experiment, the initial pH was 3.2 (Kavitha and Palanivelu, 2004; Zazo *et al*., 2007). During the reaction time, samples were withdrawn and the pH was adjusted to 10.0 using NaOH stock solution in order to stop the chemical reactions. The samples were filtered to remove the precipitated iron ions and, finally, neutralized (pH $\approx$ 7.0) to be analyzed. All the samples were diluted to a half to simulate the intrinsic dilution form the continuous activated sludge process. The analytical determinations were: Total Organic Carbon (TOC), Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) to get

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the biodegradability parameter (BOD_5/COD) and High Performance Liquid Chromatograph (HPLC) to monitor the phenol concentration and its byproducts (cathecol and hydroquinone). The results (See Figure 1) showed the effectiveness of the pre-treatment achieving 0,71 of biodegradability (BOD_5/COD) at 30min using Fenton reaction. In this condition, the wastewater could be pos-treated by conventional biological processes, resulting in a reduction in the operational costs and time reaction from both advanced oxidation and biological processes.

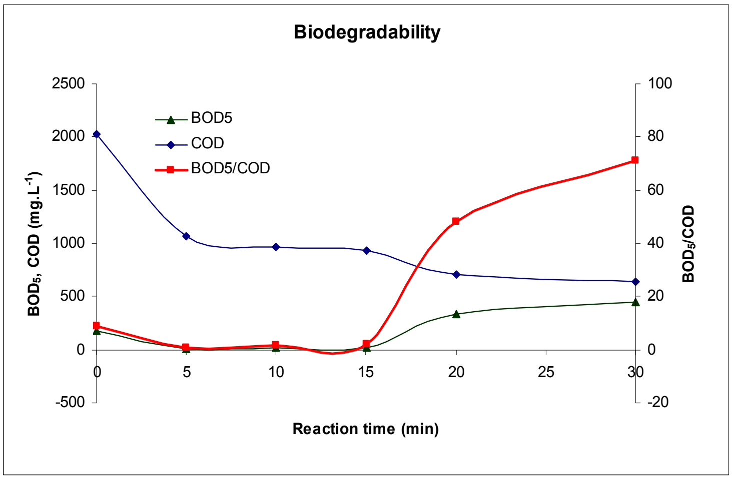

Figure 1: COD, BOD_5 and BOD_5/COD values for the sample collected of Fenton reaction ($[TOC]_{initial}$ = 1000 $mg.L^{-1}$,$[FeSO_4]$ = 1 $mg.L^{-1}$, 1000 mmol of H_2O_2 and $pH_{initial}$ = 3.2) then diluted to a half be analyzed.

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Three-phase fluidized bed bioreactor for wastewater treatment – Parameters effect

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Keywords: Model, biological treatment, refinery wastewaters, three-phase fluidized bed

The three-phase bioreactor with bacteria immobilized on solid particles is not so easy to describe mathematically, particularly in fluidized bed. In fact, the transfer phenomena are numerous and complex in bioreactors where the aerobic wastewater treatment is made by biodegradation which, consists of an oxygen absorption in liquid phase, with biological and biochemical reactions in biofilm where the substrate degradation occurs.

As we know, and thanks to their performance of pollution removal, this kind of new generation of bioreactors are more used, but they are sensitive to the hydrodynamic conditions as the interdependence between the phenomena of biofilm growth, flow and also to the kinetic effect of the biotreatment. Then, theses processes require the control of biokinetic, physico-chemical and rheological characteristics but also the hydrodynamic conditions.

In continuation of our last work (K. Allia and Coll. 2005 and L. Toumi Boumehdi and Coll. 2008), where the model proposed reposes on some assumptions, and incorporates at the same time the contribution of biomass in suspension, biofilm detachment, maintenance and the two substrates (oxygen, and organic substrate) as growth limiting factors. In addition, as phenol is the major toxic soluble organic compound in refinery wastewater, the simulation tests were achieved by considering transfer diffusion as well as biokinetic parameters relating to phenol and given by the literature. The numerical resolution of the obtained equations was solved by adopting phenol biokinetic and mass transfer constants.

The simulation results were confronted with those obtained experimentally in a semi batch three phase fluidized bed bioreactor for wastewaters treatment contaminated by hydrocarbons and the comparison revealed the difference between them. This seems to be due to the fact, that we supposed that the hydrocarbons mixture has the same biokinetic and mass transfer characteristics as phenol.

The aim of this study is to analyse the results of sensitivity analysis of the model which was carried out by studying the effect of mass transfer and biokinetic constants, substrate yield coefficient, substrate maintenance coefficient, the detachment specific rate and the operational parameters (initials conditions) on four (04) profiles relating to substrates concentrations, suspended biomass and biofilm thickness.

According to simulation results, the model predicts that biokinetic parameters effects are more important than those of mass transfer are during the time required for biodegradation.

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Impact of Operational Parameters in Activated Sludge System treating Persistent Industrial Wastewater

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Keywords: activated sludge, persistent compounds, biodegradability

The wastewater produced in a fine chemical industry has stilbene compounds as the main source of chemical oxygen demand (COD). It is diluted in the Wastewater Treatment Plant (WWTP) with other streams from different production units (whitening agents and finishing and textile processing products). Stilbene compounds are complex, with the molecular base (trans-1,2-diphenylethylene) containing replacement groups such as amine and sulfate. The characteristics of these compounds, including high molecular weight, presence of HSO_3^- groups, high salinity (12,000 mg NaCl/L, on average) and high variability of the wastewater composition, result in high recalcitrance« or low biodegradability in the WWTP influent (Poiger *et al.*, 1998).

The wastewater treatment system is based on a sequence of coagulation/flocculation processes (with the addition of aluminum sulfate and anionic flocculating agent) and activated sludge (operating with a hydraulic retention time of 48 h), producing a treated wastewater with a high COD (800 mg O_2/L, on average), which is higher than the regulatory discharge limit (250 mg O_2/L).

To analyze the impact of operational parameters in COD removal, a kinetic study of the WWTP was performed using data collected along one year of operation, based on a descriptive model of the system. Temperature revealed to have a significant influence on the WWTP performance emphasizing the relevance of the oxygen solubility in the aqueous phase, mainly in spring/summer period when the biological reactor operates with temperatures close to 40ºC, with low COD removal efficiency. Other parameters such as influent feed rate, influent COD and, in less degree, the aeration tank volume also presented influence on the COD removal. Cell growth was more influenced by the volume of the aeration tank and by the effluent outflow, indicating a possible occurrence of wash-out, corroborating to what is commonly observed in practice: formation of thin and dispersed flocs of difficult sedimentation.

In order to confirm the relevance of operational stability on the activated sludge system performance, a pilot plant simulating the WWTP biological process was constructed and installed inside the industry. It was continuously fed with effluent diverted from the physico-chemical step added of sewage (generated in the industrial facility) to supply essential nutrients (nitrogen and phosphorus) to the microorganisms. The pH of the feed stream to the pilot plant was adjusted with soda to approximately 6.6 ± 0.4 and the dissolved oxygen concentration of the biological reactor was maintained at circa 2 mg/L. The pilot unit operated for 4.5 months with different hydraulic retention times (HRTs): 24, 48, 72 and 96 h.

The performance of the plant at each HRT was evaluated through analysis of: pH and COD (influent and effluent); temperature, dissolved oxygen, VSS (into the reactor); total Kjeldahl nitrogen and total phosphorus (influent) and turbidity (effluent). The feed to microorganism (F/M) ratio, the solids retention time and the sludge volumetric index (SVI) were also determined along the operation. The analytical methods followed Standard Methods (APHA, 1992).

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Along the pilot plant operation, better results were achieved when the HRT was 72h (COD removal efficiencies of 60%). Although the temperature had been kept constant and within the range recommended for microbial activity, the better COD removal levels were not equal to those obtained in the industrial plant. These results demonstrate that other factors beyond temperature, such as recalcitrance of effluent compounds, are important for optimum WWTP performance. Increasing HRT did not result in higher COD removal levels, probably due to longer cell exposure to certain persistent effluent constituents.

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Influence of the reactor contents recirculation on methane production - anaerobic digestion of fruits, vegetables and grass waste feedstocks

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Keywords: Anaerobic digestion, Fruits, Vegetables, Grass, Recirculation

Anaerobic digestion process generates two main products: biogas, a mixture of methane and carbon dioxide, and compost. In Europe, the study of anaerobic digestion of solid wastes only started in the 1980's (Mata-Alvarez, 2002). After the energy crises of the 1970's and the global environmental considerations, such as greenhouse effect, anaerobic digestion process had a significant development, because this waste treatment produce a renewable energy source, limiting the biogas emissions that cause adverse effects and also permit organic matter and nutrients recovery, with the application of compost in soil.

Another benefit associated with the anaerobic digestion is the possibility to reduce the amount of waste sent to landfill, which is a goal expressed in European Directive 1999/31/CE.

Fruits and vegetables wastes represent a potential energy resource (Gunaseelan *et al.*, 2004). This kind of waste is produced in great quantity from markets, restaurants, agriculture and others domestic activities. Also grass is a waste produced in significant quantities from golf clubs, stadiums and gardens.

In Portugal, there are only six anaerobic digestion plants for organic solid wastes and they are quite recent. In order to fully evaluate the overall anaerobic digestion process, particular attention should be devoted to the study of specific waste characteristics and process parameters. In fact, the ability to manage a biological process is deeply related to substrate biodegradability and proper control conditions of the process. Adverse effects to the overall digestion process may sometimes occur when poorly biodegradable wastes are added to a stable processing reactor, or used in conjunction with another type of waste (Misi and Forster, 2001).

The objective of this study was to determine the influence of recirculation of the digestion mixture on methane production from anaerobic digestion of fruits, vegetables and grass. The mixture used contains 50% of fruits and vegetables and 50% of grass. This mixture was selected from previous studies carried out with a wide range of fruits and vegetables to grass ratios, as the one that yield a higher methane production.

The experiments were made with two batch reactors with 10L capacity each, using an incubation period of 35 days at a mesophilic range. One of the reactor worked without recirculation (R1) and the other with recirculation of the contents (R2).

During the experiment, the biogas volume and its composition were monitored for each reactor. The liquid phase was also analysed for COD, pH, TS, VS and volatile fatty acid determination.

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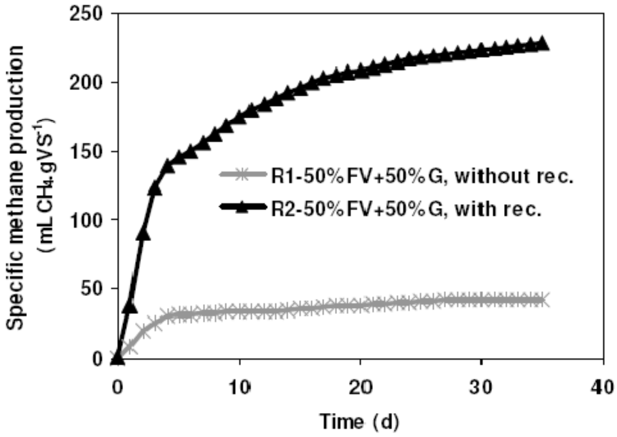
The methane production for both reactors is presented in Figure 1. Maximum specific methane production was achieved in 5 day for R1 and in 15 days for R2. R2 presents a greater methane production (223.24 mL CH4●g^{-1}VS) than R1. The average of methane percentage in the biogas produced was 45 % and 70 % for R1 and R2 respectively.



In both reactors, and during all the period of incubation, the pH of the digestion mixture do not need to be adjusted externally, since it was found that it remains in the adequate range for anaerobic digestion (Table 1).

Table 1. pH and maximum specific methane production for R1 and R2

Reactor	pH initial	pH final	Maximum specific methane production (mL CH_4•g^{-1}VS)
R1	7.42	7.38	41.54
R2	7.65	7.49	223.24

The overall volume of methane produced was much larger in R2 than R1. This is probably due to the recirculation system which ensures a good homogenization of the reactor contents. Similar results were presented by Sponza and Agdag (2004).

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Anaerobic elimination of phenol: a comparison between a two phase and a single stage digester

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Keywords: Acidogenic, biodegradation, biogas, industrial waste, methanogenic, phenol, two-phase anaerobic digestion.

Extended abstract

Phenol is derived from oil and coal and is used by many industries including the production of fertilizers, fungicides, dyes, plastics, solvents and fibre board (Veeresh *et al.*, 2005). The global production of phenol surpassed 6.6 x 10^6 t in 2000 and is mainly manufactured in Europe and the USA (Panov, 2000; Niwa *et al.*, 2002). Phenol is a priority pollutant and the latest figure shows that 3,480 t were released into the USA environment (USEPA, 2008).

The biological anaerobic treatment of phenol still remains the most attractive option. However, its inhibitory effects on methanogenesis have not been overcome. In response, a variety of single phase bench scale digesters have been proposed (Veeresh *et al.*, 2005). Nevertheless, the application of two phase anaerobic digestion (TPAD) consisting of acidogenic and subsequent methanogenic steps is largely unexplored.

The advantages of TPAD over a single stage reactor (SSR) are well recognised. TPAD reduces toxicity to methanogenesis by hosting fast growing acidogens in a separate reactor, thus preventing trophic-chain imbalances due to high rate production of volatile fatty acids (VFA's) and their low rate conversion by methanogens (Metcalf and Eddy, 2003). Besides, TPAD allows the dilution of the VFA's produced in the acidogenic phase needed into the methanogenic phase, avoiding the utilization of sources of fresh water for dilution.

This study aims to compare the elimination of phenol contained in a synthetic wastewater by utilising a two phase anaerobic digestion system (TPAD) and one single stage reactor (SSR). Performance of both TPAD and SSR was studied at dilution-batch and semi-continuous operation.

Both systems were operated in batch-dilution and semi-continuously at 35 ^{0}C. The reactors were loaded with a variable concentration of phenol and a constant concentration of a nutritional supplement. The three reactors were inoculated with sludge from an anaerobic digester treating yeast and industrial wastewater. This sludge was also utilised to produce acidogenic and methanogenic sludge for replenishing deficits due to washing out. Total organic carbon (TOC), chemical oxygen demand (COD), volatile acids (VA) and phenol (aqueous phase) were determined with Dr. Langue cuvette test kits. Volatile suspended solids (VSS) were determined by standard methods (APHA, 1998). CH_4 and CO_2 in the biogas were analysed using a gas chromatograph (GC, Varian 3400) fitted with a methanizer.

Biogas production and productivity were greater in the TPAD. The acidogenic phase enhances the biodegradability of phenol without achieving degradation of the aromatic ring. Incrementing phenol concentration (up to 1250 mg L^{-1}) was harmless to the fermentation of a readily biodegradable organic matter. Inhibition of biogas formation is prevented by dilution of the incoming load of VFA's, phenol and aromatic metabolites.

TPAD is therefore a potential wastewater treatment option to decontaminate streams containing phenol and easily biodegradable organic matter. This system enhances biogas production and allows better control of the acidogenic and methanogenic stages.

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Comparison of different control cascade strategies for anaerobic digesters using ADM1

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Keywords: Anaerobic digestion, ADM1, control, modeling, wastewater treatment.

Anaerobic digestion processes consist of a set of conversions of organic matter into methane and carbon dioxide, occurring in absence of oxygen (Lettinga, 1995). Most of the organic effluents and wastes coming from industrial, municipal or agricultural activities can be effectively processed by anaerobic wastewater treatment plants. The use of methane as an energy source, the low growth of sludge and its ability to treating wastewater with medium to high organic loads are some of the reasons for which the anaerobic wastewater treatment (AWT) has gained importance in the last few decades (van Lier et al., 2001). However, the AWT has classically been regarded as a difficult process to be controlled and managed. Different disturbances can affect an anaerobic reactor and the recovery of the normal conditions can be long and expensive. Therefore, the selection of an accurately control mechanism could give important advantages in the management of these devices helping to minimize the operational cost and maintain the plant under safely conditions.

The range of possible control systems is quite broad, and its application has been quite limited. In this sense, it is necessary to have a standard tool that allows different controllers to be compared. Several researchers have been working to standardize the use of models in wastewater treatment processes (Copp, 2002; Vrecko et al., 2006; Jeppsson et al., 2007). Particularly with respect to anaerobic digestion models, Rosen et al., 2006 discuss about the experience with the ADM1 during COST/IWA Benchmark Simulation Model (BSM2). The BSM2 is a control benchmark for plant wide modeling. Complex models such as the Anaerobic Digestion Model Nº1 (ADM1 - Batstone et al., 2002) are a good opportunity to evaluate controllers competitively, and develop operational strategies (Batstone & Steyer, 2007). In this work, the use of ADM1 according to benchmark recommendations for comparing different configurations of robust cascade controllers in anaerobic treatment of vinasse wastewater is evaluated.

A modified version of the ADM1 (Batstone et al., 2002) incorporating ethanol degradation pathways and calibrated for an USBF reactor (Ruiz-Filippi et al., 2004) was used. In this ADM1 version, an additional group of ethanol degraders was included as well as a new state variable for ethanol. The extension for ethanol not only considers the hydrogen and acetate pathways but also takes into account other controller design VFA (volatile fatty acids) pathways (propionate and butyrate) by means of the stoichiometry. Thus, VFAs are suitably predicted, including overloads or transitions between steady states.

The data was sourced from a heavily instrumented, hybrid USBF (UASB+AF) pilot plant with an overall volume of 1.15 m^3. The installed on-line measurement devices included feeding and recycling flow-meters (ABB, COPA-XE and Siemens, 7ME2531), input and output reactor pH (Cole Parmer) and temperature (Pt 100); biogas flow-meter (Brooks, 3240); infrared gas analyzer (Siemens, Ultramat 22P) for measurement of CH_4 and CO concentrations in the gas phase; electrochemical hydrogen gas analyzer (Sensotrans, Sensotox 420); on-line Total Organic and Inorganic Carbon (TOC/TIC) in the influent and effluent were determined by catalyst combustion oxidation and non dispersive infrared (NDIR) CO2 detection (Shimadzu, 4100); A new titrimetric analyzer called AnaSense® was used for the online volatile fatty acids (VFA), bicarbonate, partial and total alkalinity concentrations monitoring.

Two controllers for handling anaerobic digesters using cascade configuration are already available in literature (Alvarez-Ramirez et al., 2002; Liu et al., 2004). In addition, a novel cascade controller approach is presented and compared with the others. This new controller is multi-objective considering two operational objectives in the same simple control structure, the control on the effluent quality and the control on the maximum production of the methane gas. It requires two inputs or measures, the methane flowrate (QCH4) and the concentration of volatile fatty acids (VFA) expressed as acetic.

For a proper comparison of the three types of cascade controllers, a necessary previous step consisting of tuning the controllers was carried out. For this task sequential quadratic programming (SQP) combined with ethanol modified ADM1 for parameter optimization were required. The time required for the optimizations was 12 minutes approximately in an Intel Core Duo E6550 233GHz and 2 Gb of RAM. Some difficulties arise when trying to optimize of parameters using a model very complex as ADM1. However, the optimization is necessary for comparing controllers under the better operational conditions.

In terms of maximum methane productivity criterion, the Liu controller and the new approach presented reasonably similar performance. Nevertheless, Liu controller required a great consumption of bicarbonate for maintaining the reactor in a stable operation. This is the main handicap for applying this controller at industrial scale. Moreover, it doesn’t take into account in any case the effluent quality.

On the other hand, Alvarez-Ramirez controller doesn’t take advantage of the difference between the dynamics of the liquid and gas phase for improving its response. The consumption of bicarbonate and the gas flowrate were strongly dependent of the set-point selection. The choice of appropriate regulated variables and of appropriate set-points is therefore of crucial importance for effective controller optimization. The new controller compared with Alvarez-Ramirez controller, under quality effluent control criterion, it had a greater speed of response against organic overload perturbations.

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Treatment of waters contaminated with diesel fuel by electrocoagulation

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Keywords: diesel fuel emulsion, electrocoagulation, optimization

Electrocoagulation (EC) is one of the promising contaminated water treatments, where impurities are removed by coagulation and flotation, while coagulant is added *in situ* by electrochemical oxidation of the suitable anode material. EC is possible alternative to the metal salts or polymers and polyelectrolyte addition, for processes of the stabile emulsion breaking, adsorption, as well as the metal, colloids and soluble inorganic impurities removing[1].

In this work, efficiency of electrocoagulation process for hydrocarbon removing from the water emulsion was investigated. As model solution, emulsion of diesel fuel in distillate water with addition of sorbitan monooleate as the emulsifier was used. Process was conducted in the batch reactor with the aluminum electrodes, with addition of the NaCl for the increase of electrical conductivity of the emulsion. Inlet parameters were NaCl and diesel fuel concentrations in emulsion, current density and reaction time. Outlet parameters were total organic carbon (TOC), filtrate volume and mass loss of the anode material. Experiments were conducted in two sets, each with 16 treatments, settled by two level factorial design (TLFD) method[2]. By an optimization procedure, quantitative influence of each factor, inlet parameters and their interactions on the main responses were calculated, and presented with mathematical correlations.

Obtained results indicated very high efficiency of the electrocoagulation process, for the hydrocarbon removal from water emulsion, ranging from 98.9 to 99.5 %, calculated in respect to total organic carbon content in water, while current efficiency was in the range from 17 to 78 %. Water phase after the EC process was odorless, tasteless and colorless. As expected, it is proven that the current efficiency is increasing with increase of the electric conductivity of the emulsion and with decrease of the current density. High TOC removal was obtained even for the short periods of reaction time (5 minutes), meaning that higher current efficiencies are possible with lower current densities at prolonged reaction times.

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Application of coagulation systems coupled with adsorption on powdered activated carbon to textile wastewater treatment

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Keywords: Coagulation, Adsorption, Reactive dyes, Aluminum chloride, Reactivation

Textile industry uses and rejects high amounts of water, being the wastewaters the main way by which dyes are discharged into the environment. Textile effluents have high concentrations of organic and inorganic compounds and strong colour, caused by residual dyes that were not fixed to the fibers in the dyeing process, and present as main characteristics high variations of flow and composition, large amount of non-biodegradable compounds, presence of toxic substances, high temperature and high pH value (Cooper, 1995). Colour is one of the main contaminants of textile wastewaters. In addition, there are many different types of dyes and different dyeing manufacture processes, making difficult that only a single treatment process answers satisfactorily to the treatment requirements in all situations. In industry, the technologies that have already been applied in decolourisation of effluents are (Freeman, 1989; Metcalf and Eddy, 2003; Chakraborty et al. 2005; Solmaz et al. 2006): oxidation with ozone; oxidation with Fenton's reagent; UV oxidation; membranes filtration processes; activated carbon adsorption; ion exchange resins; coagulation/flocculation or coagulation/flotation; and electrochemical methods. The aim of the present work was to investigate the removal of reactive dyes, Reactive Black 5 and Reactive Orange 16, through of combined process coagulation/adsorption on activated carbon. A coconut-based powdered activated carbon was used as an adsorbent and aluminum chloride was chosen as a coagulant. In order to get the best removal condition for these dyes, it was verified the influence of the following parameters: dosage coagulant, aqueous solution pH, addition of sodium chloride of mixture and influence of temperature more sodium chloride. The dye removal efficiencies for 100 mg/L of Black 5 and Orange 16 by coagulation were almost 97,6% and 90% under the determined optimal conditions for Black 5 (200 mg/L coagulant dose and pH 6) and for Orange 16 (250 mg/L coagulant dose and pH 6). Coagulant was added to the dye solution and rapidly mixed (175 rpm) for 1,40 min followed by slow mixing (15 rpm) for 10 min and then allowed to settle for 1 h. Spectrophotometry was the technique used to measure the concentration of the dye remained in the fluid phase. Adsorption results of reactive dyes were analyzed by Langmuir and Freundlich isotherms models and showed good correlation. Considering the influence of sodium chloride, the presence of these ions results in significant improvement of adsorption. The influence of temperature more sodium chloride on the removal of dye from aqueous solution shows the feasibility of adsorption and its endothermic nature. The adsorption capacity (q_m) was 95 mg/g at initial pH 3 for the particle size 600 µm-1 mm and 70ºC with 6% of sodium chloride in the solution. Through the experiments of reactivation it was possible the reuse the adsorbent for more three times consecutives, beyond of the first adsorption with the virgin coal. SEMs of the native and exhausted were recorded to explore the morphology of the adsorbent. For the final evaluation of the effluent, obtained after the coagulation and adsorption process, acute toxicity tests were carried out with *Daphnia magna*, and the results obtained showed that the final effluent was not toxic. The combined coagulation/adsorption process was an excellent option for removal of reactive dyes.

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Treatment of wastewater polluted with urea by counter-current thermal hydrolysis in an industrial urea plant

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Keywords: Urea thermal hydrolysis, Liquid non-ideality, Nakamura equation state, modeling.

Abstract

In this work, the removal of urea from industrial wastewater by thermal hydrolysis is studied. The features of our model are (1) the use of Nakamura equation state and UNIQUAC equation for describing vapor and liquid equilibrium, (2) the use of multistage well-mixed for liquid and vapor flows with non-ideal rate-based, (3) the reaction assumed to take place in liquid phase. The model incorporates reaction rate of urea hydrolysis and takes into account the effects of solution non-ideality and back-mixing on the reactor performance. The rate of reaction is written in terms of activity of reactants which are determined using a thermodynamic framework for system of NH3–CO2–H2O–urea mixture.

The model provides temperature and concentration distribution of different components in liquid and vapor phases along the height of reactor. Also the effects of key parameters on the performance of urea hydrolyser such as the steam flow rate and the temperature of wastewater has been examined. The result of this work shows that an increasing of inlet temperature of wastewater and steam flow rate improves the urea removal efficiency. The predicted data of the model are consistent with the plant data indicating the validity of the model.

Introduction & Process

A urea plant produces water as well as urea. This water, which is contaminated, mainly by entrainment from the evaporation section, requires treatment if it is to be reused again. This process condensate generally contains about 2 to 9 percent by weight ammonia, 0.8 to 6 percent by weight carbon dioxide and 0.3 to 1.5 percent by weight urea.

It is now recognized by both the urea industry and the responsible governmental agencies that having urea-containing effluent streams may create possible environmental hazards. Therefore, several processes have been suggested for treating these urea-containing streams due to environmental restrictions. Which in the past decade, 100 ppm of urea was considered acceptable for plant wastewater, but today's requirements mostly call for a maximum concentration of 10 ppm (Rahimpour, 2004).

A modern wastewater treatment consists mainly out of a first desorber column, which reduces the ammonia and carbon dioxide contents. For the next column, the hydrolyser, it is important that the ammonia and carbon dioxide concentrations at the inlet are sufficiently low, in order, not to reach the chemical equilibrium. Under these conditions the hydrolisation reaction proceeds towards the ammonia and carbon dioxide side, reducing the urea content to virtually zero. Contrary to other commercial hydrolysers, this hydrolyser is operated as a counter-current bubble column to improve the efficiency; water with urea meets continuously relatively clean vapours during its way through the column from the top to the bottom. The urea outlet of the hydrolyser is decreased to ppm level. In the second desorber column again operating at low pressure, the ammonia and carbon dioxide content are also decreased to ppm level.

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There is no information available in the literature regarding the modeling and simulation of an industrial urea removal process by counter-current thermal hydrolysis. Therefore, we decided to study more thoroughly the phenomenon of urea removal from industrial wastewater (Rahimpour and Azarpour, 2005).

Reaction Scheme:

The urea first reacts with water to generate ammonium and carbamate ions, then ammonium and carbamate ions react to yield carbon dioxide and ammonia liquid:

$$NH_2CONH_2(l) + H_2O(l) \Leftrightarrow H_2NCOO^- + NH_4^+ \qquad \Delta H_{298} = -23\ \frac{kJ}{mol} \qquad (1)$$

$$H_2NCOO^- + NH_4^+ \Leftrightarrow 2NH_3(l) + CO_2(l) \qquad \Delta H_{298} = 84\ \frac{kJ}{mol} \qquad (2)$$

Reaction (1) is slow and exothermic, while reaction (2) is fast and endothermic, and goes essentially to completion under normal industrial processing (Isla, Irazoqui, and Ge

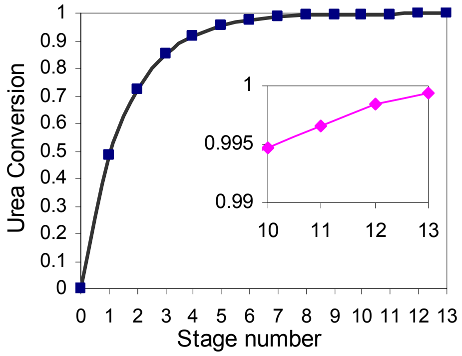

Fig. 1. Conversion profile of urea along the reactor

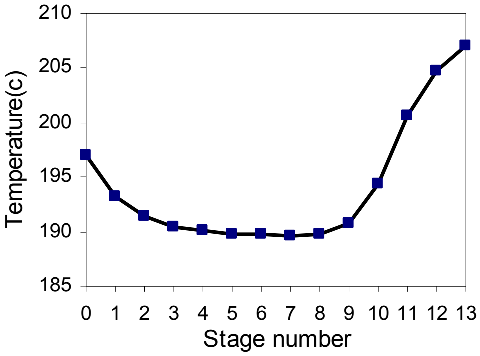

Fig. 2. Temperature profile of wastewater along the reactor

Conclusion

Urea thermal hydrolyser can remove urea from waste stream effectively, so that all components including the water can be recycled and reused in the plant. The model could be used to study the effects of process variables, such as temperature of wastewater and flow rate of steam on the reactor performance. It is also useful for a better control of the currently operating units and in the design of new hydrolysis plants.

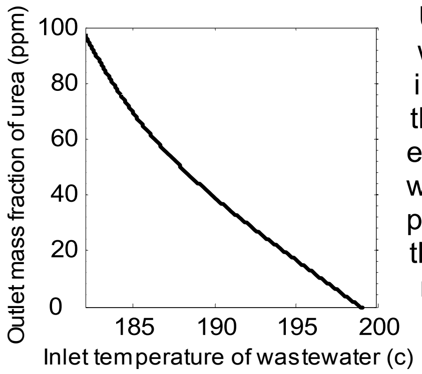

Fig. 3. Outlet mass fraction of urea along the hydrolyser versus inlet temperature of wastewater

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Tertiary treatment of slaughterhouse wastewater using ferric coagulation followed by UV or UV/H_2O_2 processes

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Keywords: Slaughterhouse, UV, H_2O_2, Tertiary Treatment.

Guidelines and regulations, with respect to the use and reuse of water in the food industry, have been created accepting or not restricting the use of non-potable water and water from direct potable reuse (Codex Alimentarius Commission, 2001). However, careful analysis are needed of each case based on a complete knowledge of the risk involved for the safety of the food product and the health of consumers. Advanced oxidative treatments (AOP) are increasingly studied to provide treated water as a source of indirect reuse with or without characteristics of drinking water, without risks to product integrity, environment and health of handlers and consumers. The main aim of this study is to evaluate the kinetics of aromatic compounds degradation using UV and UV/H_2O_2 treatment, in order to achieve the characteristics for water reuse.

Four samples of the secondary wastewater were sampled in different times on May 22nd, 2007 from wastewater treatment plant (WWTP) of a Brazilian poultry and swine slaughterhouse. The samples were kept under dark conditions at 4°C to preserve the physico-chemical characteristics. The samples showed a little variability on their characteristics, as shown in Table 1. The presence of compounds resistant to biological process treatment was identified by Liquid Chromatography coupled to mass spectrometry (Thermo Electron, Bremen, Germany). It was detected the presence of nonylphenol and octylphenol, linear alkylbenzene sulfonates, and ibuprofen in negative ion mode. In the positive ion mode, it was detected the presence of octylphenol ethoxylates (OPEO), nonylphenol ethoxylates (NPEO), polyethylene glycol (PEG), erythromycin, sulfamethoxazole and sulfadimidine.

Table 1 Range measured basic characteristic data of secondary wastewater studied

	Range	*DW*[a]		*Range*	*DW*[1]
Apparent Colour, mg.L^{-1} Pt-Co	-	15.0	*Total Coliforms, UFC/100 mL*	1000-58000	none
Real Colour, mg.L^{-1} Pt-Co	33.5-62.2	-	*Total Iron, mg.L^{-1}*	3.5-7.0	0.3
Chemical Oxygen Demand (COD), mg.L^{-1}	18.9-95.0	-	*Turbidity, mg.L^{-1}*	3.8-30.0	5
pH, at 25°C	7.3-7.6	6.0– 9.5	*UV_{254}, m^{-1}*	15.2-18.4	-

[1] Limit in drinking water in Brazil

Two different treatments were evaluated in order to achieve the drinking water parameters that are required to water reuse in food industry: (A) ferric coagulation followed by UV irradiation; and (B) ferric coagulation followed by UV/H_2O_2 treatment. Colour, turbidity, total organic carbon (TOC), chemical oxygen demand (COD), and aromatic content (UV_{254}) were the parameters followed to evaluate the performance of UV and UV/H_2O_2 processes.

The optimization of the ferric coagulation process (FSC) was performed in jar test using iron concentration in the range 5 – 25 mg.L^{-1} and pH in the range 4.5 – 7.0. The

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experimental tests using Fe(III) concentration of 16 mg.L^{-1} and neutral pH resulted in maximum turbidity removal and was chosen as optimal condition.

UV batch reactor (Trojan UV MaxTM, Model E) was irradiated by a germicide lamp (15W) with light intensity of 2.64 x10^{-3} Einstein.min^{-1}. The UV photolysis treatment were carried out in triplicate at pH 7.0 and ambient temperature. In the treatment UV/H_2O_2, the concentration of hydrogen peroxide in the beginning of the treatment was given by the Cruncher number ([H_2O_2]/DQO = 2).

The efficiency of each treatment is given in Table 2. Ferric coagulation shows high removal of COD and TOC, but low efficiency to remove turbidity and aromatic content. The irradiation with UV light increased the aromatic content and turbidity removal. UV decomposes organic matter by double bonds cleavage (Benitez *et al.*, 2003). The addition of H_2O_2, is also efficient to remove aromatic compounds.

Table 2 Percentage removals after different treatments

	A	*B*	*C*		*A*	*B*	*C*
TOC	85.4|±5.4	89.7±5.7	95.9	***COD$_{soluble}$***	33.3±33.3	54.2±20.8	78.2
Total Coliforms	99.4±0.1	100.0±0.0	100.0	***Total Iron***	83.1±9.1	-	-
Real Colour	21.4±7.1	100.0±0.0	100.0	***Turbidity***	34.1±11.3	71.0±6.5	68.9
COD	85.7±5.7	89.5±5.5	91.6	***UV$_{254}$***	31.8±6.8	94.3±0.4	94.3

A (FSC): Wastewater after ferric sulphate coagulation. B (FC+UV): Wastewater treated by ferric coagulation followed by UV treatment for 600-840 min UV exposure. C (FSC+UV/H_2O_2): Wastewater treated by ferric coagulation follwed by UV/H_2O_2 treatment for 265 min UV exposure.

The kinetics of aromatic content removal show that the addition of hydrogen peroxide increases the rate of aromatic compounds decomposition for a factor of 5 and both processes follow a pseudo-first order kinetic model. The pseudo-first order constants and the time required to reduce half of the initial concentration of aromatic content (half-life time) by UV are: $k_{UV_{254}}$ = 0.0059 min^{-1} and $t_{\frac{1}{2},UV_{254}}$ = 117.5 min. And for the treatment UV/H_2O_2: $k_{UV_{254}}$ = 0.0306 min^{-1} and $t_{\frac{1}{2},UV_{254}}$ = 117.5 min.

In conclusion, both POA tested are able to degrade organic compounds in the biological treated slaughterhouse wastewater. The combined treatment ferric coagulation followed by UV/H_2O_2 was 5.2 times faster than ferric coagulation + UV treatment, to degrade aromatic compounds, and it can be an efficient and economical choice of a treatment to reach drinking water parameters and water reuse in slaughterhouse industry.

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Membrane bioreactor and advanced oxidation processes applied to wastewater containing non-steroidal anti-inflammatory drugs (NSAIDs)

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Keywords: NSAIDs, Membrane bioreactor (MBR), Mass spectrometry (MS), Elimination.

Introduction

The occurrence of pharmaceutically active substances in the environment has become an important issue in the last few years. These compounds along with their metabolites, which can be even more harmful than the original compound, are continuously released in the environment. Recent improvements in substance specific analytical techniques today have allowed the detection of trace levels of pharmaceuticals in wastewater effluents, surface and ground waters as well as treated drinking waters (Gebhardt and Schröder, 2007).

Non-steroidal anti-inflammatory drugs (NSAIDs) belong to a class of pharmaceuticals that are extensively used worldwide and their consumption only in developed countries is estimated to be higher than several hundred of tons per year (Daughton and Ternes, 1999). To obtain the desired therapeutic results the drugs are designed to resist degradation while in parallel for renal excretion after use they are equipped with polar structures, which can be responsible for their incomplete elimination during conventional biological wastewater treatment thus reaching the environment via wastewater discharges.

These compounds of concern may induce toxic effects to aquatic organisms disturbing the ecological balances, e.g., leading to the development of multi-resistant strains of bacteria. To eliminate these compounds of concern it is necessary to improve sewage treatment because sewage treatment plant (STP) discharges are well known as predominant point sources for these drugs (Andreozzi et al., 2003). For this purpose, membrane bioreactor (MBR) and advanced oxidation processes (AOPs) have been presented as promising technologies towards the degradation of polar and persistent compounds, especially pharmaceuticals.

In this study, three NSAIDs with worldwide large consumption rates (acetaminophen, ketoprofen and naproxen) were selected for monitoring their fate over MBR and AOP treatment as well as their total removal efficiencies. The identification and quantification of the drugs along the different treatment techniques applied during this study was performed using liquid chromatography coupled with high resolution mass spectrometry (LC-(HR)MS).

Material and methods

Wastewater used as feed for MBR treatment pilot plant was taken continuously from the effluent of the pre-settling tank of the municipal STP of the city of Aachen, Germany. This wastewater was spiked continuously prior to MBR treatment with a mixture of the pharmaceuticals over a period of 4 weeks to reach a steady-state concentration of about 50 µg/L. Samples of permeate after MBR were taken and elimination efficiencies of different target drugs were determined.

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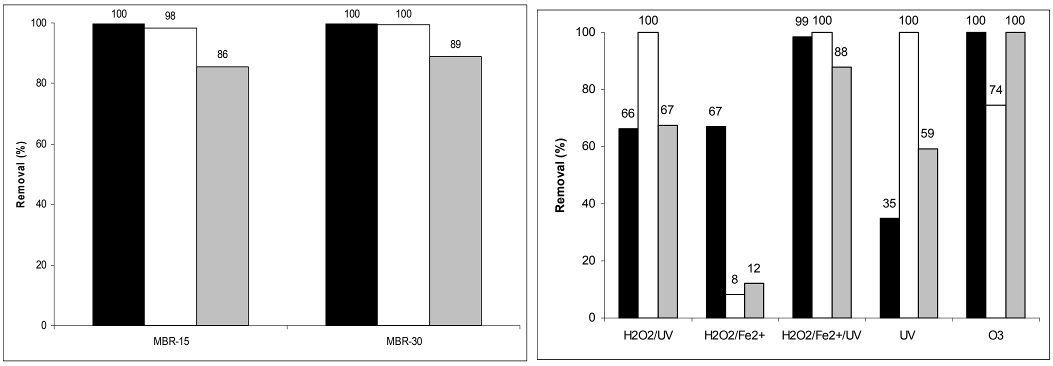
Advanced treatment was performed applying ozone (O_3), UV radiation and AOPs such as H_2O_2/UV, H_2O_2/Fe^{2+} (Fenton) and H_2O_2/Fe^{2+}/UV (photo-Fenton). The different treatment steps were performed in a temperature controled reactor (400 mL) along 30 min. A 15 W medium pressure mercury lamp (Heraeus, Hanau, Germany) was used for UV radiation. A magnetic stirrer and a Teflon coated stirring bar were used for mixing. Solutions of NSAIDs (100 µg/L of each one in Milli-Q water or in MBR permeate), were prepared prior the AOP treatments from the standard solutions. For a better comparison all treatment steps were performed under standardise conditions at room temperature and the pH was adjusted to 3.0.

Identification and quantification of spiked pharmaceuticals in MBR permeates as well as for elimination and/or degradation monitoring by AOP treatments were performed by LC-MS applying electrospray (ESI) ionisation both in positive (for acetaminophen) and in negative (for ketoprofen and naproxen) mode (ESI(+/-)) as described by Gebhardt and Schröder (2007).

Results and discussion

In Fig. 1a the mean elimination rates of target compounds during the MBR treatment are presented proving that acetaminophen and ketoprofen were eliminated completely while naproxen partly remained in the wastewater. The effect of sludge retention time (SRT) on the removal efficiency of the compounds was slightly higher toward to MBR with SRT of 30 days (MBR-30).

The results of the advanced treatment steps applied to spiked MBR permeates for the elimination of NSAIDs are presented in Fig. 1b. Analytical results demonstrated that with the application of different treatment steps, different elimination rates for each compound were achieved. The compound ketoprofen showed a remarkable sensitivity to UV radiation, however, demonstrated to be poorly or partially degraded by Fenton and O_3 processes, respectively, when compared to photo-processes. For O_3 treatment the highest elimination rates for acetaminophen and naproxen were observed, followed by the photo-Fenton process.



(a) (b)

Figure 1 - Elimination rates of NSAIDs during 4 weeks applying MBR (a) and after 30 min by advanced (b) treatments (Acetaminophen ■, Ketoprofen ☐, Naproxen ▨).

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Phosphorus and nitrogen removal using a hybrid UCT process

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Abstract

Biological phosphate removal (BPR) has become a well established process and is applied in many urban wastewater treatment plants. These processes are alternative to physico-chemical processes based on the addition of a metal salt to wastewater, in order to precipitate and insoluble phosphate salt. The reasons could be the following: lower sludge generation, chemicals savings, etc. Over the past two decades, several biological suspended sludge configurations has been proposed to accomplish biological nitrogen and phosphorus removal, and they all include the basic stages of an anaerobic zone, followed by an anoxic and an aerobic zone. From all these process the University Cape Town (UCT) or modified UCT processes are broadly used for nutrient removal in urban sewage (Ekama 1983). All the biological phosphorus removal (BPR) processes are dependent on the accumulation of bacteria capable of storing large amounts of polyphosphate inside the cells. Opposite to normal biological conversions, the BPR is not limited by a minimum solids retention time (SRT), which results from a maximum growth rate of the Phosphate Accumulating Organisms (PAO) implicated. Biological phosphorus removal systems with long SRT are less efficient for biological phosphorus removal than shorter SRT processes. Nitrification and thus nitrogen removal could be negatively affected if the system is operated at low SRT, as result of the wash out of nitrifying microorganisms with lower growing rate than heterotrophs. In this sense, the use of a carrier to promote the growth of a enriched nitrifying biofilm, could be positive to improve nitrogen and phosphorus removal in the UCT system as was also observed for COD and nitrogen removal in other high capacity hybrid bioreactors (Oyanedel 2005).

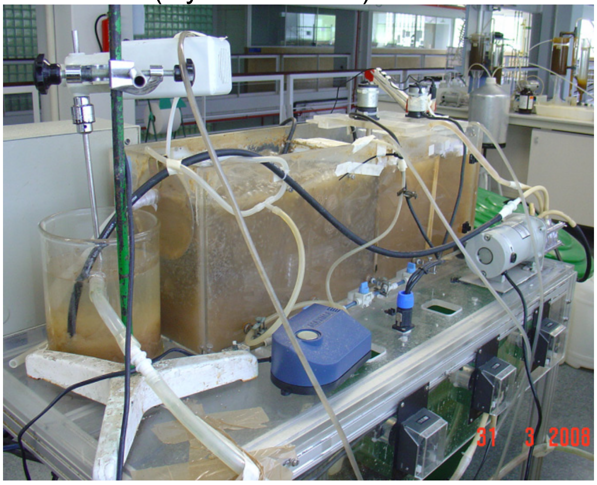

Figure 1. Image of the reactor used during the experiments

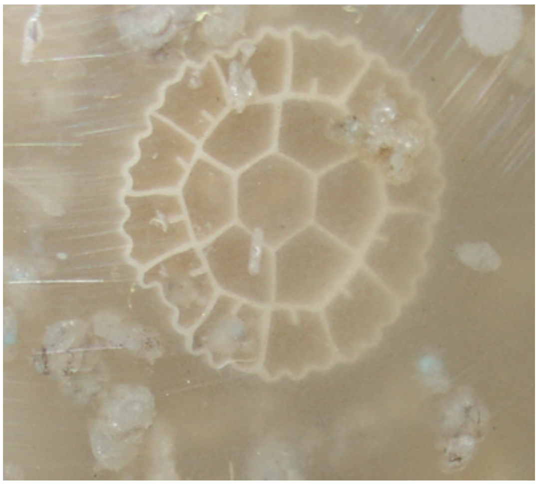

Figure 2. Image of one Kaldness K-3 ring and some particles of rough plastic support

The aim of this research was to study the behavior of simultaneous nutrient removal in a hybrid UCT system (Figure 1) using two different plastic packing materials: A rough granular support of plastic of around 3 mm diameter and the commercial product Kaldnes K-3 made of polyethylene with approximately 25 mm diameter and 10 mm long (Figure 2). These packing materials were maintained in suspension the aerobic chamber of the UCT hybrid system. The hybrid UCT system was fed with synthetic wastewater that simulates the composition of sewage. Phosphorus, nitrogen and organic matter removal efficiencies were studied at different SRT ranging from 20 to 4

days. HRT was maintained between 12 and 18 h. A high COD removal efficiency was observed, being COD in the effluent around 50 mg/L. With regard to Nutrient removal, it was observed a high phosphorus removal during a first experimental period. P in the wastewater gradually decreased from 7-10 mg/L (influent) to less than 2 mg/L (effluent). Nitrogen removal was more problematic during the first experimental days, in which only the Kaldnes K-3 was used as carrier. Ammonia removal increased as soon as the rough granular plastic support was added to the reactor. This could be a consequence of the different roughness of both packing materials. The Kaldness K-3 has a smooth surface, in which is difficult to develop a nitrifying biofilm in the presence of suspended biomass. On contrary, roughness of the granular product protect nitrifiers against detachment.

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Basic dyestuffs removal from textile wastewaters using almond hulls: Equilibrium and kinetic studies

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Keywords: Adsorption, Equilibrium, Dyestuffs, Kinetics, Textile wastewater

It is nowadays well known that there is a need of treating textile wastewaters, not only concerning primary and secondary treatments for organic matter and suspended solids removal, but also a tertiary treatment, mostly for residual colour removal.

Activated carbon is one of the most widely used adsorbents for colour removal in wastewater treatment. However, this treatment needs a high investment and leads to high operation costs, due to the high price of the activated carbon and to the high wastewater flow rate always involved, which can be greatly increased when there are no regeneration units nearby, as in the case of Portugal

Research has recently been directed towards alternative adsorbents, namely low cost adsorbents, including natural and waste materials. In this work a natural waste from almond production was used. Its culture is widespread in Mediterranean climate areas, namely in some regions of Portugal, so these wastes are easily available. This waste was tested for removal of dyestuffs from textile wastewaters.

The almond hulls were ground and sieved. All experiments were performed with particles of grain size between 0.710 and 1.190 mm. The basic dyestuff Red Astrazon FBL 200% (CI basic red 46), from Dystar is an azoic dye and is toxic to the aquatic environment. It was selected for this study based on preliminary tests. Batch equilibrium and kinetic studies were performed for the selected system.

It was verified that equilibrium was reached after 13 days contact time, at 20ºC and 100 rpm agitation. Adsorption isotherms were determined at different pH and temperature and the influence of the presence of some auxiliary dyeing agents was also evaluated.

To study the influence of temperature, isotherms at 20 and 41ºC (experiments 1 and 2), and pH 6, were performed. Both Langmuir and Freundlich models can fit the experimental results. Comparing the Langmuir maximum adsorption capacities, presented in table 1, it is observed an increase with temperature, from 15±3 to 30±11 mg/g, respectively. This increase might be explained (Asfour *et al.*, 1985) by an increase of pore size diameter due to the swelling of the almond hulls caused by heating at 41ºC, allowing the dyestuff molecules to penetrate further in the adsorbent pore structure than at 20ºC.

To evaluate the influence of pH, isotherms at three different initial pH values, 4.5 (adjusted with acetic glacial acid, an auxiliary dyeing agent), 6 and 9 (adjusted with sodium hydroxide), at 20ºC, were performed (experiments 4, 1 and 3, respectively). The experimental results were successfully fitted either by the Langmuir or the Freundlich model. Comparing the Langmuir maximum adsorption capacities, in table 1, there is an intersection of confidence intervals, indicating no significant effect of pH, which could be related to the fact that final pH was almost the same in both cases, ranging from 6.5 to 7.3. The buffer effect observed might be related with acid-base reactions with substances leached by the almond hulls.

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Table 1 Experimental conditions and Langmuir model [$q = q_s.k_L.C/(1+k_L.C)$] parameters, respective confidence intervals, square correlation to model (r^2) and variance (s^2) for each experiment.

Experiment	pH initial	pH final	T (ºC)	q_s (mg/g)	k_L (L/g)	r^2	s^2
1	6.09	6.85	20	15±3	0.31±0.14	0.979	3.42×10^{-7}
2	6.04	6.49	41	30±11	0.084±0.047	0.986	3.09×10^{-7}
3	9.05	7.31	20	16±6	0.18±0.13	0.943	6.96×10^{-7}
4	4.5	6.52	20	13±3	0.199±0.100	0.979	2.33×10^{-7}
5	4.95	5.39	20	23±26	0.033±0.054	0.896	9.35×10^{-7}

In order to approach real situations, isotherms were also performed with synthetic wastewater (experiment 5), containing acetic glacial acid, used for acidification to a 5 pH, and 0.5 g/L of trisodium phosphate. This last auxiliary dyeing agent is used as an electrolyte and has also a buffer effect in the dyeing bath. Consequently the final pH, 5.4, was similar to the initial value. Langmuir (parameters in table 1) and Freundlich models were adjusted to the experimental results and were compared with the ones obtained for the dyestuff solution without addition of other chemicals (experiment 1) and with the dyestuff solution prepared with glacial acetic acid (experiment 4), as auxiliary dyeing agent. A reduction of adsorption is observed when the dyestuff solution complexity increases, as it was expected however this is not clear due to the results dispersion in experiment 5 caused by the growth of microrganisms. The presence of organic matter, nitrogen (present in the dyestuff) and phosphorus (present as trisodium phosphate) at moderate pH, gave rise to the development of microrganisms along the 13 days of contact time. The simultaneous occurrence of biological degradation and adsorption are considered advantageous (Figueiredo *et al.*, 2000), allowing longer operation times that delay, or avoid, the replacement of the spent adsorbent.

A kinetic study was also performed with the same synthetic wastewater of experiment 5, at 20ºC, and 400 rpm shaft stirring rate. Under these conditions equilibrium was reached after 120 min and the experience lasted for 180 min. Lagergren pseudo-first order and pseudo-second order models were adjusted to the experimental results. The best fit was obtained for pseudo-second order model, using a experimental equilibrium concentration of 2.44 mg/g in the solid phase and a 80 ±14 g/(g min) kinetic constant. These model parameters can be used for comparison with different systems or different experimental conditions.

The results obtained suggest a possible application of almond hulls, an easy available natural waste, to remove the basic dyestuff Red Astrazon FBL 200% (CI basic red 46) from textile industrial wastewaters, alone, or combined with biodegradation (Figueiredo *et al.*, 2000). However there are still ways of improving the performances of this adsorbent, such as by chemical treating of the natural material (Gong *et al.*, 2005).

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Integration of emergent processes for the treatment of wastewater from nitrocellulose indutry: treatment with *Aspergillus 2BNL1* fungus and TIO_2/UV

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Keywords: delignification effluent; fungus treatment; combined processes

The aim of this work was to characterize the delignification effluent from nitrocellulose industry and evaluate the combination of fungus treatment and photo-catalytic treatment (TiO_2/UV system), for this effluent. The treatment efficiency was evaluated by color, total phenol, TOC COD, BOD, UV spectroscopy, molar weigh distribution and toxicity. All the experiments were carried out employing a delignification effluent coming from the alkaline pulping (NaOH) of the nitrocellulose production by an industry that utilizes cotton as the raw material. The fungus used in this work was isolated from activated sludge and identified as *Aspergillus* 2BNL1. This strain was selected after screening from a group of fungi for their ability to decolorize delignification effluent. The photo catalytic treatments were carried out in a glass reactor with 9.5 cm diameter cylinder (250 ml working volume), using 100 mg of free anatase TiO_2. The irradiation in the photo-reactor was obtained by a low pressure Phillips mercury lamp (without the glass cover), 125 W (λ= 254 nm) positioned inside the reactor. The temperature was controlled by water circulation. During the irradiation, the solution was magnetically stirred to provide proper mixing and air was bubbled at an air flow rate of 90 mL/min. The delignification effluent presented high color (3516 CU), total phenol (876 mg/L) and TOC (1599 mg/L) and also high toxicity even at a low concentration. The results of photo-catalysis showed that color, total phenol and TOC decreased 11, 25 and 13 %, respectively. The combined treatments showed benefits when compared with the non-combined ones. The combined processes using Fungus and photo-catalysis showed to be the best treatment, reducing the color, total phenol, toxicity (inhibition of Escherichia coli growth) and TOC by 99.5 %, 95,4%, 64% and 56 %, respectively.

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Olive mill effluents treatment by physical-chemical integrated processes

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Keywords: Agro-industrial wastewaters, Fenton process, Ozonation, Ultrafiltration, Process integration

Sustainable development is related with a good management of the effluents produced during the industrial processes. In general, the agro-industries are characterized by small familiar companies spread all over the country where the management and depuration of the final effluents is not usually efficient. Nevertheless, the growing public concern around environmental problems related to the disposal of such wastewaters brought up more restrictive legislation that is being progressively applied. In this context, the operation license of the companies is dependent on the environmental certificate which implies, among other things, the use of adequate wastewater treatment technologies.

The Olive Mill Wastewaters (OMWs) are of special concern to the mediterranic countries which are the Olive Oil world principal producers (30 million m^3 of liquid effluents are produced each year). These effluents are recalcitrant to common activated sludge biological treatments, mainly due to the presence of several compounds toxics for the microorganisms as is the case of phenolic compounds. Moreover, the mills generally operate during only a few months each year and, as a consequence, the seasonal characteristics of the effluents do not allow an efficient biological depuration treatment since constant microorganism acclimation would be required. In this context, the application of physico-chemical processes appears to be a suitable alternative, which may have higher implementation and operational costs when compared with the traditional biological processes. Nevertheless, the application of integrated systems involving several treatment processes seems to be a suitable alternative concerning both organic content degradation and operational costs (Martins *et al.*, 2008).

Within this scenario, the aim of this work is the integration of chemical oxidation processes using an ozonation stage and the Fenton's reaction with the physical treatment by ultrafiltration for the depuration of a real Olive Oil Wastewater. The ozonation process is based on the oxidant ability of ozone which reacts quickly with high electronic density molecules as phenolic acids; in the Fenton reagent the oxidant power of hydrogen peroxide is enhanced by the presence of the iron catalyst. The ultrafiltration is a membrane process suitable for separating dissolved macromolecules in solution on the basis of size and is usually used as a refining process of the other treatment technologies. After the optimization of each individual technique, different sequences of the three processes were evaluated in order to achieve the best remediation alternative for this particular effluent.

The OMWs usually exhibit a high content of organic molecules, especially polyphenolic mixtures (1-10 g/L) with different molecular weights, as well as acidity and high concentrations of potassium, magnesium and phosphate salts. Besides aromatic

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compounds, OMWs contain other organic molecules, including sugars, organic acids, and pectins that increase their organic load - COD (80-200 g/L) and BOD (50-100 g/L) (Fiorentino *et al.* 2003). The sample used was collected during the month of January of 2008 from an equalization tank and was stored at 4ºC. Table 1 shows the main characteristics of this sample. This effluent shows a low organic charge when compared with the literature values mainly due to two factors: in the equalization tank are discharged all the process wastewaters (including washing), moreover, low SST content was found.

Table 1. Characterization of industrial effluent

pH	TOC (mgC/L)	COD (mgO2/L)	BOD5 (mgO2/L)	BOD5/COD	TPh (mgeq. gallic acid/L)	SST (mg/L)	SDT (mg/L)
4.7	368±23	1485±15	216±24	0.14	106.4±5	40	1160

Indeed this is not an effluent suitable to be treated by biological processes since a low BOD5/COD ratio is observed. The low biodegradability is mainly related with the presence of bio-refractory compounds as is the case of the phenols species here measured as Total Phenolic Content (TPh) by the Folin-Ciocalteau method.

Figure 2 shows COD removal during experiments with individual ozonation and Fenton process. The results showed a lower activity for ozonation with less than 10% COD degradation after 120 min of oxidation, whereas.the Fenton process led to 55% removal after 60 min, followed by a plateau where no significant COD decrease occurs. In this context, the integration of processes allows a better effluent depuration. Moreover, the application of a final step of ultrafiltration led to the removal of small saturated organic molecules refractory to further chemical oxidation.

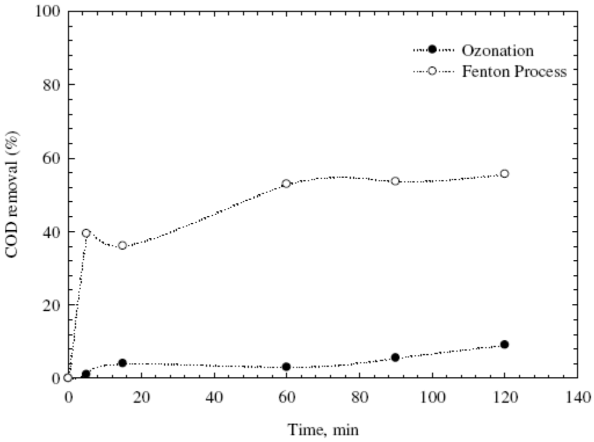

Figure 1. Preliminary results for COD removal by Ozonation (40 gO_3/m^3) and Fenton Process ($[Fe^{2+}]$ = 1000 mg/L and $[H_2O_2]$ = 7 g/L).

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Removal of formaldehyde by oxidation from phenolic resin plant effluents

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Keywords: Phenolic resin plant effluent, formaldehyde, chemical oxidation, Fenton

Phenol and formaldehyde are the main pollutants discharged by phenolic resin producing industries. Since exposure of these chemicals has potential toxic effect on living being, these chemicals must be removed from effluent before releasing it in the environment. The authors have presented some studies of C_6H_5OH and HCHO extraction from phenolic resin plant effluents with several extractants and solvents [1-3]. Cyanex 923, in an aliphatic diluent, was chosen as a selective extractant for C_6H_5OH, because it presented the highest extraction degree for C_6H_5OH among the tested extractants and the extraction of HCHO was negligible.

After the removal of phenol, the formaldehyde remains in solution and is necessary to remove it. In literature several methods are cited to obtain this objective:
- Liquid liquid extraction
- Polymerization with urea
- Oxidation in alkaline medium
- Oxidation in acid medium, advanced oxidation, Fenton reaction
- Photo Fenton reaction, advanced oxidation
- Treatment with active charcoal
- Formation of formaldehyde disulfide

Formaldehyde recovery was tested by the authors, applying liquid-liquid extraction with 1-decanol. The results were published before [1-3].

The work presented in this communication includes studies carried out to remove formaldehyde from these effluents by oxidation alkaline medium and in acidic medium (Fenton process).

Oxidation of formaldehyde in alkaline medium

Formaldehyde oxidation in alkaline medium was carried out with H_2O_2 and sodium or potassium hydroxide according to the reaction expressed by the following chemical equation.

$$2\ HCHO_{(aq)} + H_2O_{2\ (aq)} + 2\ NaOH_{(aq)} \longrightarrow 2\ NaHCOO_{(aq)} + 2H_2O_{(aq)} + H_{2(g)}$$

The results of the experiments of oxidation of HCHO show that this oxidation is very quick, a few minutes are enough to oxidize all the HCHO, and does not depend on the nature of base, NaOH or KOH.

After the oxidation of HCHO, the resultant formate was acidified with concentrated sulphuric acid and then tried to recover by liquid-liquid extraction. Two extractants namely Cyanex923 and Alamine 336 with diluents Shellsol T and Exxsol D80 were tested. The system with tertiary amine, Alamine 336, diluted in a solution of 10% 1-decanol in Shellsol T allowed to concentrate about 120 g/L of formate using 3 M NaOH as stripping phase.

Oxidation of formaldehyde with Fenton reagent

In 1894, H.J.H. Fenton discovered that by using an iron catalyst and H_2O_2, a variety of organic molecules could be easily oxidized. In acidic, aqueous solutions, highly reactive hydroxyl radicals are generated by the catalytic decomposition of H_2O_2 with Fe^{2+}.

$$H_2O_2 + Fe^{2+} \longrightarrow Fe^{3+} + OH^- + {}^{\cdot}OH$$
$$OH + Fe^{2+} \longrightarrow OH^- + Fe^{3+}$$
$$OH + H_2O_2 \longrightarrow H_2O + HO_2{}^{\cdot}$$

Organic substrates are oxidized by the free hydroxyl radicals. pH, H_2O_2 concentration and catalyst concentration are important factors to control the reaction. Other authors studied the degradation of formaldehyde with Fenton process [4,5]. They obtained almost complete removal of total organic carbon (TOC) content in 4 h. However, for industrial purposes 4 h reaction time seems long enough so the aim of the present study was to try other conditions to degrade formaldehyde in less time.

Fenton process was investigated by varying some parameters at atmospheric pressure and 25 ºC like pH and amount of hydrogen peroxide. The removal of formaldehyde was very high in most cases. It was found that ratio H_2O_2:HCHO 7 provided 97% removal of TOC after 2 h and larger amounts of H_2O_2 were necessary to mineralize around 90% HCHO in 1 h. The combination of pressure and high temperature strongly increased the kinetics of the process. Concerning the removal of formaldehyde, almost 90-100% was achieved in all cases. This data indicates that formation of intermediates during the course of oxidation are more resistent to degradation than the parent compound. Moreover, the usual presence of methanol in the solution of formaldehyde also inhibits the complete mineralization.

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Removal of linoleic acid from ethanolic solutions with ion exchange resins

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Keywords: Ion Exchange Resin, Linoleic Acid, Langmuir Isotherm, Oil Refining, Equilibrium

Introduction

The cotton oil as others one, are sensible to the several conditions that these oils are submitted when employed the traditional refining. These oils can present great losses of neutral oil and in the nutritional value (Jawad *et al.*, 1983).

The interest in cotton oil is increase because it is rich in linoleic acid, an essential fatty acid. For these reasons several investigations are performed in order to develop alternative refining methods.

The oil deacidification by liquid-liquid extraction with an appropriate solvent is one of those alternative methods (Rodrigues, 2004). But the recovery of the solvent and the essential fatty acid from the extract phase was still not investigated.

So the possibility of using anion exchange resins in order to adsorb fatty acids from the extract phase, allowing the recovery of the solvent, and the essential fatty acid was investigated in the present work.

Materials And Methods

Material. Commercial grade linoleic acid, anhydrous and azeotropic ethanol, sodium hydroxide (Merck). Strong anionic resins Amberlyst A26 OH (Rohm and Haas).

Methods. Equilibrium experiments were conducted, with resin and ethanolic solutions with different linoleic acid initial concentrations, which were vigorously stirred, until equilibrium was achieved. At the end of this period, the linoleic acid equilibrium concentration was determined using titration.

The equilibrium concentration in the solid phase was calculated by mass balance. The influences of water content in ethanolic solutions were investigated.

The adsorption isotherms were represented by q* (g of oleic acid/g of dry resin) versus C* (g of oleic acid/g of solvent) and fitted by Langmuir model, the parameters qm (maximum content adsorved on solid phase (g acid/g dry resin)) and K_d (equilibrium constant (g acid/g solvent)) were obtained.

Details about methodology are presented in Cren and Meirelles, 2005.

Conclusion

Figure 1 presents the experimental equilibrium isotherms, and these datas fitted.

The shape of the isotherms sugests that the linoleic acid has a good affinity to resin.

The results shows that the variable investigated do not significantly influence the equilibrium behavior, so a unique set of parameters can be adjusted to the whole set of experimental data.

The parameters obtained were $q_m = 1.04$ and $K_d = 1x10^{-5}$, good correlation between experimental data and Langmuir model were obteined, 5.74 % of deviation. The small value of K_d, indicates that the adsorption rate constant is much higher than the desorption rate constant, expressing the preference of the acid for the resin phase.

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The q_m value indicates that the resin range value near the maximal capacity of adsorption.

The results obteined were coerent with others references (Cao *et.al.*,2002, Antonio de Lucas *et.al.*, 1997).

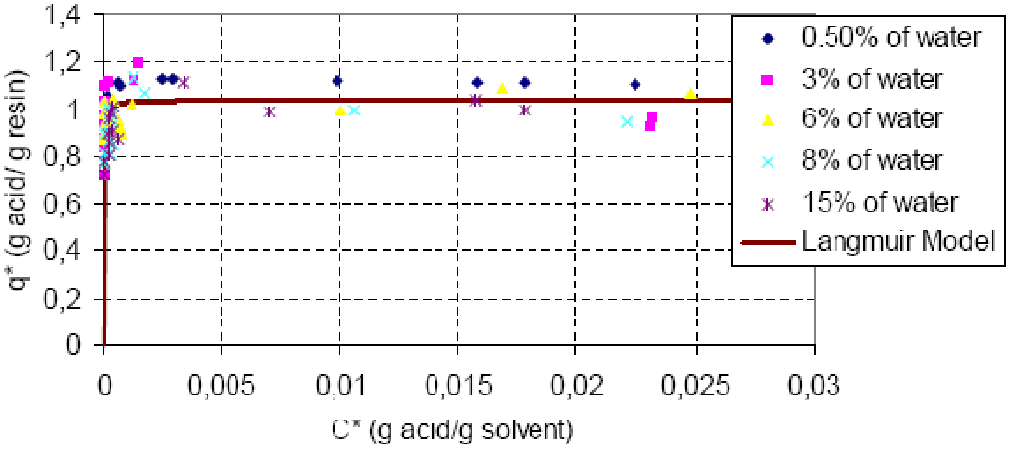

Figure 1 – Equilibrium Isotherms data with different content of water in the solvent (ethanol).

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Breakthrough curves for oleic acid adsorption in ion exchange resin

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Keywords: Ion Exchange Resin, Oleic Acid, Breakthrough Curves, Oil Refining

Introduction

Some vegetable oils presents in its composition substances with nutraceutical value or high free fatty acids content. These oils can present problems when being submitted the physical or chemical refining, which can cause great losses of neutral oil and in the nutritional value during the process (Mccaskill and Zhang,1999).

For these reasons several investigations are performed in order to develop alternative refining methods. The oil deacidification by liquid-liquid extraction with an appropriate solvent is one of those alternative methods. But the recovery of the solvent from the extract phase was still not investigated (Rodrigues *et. al*., 2004).

So the possibility of using anion exchange resins in order to adsorb fatty acids from the extract phase, allowing the recovery of the solvent, was investigated in the present work.

Materials And Methods

Material. Commercial grade oleic acid, isopropanol (Ecibra) and sodium hydroxide (Merck). Strong anionic resins Amberlyst A26 OH (Rohm and Haas).

Methods. In order to investigate the adsorption column dynamics, an experimental design (2^2) were used. Resin was put in contact (continuous system) at different temperatures with isopropanol solutions containing oleic acid. Samples were collected in column exit at different times. They were analyzed by titration to determine the oleic acid content, and the breakthrough curves were obtained.

The breakthrough curves were expressed by adimensional concentration (C/Co) versus time (minutes).

The results were analyzed by statistical study, the response surface methodology, to maximize the response efficiency of utilization (e_f), that correlates the capacity efficiently used by the resin column and the capacity used not efficiently (Sridhar *et al.,* 1994).

Conclusion

The breakthrough curves shows that at higher concentrations and temperature, the process of removal is faster, it occur because at high concentration more acid are available to ion exchange, and at high temperature the low viscosity contribute to the acessibility into resin sites (Ayoob *et. al*., 2007; Moraes and Machado,1999).

Figure 1 show the response surface, that indicate the influency of the parameters temperature and concentration of oleic acid (C_o) in the eficiency of utilization.

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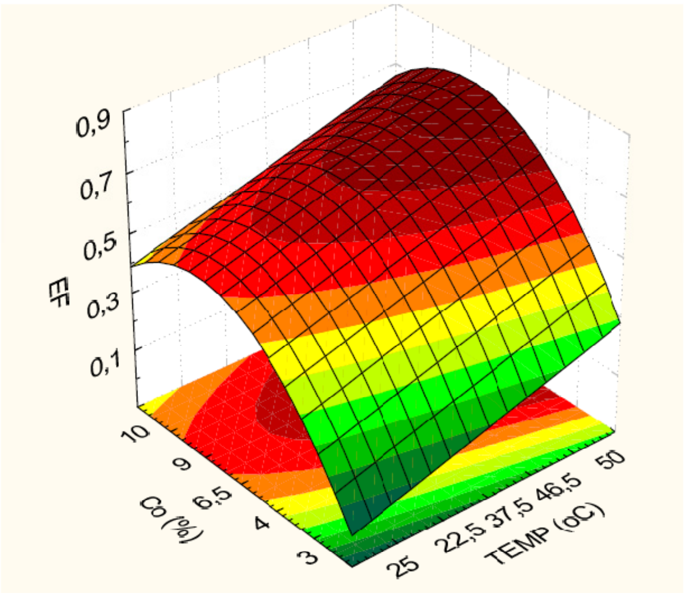

Figure 1 – Response Surface to the influency of temperature and concentration in the eficiency of utilization.

The response surface shows that the parameters optimized (temperature and concentration) had a positive influency in the eficiency of utilization, and range value around 66%. The observations mentioned above in the breakthrough curves were confirmed statistically, as higher are the temperature and concentration higher is the eficiency of utilization.

The ion exchange resin shows to have a good capacity of remove oleic acid from ethanolic solutions.

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Chromium removal by constructed wetlands: evaluating the effects of chromium rich industrial effluents

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Keywords: chromium removal, wastewater treatment, constructed wetlands

The escape of heavy metals, from anthropogenic activities, into the environment is of great concern due to its toxicological effects. Therefore, industrial metal rich effluents require special treatment before their discharge, which can be extremely expensive. Usually these wastewaters are joined to domestic waters, after some initial physicochemical treatment, resulting in the so-called urban wastewaters, which are further treated at municipal facilities. This practice may cause problems since municipal treatment plants are usually designed for domestic wastewaters.

Chromium has been selected for this study, as an example of this problem, with regard to its industrial use and potential pollution impact. Wastewaters from tannery, metal plating and many other industries contain high chromium concentrations, namely hexavalent chromium (Nriagu and Niober, 1988). Common treatment of industrial chromium rich effluents conducted at industrial plants consists mainly in physicochemical processes that transform Cr(VI) into Cr(III) with subsequent precipitation (Eary and Ray, 1988). However, these processes are not totally effective, since they often still leave high chromium levels in the water discharged.

The use of constructed wetlands (CW) for urban wastewater treatment is a rapidly growing practice, due to its economical and environmental advantages (Kadlec and Knight, 1996). CW specially dedicated to industrial wastewaters remediation has also been studied and implemented, with examples of application to heavy metals pollutants cited in the literature (Knight et al., 1999; Mant et al., 2006; Mitsch and Wise, 1998; Scholes et al., 1998; Vymazal, 2005). The effects of heavy metals in CW, used as secondary or tertiary treatment at municipal wastewater treatment plants dedicatedmainly to common urban waters, are less reported. In particular, it should be considered that both Cr(VI) and Cr(III) have been identified as plant toxics, in particular to *Phragmites australis*, species commonly used in CW (Mant et al., 2006; Shanker et al., 2005).

The aim of this work was to evaluate the effects of sporadic chromium discharges on the performance of CW normally operated for nutrients removal.

To simulate sporadic discharges of industrial chromium polluted effluents on the public frameworks using CW, small volumes of Cr(III) solutions (1 L 0,005 M $CrCl_3×6H_2O$) were injected in a pilot CW plant feed line. The CW pilot plant consists of two tanks with a working volume of 240 L, filled with lightweight aggregate (expanded clay). Tank A was planted with the emergent macrophyte *Phragmites australis*, tank B, without plants, serves for control purposes. The pilot plant has been operated continuously for more than six years, performing the treatment of a typical secondary effluent. Tank A nitrogen and 71% for COD. Tank B unplanted showed an average removal efficiency of 30%, 50% and 55%, respectively for total phosphorus, total nitrogen and COD.

After chromium injection, the tanks outflows have been monitored to pH, temperature and volumetric flow rate. The volumes collected were sampled and analysed for

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chromium contents. Mass balances calculations were performed to evaluate chromium removal. Figure 1 shows the chromium concentration at CW outflow over time.

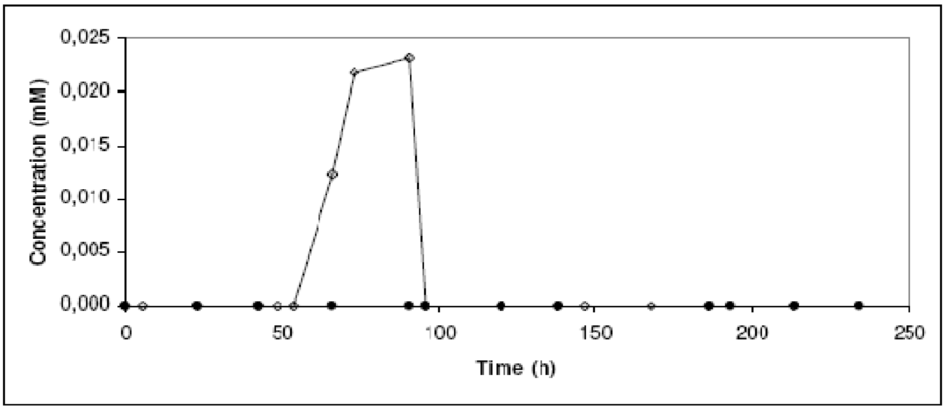

Figure 1 – Chromium concentration at pilot constructed wetlands outflow over time, after a pulse injection of a 5 mM Cr(III) solution (• tank A, planted; ◊ tank B, without plants).

Chromium removal by tank A, with plants, was verified to be 100%. Tank B, without plants, shows a removal capacity near 65%. Lab tests demonstrate an only residual chromium removal by the filter media itself. These results suggest a potential chromium depuration capacity of CW, mainly by action of the filter media microcosms, while plants uptake and adsorption to their roots and rhizomes contributes with 35% of chromium removal. Concerns may be taken about the end use of the filter media and the harvested plant materials. Under the conditions experimented, the CW didn't show any efficiency loss with respect to COD and nutrients removal function.

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Recovery of heavy metals by hybrid membrane processes

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Keywords: modified poly(phenylene oxide), ultrafiltration, dialysis, zinc, copper

Metallurgical industry, especially electroplating and metal finishing sectors, generates huge volumes of water containing heavy metal ions. For recovery and concentration attempts of these valuable components as well as for removal of undesired toxic ions from wastes applications of membrane separation processes are very useful. The membrane separation processes, consuming less energy and being more ecological than conventional separation techniques, are receiving growing interest in modern technologies. Traditional membrane processes, nanofiltration and dialysis are being replaced by such hybrid (non-conventional) processes as: polymer enhanced ultrafiltration (PEUF) [Cãnizares et al., 2002], micellar enhanced ultrafiltration (MEUF) [Juang et al., 2003], and polyelectrolyte enhanced dialysis (PED) [Marty et al., 2000].

PEUF and MEUF constitute a combination of two phenomena; binding of metal ions to a water soluble polymer (complexation) or solubilizing by surfactant micelles, respectively, and ultrafiltration. Since pore size of ultrafiltration membranes are not suitable to separate heavy metal ions, water soluble polymers are used to form macromolecular complexes with metal cations or anionic surfactant micelles are used to solubilize cations. Ultrafiltration membranes with pores smaller than both the polymeric agents and surfactants (above the critical micelle concentration, cmc) are employed; thus, the polymer-metal complexes or the micelle-solubilized metal can be retained while the simple (non-binding or non-complex) ions pass through the membrane.

During traditional dialysis ions move across nonporous ion-exchange membranes in accordance to the Donnan equilibrium principle. Dialysis is a diffusion phenomenon which is governed by the difference of metal concentration on the two sides of the membrane. The solute flux is stopped when the concentrations are equal in both compartments. The amount of metal crossing the membrane can be increased by modifying the chemical potential of the metal in one of the two compartments. If the complexing polymer is added in the receiving compartment, the interaction of the metal ions with polymer (polymer-metal complexes formation) should be responsible for increase of the amount of metal ions transferred. In the case of the stable complex formation, it should be possible to recover the total amount of metal ions in the receiving compartment [Poźniak, 2006]. In the PED there is used the porous membrane. The transport rate through porous membrane is significantly larger than the rate across solid membrane – that is the next benefit coming from the PED process application.

Poly(phenylene oxide) (PPO) is frequently used as a membrane material due to its good mechanical strength, thermo-, biological, and chemical stability, and film-forming properties. Hydrophobicity, the shortcoming of the PPO membrane, can be counterbalanced by introduction of ionic functional groups to the polymer chain. The porous ion exchange membranes form a new category of the filtration media. They proved to be very useful in the filtration of charge-bearing solutes [Bryjak et al., 2004; Poźniak et al., 2002, 2006].

The removal of metal ions, including Zn(II) and Cu(II), from aqueous solutions by: PEUF with polyethyleneimine (PEI) as water-soluble polymer, MEUF with sodium dodecyl sulfate (SDS) as anionic surfactant, and PED with poly(acrylic acid-co-maleinic acid) (poli(AA-co-MA)) as polyelectrolyte was investigated. Experiments were performed as a function of the kind of membrane: poly(phenylene oxide), sulfonated

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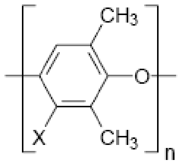
poly(phenylene oxide) (SPPO) and aminated poly(phenylene oxide) (APPO), molar concentration ratio of the water-soluble polymer to metal and pH value, and surfactant concentration.

For each evaluated membrane, the transport enhanced species was selected. The selection criterion was to keep the same charge on both bodies: the membrane and the species. For Zn(II) and Cu(II) cations recovery, APPO membrane with PEI was used in PEUF mode while SPPO membrane for MEUF mode with SDS as the surfactant.

Ionic groups were introduced to PPO (M_v=27,000, Aldrich Chemie) by sulfonation in a mixture of chlorosulfonic acid and chloroform, and by chloromethylation (mixture of methyl chloromethyl ether with $SnCl_4$) followed by amination using dimethylaminetanol. Membranes were formed by phase inversion method from polymer solution in mixture of chloroform and nonyl alcohol. Methanol was used as a coagulation medium.



$X = -SO_3^-$ or $-CH_2N^+(CH_3)_2C_2H_4OH$

In PEUF and MEUF, the stronger effect of Cu(II) interaction with PEI and better solubilization with SDS micelle were observed.

The ordinary dialysis of Cu(II) was also compared to the PED process. For the runs without a polyelectrolyte, the concentration of Cu(II) in the receiver is lower than 50%. Interaction of metal ions with poly(AA-co-MA) is mainly due to electrostatic forces and the formation of coordinating bonds. Hence, in the presence of polyelectrolyte, the metal ions concentration in the receiving solution increased to 100%.

The sulfonated and aminated poly(phenylene oxide) membranes worked more effectively that their off-charge analogue (the positive effect of ionogenic groups presence).

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Removal of dyes from the textile industry by adsorption in fixed bed columns – A sustainable process

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Key Words: Adsorption, Dyes, Textile Industry, Fixed bed, Activated Sludge.

The removal of dyes presented in industrial wastewater has received enormous attention in the last years. This is due to increasing of consciousness and most strictness on the environmental laws. The adsorption is one of the techniques that have been used with success for an effective removal of the color (Órfão et al., 2006). Although several techniques of wastewater treatment have been available, there is no simple process able to make an adequate mineralization of the colored effluents, mainly due to its complex nature (Jain et al., 2002). Then a final treatment of polishing is required, involving generally oxidative processes, with ozone, Fenton reagent, or Hydrogen peroxide + UV radiation; separation by membranes or adsorption. In the adsorption process, the activated carbon has been largely used, specially in fixed bed columns (Gulnaz et al., 2006; Kusvuran et al., 2005; Órfão et al., 2006). Due to the high cost of some conventional adsorbents, researches have been directed to the use of alternative adsorbents as ashes from the button, sludge, red mud, and others (Calvo et al., 2002; Jain et al., 2002; Netpradit et al., 2003). In this work it is investigated a new adsorbent from the sludge of process of textile wastewater treatment (Ulson de Souza et al., 2007). With the objective of determining the efficiency of the adsorbent in the removal of dyes, the adsorption isotherms were determined for the reactive dyes RR2 and RR 141, for different temperatures and salts concentrations. Defined the best experimental conditions of adsorption, through the parameter q_{max} (mg/g), obtained by the adjustment of Langmuir Isotherm for both of the dyes, the experiments were held in columns of adsorption in fixed bed, of dimensions 1.43 cm of diameter and 38 cm of height, being investigated the influence of the parameters in the removal process of mono and bi-functional reactive dyes. The initial concentration of dyes in the solution used in the experiments in columns was of 500 mg/l, and this was acidified with acid acetic 0,1M (pH 4,0). In the case of the the adsorbate RR141, 10% Sodium Chlorite (NaCl) (% in mass) was added in the solution. Breakthrough curves for the dyes RR2 and RR141 were obtained, varying the height of fixed bed from the columns with 15, 30 and 45cm of adsorbent and varying the feed flow of adsorbate in the column 8, 12 and 16 ml/min. It was also determined the capacity of adsorption in the equilibrium (saturation of column) for the different operation conditions listed previously. Due to the great capacity of adsorption of adsorbent, the breakthrough curves were obtained up to the adsorbate concentration in column outlet reached 80% of feed concentration (C/Co-0.8). The results obtained for the breakthrough curves showed that an increase in adsorbate feed flow generated a big volume of solution per unit of time through the column supplying, therefore, a minor time of operation for both the dyes studied, that means, the adsorbent will saturate more rapidly with the increase of the adsorbate feed flow. For the dye RR2, the time of saturation of the column when it operated with a height of fixed bed of 15cm and adsorbate feed flow of 16ml/min was only 55 min and for the adsorbate feed flow of 8 ml/min, the time was 3.1 times bigger. For the dye RR141 the time of saturation on the same conditions was of 160 min and

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320 min, respectively. When the column operated with a solution of dye RR2, the time was reduced, when compared to the operation time of columns with a solution of dye RR141, for the different adsorbate feed flow. The quantity of solute adsorbed in the equilibrium [q_e (mg/g)] obtained by adjustment of Langmuir Isotherm, for the dye RR2 was of 53.48mg/g and for the dye RR141, was of 78.74mg/g. For the breakthrough curves obtained in different height of bed, we can notice that an increase of the critical height of the bed imply in a saturation slower of the column due to the fact that this presents a bigger quantity of adsorbents, increasing this way, the operation time of the column. For a height of bed of 45cm and adsorbate feed flow of 12 ml/min, the time of saturation for the dye RR2 was 52,38% bigger, when compared to the height of fixed bed of 15cm and for the dye RR141. In these same conditions, the saturation time was 57,14% bigger when compared to the height of bed of 15cm. The capacity of adsorption in the equilibrium increased of height of fixed bed and decreased with the increase of adsorbate feed flow. The experimental results were compared with the data obtained by Guelli U. Souza et al. (2007), showing a great capacity of adsorption of the adsorbent in study. The adsorbent used in this work is an alternative very promising in the conception of an environmentally correct process, focusing the reduction of environmental impact of dyes from textile industries in the rivers.

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Activated carbon from coconut shells as catalyst in the CWAO of phenol in a trickle bed reactor

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Keywords: activated carbon, steam activation, catalytic oxidation, phenol

Activated carbon is a very well-known material widely used as adsorptive material for the elimination of organic compounds. It has also been used for supporting catalytic metals for dedicated processes, due to its thermal and mechanical stability. Depending on the raw material used for its manufacturing and the type of activation applied (physical or chemical), some characteristics of the activated carbon, such as surface chemistry and textural properties, could be adjusted to improve its performance as adsorbent or as catalyst support. Lillo-Ródenas et al (2005), for example, applied activated carbons, made from Spanish anthracite through chemical and physical activation, for the adsorption of benzene and toluene. The carbons obtained had different porosity and surface chemistry, and the tests demonstrated that those with low content in oxygen surface groups have the best adsorption capacity. On the other hand, modification of manufactured activated carbons can be conducted in order to change the performance of these materials. Dastgheib et al (2004) modified two commercial activated carbons by either acid wash, heat treatment under inert atmosphere, anhydrous ammonia treatment, or iron impregnation. All samples were tested in the adsorption of dissolved organic matter (DOM). In summary, acid wash dramatically decreased the adsorption capacity due to significant increases in the carbon surface acidity. Heat treatment, ammonia treatment of oxidised carbons and iron impregnation increased the DOM adsorption. These latter treatments increased the carbon basicity and the accessible surface area, as well as they promoted a favourable chemistry, which resulted in an improvement of the activated carbon performance.

Another interesting and yet not widely explored application of the activated carbon is as catalyst by itself. Fortuny et al (1998) and Tukac et al (1998) demonstrated that some activated carbons possess catalytic activity towards the oxidation of phenol both in batch and in continuous reactors. However, exact parameters responsible for this catalytic behavior has not been yet fully identified. According to some research studies, carbonyl type oxygen surface groups could be responsible for the catalytic activity shown by activated carbon in some reactions, for instance in the oxidative dehydrogenation of ethyl benzene (Pereira et al, 1999). On the other hand, an increase in the amount of acidic surface functionalities after nitric acid wash demonstrated to decrease the catalytic activity shown by a commercial activated carbon in the catalytic wet air oxidation (CWAO) of phenol (Santiago et al., 2005).

The aim of this research work is fully characterising a coal-based commercial activated carbon (ME, Merck ref. 2514) with proven catalytic properties in the CWAO of phenol, and subsequent manufacturing of an activated carbon tailored to have similar characteristics, so that it is to be expected to give the same catalytic activity. Coconut shells were used as raw material for the preparation of the activated carbon by physical activation using steam (MCS). In order to increase the metal content (specifically iron) of this carbon, iron was added during the activation procedure (MCSFe). All samples were characterised by determining its typical physico-chemical characteristics. Surface area and porosity of the activated carbons were estimated from nitrogen isotherms at 77 K. Surface area was determined from BET equation, total pore volume from the near saturation nitrogen uptake at the relative pressure of 0.98, and micropore volume from the Dubinin-Radushkevich equation. Volume of meso- plus macropores was obtained from subtracting the micropore volume from the total pore volume. Mass titration method was used to determine the point of zero charge of each sample. Boehm titration was carried out to determine the content and the distribution of the acidic surface functional groups. Thermogravimetric analyses were done to evaluate the weight change under heat treatment. Samples were heated from 100 to 900ºC under nitrogen flow at a heating rate of 10ºC/min. For measuring the iron concentration, samples were digested in concentrated nitric acid by a microwave digestion equipment. Dilute solutions were later analysed by AAS. The performance of samples in phenol adsorption were evaluated at 20ºC (± 2ºC) in oxic conditions. Specifically, solutions with phenol concentrations ranging from 0.5 to 7 g/l were left in contact with 0.25 g of adsorbent. The catalytic performance was assessed for the CWAO of phenol in a trickle bed reactor in downflow co-current. The description of the equipment and experimental procedure has been described elsewhere (Suarez-Ojeda, et al, 2005).

The characterisation of both coconut-based activated carbons, MCS and MCSFe, shows that surface area and pore distribution are comparable to those from the commercial ME. However their pH is slightly more acidic although they have similar oxygen content and distribution of functional groups. Regarding to phenol adsorption, ME and MCS give comparable capacity whilst MCSFe shows a lower adsorption due to the acidity imparted by the presence of iron. Finally, the catalytic performance of the iron-containing activated carbons seems to be related to the position of this iron in the activated carbon structure.

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Use of chemical modification to determine the binding of Cd(II), Zn(II) and Cr(III) ions by orange waste

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Keywords: orange waste, functional groups, sorption, FTIR and ^{13}C-NMR

Fruit waste materials are tipically generated in large quantities by the fruit juice industry. In the last years these materials have receive attention, because of their high quantity of pectin which contains carboxylate groups, for the removal of heavy metals.

Even though orange waste (OW) has been recently used as adsorbent in many studies, the biomass–metal binding mechanisms are not well understood. In order to investigate the adsorption mechanism, chemical modifications to OW were performed to indirectly determine the ligands on the biomass responsible for metal binding.

OW used as adsorbent was obtained from orange juice industry. The material was cut into small pieces, was extensively washed with tap water and oven-dried at 50-60 ºC until constant weight. Then the material was crushed and sieved to obtain a particle size lower than 1.5 mm. Three different treatments (Kapoor and Viraraghavan, 1997) were performed on OW within this study. ***Chemical esterification*** (treatment with methanol and hydrochloric acid at 60 ºC) was used to reduce the number of carboxyl groups, which are potential heavy metal binding sites on the biomass. ***Chemical methylation*** (treatment with formaldehyde and formic acid) was performed to block the amino groups on the biomass. And finally, ***acetylation*** of amino and hydroxyl groups of the biosorbent (treatment with acetic acid at 80 ºC) were carried out to study the effect of these functional groups in metal biosorption by OW. FTIR and ^{13}C-NMR were used to analyze the functional groups in the raw as well as chemically modified OW.

The FTIR spectra of OW exhibits a broad peak between 3200 and 3600 cm^{-1}, indicative of the existence of macromolecular assotiation (cellulose, pectin, etc.) and might be the indication of both amine and bonded OH groups. The finger print region of 1000-1200 cm^{-1} exhibits the characteristic peaks of cellulose. Bands around 1636 cm^{-1} and 1736 cm^{-1} are indicative of free and esterified carboxyl groups, respectively, which may be useful in identifying pectins. Comparing with the FTIR of OW, the streatching of the peaks around 1636 cm^{-1} in esterified OW is associated with a modification in carboxyl groups on cellulose or pectin chains. In acetylated and methylated OW, the peak observed around 3422 cm^{-1} has been changed in intensity and also in broad, suggesting significant changes in the amino groups present in the biomass and, in the case of acetylated OW, the change could be also due to hydroxyl groups.

In ^{13}C-NMR spectrum of OW, the peaks observed are similar to those obtained for other authors for this type of material (Javis et al., 1996) The characteristic bands at 105 ppm (C-1), 84 ppm (C-4), 72-75 ppm (C-2, C-3 and C-5) and 65 ppm (C-6) are assigned to cellulose. Related to hemicellulose signal at 101 ppm (C-1), 89 ppm (C-4) and 62 ppm (C-6) are observed and peaks at 101 ppm (C-1), 80 ppm (C-4) and 71 ppm (C-2, C-3) are attributed to pectins. Besides these peaks, signals attributed to carboxyl groups in their ionized (177 ppm) and esterified (165 ppm) form, methoxyl carbon (56 ppm), methylene carbon (33 ppm), acetyl carbon (23 ppm) and methyl carbon (19 ppm) were also obtained. After esterification treatment signal at 177 ppm disappears, and contrary to that we expected, the band at 165 ppm is considerably

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reduced. This unexpected result could be explained by the pectins extraction, and consequently the disappearance of carboxylic groups present in them, with this treatment. In fact, several authors have studied the extraction of pectin by heating in acid conditions (May, 1990). An increase in peaks at 38 ppm and 23 ppm was obtained with the methylation and acetylation treatment, respectivately. This fact is indicative of the presence of amine and hydroxyl groups.

Thus, correlating the data obtained from FTIR and ^{13}C-NMR spectra it could be concluded that carboxyl, amine and hydroxyl groups are present in OW and they have been modified chemically. So, all these groups might be responsible of metal uptake.

Batch sorption assays were carried out to indirectly determine the effect of several functional groups in biosorption. 0.2 g of OW were added to glass flasks containing 50 mL solution of metal ion of the desired concentration (0 – 300 mg/L). The mixture was stirred magnetically for a pre-determined time and the pH of solutions (pH = 4) was adjusted by adding dilute solutions of HNO_3 and NH_4OH. The biosorbent were then removed by filtration through glass fibre prefilters (Millipore AP40) and the filtrates were analysed for residual metal concentration by atomic absorption spectrophotometry (Perkin Elmer model AA300). The metal percentage removal and the OW sorption capacity were calculated in each case.

The removal of heavy metals by raw biomass and by modified biomass are compared in Figure 1. Biosorption of cadmium, zinc and chromium was almost inhibited with the esterification treatment, when pectins were extracted from raw materials and carboxyled groups were esterified, thus indicating the important role played by pectins and carboxyl groups in the biosorption of heavy metals onto OW. The reductions in percentage removal of metals with the esterification treatment, in comparison with OW, were higher than 79%, 66% and 82% for Cd, Zn and Cr, respectively. No significant modification in biosorption were observed in acetylated and methylated OW, so these functional groups do not seems to be involved in metal uptake by OW.

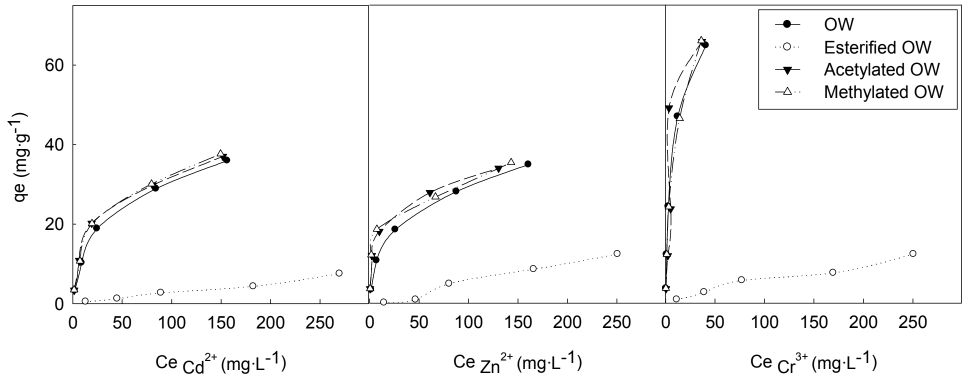

Figure 1. Effect of chemical modification of OW on Cd, Zn and Cr biosorption.

These results may be sufficient to indicate the important participation of pectins, and the carboxyl groups present in them, in the biosorption of metal ions by orange waste.

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Comparative mercury removal by ETS-4 and activated carbon from low contaminated solutions

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Keywords: Mercury, Removal, ETS-4, Activated carbon

Considered by EPA as a priority pollutant, mercury (Hg) is one of the most hazardous heavy metals present in the environment. Due to its toxic effects on aquatic life, Human beings and environment, increasing attention has been given to the removal of Hg from effluents, in such a way to satisfy the strict environmental regulations.

Microporous titanosilicates are found to be very promising materials for decontamination of mercury from aqueous solutions (Lopes et al., 2007), but are they good enough to compete with adsorbents largely used in purification processes such as commercial activated carbons?

In order to evaluate if ETS-4 titanosilicate has a higher efficiency on Hg (II) removal from solutions with low metal levels than activated carbons, batch stirred sorption experiments were isothermally (21±1ºC) carried out. Each batch experiment was performed using 8 mg of material (ETS-4 or activated carbon *Chemviron Pulsorb FG4* type) and 2 L of 50 µg L^{-1} Hg(II) solution.

The concentrations of mercury in solution along time were measured and are shown in Figure 1 together with the corresponding amounts of mercury sorbed onto each material, calculated by a mass balance.

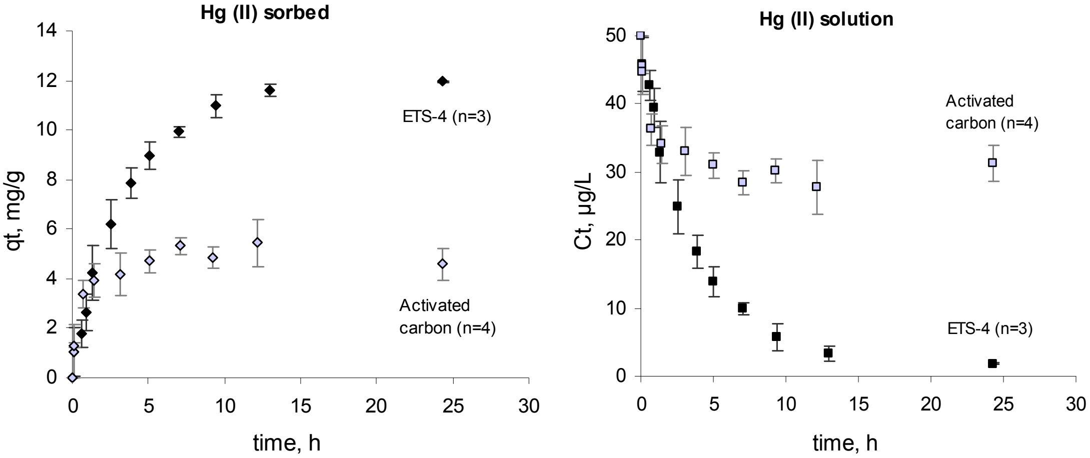

Figure 1: Mercury concentration in solution (Ct) and sorbed (qt) on ETS-4 (black symbols) or on activated carbon (grey symbols) as a function of time.

When using ETS-4 and activated carbon to remove Hg (II), the metal concentration in solution decreases with time until equilibrium, which, as it may be seen, is attained within 24 hours for both adsorbents. However, it is clear that both materials have distinct ability to remove Hg (II). The amount of Hg (II) sorbed onto ETS-4 at equilibrium is almost three times larger than onto activated carbon (Table 1).

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Table 1: Hg (II) concentration in solution (C_e, µg/L), experimental sorption capacity (q_e, mg/g) and removal percentage at the equilibrium.

Material	C_e, µg/L	q_e, mg/g	% removal
ETS-4	1,79	12,0	96,4
Activated carbon	31,4	4,60	37,2

In order to compare the kinetic performance of both materials, data fittings to the pseudo first-order Lagergren and the pseudo second order kinetic equations, which are commonly used in this kind of studies (Ho and McKay, 1999; Reddad et al., 2002; Zhang et al., 2005), were found.

For ETS-4 the correlation coefficients obtained for the two models are equal (Table 2), indicating that both of them describe the experimental kinetic results of the Hg (II) removal. However, there is a better agreement between the experimental q_e and the value given by the first-order kinetic model than by the second-order one. For activated carbon, the sorption kinetic is better described by the second-order model, supported by the correlation coefficient and by the comparison between the experimental and fitted q_e values.

Table 2: Kinetic sorption rate constants, k_1 (pseudo first-order Lagergren) and k_2 (pseudo second-order), together with experimental and calculated q_e, and the R-squared (R^2) corresponding to the fittings.

		First-order kinetics			Second-order kinetics		
	q_e-exp ($mg\ g^{-1}$)	k_1 (h^{-1})	R^2	q_e-fitted ($mg\ g^{-1}$)	k_2 ($g\ mg^{-1}\ h^{-1}$)	R^2	q_e-fitted ($mg\ g^{-1}$)
ETS-4	11.95	0.260	0.997	11.48	0.03	0.997	13.51
Activated carbon	4.60	0.772	0.858	3.22	5.46	0.992	4.78

According with the results obtained in this study, although both materials have ability to remove Hg (II) from solutions with low metal levels, it may be concluded that ETS-4 titanosilicate is much more efficient than activated carbon.

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Selection of indigenous acidophilic bacteria for the bioleaching of two SOMINCOR concentrates

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Keywords: bioleaching, acidophiles, chalcopyrite, lead concentrates

Biometallurgy, an interdisciplinary field involving geomicrobiology, microbial ecology, microbial biochemistry, and hydrometallurgy, is a novel promising technology for recovering valuable metals from industrial waste materials and for detoxifying these materials for environmentally safe deposition. Biometallurgy can be defined as the field of applications resulting for the control of natural (biochemical) processes of interactions between microbes and minerals to recover valuable metals (Morin et al., 2006).

Bioleaching exploits the ability of microbes that thrive in high acid environments, require inorganic food and energy sources and frequently display resistance to heavy metals. These unique chemolithoautotrophs are generally employed to leach sulphide ores such as chalcopyrite, an abundant but refractory copper sulphide mineral.

This work concerns to the screening of the bioleaching potential of a indigenous microbial consortium (Achada Sludge – LA), collected at the abanboned S. Domingos copper mine, for using on metals bioleaching and recovery of two different mineral concentrates: lead-zinc concentrates (Pb-Zn concentrates) and rich copper concentrates (chalcopyrite), supplied by Somincor (Sociedade Mineira de Neves-Corvo SA, Portugal).

The screening of iron-oxidising acidophiles, which are known to have a key role in the bio-oxidation of the sulphide minerals regenerating the oxidant ferric iron, was made by enrichment techniques (Johnson, 1995) at 35 ºC, pH $\approx$ 2.0 and 150 rpm, with different concentrations of substrate (lead concentrates or chalcopyrite).

The best bioleaching results for Pb-Zn concentrates by Achada Sludge were obtained for 2% substrate, with a % metals recovery of 44% Fe, 22% Cu and 100% Zn after 2 weeks of enrichment, and reaching a recovery of 65% Fe, 82% Cu and 100% Zn after 2 months of enrichment. In the abiotic controls the % metals recovery ranged from 0% (Cu, Fe) to 10% (Zn) after 2 months. For copper concentrates, the bioleaching results in % metals recovery for 2% chalcopyrite were 41% Fe, 30% Cu and 75% Zn after 2 weeks of enrichment, reaching a recovery of 77% Fe, 76% Cu and 100% Zn after 1 month of enrichment. In the abiotic controls with chalcopyrite, the % metals recovery was 3% to Fe, 14% to Cu and 62% to Zn after 1 month.

LA consortium presented an high bioleaching potential for metal-recovery from both mineral concentrates tested. Biometallurgy processes applied for Cu-recovery in copper concentrates (chalcopyrite) and for Zn-recovery in Pb-Zn concentrates can be an interesting tool to improve the added value of products. In the case of Cu concentrate this process would allow the implementation of a copper bio-hydrometallurgy to produce copper cathodes instead of selling a concentrate. For the Pb-Zn concentrates, this type of treatment could improve the economic interest of products, which constitutes a residual stream.

Furthermore, the three LA consortia, the original LA and the enriched samples either with Pb-Zn concentrates or chalcopyrite, are being used to assess the effect of combined contamination of heavy metals on soil bacterial communities using genetic community fingerprinting by 16S rDNA profiles and heavy-metal resistant genomes.

Acknowledgements

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Kinetic constants of phenolic compounds oxidation by Fenton's reagent

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Keywords: Fenton reagent, kinetic constants, phenolic compounds, AOP

Pulp and paper industries, wine production, distillery industries, olive-oil extraction and table olive production are examples of industries and agro-industries that produce highly contaminant wastewaters. These wastewaters are characterized for being rich in organic substances (phenols, polyphenols, organic acids), high COD values and a remarkable recalcitrant character due to the presence of phenolic compounds.

As result of this situation and the more restrictive legislation concerning effluents releases into rivers and streams, new technologies have been developed to reduce these refractory contaminants. For example, Fenton's reagent oxidation is a homogeneous catalytic oxidation process based on the generation of very reactive oxidising free radicals, especially hydroxyl radicals, through the combination of hydrogen peroxide and ferrous ions (Parsons, 2004).

Hence, the main objective of this study is to evaluate the oxidation of different phenolic compounds by Fenton reagent and to calculate the kinetic constants of 10 phenolic compounds normally present in these industrial wastewaters. The 10 phenolic compounds were: *p*-coumaric acid, caffeic acid, veratric acid, ferulic acid, protocatechuic acid, vanillic acid, syringic acid, β-resorcylic acid, 3-(4-hydroxyphenyl)-propionic acid and 3,4,5-trimethoxybenzoic acid.

A series of experiments were developed to determine the rate constants for the reaction between each individual phenolic compound and hydroxyl radicals generated in Fenton process. This determination could be performed under certain specific conditions, by means of a competitive kinetic model. In this case, was used a mixture of a reference compound (R), *p*-hydroxybenzoic acid, and another phenolic compound (B). A first-order reaction with respect to the radical concentration and the phenolic compound concentration was used in our model.

$$\ln\frac{[B]_0}{[B]} = \frac{k_B}{k_R}\ln\frac{[R]_0}{[R]}$$

In this expression k_R is the rate constant for the reference compound, i.e., p-hydroxybenzoic acid with $k_R = 2.19 \times 10^{-9}$ L/(mol.s) at 30ºC and k_B is the rate constant for each one of the phenolic compounds. A plot of $\ln([B]_0/[B])$ versus $\ln([R]_0/[R])$ yields a straight line, whose slope is the ratio of rate constants k_B/k_R, as showed as an example in Figure 1.

After regression analysis, the slope k_B/k_R for each compound was deduced using the known k_R and the k_B rate constants determined for the remaining phenolic compounds The values are exposed in Table 1.

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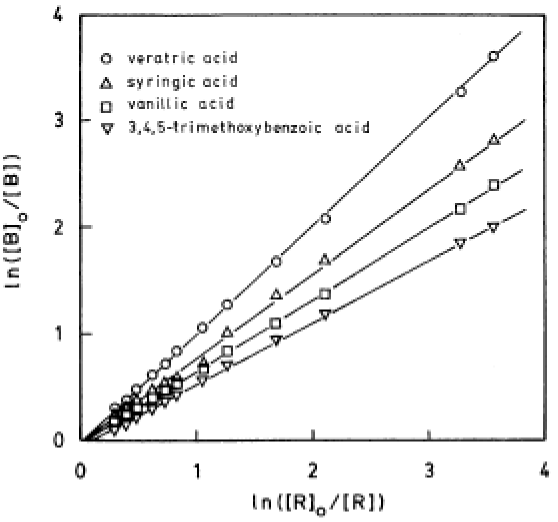

Figure 1 - Plot of ln([B]$_0$/[B]) versus ln([R]$_0$/[R]) for some phenolic compounds

Table 1 - k_B/k_R and k_B values for each phenolic compound

Phenolic compound	k_B/k_R	$k_B \times 10^{-9}$ (L/mol.s)
ferulic acid	1.754	3.84
3-(4-hydroxyphenyl)-propionic acid	1.172	2.57
p-coumaric acid	1.155	2.53
veratric acid	1.011	2.21
p-hydroxybenzoic acid	1.000	2.19
β-resorcylic acid	0.991	2.17
caffeic acid	0.821	1.80
syringic acid	0.791	1.73
vanillic acid	0.672	1.47
3,4,5-trimethoxybenzoic acid.	0.567	1.24
protocatechuic acid	0.307	0.67

These values are concordant with the deduced values for hydroxyl radical rate constants in aqueous solution for other similar compounds in an order of 10^9 L/(mol.s) (Abad *et al.*, 2007).

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Study of the behaviour of the azeotropic mixture ethanol + water + 1-butyl-3-methylimidazolium methylsulfate

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Keywords: VLE, ternary system, 1-butyl-3-methylimidazolium methylsulfate, correlation, azeotrope.

Ionic liquids (IL) are substances formed by ions and they show a negligibly vapour pressure at normal temperature and pressure conditions. This and other properties, such as their relatively low viscosities, their thermal stability and their capability as solvents, make the ionic liquids a new alternative for different processes. They can be applied as replacement for conventional toxic, flammable and volatile organic solvents. The use of IL in separation processes is one of the multiples alternatives.

The prediction of the ideal IL for each separation process is nowadays impossible, since there is not enough information about the influence of the structure of IL in its physical and solvent properties. We have to take into account that there are many possible ionic liquids by combination of ions (Holbrey and Seddon). Besides, experimental phase equilibrium data is required for developing thermodynamic models and for understanding their thermodynamic behaviour.VLE data permit checking the potential of g^E-models which are applied for the description of the real behaviour of systems with ionic liquids.

In this work, we study the possibility of separating the azeotropic mixture ethanol-water ($w_{1(az)} \approx 0.96$) using an ionic liquid, the 1-butyll-3-methylimidazolium methylsulfate ([Bmim][$MeSO_4$]). For this purpose, experimental data were determined and correlated.

Experimental data of isobaric vapour-liquid equilibria for the ternary system ethanol + water + [Bmim][$MeSO_4$] was carried out at 101.3 kPa. Experimental data of ternary system was correlated using the NRTL equation (Renon and Prausnitz,1999). With the parameters obtained in the correlation, we can study the behaviour of the azeotropic mixture ethanol-water with different amounts of different ionic liquids.

Apparatus and procedure.

A glass Fischer Labodest apparatus model 602/D was used in equilibrium determinations. The equilibrium vessel is a dynamic recirculating still and it is equipped with a Cottrell pump. A thermometer Yokogawa model 7563, with an accuracy of ± 0.01K was used to measure the equilibrium temperature. For the pressure measurement, a digital pressure controller Ruska model 7218 with an accuracy of 0.001 kPa was used.

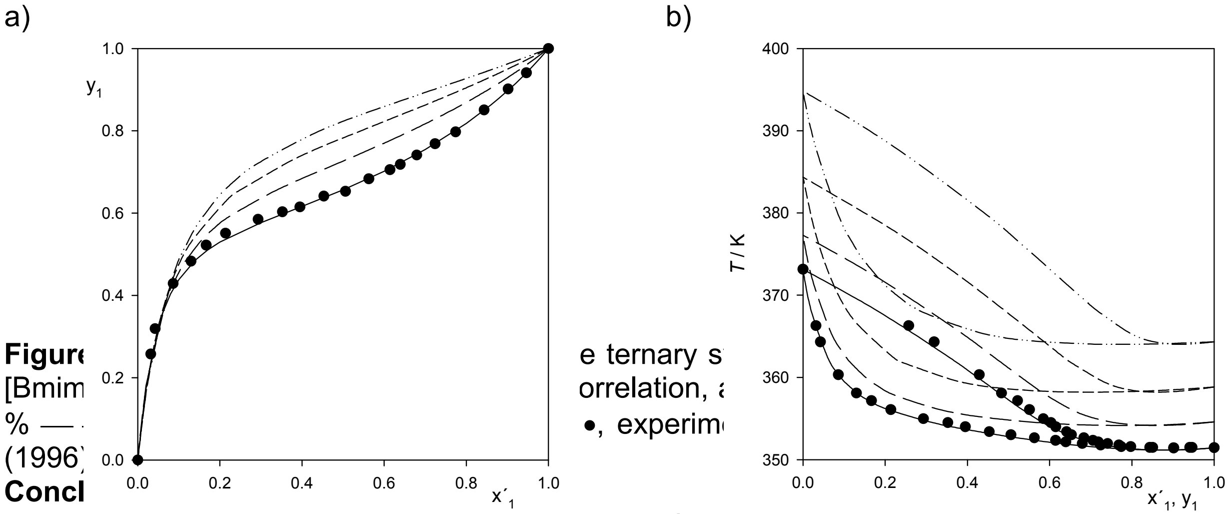
The densities and speeds of sound of pure liquids and mixtures were measured with a densimeter Anton Paar DSA-5000, with a precision of $\pm 2 \cdot 10^{-6}$ $g \cdot cm^{-3}$. To measure refractive indices, an automatic refractometer Abbemat-HP Dr. Kernchen with a precision of $\pm 4 \cdot 10^{-5}$ was used.

Results and discussion

To correlate the experimental phase equilibrium data of the ternary system, the NRTL equation (Renon and Prausnitz, 1968) was used, treating the ionic liquids like no dissociating components and assuming ideal behaviour of the vapour phase. In this case, the objective function to minimize is defined by:

$$\mathrm{O.F.} = \sum_{i=1}^{np} (T^{exp} - T^{calc}) \qquad (1)$$

With the obtained parameters, we can predict the behaviour of the ternary system depending on the quantity of ionic liquid present in the mixture. In Figure 1 the predicted VLE containing the azeotropic system EtOH-H_2O and the ionic liquid [Bmim][$MeSO_4$] are presented in a pseudobinary way. In these figures, the liquid phase composition of the low-boiling component is the amount of this substance in the volatile part of the liquid phase. The ionic liquid concentration is stated for each curve separately. As it can be observed in these figures, the addition of [Bmim][$MeSO_4$] to this binary azeotropic mixture leads to a noticeable decrease in the molar vapour fraction of ethanol.



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Concl

Ionic liquids are becoming a new alternative for separation processes. The studied IL [Bmim][$MeSO_4$] is capable of breaking the binary azeotrope EtOH-H_2O, opening a new possibility as entrainer for this system. It is noteworthy that, because of the nonvolatility of the IL, this can be regenerated by stripping, evaporation or drying.

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Model-based optimization of a semi-batch industrial polymerization process

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Keywords: vinyl acetate, polymerization, modeling, control

The research work presented in this paper considers the polymerization plant in MITOL chemical factory in Sežana, Slovenia. This is the leading glue manufacturer in the country with production rate more than 90% of its capacity. One of the challenges now is to increase the production rate, while keeping the quality parameters at the desired values. A possible approach to reach this goal is by applying better control strategies that will decrease the batch time. The underlying research is aimed at the design of the process model and its application in the search for more optimal plant operation.

The process considered is a polymerization of vinyl acetate by using potassium persulfate (KPS) as initiator and polyvinyl alcohol as a protective colloid. The production process is semi-batch with the monomer added continuously during the batch. The current batch control is done by following a recipe derived from experience and that fact also takes our attention. The most influential factor attributing to reaction rate is the temperature in the reactor that should be kept within certain limits. The temperature is controlled by manually adding initiator every time the temperature starts to decrease.

We are facing the challenge to design optimal control actions required to reach the final product with specified quality parameters in as short time as possible. For this purpose, a process model consisting of differential and algebraic equations (DAE) has been already constructed (Aller et al., 2007). The model predicts four outputs, i.e. conversion, particle size diameter, solids content and viscosity. While this model can take over the role of a soft sensor in on-line monitoring and control, it is unable to predict the temperature changes in the reactor from the change of operating conditions. To improve the predictive capability, the model was extended with additional differential equations that estimate the reactor temperature profile as a function of the concentrations of reacting chemicals. This paper presents the extended model using the modeling from first principles (energy balance). The energy balance is based on reaction heat capacity, and the energy produced and consumed during the reaction. The latter includes the following:

- heating of the reactor though the heating jacket,
- producing the heat in the exothermic reaction,
- heating the incoming monomer to the reactor temperature,
- cooling of the reactor by a reflux though a condenser,
- heat losses to the surrounding.

The so obtained model was evaluated on 5 industrial batches (Fig. 1, Table 1), for which the process outputs were measured purposely at the end of each batch. In addition, the model was validated statistically on a larger set of industrial batches where output quality parameters are measured after mixing several batches in a storage tank.

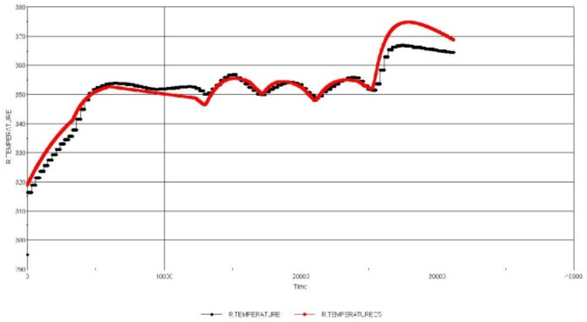

Figure 1 *Real plant temperature profile and estimated temperature profile*

Table 1 *Output variables – real data and estimated data*

Batch N	Solid Content		Viscosity		Conversion	
	Estimated	Data Mitol	Estimated	Data Mitol	Estimated	Data Mitol
1192	44.6	46.4	33888	37520	93.93	99.95
1203	43.9	45.9	32617	31200	92.18	99.90
1214	36	45.4	26180	25040	75.00	99.89
1253	42.5	46.9	31781	34000	88.48	99.13
1256	42.5	46.4	31754	30160	89.34	99.37

Having the temperature profile we can apply control strategies to observe the changes in the profile (and consequently the process outputs) by supplementing different amounts of monomer and initiator to decrease the batch time and to increase the production rate. In the paper, the model is used to analyze the effect of tighter temperature control on the shortening of batch time. Simulation results, derived in gPROMS environment, are presented.

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Reacting media models building optimization

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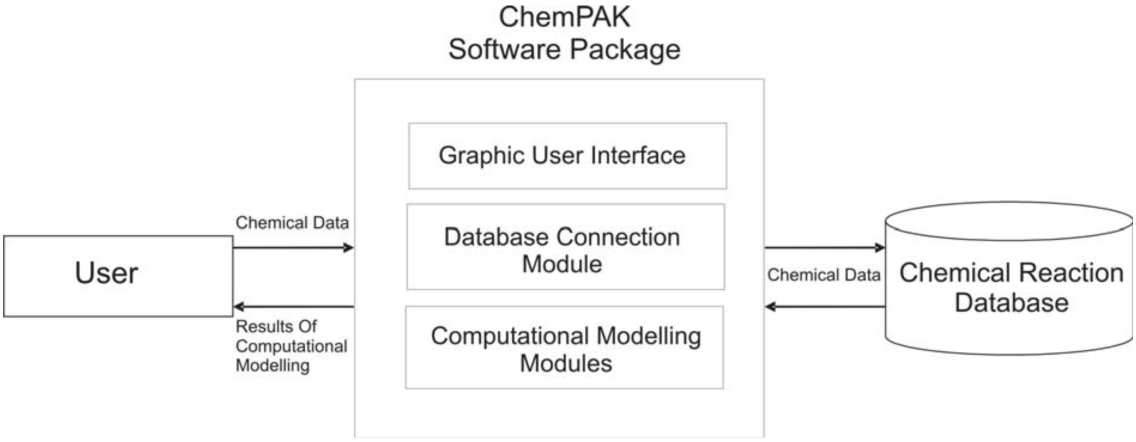
Keywords: Chemical Kinetics, Catalysis, Reaction Engineering, CFD & chemical engineering

The optimization of kinetic scheme of chemical process is the important stage of reacting media models building. This iterative method consists from the next steps: proposition of scheme from theory, translation scheme to the system of ordinary differential equation (ODE), finding solution of this system, comparison results with experimental data, modification of kinetics scheme.

ChemPAK software package was created for automation of this process. ChemPAK consists of network database, computational modules and visual interface. User can create and edit systems of chemical equations with unlimited number of equations with easy-to-use interface, translate systems of chemical equations to systems of ODE, add some new equations to the translated system and solve this system by using one of ChemPAK computational modules.



The problem of modeling kinetics of Butlerov reaction in circumstellar disk was solved with using of ChemPAK. Butlerov reaction consists of 44 reversible chemical equations.

Also the problem of homogenous pyrolysis of C2-C3 hydrocarbons under CO2-laser-induced heating was solved. Reaction scheme for this task consists of more than 100 chemical equations.

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Multiobjective optimization of the benzene nitration process

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Keywords: Multiobjective Optimization, Goal Attainment Method, Multivariate Analysis, Benzene Nitration

Benzene nitration with mixed acid is a liquid-liquid reaction where the degree of conversion to mononitrobenzene (MNB) and selectivity, depend on a wide range of parameters. Quadros (2004, 2005) studied this system in a pilot plant under operating conditions in the range of those used in the industrial process. The experimental data thus collected are here used to establish the trade-off between two conflicting objectives: to maximize both production of MNB simultaneously minimize the production of by-products, dinitrophenol (DNP) and trinitrophenol (TNP), consequently reducing the environmental footprint of the process. Quadros (2005) confirmed that, if inlet conditions are selected taking into consideration only the maximization of MNB production, the concentration of both DNP and TNP will also increase. So, to improve the selectivity of the process, a compromise must be reached.

Searching for these “optimum noninferior solutions”, or Pareto optimum solutions (Censor, 1977), involves formulating a Multiobjective Optimization Problem (MOOP) and selecting a method to solve it. Generating the MOOP requires not only objectives definition but also the establishment of an accurate region of feasible solutions for the optimum search (Lim, 1999). The constraints used are the predictive models for the outlet concentrations of MNB, DNP and TNP as well as variables constraints. Such constraints were introduced in order to prevent convergence to low probability or even impossible variable combinations.

The reaction mechanisms involved in the formation of nitrophenols are complex, and physical and chemical data required for the development of mechanistic models are not available. Therefore, a statistical multivariate analysis approach was adopted to develop data-driven empirical models to predict the formation of these compounds. The variables selected as potential regressors were: the temperature in the reactor (***T***), the ratio of inlet molar flow rates of benzene and nitric acid ($\boldsymbol{F_B/F_N}$), the nitric (***NA***) and sulfuric (***SA***) acids inlet weight fractions, the interfacial area (***a***) and the residence time ($\boldsymbol{\theta}$). The regressors selection was driven by the aim of developing models valid over a wide range of MNB production. The multivariate linear regression (MLR) methodology (Chatterjee, 1991) with the forward stepwise variable selection method, implemented with the Statistica® software, was used to obtain the models. The procedure followed is described in Quadros (2004, 2005).

Two of these variables, ***a*** and ***T***, are not process inputs and can not be set independently from the others. Nevertheless, it is known (Quadros, 2005) that ***a*** is related to the ratio of organic to acid phases, here represented by $\boldsymbol{F_B/F_N}$, and to the physical properties of the liquid-liquid mixture which, in turn, are sensitive to ***T***. By checking graphical representations of these variables, a relation between ***a***, $\boldsymbol{F_B/F_N}$ and ***T*** was established using a Mahalanobis distance (MD) approach (Chatterjee, 1991), which is essentially a covariance weighted distance to the centroid calculated from experimental data. Points with MD less than a threshold belong to an ellipsoid region, whose axis ratio depends on the covariance matrix. Such threshold was set with resource to the Hotelling's T^2 statistic, using a confidence level of 95%. A similar

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procedure was followed for modeling the relationship between ***NA*** and ***SA***. All the upper and lower limit bounds, except for θ, were relaxed so that they are not active constraints in the optimization process, therefore allowing the MD based constraints to freely play their role.

The MOOP was solved using the Goal Attainment Method (GAM) developed by Gembicki (1974) and implemented in the *fgoalattain* routine from the Optimization Toolbox of Matlab®. This method involves expressing a set of design goals, F*, associated to a set of F objectives. The problem formulation allows the objectives to be under or overachieved and the degree of goals achievement is controlled by a vector of weighting coefficients, W. This is reduced to a standard optimization problem where a parameter φ is minimized with a given set of additional constraints formulated as a weighted flexible distance from the goals, $F_i(x)-w_i\,\varphi \le F_i^*$. This problem is addressed by GAM as a nonlinear programming one exploiting the Sequential Quadratic Programming (SQP).

For the multiobjective optimization of the benzene nitration process it is legitimate to set as goals 100% of MNB and 0 ppm of DNP and TNP. Figure 1 illustrates the trade-off results between these two conflicting objectives, confirming that a decrease in byproducts formation can only be achieved if MNB production is reduced. Furthermore, a unit reduction on MNB leads to a three fold decrease on DNF+TNF. It was also confirmed that the degree to which byproducts can be reduced depends on the operating conditions, Figure1

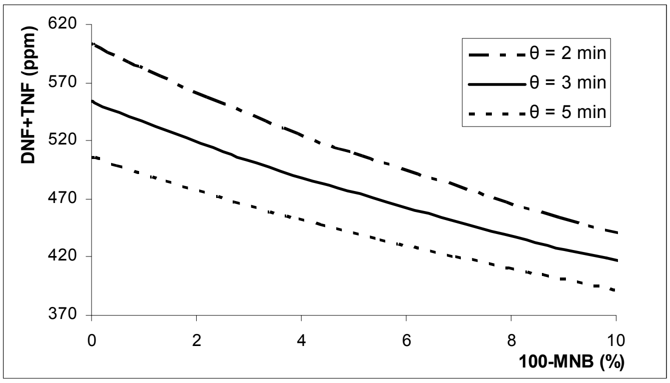

Figure 1 – Pareto front obtained for interest region of MNB>90% depending on the θ upper limit constraint.

Acknowledgments

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Dynamic optimization of fed-batch alcoholic fermentation

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Keywords: fermentation, fed-batch, dynamic optimization, ethanol, *Saccharomyces cerevisiae*, alcohol.

The dynamic optimization of the fed-batch fermentation consists in the determination of the optimal profile of the control variable that maximizes the yield or the productivity, subject to inequality and equality constraints. The numerical solution of this problem has been intensively investigated through indirect [1-3] and direct [4-6] methods, genetic algorithms [7-10] and dynamic programming [11-14]. Previous studies are related to *Zymomonas mobilis* or adopt operational restrictions and kinetic models that do not represent the conditions adopted by Brazilian industries, some of them presenting yield higher than 0.511[g/g] for glucose as substrate, justified by less accurate kinetic models [15-17]. Several experimental studies confirm the yield and productivity increments of the ethanol in the fed-batch fermentation compared with the batch one and evaluate the effect of variable substrate feeding. However, the results are highly dependent of the microorganism and substrate, filling times, feed profile and substrate concentration and are applicable only to restrict conditions [17-21]. The challenge to solve a singular problem with differential index fluctuation is added to the development of accurate and simple enough mathematical models of a nonlinear process with complex metabolism, for control and optimization purposes. Furthermore, it must guarantee that the open-loop implementation of the calculated optimal trajectories results in the desired optimization in the presence of disturbances [22-23]. In this work, the optimal profile of the substrate feed rate is calculated considering conditions and constraints adopted industrially. The parameters of kinetic models were estimate by Differential Evolution for high fed substrate concentrations using *Saccharomyces cerevisiae* from batch and fed-batch experiments. The resulting differential-algebraic dynamic optimization problem is solved by the control and state parameterization method considering the maximization of productivity [24]. The control and state variables profiles are presented and the influence of factors like substrate and ethanol concentration, fermentation time and filling rate on the optimal profiles are evaluated.

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Control and optimization algorithms proposed for two layer approach: application to a three-phase catalytic reactor

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Keywords: Genetic Algorithms, Three phase reactor, DMC controller, Two layer approach, One layer approach.

Control and optimization algorithms can be used separately or can be alternatively integrated arranged in a hierarchical strategy, leading to real time integration in order to solve on-line optimization and control problems (Rezende, 2007).

Integration of control and optimization in real time can be carried out basically through two main strategies, usually named one-layer approach and two-layer approach.

In the case of two-layer approach, the upper level is the optimization one, which determines the optimal set-points of the variables at the steady-state which are then used in the control of the system (González et al., 2006). In the one-layer approach, the economical optimization problem and the problem of control are solved simultaneously.

The case study employed in this work is a three phase catalytic reactor in which the reaction of hydrogenation of o-cresol producing 2-methyl-cyclohexanol occurs, as is showed: $3\,H_2(g) + C_6H_4OHCH_3(l) \rightarrow C_6H_{10}OHCH_3(l)$.

This reactor is characterized by a high dimensionality and non-linearity model, which is difficult to be optimized by conventional methods, since not always convergence is achieved (Rezende et al., 2008). This justifies the use of an evolutionary method, based on the Genetic Algorithms (GAs), to deal with this process. In this way, in order to optimize the process, the Genetic Algorithm code is coupled with the rigorous model of the reactor.

Genetic Algorithms showed to be robust and efficient, since they found the optimal set of variables that conducted the reactor to the optimized steady state, translated in terms of exit concentration of o-cresol in the liquid phase (Bl) and reaction medium temperature at the exit of the reactor (T). A computational time required was suitable for real time applications.

The variables optimized by Genetic Algorithms are used as possible manipulated variables and as set-points in the control of the reactor with the aim of maintaining the reactor on the optimized steady state. In order to control the reactor, the algorithm employed is the Dynamic Matrix Control (DMC) that is a type of Model Predictive Control (MPC). DMC makes use of a linear model, the convolution model, which is obtained through step disturbances in the input variables (Rezende, 2007).

Figure 1 presents a schematic drawing that shows the input variables optimized by GAs, to know, which are linear velocity of gas (ug), linear velocity of liquid (ul), linear velocity of coolant (ur), feed concentration of hydrogen in both the gas phase (Agf) and in the liquid phase (Alf), feed concentration of o-cresol in the liquid phase (Blf), reactor feed temperature (Tf) and feed coolant temperature (Trf) These input variables generate outputs to the optimized steady state. Such variables are, respectively, possible manipulated variables and set-points of controlled variables that are sent to the control layer.

The controlled variables (exit concentration of o-cresol in the liquid phase and reaction medium temperature at the exit of the reactor) return to the set-point optimized by Genetic Algorithms when load disturbances occur.

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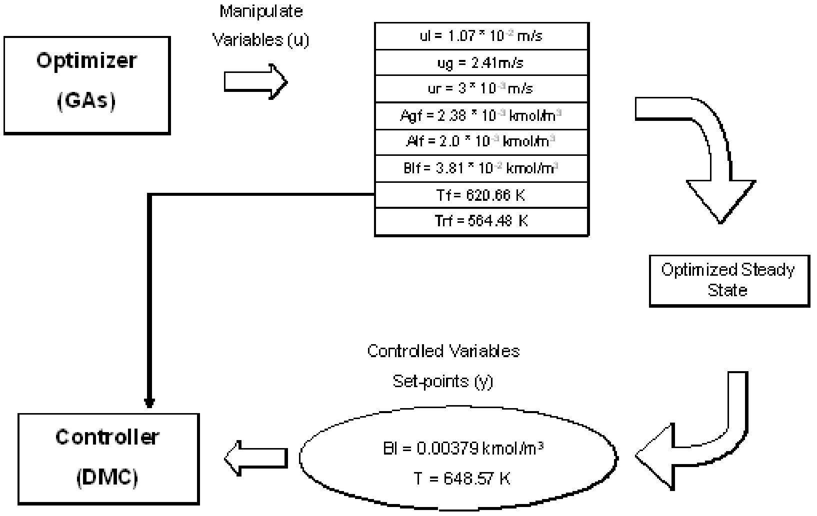

Figure 1 – Schematic drawing of the proposal of two layer approach for the reactor.

Figure 2 shows the DMC controller acting in the set-point, for instance, of the reaction medium temperature at the exit of the reactor

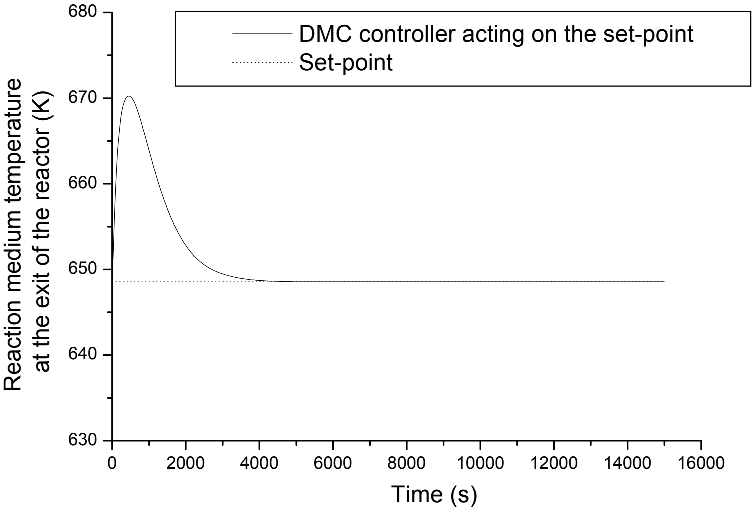

Figure 2 – DMC controller acting in the new set-point of the controlled variable determined by GAs.

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Selection of controlled variables for an industrial distillation column with heat pump

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Keywords: Control configuration selection, Distillation columns, Plantwide control, Self-optimizing control

In this work, self optimizing method is used to determine control configuration of a distillation column with heat pump. The column is an industrial C3 splitter that mainly separates propylene and propane. The column overhead is pressurized by a compressor and condensed in the shell-side of the reboiler. Several control scenarios were examined to find controlled variables having self-optimize characteristics (i.e., when they are kept at constant setpoints in face of disturbances, indirectly maintain the plant operation near an economic optimum). The results show that the ratio of reflux flowrate to boilup flowrate has good self optimizing control property.

INTRODUCTION

Distillation columns used for polymer grade separations of close boiling mixtures are highly energy consuming. Low relative volatilities and high product purity lead to very high reflux ratios and consequently large amount of energy. Some approaches have been recommended to improve such columns with respect to operating cost and heat integration. One approach is a distillation column with heat pump as shown in Fig. 1.

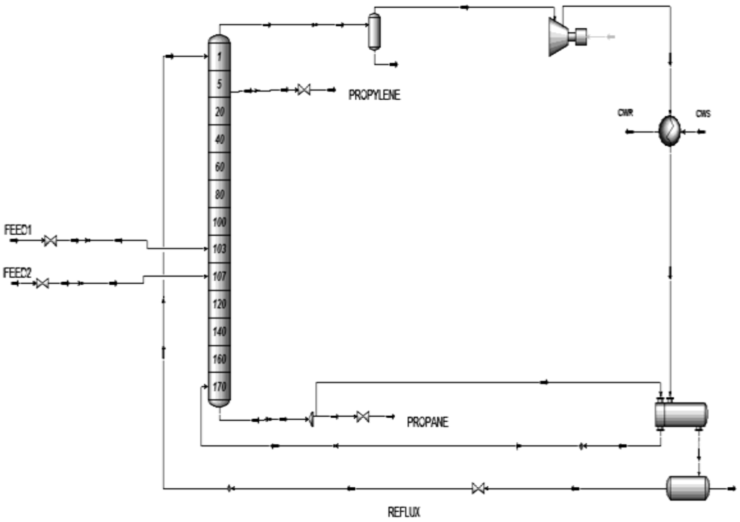

Fig. 1 flowsheet of the process.

A self-optimizing control method presents a mathematical procedure for selection of controlled variables [1]. In this method first, a cost or profit function is formulated as quality of operation that would be minimized or maximized. Those measured outputs are selected as controlled variables that while keeping them at their optimum setpoint values, the disturbances have the least effect on the optimum operation of the process and the lowest loss. If the cost function and loss are shown with J and L respectively, then the loss is defined by:

$$L(c_s,d)=J(c_s,d)-J(opt) \qquad (1)$$

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Where cs indicate the setpoint of desired controlled variable and d indicates the disturbance occurring.

Self-optimizing control method

The self-optimizing method has the following six steps: (1) Determination of degrees of freedom, (2)Definition of cost function and constraint, (3) Steady state optimization, (4) Identification of sets of candidate controlled variable, (5) Evaluation of the loss. From the five available degrees of freedom, four degrees were consumed to maintain two holdups and two active constraints (i.e. column pressure and distillate product purity).
The expressions developed for the cost function is defined as the following.
J = - (propylene price*distillate rate + propane price*bottom rate – 1st feed cost*feed rate – 2nd feed cost*feed rate- compressor energy cost*energy rate- cooling water cost*cooling water rate)

$$J = -(p_D D + p_B B - p_{F_1} F_1 - p_{F_2} F_2 - p_s W_s - p_{cw} V_{cw}) \qquad (2)$$

The various controlled variables being evaluated through self-optimize procedure are shown in Table 2. To select the best controlled variable, the loss is calculated through keeping the candidate controlled variable at its setpoint and implementing active constraints while the disturbances are being applied. The results of the average values of losses are presented in Table 2. The comparison of economic losses shows that control of V/L leads to the smallest economic loss.

TABLE 2 AVERAGE LOSS CALCULATION RESULTS

Variable	Variable description	*Average loss*
L	Reflux rate	29.18
V/B	Boilup rate to bottom rate	28.5
1-x_B	Bottom purity	27.7
T	130'tray temperature	28.1
V	Boilup rate	22.7
D/L	Distillate to Reflux rate	22.4
B/L	Bottom to Reflux rate	29.0
V/L	Boilup to Reflux rate	12.7
V/F_1	Boilup to 1'st feed rate	24.6
V/F_2	Boilup to 2'st feed rate	28.3
Q	Heat exchanger duty	27.9
DT	Two products temperature difference	22.2
L/F_1	Reflux to 1'st feed rate	18.8
L/F_2	Reflux to 2'st feed rate	29.9

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An SPC inspired MPC formulation for quality variables

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Keywords: MPC, state-space model, stochastic optimization, quality cost.

It is rather common nowadays the chemical production plants have an automation level that includes the acquisition of hundreds or thousands of variables per second by measurement devices installed in the field, and its storage in large data basis. This information is able to be used for several purposes, from fault detection to data-driven modelling, and knowledge extraction. The modelling of chemical units behavior based upon fundamental principles is nowadays well established, and the phenomenological understanding/description of physical and chemical mechanisms is perfectly established for a large range of units. Although, there is still process units, which due to the intrinsic complexity, the status of development and solution of first principle based models is still insufficient to make them useful for model predictive control purposes. On these group of systems one may refer some particulate based processes.

Another aspect not completely dominated regarding first principles modelling is mathematical representation of some quality variables. It is common the lack of knowledge to represent the relation between first principles primary variables and quality variables, such as brightness, dirt count, and d_{50}. Moreover, reliable on-line measurement devices for them are sometimes not available or involve large time delays that can reduce the controllers performance.

The combination of these aspects makes the data-driven modelling approaches suitable to achieve an acceptable model representation in a priori confined operation regions. Several tools devoted to this task are employed, from artificial neural networks to multivariate statistical projection based approaches, and time discrete models. The linear discrete state-space framework offers a simple representation of system dynamics incorporating a stochastic component to describe the parameters/inputs/measurement devices uncertainty either non-stationarity features (Durbin and Koopman, 2001).

Here we propose anMPC formulation based on discrete linear state-space models identified from process data to control quality variables inside desired regions. The model identification is carried out employing a subspace projection algorithm (Van Overschee and DeMoor, 1994) combined with Akaike criterium to determine the order, and considers explicitly missing observations in the data set used (Gândara et al., 2006). The model is to be used to forecast the dynamics of quality variables represented by probability distribution functions (pdf) at each discrete time instant of the control horizon. The average value and uncertainty, described by the pdf second moment, are projected in the control horizon employing the state-space model. The quality variables uncertainty is described by normal pdf resulting from the combination of initial state and inputs uncertainty.

In recent years a new formulation of MPC was developed to account for the uncertainty of inputs, with the outputs controlled within a previously defined region - the so-called chance constraints MPC (Li et al. 2002) determining the optimal sequence of

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actuations to keep the output within a desired region with a predefined probability level. The formulation involves an objective function only dependent on inputs, and probabilistic constraints for the outputs. Our approach is related with the chance constraints formulation. Indeed, inspired on SPC and Taguchian loss paradigms according which the probability of a quality variable falling outside the control limits region represent a potential loss quantified by a cost, we allow the quality variables to be outside the control region. However, a penalty cost proportional to the probability of being out of the control region is applied, intrinsically accounting for the cost of producing out of quality during elementary time discrete intervals.

The formulation gives origin to a NLP problem characterized by a non-linear objective function and a set of linear constraints representing the process model. The problem is solved at each time instant using GAMS/CONOPT, determining the optimal actuations profile in the control horizon. A Matlab procedure is employed to sequentially iterate the MPC algorithm by calling the GAMS procedure. The formulation is applied to the control of d_{50} of the product of an industrial precipitated calcium carbonate process.

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The Importance of the simulation in refinery processes

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Keywords: Simulation, Process simulators, Performance models

The main tool of the computer aided process engineering is the process simulation (Biegler, 1989). One of the main objectives of the simulation is allowing the creation of models in steady-state or in transient regime of the performance of a plant, under defined conditions and through the developing of process material and energy balances (Glasscock and Hale, 1994).

In mathematical point of view, a simulation problem corresponds to obtain a solution of a high number of equations which represents the performance of the unit operations and the behaviour of the chemical compounds.

The simulation is very relevant in several working areas of the refinery processes, such as:

Conception/Design – Study of different design alternatives and determination of the operation values, to select the best solutions, using economic drivers, safety rules and/or environment control;

Start up – Prediction of operational conditions during the start up of the processing units;

Operation – Study of limit operation conditions attending to safety rules and the possible modifications in the project specifications;

Optimization – Modification of the operational values with the objective to evaluate the operational conditions which minimizes the economic, energy and environmental requirements. In this case, the simulation has been demonstrated as a good strategy to maximize the profit through the reduction of consumption or by increasing the production, considering the installed equipment.

The main steps in simulation are: i) the problem definition; ii) the development of the process model; iii) the collection of additional data; iv) the resolution of the model equations, and finally v) the analysis of the results.

One simulation problem is defined through a sequence of steps which are: i) the selection of components, ii) the selection of the appropriate unit operations, iii) the creation of the process flux diagram, iv) the selection of the correlations to evaluate the thermodynamic properties, and iv) the introduction of the information related with streams, equipment and operation.

The study, here reported, corresponds to the utilization of a process simulator to develop a model for the distillation unit of the fuel plant of Petrogal Porto Refinery. Figure 1 presents a schematic diagram of the installation. The study was developed in two phases: i) in the first phase, the objective was to obtain a performance model for the unit, comparing the results obtained by simulation with the operational values; and ii) in the second phase, some modifications in equipment were introduced, to evaluate the global performance of the unit for the processing of a light crude oil (Figure 2).

The main goal was to evaluate different operational scenarios which allow the increase the gas oil yield and the reduction of energy consumption.

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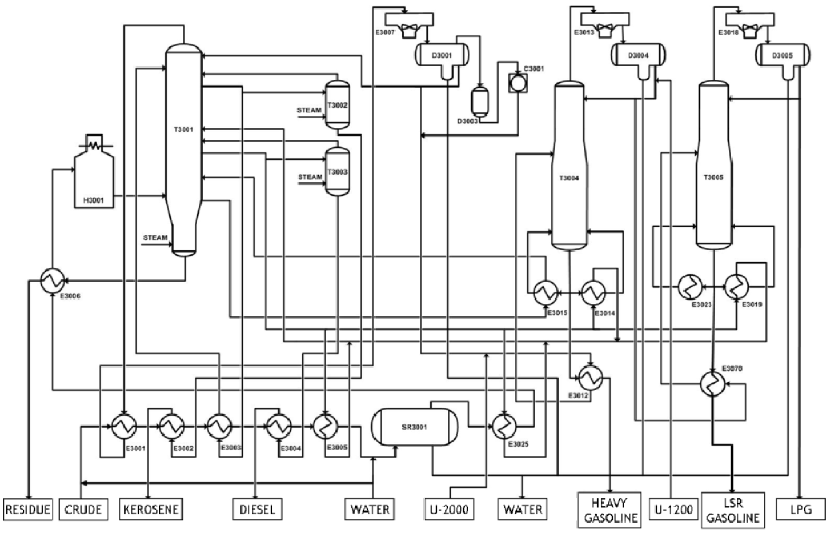

Figure 1 – Diagram of the fuel plant distillation unit.

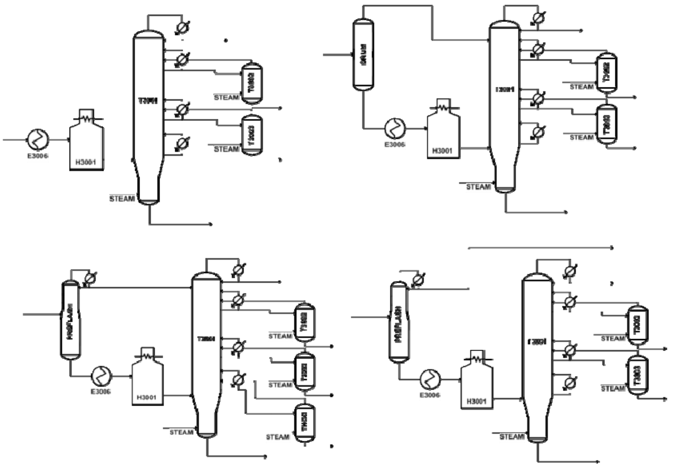

Figure 2 - Study cases with modifications of installed equipment.

The results of the studies performed with the process simulator demonstrated that these tools allow developing models applied to the reality and are very useful to evaluate different operational scenarios.

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Dynamic simulation of CO_2 absorption from post combustion gases

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Keywords: CO2 capture ; gas absorption ; dynamic simulation

CO_2 capture from combustion flue gases is currently an issue of main interest due to the high taxes being imposed on industrial companies as a measure to achieve CO_2 emissions abatement.

CO_2 capture can be efficiently achieved by gas absorption using amines, namely monoethanolamine (MEA). Based on previous experimental work, this study considers the development of a dynamic model of an absorption column for post combustion CO_2 capture, validated through a simulation procedure.

This study comprises also: a dynamic model of the temperature swing de-absorption process; the integration of both models for absorption and de-absorption (with the introduction of heat exchangers, pumps and other ancilliary equipment); and also the reaction kinetics of amine decomposition. The developed model was also refined and tested for other amine solutions.

A control system for the process was also devised, comprising the pertinent sensors, and various control structures for the absorption column were tested and compared, such as single loop PID controllers (including the pairing problem) and MPC.

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Three-dimensional gas-solid fluidized bed simulation based on kinetic theory of granular flow

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Keywords: Fluidized bed, CFD, Multiphase flow, Kinetic theory

Fluidized beds are widely used in many operations in chemical, metallurgical, energy generation and especially in petrochemical industries. Major applications are fluid catalytic cracking (FCC) risers and CFB combustors. No analytical tools that describe the influences of complex geometries, chemical reaction, internal reflux and heat transfer on the flow pattern in fluidized beds are available. With the increase in the availability of computers, the application of mathematical models to predict the behavior of a fluidized bed follow the same trend, and several models have been proposed.

In this work, a three-dimensional two-phase flow model based on kinetic theory of granular flow (KTGF) was used to predict the behavior of a gas-solid fluidized bed. The model is based on a Eulerian description of the two phase, gas and particles, and is composed of a set of mass conservation and momentum equations for each phase. In this model, turbulence k-epsilon model and multiphase mixture are contemplated. In order to describe the behavior of several particles in a continuum, the kinetic theory of granular flow was used. This approach has gained attention and its use in eulerian simulations has increased. The kinetic theory of granular flow is based on the kinetic theory of gases, first developed by Chapman e Cowling (1970). In this theory, the inter-particle interactions are taken into account, by adding the contribution of collisions between particles, Jenkins e Savage (1983), which are the main mechanism of transport due to particulate phase properties.

In this work, the geometry and numerical mesh were generated using ANSYS ICEM CFD software and the set of partial differential equations was discretized and solved using CFX. Simulation data were verified against data found in Samuelsberg e Herjtager (1996), who used a cold flow laboratory circulating fluidized bed and simulated a two dimensional model. The particle velocity was measured using a laser Doppler anemometry in that experimental study.

Results using this model have shown that the model agrees with the experimental data, and predicts a flow behavior similar to that found experimentally. It predicts the core annulus flow, wich is known from literature. The simulations were performed in three different values of velocity.

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Writing a software package for simulation of the three-dimensional structure of paper

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Keywords: Modeling of the three-dimensional structure of paper, Cellular automata, Implementation.

Although theoretical paper models have existed in the literature for over two decades, most experimental research work encountered in the field of pulp and paper is mainly of empirical nature. One of the primary reasons that have hindered the generalized use of modeling in this area is the lack of easy understood models and available computational implementations.

In this article we aim to render the barrier to the use of modeling less binding by describing key building blocks of a MATLAB implementation for the KCL-PAKKA model (Niskanen and Alava, 1994; Nilsen et al., 1998). This is a stochastic simulation sedimentation model to generate the three-dimensional network structure of paper which gives realistic predictions for many paper properties.

To form a paper web, *flexible* straight fibers are laid down, one at a time, onto a flat substrate. Fibers are positioned and oriented at random in the in-plane (x and y) directions and placed parallel to the substrate on top of the underlying network. Thereafter the parts of the fiber that do not touch the network beneath them undergo bending in the vertical direction such that they either do contact with previous adjacent fibers or the largest deflection allowed by the bending stiffness of fibers is reached (see Figure 1).

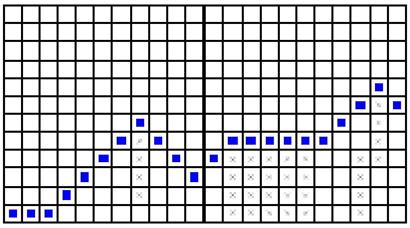

Figure 1: Bending mechanism of fiber generated by computational simulation. The crosses represent parts of previously deposited fibers and the squares denoting the bottom layer of the bending fiber.

Space is discretized into a Cartesian *uniform* grid of cells so that each fiber in the model is represented by a single vertical planar strip one cell wide. Any two nearest neighboring cells on the fiber can make at most the maximum vertical lattice steps allowed (flexing rule).

This approach allow us to exploit the potential of *cellular automata* (see the book by Schiff, 2008). A cellular automaton is a spatial discrete time model. Each cell can exist in different states chosen from a finite set. At each time step each cell changes its state based on a set of rules that represent the allowable physics of the phenomena. Figure 2 shows an example of a 3D fiber network constructed with the sedimentation model.

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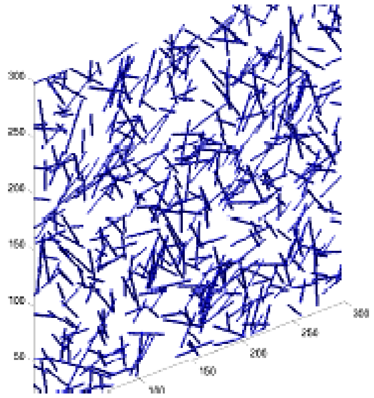

Figure 2: Simulated 3D fiber network.

The tendency of fibers in the suspension to concentrate around drainage sinks (those parts of the deposit that have less fibers per unit area) leads to a smoothing mechanism of a paper sheet. This effect is simulated by the particle deposition rule of Provatas and Uesaka (2003) as can be seen in Figure 3.

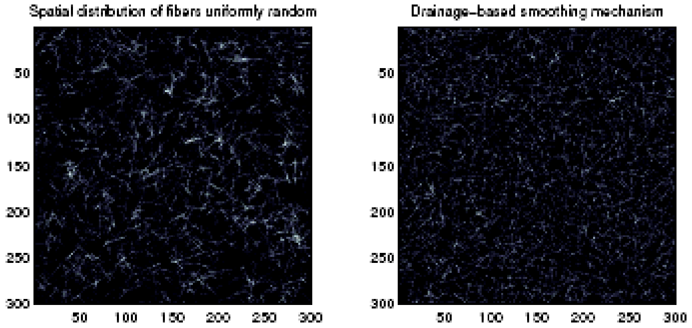

Figure 3: Simulated X-ray image of the (x, y) plane for paper handsheet as generated from the deposition rule.

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3D simulation of multiphase flow in large-scale venturi scrubbers

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Keywords: Venturi, Multiphase flow, CFD

Venturi scrubbers are used in various industrial plants to remove particles from gas streams due to their high collection effectiveness, particularly for small, low inertia particles.

They have been extensively studied in order to foster their understanding and modelling. The flow of gas and water in a venturi scrubber can be described as annular two-phase flow with further complexities due to the venturi geometry. Different models have been proposed in the literature and they range from simple correlations to more complex models (Azzopardi et al., 1991; Cruz et al., 1997; Ananthanarayanan and Viswanathan, 1999).

Pressure drop is a major parameter in the performance of venturi scrubbers as the energy costs associated with gas pumping are the main drawback of these systems. It has been observed that an improvement in the efficiency of the equipment is always related to this parameter (Gamisans et al., 2002).

In this study, a Computational Fluid Dynamics (CFD) model for the flow in a large-scale venturi scrubber has been developed using Fluent 6.2 version, which is based upon the finite volume method, to solve the conservation equations. The air flow is considered incompressible Newtonian and turbulent. The turbulence was modelled by the k-ε standard method. An Eulerian-Lagrangian approach has been taken in simulating the two-phase flow.

For the domain discretization, flow symmetry is assumed along the cross sections of the scrubber. Hence only one-fourth of the scrubber volume is modelled. The resulting grid is defined using a Hexahedral/Wedge Cooper mesh generation algorithm in Gambit. The 3D final mesh results in 15229 hexahedral elements (Figure 1).

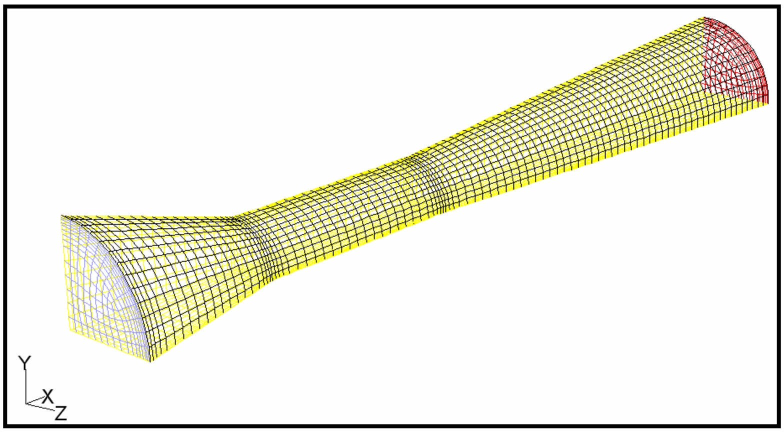

Figure 1 – Computational grid for the domain (Gambit).

The experimental validation of these more complex models is always important but experimental data, in large-scale venturi scrubbers, are scarce in the literature.

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A large scale cylindrical venturi was built for measuring the most relevant operating parameters for industrial applications and to provide a reliable experimental data base that can be used for model validation.

The experimental measurements include: the pressure drop along venturi, the droplet size, measured by laser diffraction based instruments, and the performance of the venturi scrubber in the collection of fine powder (Silva et al., 2008), for different operating parameters such as gas velocity and liquid flow rates. The liquid mass flows tested are from 0.013 to 0.075 kg/s and the throat gas velocity varied between 34 and 70 m/s.

Data for the experimental pressure drop along the venturi, for a gas velocity of 70 m/s at the throat, normalized by the pressure at the convergence inlet are compared with the numerical results in Figure 2. The effect of the dispersed phase with a liquid mass flow of 0.038 kg/s is also presented and the results show a very good agreement.

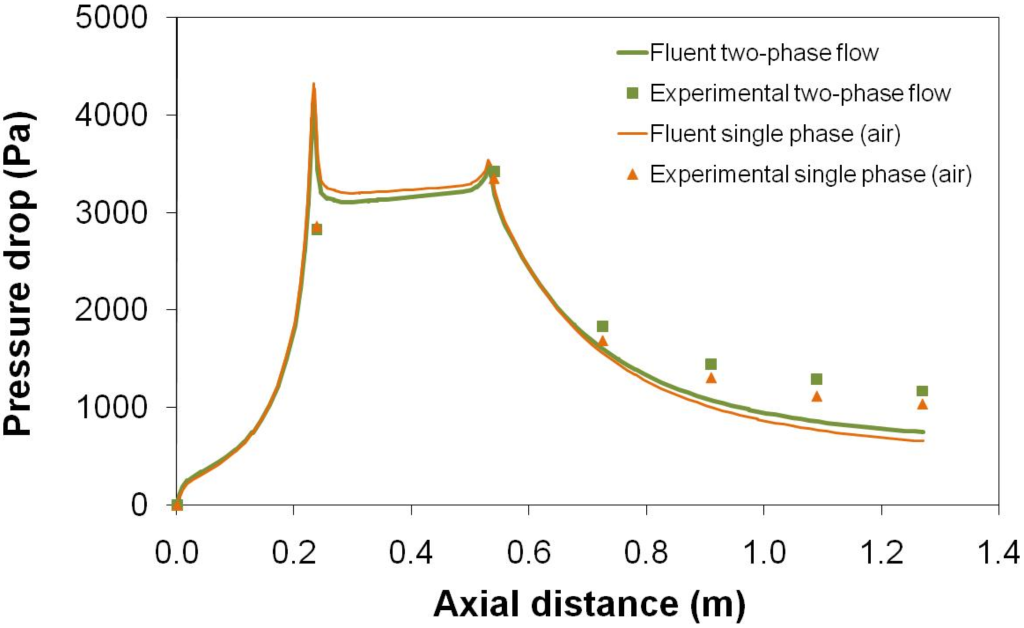

Figure 2 – Comparisons between experimental and predicted pressure drop along venturi.

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Numerical simulation of supercritical extraction processes

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Keywords: method of lines, method of characteristics, finite differences, orthogonal collocation, finite elements.

The supercritical fluid extraction (SFE) of natural compounds using CO_2 is a promising separation technique for recovering high added value products with minimum degradation, with environmentally friendly solvents and higher selectivities.

An important related task is the modelling of the SFE and subsequent determination of the key parameters involved, in order to simulate the process and finally optimize the interested operating conditions. This work focus on the modeling of SFE of seed oil and the numerical solution of the corresponding differential equations.

The model comprehends a set of coupled material balances distributed into three control volumes: one mass balance to the fluid phase (extractor bed), another one to the freely available oil in the seed, and a third mass balance to the tied oil existent inside the core of the seed. Several authors propose different mechanisms for the transport kinetic in the solid particle as mass transfer in parallel or in series (Marrone *et al.*, 1998; Sovová, 2005). Both approaches will be analyzed and compared.

Whichever the model adopted, a set of partial differential equations of the hyperbolic type results, which may be solved by classical numerical techniques: the Method of Lines (Shiesser, 1994) and Method of Characteristics (Acrivos, 1956; Aris and Amundson, 1973; Perrut et al., 1997). The axial spatial coordinate is discretized using upwind finite differences and orthogonal collocation on finite elements, both methods having only local dependencies. The library of Shampine (2005) is used for solving the same model, based on central finite differences with flux correction, which is a versatile and specialized numerical library for hyperbolic systems of equations.

All these libraries and routines are implemented in Matlab. The solutions obtained are compared with experimental data provided in terms of extraction curves, i.e. extraction yield *versus* time. The concentration wave fronts inside the extractor are compared and computation time reported, based on the profiling tools available.

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Influence of a heated brine spray system on the dynamic model of an integrated recrystallization industrial process

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Keywords: Dynamic modelling, Simulation, Process integration, Industrial case study, Cogeneration, Spray system, Crystallization process

The aim of this study is to carry out a dynamic model of a Portuguese industrial integrated system in order to analyse the integration and feasibility of the different units, maximizing its efficiency and minimizing its environmental damage, and to study the effect of some operational and atmospheric conditions on the system.

This integrated system, located at Carriço, Pombal (Portugal), includes the following three separated processes (Moita et al., 2004):

- A gas turbine cogeneration system;
- A salt recrystallization process;
- A natural gas salt caverns storage.

The leaching programme to obtain the cavern for future Natural Gas storage generates the needed brine to feed the salt recrystallization ponds. The cogeneration system provides the electrical power to export to the regional net, and its associated thermal energy is used in the salt recrystallization process improving the global efficiency.

The simulation of the integrated process, which includes the cogeneration system, the plate heat exchangers used to heat the fresh brine dumped into the recrystallization ponds and the salt production process, is made through first principles dynamic models. These consider the phase equilibrium thermodynamics (solid, liquid and gas equilibrium) as well as other areas of scientific knowledge such as fluid dynamics, crystallisation, heat and mass transfer, simultaneously heat and power production, etc.

The integrated system model was simulated through *gPROMS 2.3.7* of the Process Systems Enterprise, Ltd. This complete distributed model gives the dynamic behaviour of several variables such as the brine temperature, concentration and ponds level profiles as well as the thermal efficiency and the final salt production inside the recrystallization ponds, using different atmospheric and operational conditions as state variables (Moita et al., 2005).

In this paper an extension of the model previously presented (Moita et al., 2004, 2005) is developed to include a new heated brine spray distribution system model. This new spray model, which is based on the ballistics theory, allows predicting the brine drops position and velocity, as well as, for the all spray system, the global water evaporation rate, brine and salt flow rate, brine temperature, brine concentration, and energy losses (Moita et al., 2008). These spray variables are then included on the integrated system model mentioned.

The integrated process operation with and without sprays will be compared through some performance indicators, such as, the salt production, the water evaporation rate and the process global efficiency, to analyse its benefits, by using different operational and atmospheric scenarios. Due to brine air drift issues some restrictions should be considered to define the on-off sprays working periods. The appropriate wind directions time profile is considered in the model using some quasi-random time profiles for the allowed operation of the spray system (Moita et al., 2007). It will also be determined the number of sprays and the appropriate heated brine recycled flow rate split between the direct flow to the taps on the working ponds and the spray distribution system. This

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operational management is obtained to maximize the global salt production and to guarantee the nominal circulating water temperatures values, which act as the interface between the recrystallization process and the cogeneration system.

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Non isothermal phenomenological model of an enological fermentation: modelling and performance analysis

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Keywords: Non-isothermal model, Enological fermentation, Killer yeasts, Performance Analysis.

Introduction

High-quality wines are the market segment that offers the best advantages for wine production in the provinces of Cuyo (Argentina). At this time in the regional wineries, varietal musts and selected yeast starters are used and usually fermenters are operated on batch mode with limited control on its main operation variables. Therefore, a strict process monitoring and control in winemaking is required (Vallejo *et al.*, 2005; Ortiz *et al.*, 2006). Some of the selected strains possess killer properties, which in this case represent a guarantee for their implantation in the fermentation (Zagorc *et al.*, 2001).

The alcoholic fermentation constitutes the core of winemaking processes. At this stage occur complex changes physical, chemical and biological that determines the final quality of wine. Such characteristics and the presence of non-constant parameters and high non-linearity produce complex dynamic responses of the bioprocess. Although many of these problems have been widely studied (Boulton *et al.*, 1996; Fleet, 1993), it still exist several that need to be solved.

Non-isothermal phenomenological model

A non-isothermal model for a batch alcoholic fermentation is presented. The model couple mass and energy balances predicting satisfactorily the behavior of the main output process variables: viable cells, substrate (sugar) and ethanol concentrations, carbon dioxide released, and the bioreactor temperature. The proposed model is based on the one developed by Scaglia *et al.* (2007), that possesses an excellent performance for isothermal fermentations. This one avoids possible discontinuities, allows a more accurate and smooth approximation to the experimental data. In this case, the main model objective has been posed on temperature, due to its control is critical to produce fine wines with constant quality and to reduce the winery cooling requirements (Aballay *et al.*, 2006).

The non-isothermal kinetic model proposed, consists of a set of ordinary differential equations which calculate the before mentioned balances, including the heat transferred between the reactor and its cooling jacket. Balances have been coupled by means of the Arrhenius's equation that describes the temperature influence on the cell growth rate (Aballay *et al.*, 2006; Phisalaphong *et al.*, 2006).

Kinetic parameters were adjusted using experimental data obtained from anaerobic lab-scale cultures of *S. cerevisiae* (killer), and/or *Candida cantarelli* yeasts in Syrah grape must. In the case of the specific parameters in Arrhenius's expression, they were adjusted by the least-square method (Aballay *et al.*, 2006). Further details about experimental procedures were described by Toro and Vazquez (2002).

The obtained results from simulation with the model proposed shows acceptable agreement with own experimental and published data, i.e., with those presented by Martínez *et al.* (1999). Moreover, it predicts in advance the fermentation evolution without appreciable delays what allows to take proper control actions on the process.

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Disturbance analysis

Due to in practice, the temperature in the bioreactor must be maintained constant at 23±1 ºC to avoid the quality product decrease, a wide performance analysis by using the dynamic non isothermal model obtained was carried out. The most frequently found disturbances in real plants are applied on temperature, one of the main operation variables, and the corresponding responses for the output process variables considered in the model are showed. Finally, some conclusions about possible control strategies to be applied are outlined.

Conclusions

A previously developed isothermal model has been expanded for non-isothermal fermentations, coupling the mass and energy balance by means of the Arrhenius' law. It has been validated with own and published experimental data and, a complete disturbance analysis was performed. The performance shown by the model will allow its application in advanced optimization and control strategies. A non-isothermal model for wine fermentations with an optimum temperature trajectory will be addressed in next works.

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Model aided design of gas-lift reactor for oxidation reaction with fast reversible catalyst deactivation

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Keywords: Gas-lift reactor, Model, Glucose, Palladium, Catalyst deactivation

Abstract

Tanks-in-series based model was applied to aid design of gas-lift reactor (GLR), where oxidation reaction along with catalyst reversible deactivation proceeds.

Heterogeneously catalyzed wet air oxidation of glucose dissolved in aqueous alkaline solution served as model reaction. Commercial 2.8% Pd/C catalyst was used in this study. Its advantage is activity at mild conditions with high selectivity towards salt of gluconic acid. But it gets deactivated by the action of oxygen quickly during the reaction course.

Multifunctional gas-lift reactor allows the desired reaction, as well as the catalyst reactivation. Optimal design of the gas-lift reactor makes it possible to keep high and stable activity of the catalyst.

Mathematical model

Our goal in this study was to design a target GLR with optimal geometry and performance. In the target reactor the oxidation reaction with the catalyst reversible deactivation proceeds. For the design purpose, GLR mathematical model was derived and applied. Tanks-in-series model (as schematically presented in Figure 1) is employed in the GLR mathematical model to grasp the way of mixing in real GLR. Every single tank of the tanks-in-series model is described by a set of equations combining hydrodynamics and chemistry that are supposed to hold for the individual section of GLR reactor. The number of tanks that form the sections of the tanks-in-series model refers to the extent of axial dispersion in the corresponding sections of real GLR, and is set as one of input parameters.

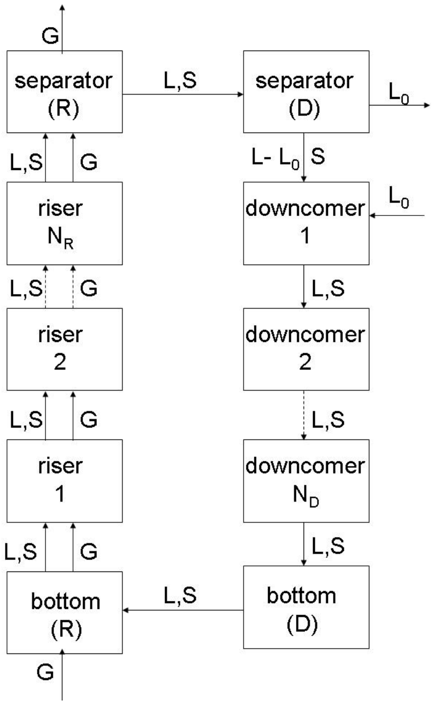

Figure 1: Scheme of tanks-in-series model employed in the GLR mathematical model. The tanks-in-series model is employed in conti-nuous mode of operation See the ref. [1] for the GLR model detailed description.

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Results and discussion

Simulations aimed to find optimal geometry of the target reactor and to see its performance were run using commercial software Matlab. Requirements on the GLR geometry were determined by the simulation input parameters. For each set of the input operating parameters the location of optimum geometry shifts as a consequence of change in deactivation extent of the catalyst after it passes riser of the GLR. For any deactivation extent always an optimum activation time exists. If the reactivation time is shorter, the reactivation process is insufficient. If the reactivation time exceeds its optimum, the time the main reaction could have proceeded is wasted and the reactor productivity drops down.

Gas superficial velocity, U_{GR}, is the parameter that primarily determines the GLR hydrodynamics. It has strong influence on the target reactor geometry. In effort to keep the reactor productivity (represented by the reaction rate) highest possible, the internal-loop GLR optimal geometry changes with change in U_{GR} (see Figure 1).

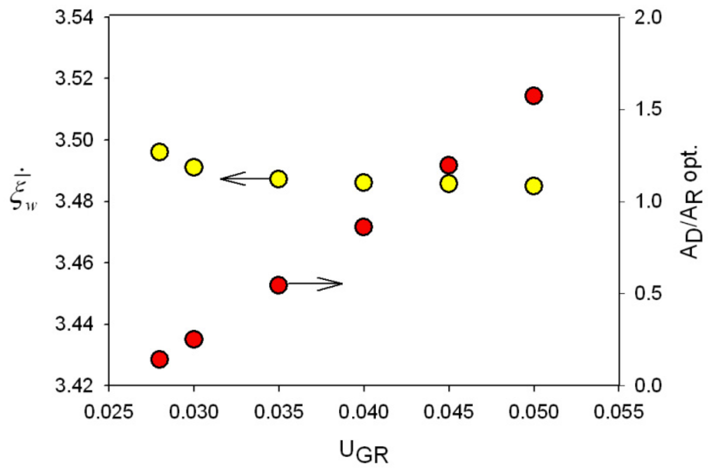

Figure 2: Influence of U_{GR} on the target GLR geometry, on condition that the GLR productivity must be the highest possible.
Simulation conditions: $c_{0,Glc}$= 100mol/m^3; $c_{0,Glcac}$=0mol/m^3; $Y_{feed,O}$=0.21; X_{Glc}=0.02.

Conclusions

GLR mathematical model was used to aid design of target reactor for reaction of heterogeneously catalyzed glucose oxidation. The derived model helps to overcome the problem of the catalyst's fast reversible deactivation by appropriate calculation of the target GLR geometry for any given input conditions.

List of symbols

A	cross-sectional area (m^2)
c	concentration (mol.m^{-3})
$\dot{\xi}_w$	cycle-time-averaged specific reaction rate (mol.kg^{-1}.s^{-1})
U	superficial velocity (m.s^{-1})
X	conversion (–)
Y	molar fraction (–)

indexes and abbreviations:

D	downcomer
feed	feed
G	gaseous phase
Glc	glucose
Glcac	gluconic acid
GLR	gas-lift reactor
i	component
L	liquid phase
O	oxygen
opt	optimum
R	riser
S	solid phase

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A new hybrid modeling methodology based on delayed differential equations: application to antibody expression by *Pichia pastoris*

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Keywords: Hybrid Modeling, Biosystem Dynamics, Delay Differential Equation, AR(X).

Here a novel methodology for dynamic Biosystems modelling is presented incorporating discrete time series, namely AutoRegressive (eXegenous) models into the nonparametric/parametric hybrid modelling framework, proposed earlier by Oliveira (2004). This results into a set of Delay Differential Equations (DDE) describing the material balances of a bioreactor system in which the dynamic kinetics are mimicked by a parameteric/nonparametric submodel. The idea is to display better consistency with the nature of biological processes by ascribing the dynamics of cellular metabolism to the parameteric/nonparametric subsystem.

Time delays have been experienced in many bioprocesses and, as well known, can be source to instabilities and oscillations. In most cases, however, only a certain time delay between the substrate uptake, biomass growth and product formation is observed as for example in the growth phase of fed-batch fermentations of *Saccharomyces cerevisiae* or *Pichia pastoris* (Ren et al, 2003). Optimization of such processes, namely the optimisation of feeding strategies, requires a modeling technique that accounts for the observed time delays in order to prevent instabilities and oscillations.

Time-delay biological processes have been well studied and modelled before. Such phenomenological models usually base on the general concept of retarded functional differential equations, Bocharov and Rihan (2000). Under some simplifications, models considering either discrete delays (Wolkowicz et al, 1997), distributed infinite delays (Daugulis et al, 1997, Wolkowicz and Xia, 1997) or ordinary differential equations (ODE) of kinetic rates (Ren et al, 2003) can be obtained. On the one hand these models are capable of explaining stability of processes and suitable for estimation of process key variables, but on the other hand, application to other cell systems is limited and their development is cost expensive. Consideration of delays in hybrid models can generally be carried out in a similar way as with phenomenological models. However, integration of ODE of kinetic rates into the hybrid model is not suited as neither a kinetic function nor the kinetics are directly known. Modelling with distributed delays is as well rather unlikely to be successfully applicable. Some mathematical postulation of arbitrarily large delays for unknown weighting functions of the delayed variable would have to be assumed and this mathematical convenience is in limit biologically unrealistic (see Bocharov and Rihan, 2000). Instead, the concept of discrete delays was chosen for incorporation into the hybrid model. This has no limitation for application as it is mathematically clear that a discrete time series of one variable is somehow analog to a differentiation and thus analog to the concept of distributed delays. Further the choice of discrete delays is confirmed by good prediction capabilities of the proposed hybrid structure for the model proposed by Ren et al (2003) and by two simulations using discrete and distributed delay models. For the

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latter fig. 1 shows an example of the results obtained with a simulated fed-batch. The same quality of results was obtained for all simulated models.

The present study is also supported by fed-batch experimental data of the antibody expression by recombinant Pichia Pastoris. Plots of measurements of biomass, product, pH, temperature accumulated mass of glycerol and methanol over time reveal time delays among each other. Firstly, the time delay was roughly estimated and then systematic studies of the influence of the roughly estimated and of additional time delays on the prediction capacity of the proposed hybrid model were studied. It is shown that the proposed DDE hybrid model is capable to model discrete and distributed delays in data and further outperforms the hybrid structure originally proposed by Oliveira (2004).

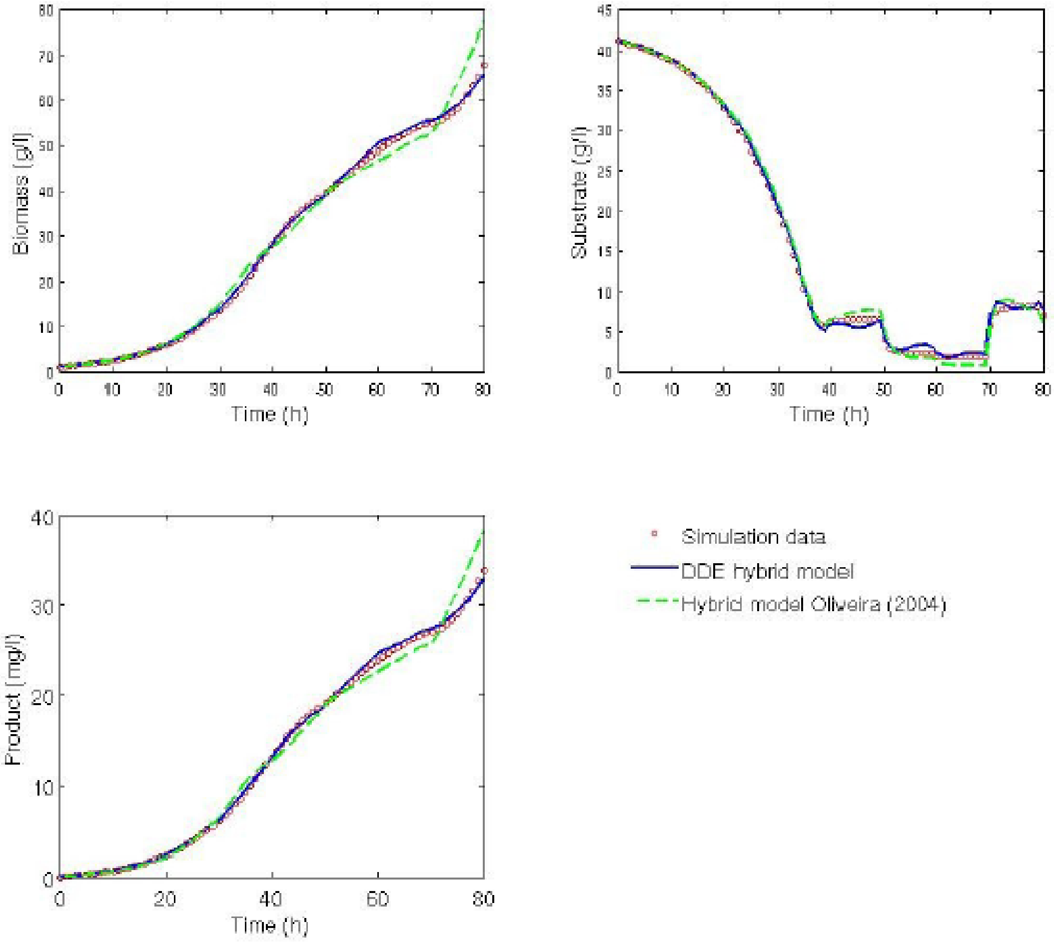

Figure 1. Plot of concentrations over time for data obtained with DDE hybrid model (solid line, blue), the hybrid model Oliveira (2004) (dashed line, green) and the true simulation using the distributed delay model (o, no line, red).

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Adaptive evolutionary design of extractants for the separation of organic compounds from aqueous streams by liquid extraction

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Keywords: Genetics algorithms, molecular design, Unifac, CAMD, extraction agents.

Industry poses different separations problems for diverse compounds recovery. Separation stage is very important on the product cost, especially for the biotechnological products. Liquid-liquid extraction is a widely used separation operation; however it requires separation agents that must fulfill conditions such as low toxicity, high distribution coefficient and selectivity, among others. Therefore, different approaches have been developed to tackle this problem, being computer aided molecular design the most promising alternative. It is an optimization process looking for the best group combination to successfully perform some objective in a given process, including some physical, feasibility and process restrictions. To solve this problem we present a new genetic algorithm that does not show some limitations present in early works.

The computer aided molecular design is an inverse engineering methodology, it has been successfully used by some authors for the generation of different molecules. The molecular design could be understood as an optimization process with various kinds of restrictions: physical, structural properties, etc, defined in function of the structural groups. The CAMD methodology is based on different property estimation methods coupled with an optimization method. The more used alternative for property estimation are group contribution methods, in despite of its limitations.

The optimization problem involved in molecular design is formulated as a mixed integer non linear programming model (MINLP) (Churi & Achenie, 1996, DUVEDI & ACHENIE, 1996, Stefanis, et al., 1996, Hostrup, et al., 1999), but some authors use non linear programming. Due to the high level of complexity, deterministic methods not always find the global optimum and are very susceptible to get trapped in local minima In contrast, stochastic methods have a greater possibility finding the global optimum and their form of use is very flexible. These are the reasons that lead to the use of genetic algorithms in this work.

Thanks to the advantages of the genetic algorithms over the other stochastic methods, authors as van Dyk (van Dyk & Nieuwoudt, 2002), Venkatasubramanian (Venkatasabrumanian, et al., 1994) and Xu (Xu & Diwekar, 2005) has used them for solvent design. However, their implementations have some limitations: a) the molecules representation is diverse and often allow the construction of molecules with a low complexity (because of the representation and the low maximum number of groups that form the molecule); additionally, almost in all cases the representation permits multiple molecules b) there is a non uniform use of the restrictions of the problem, especially the structural feasibility restrictions, and it is unknown the effect of the problem formulation on the results, c) in despite of the genetic operators used are similar, their probability of use in the algorithm is a parameter to be adjusted by the user, no warranting convergence nor optimality, and d) the use of different property estimation methods makes difficult the results evaluation and introduces a high uncertainty level in the problem that could change the results.

This work presents an alternative software that aims to alleviate the aforementioned difficulties.

SOLVENT DESIGN

The CAMD software for solvent design is divided in the following way: a routine to construct molecules structurally feasible, another one to estimate properties, and a final one that encompasses the others and makes the optimization.

Molecule's Construction

We used a novel molecules construction technique; it allows the design of aliphatic, aromatic and cyclic compounds. In all cases the maximum number of functional groups is restricted to three, considering easy of synthesis heuristic. Constructed molecules must fulfill the Odele-Machietto restrictions (GANI & BRIGNOLE, 1983).

Properties Estimation

The principal properties needed for the estimation of solvent performance are distribution coefficient and selectivity. The Unifac-Dortmund model (Gmehling, et al., 1993, Gmehling, et al., 1998, Gmehling, et al., 2002) is used for the calculation of activity coefficients. Boiling point, density and Gibbs free energy are used as restrictions in the design process. For its estimation the last version of the GCVOL method for density (Ihmels & Gmehling, 2003) and the Constantinou and Gani methods for melting, boiling point and Gibbs free energy are used. To include these restrictions in the design, the method of fitness function penalization is used.

Genetic Algorithm

For the solution of the problem, an adaptive genetic algorithm called HAEA [42] is used. Every molecule is represented in two ways, genotype and phenotype. Molecule genotype is a tree representation, in it every node represents a group and the branches, the bonds of this group with others groups. Phenotype is an array of groups of the same molecule; it is used for properties estimation. An initial population of 100 individuals was used. The molecules have variable length and a maximum length of 30 groups. In order to achieve a good diversity of the initial population, 10 % of the molecules created are cyclic, 10 % aromatics, and the others aliphatic. For the generation of new individuals four genetic operators were used: mutation, crossover, group insertion y deletion. The operator's probabilities were adjusted with the problem using the adaptive algorithm HAEA. The individual's selection was performed by tournament.

We applied our software for two applications: a literature model case, the acetic acid extraction of and a biotechnological problem, lactic acid extraction. Our results are in accordance with literature results and allow us to design novel molecules

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Heuristic methods to scheduling operations in parallel machines

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Keywords: Parallel Machines, Scheduling, Heuristics, Dyeing

Processing in parallel machines is frequent in the textile industry, particularly in the dyeing sector. In this paper, a scheduling problem of identical parallel machines is presented in that the orders, with due dates, have different processing times and different priorities. It is still considered the existence of dependent setup times of the sequence. The objective is to minimize the total weighted tardiness of delivering the orders.

This is a difficult problem to solve. It belongs to the NP-hard problems. By that, the use of heuristic methodology is justified. The objective of this study is to test and evaluate the efficiency and the effectiveness of the heuristic methods developed specifically for this problem, and to extend their use in general situations.

The problem of scheduling parallel machines is defined by the set of tasks T to be processed and by the set of machines M available for their processing. In this problem the aim is to find a solution that assigns the tasks in T to a machine in M in such a way to complete their processing while respecting the set of imposed constraints. Scheduling problems have the following two main constraints to respect: (1) a machine can only process one task at a time; and (2) a task can be processed only in one machine at a time. Also, it is assumed when a task is started, their processing cannot be interrupted until completed. The machines are identical and parallel; they perform the same type of operations and have equal processing speed.

In this work, a heuristic of constructive type is presented, that is based in the processing list that assigns the tasks in sequential order to the machines that are available earlier. This list is an ordering of all tasks. Sequentially, the tasks will be assigned one by one to the machine that is available earlier by attending to the ordering defined in the list of processing. In this constructive algorithm, the processing list is defined by the dispatch rule Earliest Due Date (EDD).

To improve the quality of the results obtained by the constructive algorithm, a local search procedure is implemented. Starting from an initial complete solution, replacing the current solution by the best solution existing in the neighbourhood of the current solution will be tried iteratively.

The initial solution is obtained by the constructive algorithm based in EDD rule. Starting from the current solution, a swap process is initiated within the list by which the tasks are assigned to the machines. The swap procedure consists in changing the order of two consecutive tasks in the processing list. From this swap results a new solution that is evaluated by the constructive algorithm. Now, instead of using the EDD rule, the tasks are sequenced by the new order defined in the list that respects the operations exchange.

Given a current solution, all solutions that are possible to be obtained by the sway procedure are generated. The solution that produces the lowest value of total weighted tardiness becomes the current solution if it is better than the actual current solution; if not, the search ends, giving the current solution as the local optimum solution. When a current solution is replaced by a new one, the procedure for a generation of a new set of solutions is initiated. This strategy is defined as the best improvement. The adoption of this strategy is justified by the number of new solutions that is possible to be

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generated by the swap procedure of consecutive tasks. This strategy allows obtaining acceptable computing times.

Instances were used based in the instances for the single machine problem available in OR Library from J.E. Beasley. The information for the setup times was added using random values based on the following proportions: 75% of tasks use common chemist processes to clean the dyeing machine; and 25% of tasks use a different process that takes more time to setup the machine.

The due date information was updated, dividing by the number of parallel machines. This option intends to keep the same level of congestion that exists in the single machine instances. There were selected instances of size 40, 50, and 100 tasks with different levels of congestion. Also, a small instance with 6 tasks was added. In all greatest instances, it was considered 6 parallel machines; and only 2 machines for the smallest one.

On average, for the used instances, the local search procedure allows for 56% of the total weighted tardiness to be reduced; and for some instances, the local search obtained the optimum solution. Furthermore, the increasing in computation time was not significant.

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Multiproduct pipeline with inventory management: the CLC's case study

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Keywords: Pipeline, Inventory Management, MILP, Decision Support System.

Supply chain management has continually attracted much attention from several service and industry organizations; due to the fact that companies are constantly looking for new opportunities to cut costs, improve profit margins while maintaining customer satisfaction. Being the oil supply chain one of the most important entities in world wide economy, it is crucial to develop advanced decision support tools to efficiently manage such system. This chain comprises several types of operations, several sites and several workers, at different levels. If, on the one hand, integrated planning and scheduling provides solutions with a wide vision, on the other hand such management tool would be rather complex to build and to use over the complete chain.

Several authors have proposed methodologies addressing different business areas, from exploration to distribution. Refinery operations remain as the focus area, but the remaining functions are crucial to take the maximum benefit from the chain.

Pipelines are widely used, being a reliable equipment for oil and oil products transportation, with reduced costs for a long term solution. Pipeline transportation scheduling has been addressed by some authors through both continuous and discrete approaches, such as Rejowski and Pinto (2008), Magatão et al. (2004), Cafaro and Cerdá (2008) In these works, pipelines are already fairly represented, but no effort has been done to represent tank farms with all physical tanks and mandatory inventory management procedures, which was first proposed by Relvas et al. (2006 and 2007).

This work addresses a system comprising a pipeline transporting several oil products between one refinery and one distribution centre located in a local market. The transportation within the pipeline has to respect possible sequences of products and flowrate constraints. At the distribution centre each tank as fixed product service. Each new batch discharged from the pipeline has to settle for a minimum amount of time, varying with the product. Clients are satisfied on site and forecasts are available on a daily basis. The pipeline schedule should accomplish the availabilities at the distribution centre, and satisfy the complete set of demands. In this way, two MILP (Mixed-Integer Linear Programming) models were developed to describe all system operations and constraints. These models use a continuous time formulation, through the implementation of sequencing variables.

The main difference between models is the representation's detail level of tanks at the distribution centre. The Aggregated Tank Formulation (ATF) MILP model considers that each product is stored in a big-single tank. Conversely, the Disaggregated Tank Formulation (DTF) MILP represents tanks as single entities with the real capacity.

The difference on the applicability of both models is the time horizon. The ATF model is able to produce results on a medium term horizon and feed the monthly refinery's production plan. The DTF is applicable to short term horizons, but will provide a complete schedule for inventory management at the tank farm.

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The implementation of such models in real world scenarios requires attention on two essential points: system dynamics and results in real time. System dynamics motivates the development of a reactive scheduling procedure that receives an unforeseen situation and integrates it in a new schedule with minimum deviation from the previous one. On the other hand, to obtain results in real time it is essential to explore support techniques to reduce complexity, minimising the solution search effort, such as a initialization heuristic, that provides product sequences.

The integration of the current models enables the development of the decision support system architecture presented in Figure 1. Information can be used in a shared way to take advantages from the synergies between tools.

The real world scenario of CLC – Companhia Logística de Combustíveis has been used as a case study. This company transports six oil products from Galpenergia Sines' Refinery and distributes them in the central region of Portugal The distribution centre comprises twenty nine tanks, whose product service is fixed. The MILP models were implemented in GAMS/CPLEX and results were obtained for medium and short term horizons. The reactive scheduling has been used to address several types of occurrences, while a resource allocation heuristic provides pumping sequences. This framework is currently under development and testing, through a technological cooperation between Instituto Superior Técnico (IST) and CLC.

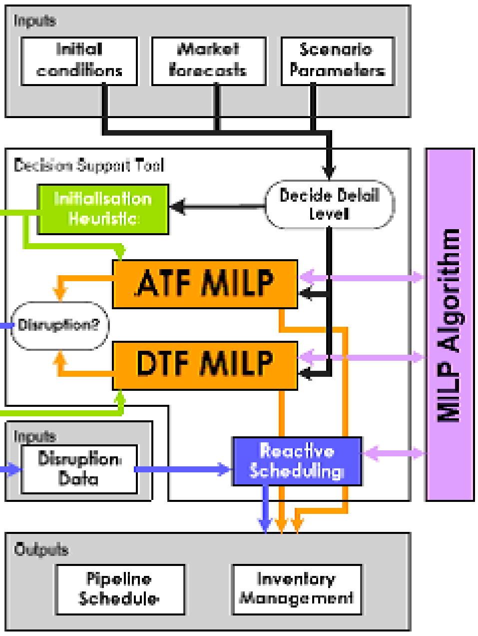

Figure 1 – Decision Support System Architecture

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Water use optimization in wine industry. Design of the water and the wastewater treatment network

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Keywords: water optimization, winery industry, effluent treatment, integer linear programming, water pinch

Nowadays water has become a very valuable resource, not only in those regions where it is scarce, but also in those where water is abundant. The origin problem is due to the contamination of natural water sources. In this context it is necessary to develop tools to allow for the design of urban and industrial water networks that optimize water use.

If the water requirements are minimized, the water effluent generation will decrease and natural water course contamination will be reduced.

In the last two decades, important research on water use optimization has been carried out Dhole et al.(1996), presented an alternative graphical method in which each operation can have one or more input and output stream with different flows and contaminant concentrations *(water source and demand composite curves)*. They called this water pinch analysis. The methodologies developed at that moment could not be applied to time dependent processes.

In 1999, Y.H.Yang et al. developed a non linear mathematical model to synthesize optimal effluent reuse networks. The necessary conditions to achieve the optimal water network design for single contaminant systems were introduced by Savelski and Bagajewicz in 2000. After that, Bagajewicz et al. developed an algorithm that combines the mathematical programming with the optimal statements, to solve water network design problems for multiple contaminant systems. Y.L.Tan et al., presented a new procedure implementing a *water cascade table*, to establish the minimum water requirement (MWR) and the minimum effluent generation. They also developed a systematic procedure to design water networks for batch processes.

In the recent past, research in batch processes has received increased attention. In 2005 Majozi presented a mathematical formulation for freshwater and wastewater minimization in multipurpose batch plants.

Batch processes are a common encounter in the production of specialty food and beverages, which are usually characterized by similar recipes that allow for equipment sharing. In San Juan, Argentina, one of the main industrial activities is the agroindustry, producing olive oil, wine, dried fruits, sweetmeats and canned goods. All of them are seasonal activities, characterized by multibatch processes, with peaks of water and energy requirements.

This study presents two parts: the design of the water and wastewater network. These proposals have been carried out in a winery of San Juan, Argentina. This industry is the most important of the region and has historically been a handicraft activity. In order to gain foreign markets, international norms and environmental regulations must be met. Wineries use large quantities of fresh water for cleaning tasks especially during the wine vintage, the season in which the largest amount of water is consumed in the entire region takes place. In addition to this, the fact that San Juan is arid, justifies the development of tools that lead to water savings.

The last investigations developed for batch process could be difficult to apply in wineries, at least in this region, where little engineering has been developed to this industry and the water-using operations are programmed only within the previous 24 hours, starting and finishing times of these activities are random.

This work presents a combination of the water pinch technology, which is a graphical tool, with the mathematical modelling, to synthesize the winery water network.

Firstly, the MWR and the water reuse possibilities are determined using the water pinch analysis. The water pinch analysis, allows the water use integration among the water using operation of the process and the determination of industrial water reuse possibilities. The results obtained are real only if the process is continuous, but as the case under study is a batch process, the results are not valid. In order to design a water network that can be apply on an industrial scale, those results are introduced in the mix integer linear programming (MILP) and finally the solution allows the optimal water network design.

A series of considerations, made to adjust the water pinch analysis results to discontinuous processes, yields the MILP. It is important to explain that among the four options to reduce the industrial water consumption, *process changes, reuse, regeneration–reuse and regeneration–recycle*, the only one considered in this study is *reuse*. The wastewater can be reused in other operations whenever the pollutants do not affect the reuse operations. The reuse diminishes the pure water consumption and the effluent generation but, does not modify the contaminant mass load. The results proved that a water reduction of almost 30 % can be achieved and, that by minimizing the water use, the effluent generation decreased.

Taking into account the wastewater and the results obtained in the previous work, in a centralized treatment system, even if two streams require different treatment operations, they are combined, and both streams undergo each treatment, increasing both capital and operating costs. In a distributed effluent treatment system, in contrast, the streams are segregated and treated separately (Mann G. and Liu Y., 1999). This part of the work attempts to demonstrate that a distributed effluent treatment network leads to reduction of the effluent flowrate to be treated in order to fulfill the permitted contaminant level for discharging in the sewage or a natural water course.

The water used in the food industry for cleaning and disinfection is contaminated to a greater or smaller degree, whereas the water used for heat exchange, remains almost the same as before. Therefore, it is convenient to separate the contaminated streams from the clean ones, to avoid useless and costly treatments of huge amounts of water. The present work combines this technology with an equation system that leads to design a distributed effluent treatment network for batch processes. The obtained network reduces the global treatment flowrate by collecting streams in different groups according to their contaminant concentrations and processing them separately.

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Optimal heat exchanger network synthesis including the detailed equipment design using particle swarm optimization

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Keywords: Pipeline, Inventory Management, MILP, Decision Support System.

Heat exchanger network (HEN) synthesis has been a well-studied subject over the past decades. Many studies and methodologies were proposed to make possible the energy recovery between process streams, minimizing the utilities consumption, the number of heat transfer equipment and the gaseous and liquid pollutant emissions from the combustible burn and water usage.

Most of these formulations, however, assume constant heat-transfer coefficients and countercurrent arrangement for all stream matches, which can lead to non-optimal results. Heat transfer coefficients are strongly influenced by the heat exchanger geometry and operational constraints given by standard design codes.

Some recent papers (Ravagnani et al. 2005a, Ravagnani et al. 2005b, Ravagnani et al. 2006) use Genetic Algorithms to solve the HEN synthesis problem and good results are found but with large computational effort.

In this contribution an optimization procedure for the synthesis of HEN including the detailed design for each heat exchanger in the network is proposed, based on the model presented by Ravagnani and Caballero (2007). The model takes into account the optimization of the equipments and their geometric and operational constraints from the synthesis stage. Shell and tube pressure drops and fouling are considered, as well as mechanical aspects, like shell and tube bundle diameters, internal and external diameter of tubes, number of tubes, number of baffles, baffles spacing, number of shells, tube length, tube pitch, tube arrangement and the fluid allocation in the heat exchanger.

As presented in Silva et al. (2008), the approach is based on the use of Particle Swarm Optimization to determine the HEN that minimizes the total annualized cost, accounting for capital costs of heat exchangers, and the energy costs for utilities and pumping duties, following the model proposed by Ravagnani et al. (2008). The algorithm combines two distinct models, a superstructure simultaneous optimization model for the HEN synthesis considering stream splitting and by-pass without isothermal mixing and a model for the detailed equipment design, following rigorously the standards of the TEMA.

Some examples from the literature were used to show the application of the proposed method, and the results confirm the achievement of the optimum HEN configuration with the detailed heat exchangers design, following the TEMA standards.

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Neural network control for riser- slugging suppression

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Keywords: Slug Flow, Neural Network, Nonlinear Model, Control Structure Design

The challenge of handling intermittent flow with liquid slugs followed by gas pockets in multiphase flow lines becomes more important when the number of satellite fields increases. Oil, gas and water are transported from the wells in some km long flow lines along the seabed and up through a riser to the oil rig. Slugging may have undesirable effects on the oil and gas production process. Severe slugging will affect the inlet separator liquid level, may give poor separation, and in some cases lead to separator flooding. The oscillating pressure may cause wear on processing equipment and will reduce the lifetime and increase the maintenance costs compared to production with even flow (Godhavn et al.,2005).

However, designing the topside facilities to accept these transients may require large and expensive slug catchers with compression systems equipped with fast responding control systems. Therefore, system design and methodology used to control or eliminate severe slugging phenomenon become very crucial when considering the safety of the operation and the limited available space on the platform (Mokhatab et al.,2007).

Fard et al.(2003) show that a cascade-control strategy with feedback from bottom pressure and flow rate at the top of the riser is best capable of suppressing the slugs. However this control strategy requires pressure measurements at the bottom of the riser. A simple nonlinear dynamic model with only three dynamic states is developed by Storkaas and skogestad (2002). This model covers both riser slugging, and, more importantly, the unstable but preferred non-oscillatory flow regime that exists at the same boundary condition. There is a great economic potential if slugging can be reduced or removed completely.

Artificial neural network designed is analytical tool that imitates the neural aspect of the human brain, whereby learning is based on experience and repetition rather than the application of rule-based principles and formulas. ANN provides capabilities such as learning, self-organization, generalization (response to new problems using incomplete information), and training (Hagan et al.,1996).

We have developed ANN control system for handling and suppressing riser-slugging and simulated this structure and compared the results with Storkass work.

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Use of Kohonen neural network in the sensorial analysis of *Malpighia glabra* L. wines

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Key-words: neural network, wines, sensorial analysis, Kohonen algorithm, *Malpighia glabra* L.

Artificial neural networks technology had been applied in simulation and optimization of several industrial processes (Kohonen, 1989). Actually, Kohonen algorithm was applied to help in the pediatrics diagnosis area, considering particular issues noticed in newborns and suckling, usually with non-specific clinical trial (Dias and Radonsky, 2003). This paper presents an approach of artificial neural networks to predict the sensorial quality of wines. A Kohonen network has been used as a software tool in order to increase the human skills in this kind of application. An initial prototype was implemented using the artificial neural networks technology, together with the Visual Basic software from Microsoft, for evaluation of sensorial qualities of seven samples of acerola (*Malpighia glabra* L.) wine. Fresh acerola juices were fermented by *Saccharomyces cerevisiae* yeast for 15 days. Sucrose and acerola mass were varied to obtain seven acerola wines with different compositions (Evangelista et al., 2005). 50 consumers chosen to perhaps had been used to obtain the sensorial data using a hedonic scale of 1-9 times (Teixeira et al, 1987). Sensorial values of flavor, aroma and appearance obtained of the hedonic dating were compared. The characteristic of wine, as flavor and color were similar to the acerola fresh fruit. The consumers evaluated the wines as very good, all sensorial qualities were more than 5 in hedonic scale. Results showed that Kohonen network classified the acerola wines in distinct group, for frequency among yours sensorial responses. Kohonen network results were similar to *t Student* classification, this show that the use of Kohonen algorithm in the sensorial analysis of wines is valid. Kohonen algorithm is very good in clustering the acerola wine samples and it uses in sensorial analyses of wines is promises.

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Study of aromatic recovery by extractive distillation

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Keywords: Sulfolane, Extractive distillation, Aspen Plus, NRTL, Aromatics

Abstract

The feasibility to use an extractive distillation process to replace liquid-liquid extraction unit (Arosolvan) at Galp Energia plant in Leça da Palmeira was studied. The simulations were performed using Aspen Plus® simulation modules, and NRTL model was used to predict thermodinamic data. Simulation results allowed to conclude that aromatics recovery increases comparatively to actual operation data. An economic study was performed determining the investiment and operating costs.

Introduction

Nowadays, Chemical and Petrochemical industries are facing a growing need for cheaper and higher quality raw materials. Aromatic compounds, such as benzene and toluene, are examples of such important feedstocks. Traditionally, these substances are produced from petroleum distillation cuts by liquid-liquid extraction, but extractive distillation has become a good alternative. Extractive distillation is the state of art of distillation since it allows to optimize the production and purity of the required products with inferior operation costs. The main difference between this technology and liquid–liquid extraction is the addition of a third component, a highly selective solvent that changes the separation between the substances to be separated, increasing relative volatility values.

The ultimate challenge for extractive distillation is to develop even more efficient solvents, as a way to maximize production capacities with reduced operation costs and lower environmental impacts. New solvent production technology has improved substantially, leading to the availability of new solvent mixtures and making the implementation of extractive distillation more practical and atractive.

The purpose of this project was to verify the aplicability of extractive distillation in Arosolvan Unit at the Aromatics Plant of Leça da Palmeira's refinery (Galp Energia), using Aspen Plus® simulator. A comparison between simulator's data and operating data from the refinery was also performed.

Simulation

The implementation of a extractive distillation process begins with the choice of an appropriate solvent. The main parameters to consider are its selectivity, stability and toxicity. After a literature review, the solvent *Sulfolane* was chosen. This solvent is already used by UOP company (www.uop.com) and it has the particularity to promote a good separation at acceptable costs for an industrial application.

The extractive distillation was designed and simulated using Aspen Plus® simulator, one of AspenONE ™ Process Engineering modules, developed by the AspenTech. The thermodynamic model used was the Non-Random-Two-Liquid (NRTL), found (Ko *et al.*, 2002) to give a better aproach to a real system .

The basic configuration of a extractive distillation process like shown in Figure 1 requires two separation columns, while the existing liquid–liquid extraction unit (Arosolvan) has three columns.

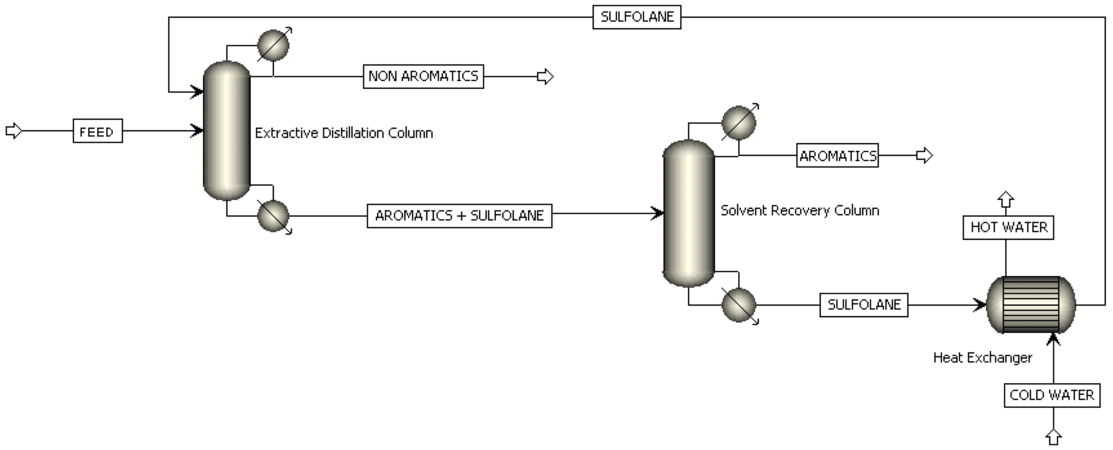

Figure 1 – Flowsheet of the extractive distillation unit, with *Sulfolane* solvent.

The first column (extractive distillation column), is where the separation of aromatics from the non-aromatics occurs. In the second column (Solvent recovery column), the separation of the aromatics from the solvent is achieved.

As a way to estimate the dimensions of the columns, DSTWU model was used. This module estimates reflux ratio for a known number of trays, as well as the feed tray and energy consumptions in the condenser and reboiler. After this initial simulation, products composition were determined and operation conditions were optimized, using the rigorous model RadFrac.

From the computed results, the aromatics recovery percentage was calculated and compared to actual data from the refinery. Table 1 shows the recovery fractions.

Table 2 – Recovery fractions.

Compound	Total Recovery [%]	
	Actual	Proposed alterations
Toluene	90,026	99,996
Benzene	88,215	99,339
N-heptane	57,062	99,138

Conclusions

The Sulfolane process was simulated with Aspen Plus, a sequential modular simulation software, using NRTL model. With the proposed process we achieved a 99% compounds recovery with a total investment amounting to 6 000 000 euros.

Acknowledgment

This work was carried out under the gratefully support and cooperation of Galp Energia.

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Pore size distribution of baker's yeast evaluation using x-ray micro-tomography

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Keywords: Yeast, drying, microtomography, porosimetry.

Granular baker's yeast is industrially dried to reduce transport costs and augment conservation time. However, this process is energy intensive and may cause viability loss of the product (Bayrock and Ingledew (1997a)). Therefore drying has to be well understood and controlled. Most modelling approaches applied to yeast drying hide inner-grain drying in fitting coefficients or in desorption isotherms. Such approaches neither allow correct understanding of viability loss mechanism nor offer a general explanation of the observed drying rate curves (Debaste et al. (2008)). Inner-grain phenomena modelling, whether trough continuous or discrete approaches, requires knowledge of the porous structure of the solid.

For *Saccharomyces cerevisiae*, a living organism, classical intrusive pore size identification methods, such as mercury porosimetry, gives doubtful results. Indeed, although yeast is known to survive to very high pressures (Espinasse et al. (2007)), little is known about its reaction to contact with high pressure mercury.

In this work, identification of a network representing accurately the solid is attempted. The goal is to have a network suitable for pore network simulation of the drying in grains (Metzger et al. (2007)). However, in this work, the network is used to compare microtomographic results to mercury porosimetry.

The construction of the network is based on a x-ray microtomographic investigation of a yeast grain. This technique has already been shown to be an alternative to mercury porosimetry for foam materials shrinking under the pressure (Léonard et al. (2008)). This analysis method offers a non-intrusive three dimensional image of the grain. The image treatment used to deduce a complete network includes several steps (Al-Raoush and Willson (2005)). First of all, image has to be segmented: every pixel, depending on its grey level, has to be identified as belonging to solid or void. Then network topology is deduced by calculating the medial axis of the void space. Pores (volumes of fluid) and throats (locations of the resistance to flow) dimensions are finally calculated. This complete algorithm and its different parameters are tested on a virtual void space, composed of the overlapping of octahedron of distributed size.

Thereafter, yeast porous network is calculated. The results directly offers pores and throats size distributions. Also the network can be used in pore network modelling to simulate the porosimetry experiment (Kamath et al. (1998)). Comparison between experiment and simulation is presented on the figure 1. The two curves are totally different. The mercury porosimetry shows continuous invasion for 3 orders of magnitudes of pores while the model based on the x-ray microtomograpic measurements presents a sharp s-curved characteristic of a uni-modal distribution. This last distribution suffers from the limits of resolution of the micro-tomographic apparatus used. Although further analysis with micro-tomography should be undergone to fully describe the porous morphology of the sample, the presented results already assess the inadequateness of the mercury porosimetry for evaluating the granular structure of yeast. The network may also be used to realize grain drying simulations.

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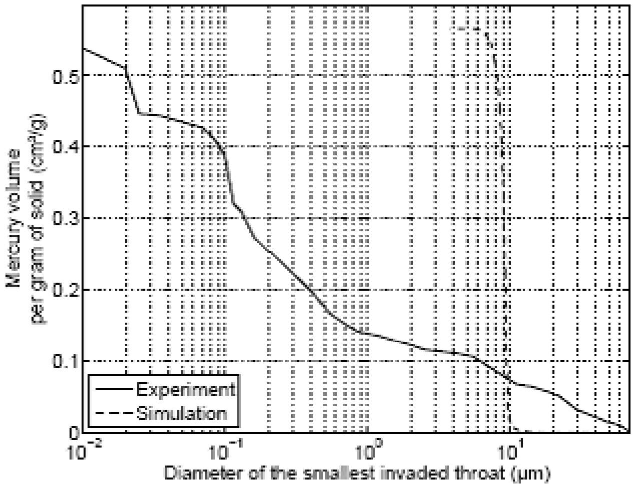

Figure 1: Comparison between experimental and simulated mercury porosimetry.

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Drop distribution determination in a liquid-liquid dispersion by image processing

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Keywords: liquid-liquid systems, image processing, drop size distributions

Image processing is a very relevant area of computer science with applications in many domains. Quantitative analysis and interpretation of digitized images is currently an important tool in several scientific domains.

Namely in multiphase systems modelling in Chemical Engineering, the acquisition and treatment of images of particulate phases become essential for the calculation of particle size and shape distributions. In the modelling of liquid-liquid systems these are of major importance either for hydrodynamic and mass transfer (with or without chemical reaction) modelling and validation. From these arise simulation, interpretation and performance predictions of multiphase reactors

Often, such a treatment is undertaken by visual/manual techniques which imply high costs, intensive labour, weariness build-up and consequent high error rates, while a fully automated computational approach has a definite potential for better performance.

The aim of the present work is the development of shape discrimination and size classification algorithms for liquid drops in monochromatic digitized frames.

Image frames were obtained at our SIPROM (Modelling and Simulation of Multiphase Systems) Laboratory with a digital video camera + microscope + illumination set-up from a dispersion of toluene in water within a transparent mixing vessel (Ribeiro, 2002). Experimental conditions (mainly phase ratio and agitation speed) led to frames of deeply focused fields with partially overlapping drops and high background noise. Since non-invasive image acquisition and lighting were our uncompromising starting option, no significant improvements were obtained although all available image acquisition and cleaning techniques were used within this fundamental constraint. Thus, partial images and ill-defined drop boundaries led to all low-cost, market-available image processing software packages requiring a high number of frames in order to secure statistically significant drop samples which, again, made for greatly time-consuming, unreliable procedures

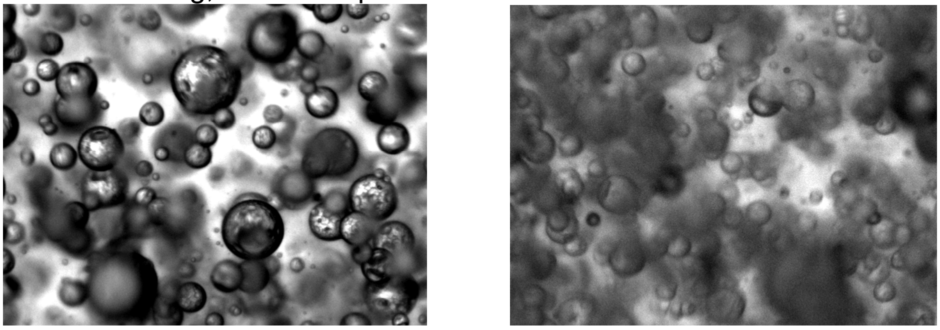

Figure 1 - Typically images [A - very good pattern image (12 drops measured) and B - difficult pattern image (4 drops measured)].

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This led us to develop our own image processing software for which a preliminary test and calibration stage was performed on archive images and drop size distributions from a previous semi-automated procedure (Ribeiro et al. 2004). This has shown that scanning and processing a high definition frame for drops of all sizes between frame and a few pixels size was still a lengthy process, not much faster than the traditional visual/manual ones, human fatigue saving being the single significant advantage.
We are now testing a promising approach (implemented in *Matlab*), which, given one of these photographic images of a dispersion, automatically identifies the contour of existing drops and classifies them according to their diameter.

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Bright field versus phase contrast microscopy in activated sludge image acquisition methodologies

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Keywords: Activated sludge, image analysis, bright field, phase contrast, aggregates, filaments

An activated sludge system includes a complex ecosystem composed of different types of microorganisms such as protozoa, metazoan and filamentous or zoogleal bacteria, and a good balance between the different types of microorganisms is essential to guarantee good settling properties and a clear supernatant. Generally, the evaluation of aerated tanks may be estimated by visual inspection under an optical microscope coupled to automated image analysis methodologies. As a matter of fact, in recent years, activated sludge processes have been monitored through microscopic observations for aggregates contents and morphology and protruding filamentous bacteria contents determination (da Motta *et al.*, 2001).

Automated image analysis is considered to be a feasible method to characterize quantitatively both aggregated and filamentous bacteria, with two image acquisition methodologies standing out: phase-contrast microscopy as proposed in the works of Cenens *et al.*, 2002 and Jenné *et al.*, 2007 among others; and bright field microscopy as in the works of da Motta *et al.*, 2001, and Amaral and Ferreira, 2005. In comparison, bright field microscopy is the cheapest and simplest activated sludge examination methodology, whereas the phase-contrast microscopy requires more expensive equipment and a skilled operator. Furthermore, the inner nature of the phase contrast microscopy causes the aggregates borders to become ill-defined, as the objects hallo hinders the assessment of their boundaries. However, this methodology presents the advantage of a more precise determination with respect to the protruding filamentous bacteria contents. As a matter of fact, the high transparency of the filamentous bacteria poses a contrast problem in bright field microscopy acquisition, opposite to the clear filaments/background distinction in phase contrast microscopy. Therefore, it comes as no surprise that studies have already been performed using bright field acquisition methodologies to survey aggregated biomass and phase contrast acquisition for filamentous bacteria assessment (Amaral, 2003).

In order to determine the best image analysis acquisition methodology of activated sludge samples, the present work aims to determine both protruding filamentous bacteria and aggregated bacteria contents using, on one hand, bright field microscopy and, on the other, phase contrast microscopy. For that effect, activated sludge samples were collected from the aeration basins of seven domestic wastewater treatment plants located in the North of Portugal

Bright field microscopy: All images were acquired in a *Leitz Laborlux S* optic microscope (*Leitz*, Wetzlar), with 100x magnification, coupled to a *Zeiss AxioCam* (*Zeiss*, Oberkochen). Image acquisition was performed in 1300 x 1030 pixels and 8-bit format through the commercial software *Axio Vision 3.1* (*Zeiss*, Oberkochen).

Phase contrast microscopy: All images were acquired in a *Diaphot 300* microscope (*Nikon Corporation*, Tokyo) with 100x magnification, coupled to a *Sony CCD AVC–D5CE* (*Sony*, Tokyo) grey scale video camera. The images were acquired in 768×576 pixels and 8-bit format by a *Data Translation DT 3155* (*Data Translation*, Marlboro) frame grabber using the commercial software package *Image Pro Plus* (*Media Cybernetics*, Silver Spring).

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The analysis of bright field and phase contrast microscopy raw data allowed establishing the best acquisition methodology for both aggregated and protruding filamentous bacteria by comparison of the total filaments length per volume (TL/Vol) and total aggregates area per volume (TA/Vol) values for each methodology. Furthermore, and supported on the study of Amaral and Ferreira (2005), two parameters characterizing the aggregates and filaments dynamics were also included in this study: total filaments length per total aggregates area ratio (TL/TA) and total filaments length per total suspended solids (TL/TSS). Figure 1 represents the relationship between TL/Vol and TL/Vol for both bright field and phase contrast methodologies.

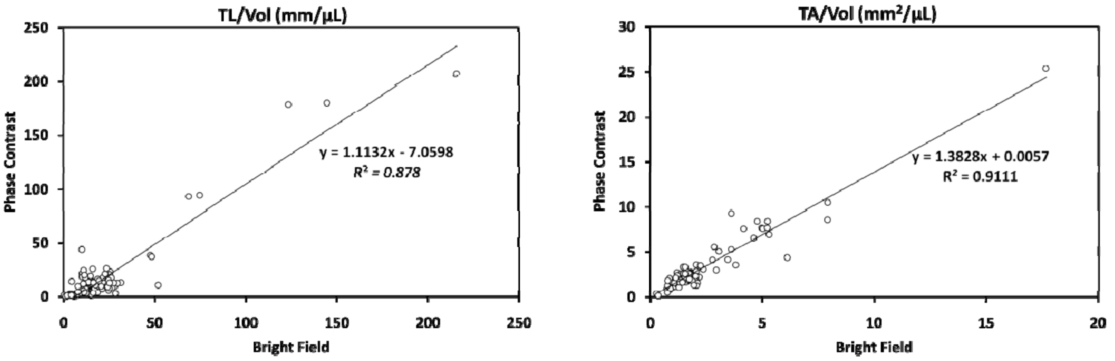

Figure 1. Bright field and phase contrast results for TL/Vol (a) and TA/Vol (b).

The results herein reported in Figure 1, revealed satisfactory correlation coefficients for TL/Vol (0.9370) and TA/Vol (0.9545), with a slight dispersion between bright field and phase contrast assessment. Taking into consideration other studies developed by our research group, the inclusion of TL/TSS and TL/TA parameters is of utmost importance, allowing to assess filaments and aggregates contents collected from different aerated tanks. Once again, a satisfactory correlation coefficient of 0.9229 was obtained for TL/TSS ratio. However, the poor 0.8373 correlation coefficient achieved for TL/TA might be due to the cumulative sum of impreciseness both in terms of TL and TA, resulting also in a strong overestimation of this parameter in phase contrast acquisition.

This study clearly demonstrated that, with respect to the protruding filamentous bacteria, the bright field acquisition results mimic, at a certain extent, the phase contrast results. However, the inverse relationship does not hold true for the aggregated biomass contents assessment for the phase contrast acquisition. In fact, it was found that this methodology overestimates by 38% the results obtained by the bright field approach. Thus, considering the advantages and disadvantages of each acquisition methodology and the obtained results, bright field microscopy proved to be not only more simple and inexpensive but also providing the best overall results.

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Multivariate monitoring of an activated sludge process for biological treatment of a synthetic wastewater effluent

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Keywords: activated sludge, monitoring, principal component analysis, spectroscopy

To face the steadily increasing global water problems, the search for alternative effective ways to monitor biological wastewater treatment processes is essential when changes should be detected as soon as possible so measures could be taken accordingly to avoid major damage to the biological process and to the environment. Besides the need for rapid monitoring, these techniques should have no addition of chemicals, avoiding its consumption, its emission to the wastewater and transportation for adequate treatment of the residues resulting from analysis.

Multivariate statistical tools and spectroscopic techniques are among the present valuable alternatives for these purposes. Besides the advantages already referred, spectroscopic techniques can be used without any sample pre-treatment, what makes them interesting for online monitoring. In this work they were applied to monitor a lab scale plant treating synthetic wastewater. The system was disturbed to promote changes that simulate real possible scenarios in order to verify if the technique can efficiently detect the induced variations.

Experiments were carried out in an activated sludge plant consisting of a 25 L tank with 17 L of suspended biomass and a cylindrical settler of 2.5 L, with recirculation of biomass from the settler to the tank using an air pump. The complete mixing inside the reactor is guaranteed by the diffusion of air from its bottom. During the monitoring period the concentration of dissolved oxygen was maintained in excess. The reactor was inoculated with biomass collected from a municipal WWTP designed to a population of 214.605 inhabitants. Synthetic wastewater was prepared with a mixture of peptone and meat extract as carbon source, urea, K_2HPO_4, NaCl, $CaCl_2$ and $MgSO_4$.

During the monitoring period, influent flow (Q_{in}), COD_{in}, COD_{out}, TSS and VSS in the reactor and in the effluent, $N\text{-}NO_3^-$ in the effluent and pH in the reactor were measured.

$N\text{-}NO_3^-$ was measured using HPLC and remaining parameters were analyzed according to Standard Methods. Each sample was analyzed in triplicate. An immersible probe and a USB4000 portable dispersive UV-Vis detector, both from Ocean Optics, were used to acquire spectra in the settler in the wavelength range from 230 to 700nm. In order to create system imbalances the process was disturbed by i) slight increase of the organic load (with simultaneous decrease of HRT and increase of COD_{in}); ii) a systematic removal of sludge from the reactor after a long period without purging biomass from the system; and iii) sudden decrease of HRT, by this order. Principal Component Analysis (PCA) was used to analyze all data. PCA is a linear mathematical method used to find patterns in large data matrices. The algorithm reads data as vectors in a multidimensional space and seeks the direction (vector) in which the variance is the largest. These vectors are designed principal component (PC) vectors and are usually numbered (PC1, PC2, ...) in order of importance. Typically, the variance in the data set is modeled within the first few PCs, depending on the complexity of the sample matrix. This operation reduces the number of vectors needed to describe the variance in the matrix. The resulting PCA matrix can be plotted and studied in order to identify clusters of samples and time dependent variations.

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PCA was applied to the auto-scaled set of eleven variables measured during the monitoring period such as: Q_{in}, COD_{in}, COD_{out}, Organic Load, TSS_r and VSS_r in the reactor, TSS_{out}, VSS_{out}, food-to-microorganisms ratio (F/M), $pH_{reactor}$ and $N\text{-}NO_3^-{}_{out}$. With two principal components (PCs) it was possible to explain 71.70% of the variation in the data. Figure 1a shows the biplot obtained presenting in the same graph the samples and the measured variables.

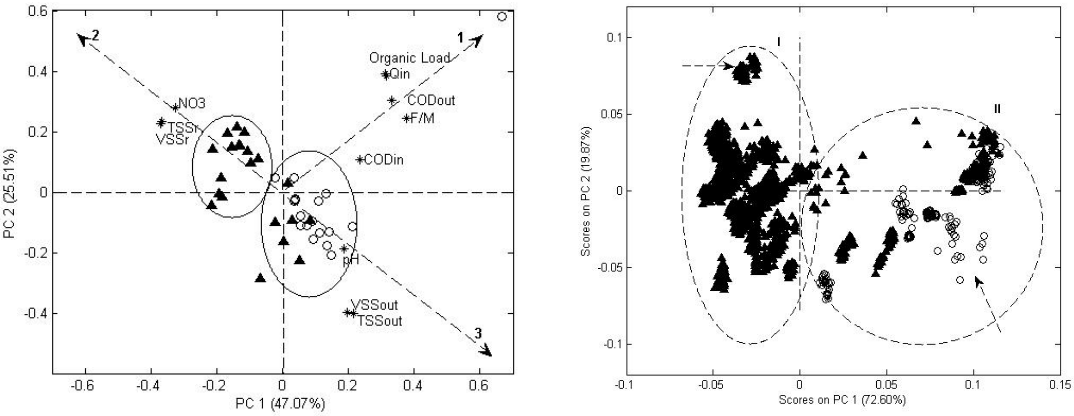

Figure 1. (a) Biplot representing simultaneously the samples and the variables measured during the monitoring period. (b) Score plot representing the UV-Vis spectra variation during the monitoring period. Symbols are explained in the text.

The major advantage of the biplot representation is the possibility of establishing relations between samples and variables. Moreover it is possible to identify how the different variables are related with each other. In this case samples are divided in two main groups – I and II – corresponding to the first and to the second monitoring period respectively. Group I is characterized by a higher nitrification rate, when disturbances i) and ii) were imposed, while group II is associated to a low or even inexistent nitrification process, when disturbance iii) was applied and purges were more frequent. It is possible to identify some samples measured during the first monitoring period in group II. These samples correspond to the analysis performed during the system start-up and after purging biomass from the reactor. In both cases the nitrification rate was low what explains this displacement. Attending the variables, these are also divided in groups according to their influence in the system. It is interesting to notice how PCA can easily identify the close relation between nitrate and biomass concentration in the reactor and how the presence of nitrate is inversely related with the pH in the reactor. The monitored samples are placed along the arrows #2 or #3 depending if they correspond to a high or low nitrification period, respectively. The organic load increase induced during the first period is only slightly noticed by a displacement of the samples in the direction of the arrow #1. However, the influent flow increase during the second monitoring period is clearly identified in the right upper zone of the figure. This result can be explained by the fact that the disturbance imposed to the process in the first period was less significant when compared with the one induced in the second monitoring period. Similar analysis is achieved when spectra are also analyzed with PCA. Spectra were pre-treated by applying a first derivative and mean centering the raw data. With two PCs it was possible to explain 92.5% of the data. The score plot obtained is shown in Figure 1b. Once more the two periods mentioned earlier are clearly distinguished. Spectra acquired during the two different organic load conditions induced to the system – i) and iii) – are indicated by dashed arrows in the score plot. This study points out that spectroscopy associated with chemometrics are potential tools to efficiently, rapidly and intuitively follow-up variations in the system in an equivalent way to the use of different monitoring parameters.

Usefulness of PCR-DGGE for monitoring lactic acid bacteria succession during fermentation of grass silage

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Keywords: PCR-DGGE, silage, LAB, *Lactobacillus plantarum*

Introduction

Ensiling herbage with the aid of lactic acid bacteria (LAB) is a widely used practice for conserving forages to produce feedstuff for animal consumption. In fact, there are many starter cultures comercially available to improve naturally occurring fermentation. These products usually contain *Lactobacillus plantarum*, a homofermentative LAB that occurs naturally on herbage, which is expected to control microbial shifts during the ensiling process (1).

Although numerous studies had been carried out to follow changes in microbial population, they are based on the utilization of conventional cultivation methods which are laborious and time-consuming. For these reason, molecular based methods, such as PCR-DGGE, have provided a powerful tool to analyse complex populations without using culture-dependent methods.

In this work a procedure based on PCR-DGGE method is presented to follow the diversity and the dynamics of the LAB population in ryegrass silages inoculated with and without *Lactobacillus plantarum* at different fermentation times.

Materials and methods

Silage preparation and herbage inoculation

Silages were obtained by packing 100 grams of fresh chopped ryegrass (*Lolium perenne*) into polythene bags, which were then vacuum-sealed using a vacuum-packaging machine at its maximum speed. Samples were taken at 1, 2, 5, 8, 15 and 35 days of ensiling, after incubation at 25±2ºC.

Pure cultures of *Lactobacillus plantarum* 8.01 were prepared in MRS broth (Cultimed, PANREAC), at 30ºC for 12 h, from freezed cells after two successive transfers. Cultures were then centrifuged and washed twice with sterile physiological saline solution and finally resuspended in an identically volume of saline solution.

The inoculum was applied as a fine spray and the herbage was thoroughly mixed before packaging. A batch sprayed with saline solution was also prepared in order to serve as a control of the spontaneous fermentation.

DNA extraction and PCR amplification

The total DNA of the fermentated products was extracted using a method based on the phenol:clorophorm isolation method, from 10 mL of silage homogenate which was centrifuged and finally resuspended in 1 mL of PBS (phosphate buffer saline).

The extracted DNA was used as the template in PCR amplification and the kit Pure Taq Ready-To-Go PCR Beads (Amershan Biosciences) was utilized to minimize the variation among the different samples. The primers used for amplifying the V3 region of 16S rDNA were 357F-GC and R518.

Initial DNA denaturation was performed at 95°C for 5min, followed by 35 cycles of denaturation at 94°C for 30s, annealing at 56°C for 30 s, and elongation at 68°C for 40s, and then a final elongation at 68°C for 10min.

Denaturing gradient gel electrophoresis (DGGE) analysis of PCR products

We performed DGGE on a Dcode system (Bio-Rad). 16S rDNA fragments were loaded onto a 8% polyacrylamide gel, which was made with a denaturing gradient ranging from 35% to 55% (previously optimized). The denaturant (100%) contained 7 M urea and 40% formamide. Electrophoresis was run in 1xTAE buffer at 60ºC, for 16 hours at 75 V. After electrophoresis, gels were stained with ethidium bromide.

Results

Gels were digitally photographed with UV transillumination and analized using the software AlphaEaseFC Imaging (Alpha Innotech).

The appearance and disappearance of the amplicons in the DGGE profile indicate important shifts in the microbial community structure (2). In general, the profiles obtained showed a significant reduction on the number of species observed at the beginning of fermentation, independently from the type of silage considered. In fact, on inoculated silages the population was basically reduced to *Lactobacillus plantarum*, which was the predominant specie. This fact can be due to the acidification of the environment which causes that only certain species, resistant to low pH, survive leading to a reduction on the total microbiota.

The intensity of an individual band is a semiquantitative measure for the relative abundance of this sequence in the population (3). Thus, the great intensity of the band corresponding to *Lactobacillus plantarum*, even on spontaneous fermentations, shows that this was the most abundant specie strongly suggesting its direct participation on the fermentation, being probably responsable of the drastic pH drop observed. We also found that the relative intensity of this band increased during the ensiling process which can be correlated to the increase on the LAB counts (data not shown), mainly on inoculated silages with Lb 8.01.

Both facts, the prevalence of *Lactobacillus plantarum*, which was a minoritary group on the fresh herbage, and the dissapearance of some species which were initially present on the grass, show the shift of certain initial microbiota caused by the presence of *Lactobacillus plantarum*. This is a well known fact obseved on spontaneous lactic acid fermentations, where a minoritary population, by a complex mechanism, becomes the predominant specie.

In conclusion we found that, as PCR-DGGE analysis has a high sensitivity for detecting sequence differences, it is also an useful tool to study the diversity of lactic acid bacteria on complex microbial populations such as silages. It is also helpful to asses the presence of a specific bacteria or group of bacteria and to follow its behaviour during the ensiling process.

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Thermolithography, a rapid prototyping process: Influence of physical and operational parameters

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Keywords: Rapid Prototyping, Thermolithography, Heat Transfer, Simulation.

Rapid Prototyping (RP), or solid freeform fabrication as it is also known, is a technology that fabricates models and prototype parts from geometric computer data, building parts by selective accretion of material layer by layer. The layers are obtained by converting a 3D CAD model into thin parallel slices bounded by their contours. With the RP technology, a part can be produced directly from the CAD data much faster than conventional methods.

Thermolithography (IRTL) is a Rapid Prototyping process which applies infrared laser to cure thermosensitive resins in order to get three-dimensional objects. IRTL is original from State University of Campinas (Unicamp), Brazil The IRTL apparatus is shown in Figure 1. The initial phase of IRTL consisted on the production of small three-dimensional pieces with no contraction and eliminating the post-curing extra treatment (Scarparo *et al.*, 1992; Barros, 1993).

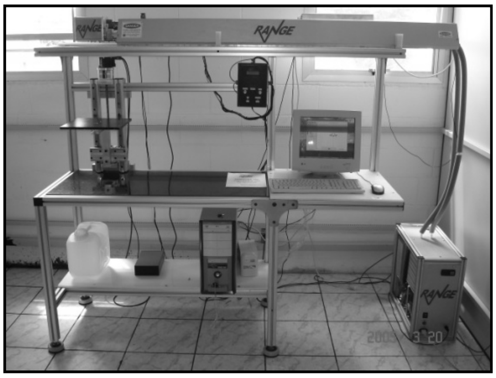

Figure 1. Nowadays IRTL apparatus.

Many cases can present difficulties in producing models because of the contraction suffered by the prototype and of the additional treatment of post-curing, besides the high cost of equipments and materials used. However, the Thermolithography prototypes show good spatial resolution, do not need post-cure treatment, absence of contraction, benefiting the localized cure and final shape of the projected object, besides high mechanical stiffness. The employed laser is CO_2 type with wavelength (λ) equals to 10.6μm.

The quality of final IRTL prototype is guaranteed if the cure phenomenon is localized during the energy transfer from laser to the standard sample. The localized cure consists on the solidification, at the right proportion, only in the region of the polymeric sample irradiated by the laser beam comprehended within the area defined by the beam diameter with an optical absorption depth δ in the resin, generating a reached volume. The standard sample is composed by epoxy resin, diethylenetriamine and silica in an exact stoichiometry. The heat transfer of this process has been studied. A mathematical model containing the equations that govern the heat transfer problem in the material (see equation 1), an overview about the involved parameters in the process and the contour conditions that are applied and analyzed (Scarparo *et al.*, 1992) has been explored. This model was adapted to ANSYS syntax and simulations were performed to show the influence of laser parameters (operational) and of sample composition (physical) variations as in Figures 2 and 3 (Rezende, 2006).

$$\rho \cdot c_p \cdot \left(\frac{\partial T}{\partial t} + V_x \frac{\partial T}{\partial x} + V_y \frac{\partial T}{\partial y} + V_z \frac{\partial T}{\partial z} \right) = \overset{\bullet\bullet\bullet}{q} + \frac{\partial}{\partial x}\left(k_x \frac{\partial T}{\partial x} \right) + \frac{\partial}{\partial y}\left(k_y \frac{\partial T}{\partial y} \right) + \frac{\partial}{\partial z}\left(k_z \frac{\partial T}{\partial z} \right) \quad \textbf{(1)}$$

where,

ρ	Specific Mass of the material
c_p	Specific Heat
T	Temperature
t	Time
$\overset{\bullet\bullet\bullet}{q}$	Heat Generation Rate
k_x, k_y, k_z	Conductibility of material in directions x, y, z

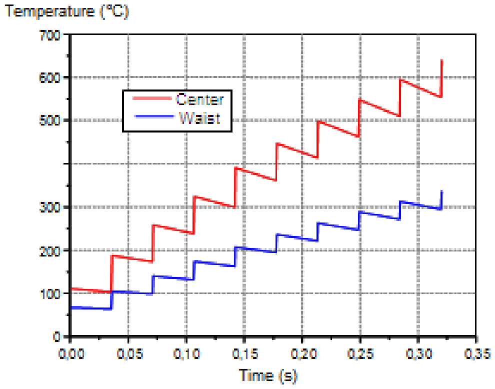

Figure 2 – Laser beam diameter d=0,8mm and laser beam scan v=1,60m/s.

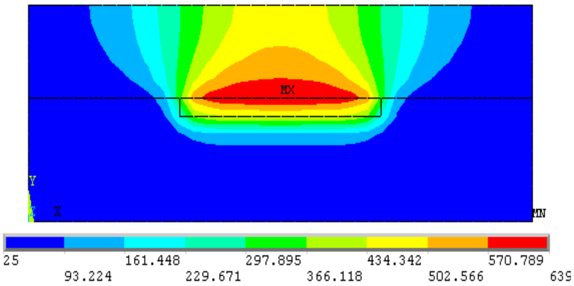

Figure 3 – The respective temperature spatial distribution profile.

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Functional screening of a metagenomic library obtained from soils and water of S. Domingo's mine

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Keywords: metagenome, heavy-metal resistance, 16S rDNA

Despite the dominance of microorganisms in the biosphere, relatively little is known about the majority of environmental microorganisms, largely because of their resistance to culture under standard laboratory conditions. As such, alternative approaches are required to assess the large amount of information in the environmental metagenome.

An often-cited estimate is that as much as 99% or more of microbial life remains unculturable, and therefore cannot be studied and understood in a way that microbial ecologists have become accustomed to over the past century. Although the portrait of the microbial world was revolutionized by analysis of 16S rRNA genes, such studies yielded only a phylogenetic description of community membership, providing little insight into the genetics, physiology, and biochemistry of the members. Metagenomics provides a second tier of technical innovation that facilitates study of the physiology and ecology of environmental microorganisms. The composition of microbial populations of tailings waste at the abandoned São Domingo's copper mine was investigated, and the ability of pure and mixed cultures of indigenous microorganisms to reduce sulphates was examined. These approaches revealed new genes and gene products that may have a major role in biomining processes.

Materials and Methods

Samples of soil and water from an abandoned cupric pyrite mine (Mina de S. Domingos, Southeast Alentejo, Portugal) were used in batch assays to study the sulphate reducing ability of microbial population in presence of zinc, copper and iron. The inoculum (OI) (200 ml) was grown 15 days in (g l^{-1}): 1.2 Na_2SO_4; 0.16 $CuSO_4.5H_2O$; 1.245 $FeSO_4.7H_2O$; 0.33 $ZnSO_4.7H_2O$; 0.3 $Na_3C_6H_5O_7$, pH 6.0, final volume 600ml (BI). This culture (200 ml) was then subjected to a double concentration of the metals (same volume) used previously, during 1.5 months (BII).

Separation of whole DNA from samples of each batch assay including original inoculum (OI, BI, and BII) was performed using UltraClean soil DNA isolation Kit (MO BIO).

After isolation of gDNA, PCR was performed using 16S rDNA primers for bacteria and archaea, primers 1510R and 1492R respectively, (Hugenholtz and Goebel, 2001). The amplicons were inserted in pJET plasmid (CloneJET PCR cloning kit, Fermentas) and a metagenomic library was constructed with recombinant DH5a cells with clones with partial 16S rDNA gene that were sequenced by an ABI PRISM 310 sequencer (Applied Biosystems).

In order to assess the effect of combined contamination of heavy metals on soil bacterial communities using genetic community fingerprinting by 16S rDNA profiles and heavy-metal resistant genomes, PCR with primers specific for heavy-metal resistance genes was carried ou - sections of mercury resistance determinant on the transposon Tn501, *mer*1, the *czc* gene cluster of plasmid pMOL30, that ensures resistance to Cd $^{2+}$, Co $^{2+}$ and Zn^{2+}, *czc*A,B, system of copper sequestration, with copper metallothionein *CuBP* and the copper resistance determinant on the plasmid pRJ1004, *pco*A and finally the genetic system for arsenate resistance, arsenate reductase, arsC.

Results

Results obtained showed the amplification of genes correspondent to *CuBP* and *ars*C in all batch assays. However, analysis the sequences obtains from the metagenomic library constructed with each pool of DNA revealed that microbial population suffers adaptive events that have reduced the strain heterogeneity of the community. These results can be explained by the effect of selective pressure caused by metal concentration of culture media, in the dynamics of microbial population. Analysis of sequence data is still being processed.

Samples	Primers		Homology (%)
BI	*ars*C	*Nitrobacter hamburgensis*	89
		Delftia acidovorans	80
		Ralstonia pickettii	81
	CuBp	*Novosphingobium aromaticivorans*	92
		Sphingomonas sp.	88
		Pseudomonas sp.	79
		Burkholderia multivorans	78
BII	*arsC*	*Ralstonia pickettii*	82
	CuBp	*Pseudomonas sp*	75
		Gluconacetobacter diazotrophicus	
BI	*1510R*	*Syntrophus aciditrophicus*	92
	1492R	*Mannheimia haemolytica*	79
BII	*1492R*	*Desulfovibrio desulfuricans*	82

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Computer-assisted structural and adsorptive characterization of carbon nanotubes for adsorptive and environmental applications

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Keywords: nanotechnology, gas adsorption, SWCNT characterization, molecular simulation

Many potential applications have been proposed for carbon nanotubes, including conductive and high-strength composites; energy storage and energy conversion devices; sensors; field emission displays and radiation sources; hydrogen storage media; and nanometer-sized semiconductor devices, probes, and interconnects (Baughman et al., 2002). Some of these applications are now realized in products. Others are demonstrated in early to advanced devices. Nanotube cost, polydispersity in nanotube type, and limitations in processing and assembly methods are important barriers for some applications of single-walled nanotubes (SWNTs). In particular, the application of SWNTs as adsorbents, catalyst supports, membranes and related materials, may be realized by availability of more detailed information on the porosity and adsorption mechanisms of these materials.

Despite considerable effort, the interaction between organic molecules and nanotubes remains largely unexplored. We have successfully investigated the adsorption mechanisms of various organic molecules on single-walled carbon nanotube (SWNT) bundles by molecular simulation and experimentally, including adsorption equilibrium measurements and Raman spectroscopy (Agnihotri et al. 2007, 2006, 2006a).

Here we report quantitative data on several structural and surface characteristics of SWNT samples, such as specific external surface area of the bundles, hydraulic mean diameter of the bundles, fraction of nanotubes which are open-ended and unblocked, and discuss their specific influence on the adsorption mechanisms for several gaseous adsorbates supported on experimental measurements of their adsorption equilibria. Our analysis has been thoroughly validated experimentally for various probe molecules such as nitrogen, alkanes, alkenes, and other organic molecules.

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Equilibrium and kinetics modelling of separation of Cr(III)/Cu by a chelating resin from industrial effluents

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Keywords: Cr(III) removal, Chelating resin, Equilibrium, Kinetics

The environmental contamination with heavy metals may create acute or chronic toxicity problems, which should be avoided when ever is possible. Indeed, although heavy metals could be found naturally in soils, sediments, water and even in living organisms, anthropogenic releases can increase its concentration to higher concentrations. The wastewater from electroplating industry was tested in this study, since it may contain large number of metals, including chromium, copper, nickel, zinc, manganese and lead. The specific contaminants addressed in this work were chromium and copper.

The chromium was selected mainly due to its high concentration in electroplating effluents, commercial value and also based on health concerns (hexavalent chromium is potentially carcinogenic when inhaled). Copper is in general a heavy metal of serious concern because of its toxicity to aquatic life.

This study is part of a more global project which has as main objective the integration of ion exchange process with nanofiltration in order to recover at least one valuable metal (Cr), and produce low level of liquid effluents.

The ion exchange process showed to be a promising alternative technique to the traditional ones (precipitation coupled with filtration) for removal and recovery of valuable Cr. In this study the performance of a chelating exchange resin (Diaion CR11) was evaluated for a system with two species: Cr(III) and Cu. In order to assess the sorption capacity of the resin some equilibrium experiments were carried out, as well as transient column tests for understanding the kinetic behaviour of the process. The experimental results were very motivating, and are now complemented with the modelling approach, since it allow the enhancement and the understanding of the global process.

In this study, the Langmuir model was used for describing the experimental equilibrium ion-exchange data obtained in batch experiments.

For describing the dynamic behaviour of the fixed bed adsorption process, a model was developed for the saturation step, in order to predict the species concentrations in the solution, at the outlet of the column, as a function of time (breakthrough curve). The mathematical model includes pore diffusion, film mass transfer resistance and axial dispersion. The numerical solution of the system of equations, subject to initial and boundary conditions, was performed by discretizing in the radial direction the equations representing the mass balances inside the particles using orthogonal collocation in NE finite elements with cubic Hermite polynomials as basis functions[1]. This led to a new boundary value problem of N_{comp} (2NE +1) [N_{comp} is the number of components] parabolic PDE's in the independent variables (reduced axial coordinate and reduced time) that was numerically integrated with the PDECOL package.

Figure 1 (a)-(b) shows that Langmuir model fits very well the equilibrium experimental data, for the conditions tested and for both metals (Cr and Cu).

Figure 2 illustrates as an example that the mathematical model tested predicts satisfactorily the observed experimental results, even taken into account the additional complexity of considering multicomponent system with two species (Cr and Cu).

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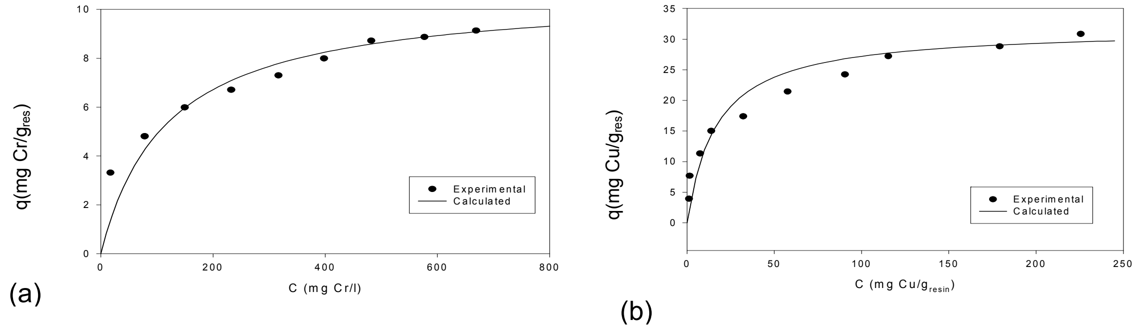

(a)

(b)

Figure 1- Adsorption equilibrium model prediction based in Langmuir equation of experimental data obtained in batch experiments for (a) – Cr(III) ; (b) – Cu.

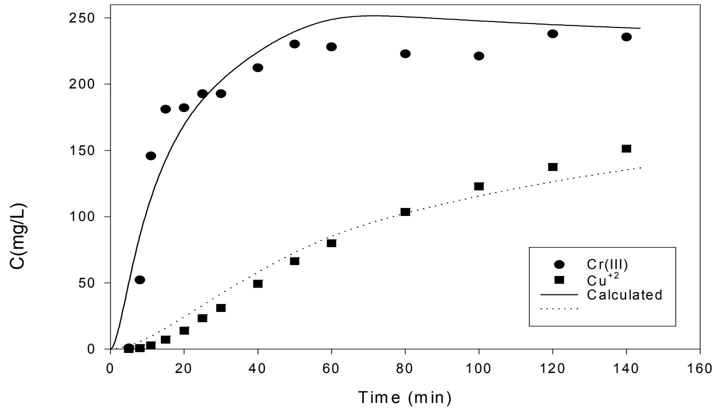

Figure 2 – Prediction of dynamic behaviour of the fixed bed adsorption process for Cr (III) and Cu.

Acknowledgement

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Hydrogen production from methane steam reforming: parametric optimization of a membrane reactor

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Keywords: Modeling and simulation, sensitivity analysis, reforming reactions, hydrogen.

Methane steam reforming is the predominant industrial method used for hydrogen production. Since reforming reactions are highly endothermic, the reactor must operate at elevated temperatures in order to achieve reasonable conversions. The use of membrane reactors can promote the displacement of equilibrium through selective and continuous removal of H2, which may allow the reaction to be carried out at milder temperatures (Uemiya, 2004).

This work aims to carry out the mathematical modeling of a membrane reactor for methane steam reforming, to validate the model with experimental data, to verify the influence of important process variables and to find out better reactor operating conditions. The mathematical model was developed to a small-scale membrane reactor and takes into account the energy and mass balances, as well as, the pressure drop along the reactor. Real gas behavior was considered by the use of Virial equation of state with van der Waals mixing rules. Ergun equation was used to describe reactor pressure drop. Three methane steam reform reactions were taken into account:

$$CH_4 + H_2O \rightleftharpoons CO + 3\,H_2; \quad -\Delta H_{298K} = -206,1\,kJ/mol \tag{1}$$

$$CO + H_2O \rightleftharpoons CO_2 + H_2; \quad -\Delta H_{298K} = 41,15\,kJ/mol \tag{2}$$

$$CH_4 + 2\,H_2O \rightleftharpoons CO_2 + 4\,H_2; \quad -\Delta H_{298K} = -165,0\,kJ/mol \tag{3}$$

Two available intrinsic kinetics from the literature were considered (Xu and Froment, 1989; Hou and Hughes, 2001), but the results shown here were obtained using only kinetic expressions and parameters from the former author. The solution-diffusion mechanism was used to model the palladium-based membrane. Membrane thickness was considered to be thick enough to allow the Sievert's law use. The non-isothermal, non-isobaric mathematical model developed is a set of ODEs, of initial value type, integrated using the package DLSODE (*Double precision Livermore Solver for Ordinary Differential Equations*) in FORTRAN. The validation step was performed by the comparison between experimental data from the literature (Shu et al., 1994) and simulated date from the model, at same conditions. They were compared reaction pressure, molar steam-to-methane feed ratio and reaction temperature. A good agreement was found between experimental and simulated date, as shown in the Table 1.

A parametric optimization study was done using the developed and validated model. It was investigated how reactor pressure (*P*), reactor temperature (*T*), molar steam-to-methane feed flow ratio (*M*), inert feed flow rate (*FI*) and methane feed flow rate (*FCH40*) affect both methane conversion and hydrogen recovery. A standard or reference operational condition for membrane reactors in methane steam reforming is, according to Shu et al. (1994): *P* = 136.00 *kPa*, *T* = 773.15*K*, *M* = 3, *FI* = 2.75 x 10^{-5} mol/s and *FCH40* = 2.75 x 10^{-5} mol/s. Based on these standard values, a parametric sensitivity analysis was performed, changing parameters in –10% and +10%. The results for M and T are shown in Figure 1(a) and 1(b), respectively, in which methane

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conversion and hydrogen recovery profiles are plotted against reactor length. It can be seen that increasing temperature, conversion increases, while increasing For *Saccharomyces cerevisiae*, a living organism, classical intrusive pore size molar steam-to-methane feed flow ratio, conversion decreases. Therefore, in order to maximize both methane conversion and hydrogen recovery, a trade off between high temperature and low molar steamto-methane feed flow ratio must be accomplished. Based on individual parametric sensitivity analysis, the best conditions were found to be: P = 506; 625 *kPa*, T = 873:15*K*, M = 4, FI = 0.0001375 mol/s, $FCH40$ = 0.000005 mol/s, leading to methane conversion and hydrogen recovery above 99,8% and 93,2%, respectively. Based on these results, optimal policy operational conditions, for laboratory scale membrane reactor, can be established.

Table 1: Membrane reactor performance: simulated and experimental data at different conditions

			Methane conversion		
M	P[kPa]	T [°C]	This work	Shu et al. (1994)	Error (%)
3	136	500.4	49.25	51.12	3.66
3	136	400.6	22.53	26.48	14.92
3	136	300.5	6.15	7.12	13.62
3	150	500	49.13	49.69	1.13
3	170	500	49.23	48.7	1.09
3	184	500	49.46	46.9	5.46
3	203	500	49.93	45.1	10.71
2	136	500	40.56	36.61	10.79
2.5	136	500	45.17	42.55	6.16
3	136	500	49.25	49.21	0.08
3.5	136	500	52.95	52.75	0.38
4	136	500	56.35	58.07	2.96

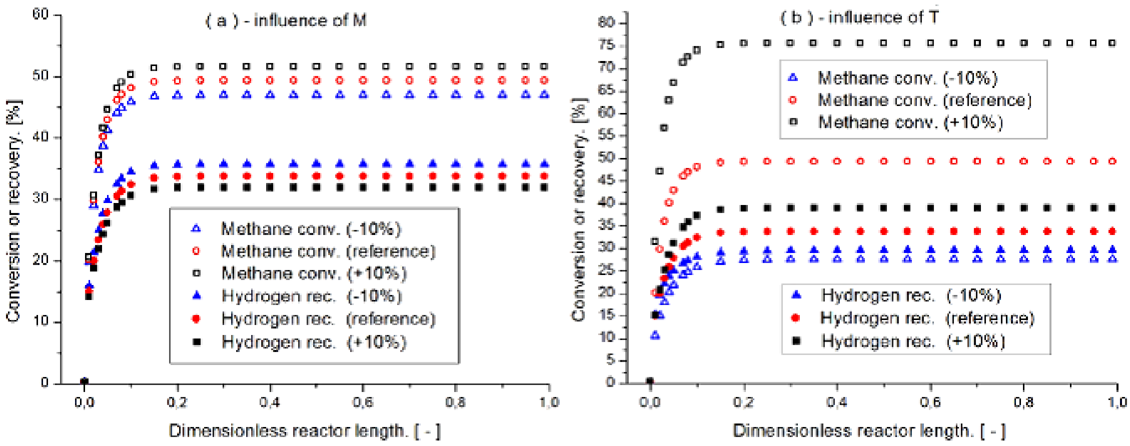

Figure 1: Methane conversion and hydrogen recovery profiles along the reactor lenght: influence of M and T.

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Evaluation of redundant measurements on the air quality monitoring network of Lisbon and Tagus Valley

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Keywords: Principal component analysis, air quality, monitoring networks.

The aim of this study was to evaluate redundant measurements in the air quality monitoring network (AQMN) of Lisbon and Tagus Valley (LTV). With this purpose, the minimum number of monitoring sites was achieved using principal component analysis (PCA). PCA is a statistical method which identifies patterns in data, revealing their similarities and differences (Shrestha and Kazama, 2007). The principal components (PCs) are uncorrelated variables being obtained by linear combinations of the original variables. The influence of each original variable on the PCs is calculated by a rotation algorithm (Varimax rotation in this case), to achieve the rotated factor loadings which represent the contributions of each variable on a specific PC (Pires et al., 2008a, 2008b).

PCA was applied in many studies, aiming the management of water monitoring networks (Mendiguchía et al, 2004; Shrestha and Kazama, 2007; Singh et al., 2004). However, concerning the management of air quality monitoring networks, only our previous publications (Pires et al., 2008a, 2008b) applied this technique. In this study, PCA was used as a classification method, grouping the monitoring sites with redundant measurements in AQMN of LTV. The air pollutants analysed were CO, NO_2, PM_{10} and O_3. The pollutant concentrations data were colected in twenty monitoring sites: Alfragide (S1), Arcos (S2), Avenida da Liberdade (S3), Beato (S4), Camarinha (S5), Cascais (S6), Chamusca (S7), Entrecampos (S8), Escavadeira (S9), Laranjeiro (S10), Lavradio (S11), Loures (S12), Mem-Martins (S13), Odivelas (S14), Olivais (S15), Paio Pires (S16), Quebedo (S17), Quinta do Marquês (S18), Reboleira (S19) and Restelo (S20) (QualAr, 2008). Figure 1 shows the location of these monitoring sites. The period considered was from January 2006 to December 2006.

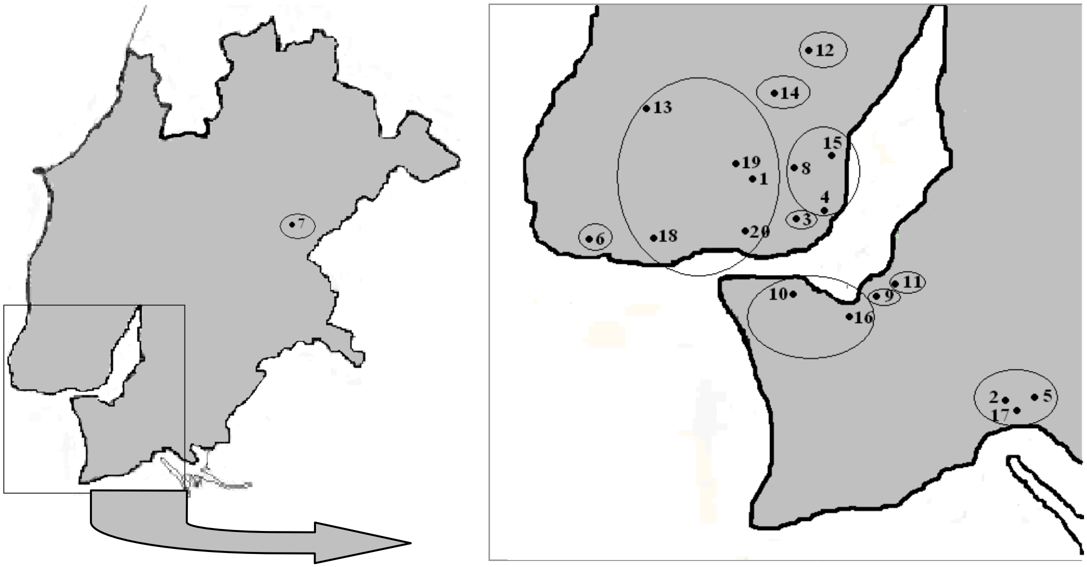

Figure 1. Location of monitoring sites and the PCA results for NO_2 data.

The air quality data was organized in such a way that each column had the hourly average concentrations of one air pollutant at one monitoring site. To apply PCA, the air pollutant concentrations need to be available at the same time for all monitoring

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sites. Concentrations were Z standardized to have zero mean and unit standard deviation. The first step to apply PCA is the selection of the number of PCs. Kaiser criterion, which selects the PCs with eigenvalues greater than 1, is commonly used for this purpose (Pires et al., 2008a, 2008b). However, in this study, the PCs were selected representing at least 95% of the original data variance. The PCs selected by this criterion have more information about the air pollution data, increasing the confidence in the PCA results.

Table 1 shows the grouped sites with redundant measurements that resulted from the application of PCA. Additionally, Figure 1 also shows, as an example, the results of the application of PCA to NO_2 data. From twenty studied monitoring sites, only ten for CO, eleven for NO_2, five for O_3 and nine for PM_{10} were needed to characterize the region. The air pollutant analysers corresponding to the redundant measurements can be installed in non-monitored regions, allowing the enlargement of the air quality monitoring network.

Table 1. PCA results for all air pollutants considered in this study.

CO (1)	**PC1** – S3, S4, S8, S15; **PC2** – S6; **PC3** – S9, S10, S11, S16; **PC4** – S1, S19; **PC5** – S2, S17; **PC6** – S20; **PC7** – S14; **PC8** – S13; **PC9** – S12; **PC11** – S5.
NO_2 (2)	**PC1** – S1, S13, S18, S20; **PC2** – S11; **PC3** – S2, S5, S17; **PC4** – S7; **PC5** – S6; **PC6** – S3; **PC7** – S4, S8, S15; **PC8** – S12; **PC9** – S14; **PC10** – S9; **PC11** – S10, S16.
O_3 (3)	**PC1** – S4, S8, S15; **PC2** – S7; **PC3** – S1, S9, S10, S16, S20; **PC4** – S12, S13, S14, S18, S19; **PC5** – S2, S5.
PM_{10} (4)	**PC1** – S1, S13, S18, S20; **PC2** – S11; **PC3** – S7; **PC4** – S6; **PC5** – S3; **PC6** – S5, S17; **PC7** – S12; **PC8** – S13, S18, S19; **PC9** – S9.

(1) – CO concentrations were not measured at S7 site; (2) – S19 site did not present 75% of the annual data for NO_2 concentrations; (3) – O_3 concentrations were not measured at S3, S6, S11 and S17 sites; (4) – PM_{10} concentrations were not measured at S2, S4 and S16 sites (S1 and S20 sites did not present 75% of the annual data).

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Comparison of dynamic data reconciliation estimators and gross errors detection strategies

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Keywords Data reconciliation, Kalman filters, MHE approach, Gross Error Detection

A real-time on-line optimization provides set-points to the distributed control system and therefore maintains the process near its optimum operating conditions. This optimization requires an accurate process model and reconciled process data needful to specify the current status of the plant model and to estimate model parameters for plant-model matching. The main goal of data reconciliation is to adjust process measurements with random errors by having them satisfy material and energy balance constraints. and is a way to improve the quality of the measurements taken from a process via DCS or any other means of data collection.

It is natural to assume the presence of random, normally distributed errors with zero mean and know variances, to be present in the process data. However, a different type of error – gross error – sometimes is present in the data. The presence of gross error invalidates the statistical basis of data reconciliation procedure and, therefore, it should be removed or compensated from the measurement data set. When flawed information is used for state estimation and process control, the state of the system is misrepresented and the resulting control performance may be poor and can lead to suboptimal and even unsafe process operation because the reconciled values will exhibit '*smearing*' when compared with the true values (Liebman, Edgar & Lasdon, 1992). The elimination of less-frequent gross errors is achieved by gross error detection techniques.

The purpose of this work is to compare dynamic data reconciliation estimators and algorithms for detecting gross errors. The problem of dynamic data reconciliation is treated using five different Kalman filters formulations and a Moving Horizon Estimator (MHE) approach. The problem of data reconciliation is treated using five different Kalman Filters formulations and a Moving horizon estimator approach. The estimators are compared using a nonlinear dynamic example, in transient situations. The Kalman filters formulations are:

Extended (EKF): The prediction stage consists of the integration of differential equations for the process model and the error covariance matrix.

Constrained extended (CEKF): The integration of error covariance matrix is not carried through with differential equations of the system. In the correction stage, the constraints appear in the optimization problem.

Discrete extended (DEKF): The integration step is done using the discretized form of the dynamic model and the prediction of the states is done using the discretized states jacobian.

Modified discrete extended (MDEKF): The error covariance matrix is not updated in the correction stage. The update is done on the prediction stage with the discrete form using the correction equation of CEKF.

Continuous extended (CtEKF): The error covariance matrix is not updated in the correction stage and the integration of error covariance matrix is carried out in a different way.

The detailed formulations are showed in Brown and Hwang (1996) and in Salau et al. (2007).

The MHE formulation is similar to a nonlinear model-predictive control (NMPC). The main difference between those algorithms is the NMPC optimizes and predicts forward

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in time, while MHE optimizes and smoothes backward in time. Additionally, in the MHE, the estimates of the past measured state and input variables over finite history horizon are adjusted to optimally satisfy the modeling equations. Only most recent set of estimates are saved and the optimization is repeated at the next time step.

The problem of gross error detection and bias estimation is also treated using two different algorithms combined with the global test: The GLR, proposed by Narashiman and Mah (1988), and a bias detection method, based on the moving horizon strategy, proposed by McBrayer and Edgar (1995). The algorithms were compared using two different estimators: Extended Kalman Filter and the MHE.

The data reconciliation results show there are not significant differences in quality of the reconciled states obtained by the different estimators. On the other hand, the time spent in the data reconciliation step differs among the estimators. The time spent using MHE was significantly higher than the other ones. This increase of time spent is caused by the use of a time horizon. This procedure, to estimate the past state, implies to integrate the model during a longer time which requires an extra computing effort.

Another important conclusion is the computing time improvement achieved by the implementation of MDEKF. The main advantages of this formulation are the use of analytical solution for the optimization problem in the estimation and the prediction step of the error covariance matrix obtained using discrete form. Comparing to EKF, which also uses the analytical optimization, the error covariance matrix has to be integrated in a numerical way every time step demanding considerable computing effort. If compared to CEKF, the MDEKF has the advantage to set the optimization problem in an analytical way differently of CEKF, which set the problem through a numerical algorithm, in this work, a quadratic programming technique.

The implementation of the proposed gross error detection strategies shows the GLR is simpler and less computation time spending than the algorithm proposed by McBrayer and Edgar (1995). In this work is showed an alternative implementation of the combined use of the global and the GLR tests which consists in evaluate the global test for definite number of times and not in a cumulative way, as proposed in the original GLR.

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Robust design and operation of two- and three-Column compact SMB process for binary and ternary separations

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Keywords: SMB, robust optimization, low-column schemes, parameter uncertainty

Over the last years there has been intensive research on simulated moving-bed (SMB) chromatography. As a result, several new operating procedures and schemes have been developed to improve the separation efficiency, thus allowing for increased productivity, unit downsizing, or simplification of the process flowsheet (Kloppenburg and Gilles, 1999; Ludemann-Hombourger et al., 2000). Further operating alternatives have been developed, expanding the domain of the SMB from binary to the ternary separations (Xie et al., 2002; P.C. Wankat, 2001).

In this work we report a class of simple SMB schemes, using only two and three columns, to achieve binary and ternary separations. These schemes have the advantage of employing fewer columns, and requiring only two or three pumps, which renders the system more economic; the set-up is also simpler to build (Rodrigues et al., 2008).

However, these compact SMB schemes expand the degrees of freedom for operating the cycle, which renders them more sensitive to process disturbances, namely flow-rate stability, and parameter uncertainty, such as packing variability, approximate isotherm modeling, and extra-column volumes. Therefore, a robust design of the system is needed, particularly for production-scale (Mota et al., 2007).

A robust design of the proposed two- and three-column processes has been developed for protection from flow-rate, dead-volume, and isotherm uncertainties. The optimal cycle parameters are chosen only among candidate solutions that are robust feasible, that is, remain feasible for all perturbations from the multidimensional uncertainty domain. This gives rise to a robust approach to optimal design in which the nominal problem is replaced by a worst case problem. The nominal optimization problem and its robust counterpart are formulated using a full-discretization approach for steady periodic dynamics. The resulting nonlinear programming problems are solved by an efficient interior-point solver (Kawajiri and Biegler, 2006).

The procedure is successfully employed to find robust operating conditions for the linear separation of nucleosides mixtures by reversed-phase. The robust schemes are validated experimentally. Emphasis is given to the pros and cons of running the processes under nominal and robust operating conditions.

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Simulated Moving Bed Chromatography for environmental gas-phase applications

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Keywords: Simulated Moving-Bed (SMB), gas chromatography, gas separation

Simulated moving-bed (SMB) chromatography is a well-established adsorption process for obtaining high-purity products at a similarly high recovery. It has been increasingly applied for the separation of pure substances in the pharmaceutical, fine chemistry, and biotechnology industries (Juza et al., 2000). Its advantages with respect to discontinuous batch chromatography (Nicoud, 1992) are higher product purity, less solvent consumption, and higher productivity per unit stationary-phase (Jupke, 2002; Strube, 1998; Wankat, 1986).

The SMB is a practical way of implementing a counter-current chromatographic process. N identical chromatographic columns are connected in series to build a closed loop. By moving the input and withdrawal ports one column ahead, in the direction of fluid flow, at fixed intervals, the counter-current contact adsorbent/fluid is simulated. The SMB has been mainly employed at liquid-phase, although there are a few limited applications of vapor-phase SMB chromatography (separation of xylene isomers, linear/nonlinear paraffins, and volatile inhalation anesthetic enantiomers). The SMB principle can be applied to separate a gas mixture of two competing adsorbates using a weakly adsorbed gas as eluent (Fig. 1).

The cyclic steady-state of continuous multicolumn gas chromatography, operating under isothermal conditions, can be reproduced experimentally using a single-column setup. The experimental procedure is based on a mimetic single-column chromatographic model in which the part of the outlet stream that is not recovered as product is recycled back to the column with a lag of $(N-1)\tau$ time units, where N is the number of columns of the multicolumn unit and τ is the switching interval.

The idea is to reproduce on-line the gas composition profile at column inlet from measurements of the outlet gas composition taken $(N-1)\tau$ time units before, while at the same time satisfying the imposed mass flow rate profile. In addition to on-line monitoring of the outlet gas composition, the experimental setup requires three mass flow controllers, which feed the column at variable mass flow rate with the three gases: the two adsorbates and the gaseous eluent. The three mass flow rates are continuously manipulated so that the composition and mass flow rate of the combined inlet stream are the same as those for the ideal single-column chromatographic model.

During the first $(N-1)\tau$ time units of operation the lagged outlet composition profile is pre-computed by process simulation using the model of the chromatographic unit; at later times it is determined from the on-line composition measurements of the gaseous outlet stream.

Our implementation is successfully applied to the separation of carbon dioxide and methane on activated carbon in the Henry's law region using nitrogen as purge gas (Mota and Esteves, 2007, 2007a). It is shown that by correctly selecting the step within the cycle for process start-up, the steady periodic state can be achieved in a minimum number of cycles. When the SMB process parameters are correctly chosen, the state of the column at the end of the switch interval during which it was partially fed with gaseous eluent is very well approximated by that of a clean column; the subsequent switching interval is, therefore, the preferred one for process start-up, because a clean column is already very close to its initial state for that switching interval when the system is under cyclic steady-state conditions. Hence, the quantity of gaseous

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adsorbates and eluent required to experimentally reproduce the periodic state of the SMB process can be reduced by a factor of about 50. This may be an economic, optimal manner of experimentally testing a set of operating conditions to achieve a given separation performance for a new continuous chromatographic application.

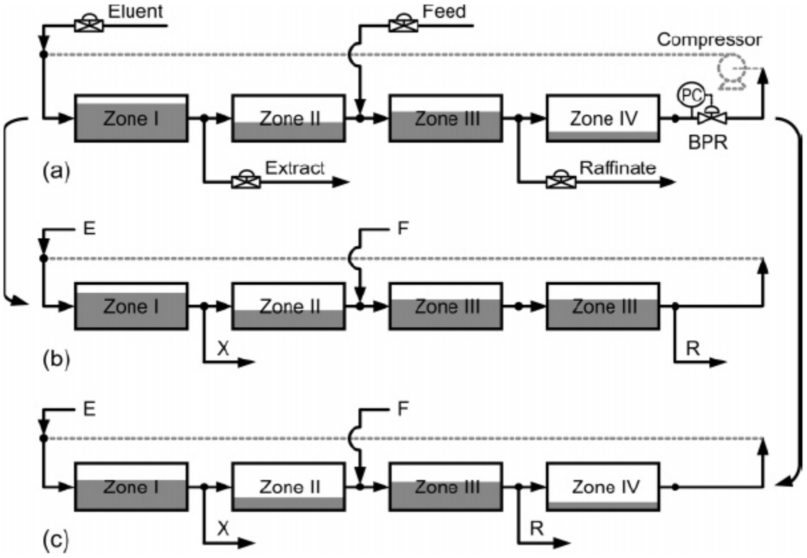

Figure 1 – Flow diagram of 4-zone (a→a) SMB, (a→b) Varicol, and (a→c) PowerFeed gas-phase processes. The dashed lines symbolize the species that are removed when the unit is operated in open loop. E, F, X and R denote eluent, feed, extract and raffinate ports. The height of each shaded area is proportional to the flow rate in the corresponding bed. For simplicity, all schemes are assumed to have the same configuration and flow rates, as depicted in the top diagram, at the start of the step. The usual SMB keeps the configuration (a) constant over the whole step; all ports are then switched one column forward. In Varicol, the ports are switched asynchronously; e.g., after a fraction of the switching interval, the position of the R port is moved forward by one column, giving rise to configuration (b), which has increased zone III by one column and removed zone IV; at the end of the step, all ports have moved forward by one column. In PowerFeed, the flow rates are varied over the duration of the switching interval but the ports are only moved forward by one column at the end of the step.

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A streamlined two-column SMB process for chiral separation

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Keywords: SMB, chiral separation, Enantioners, two-column process, open-loop configuration

Simulated moving bed (SMB) chromatography is a powerful tool for chiral separation, because of its many advantages with respect to discontinuous batch chromatography, namely higher product purity, less solvent consumption, and higher productivity per unit stationary phase. Newly developed cyclic operating schemes and column configurations are pushing the trend toward the use of units with fewer columns and more economical set-ups.

Despite the lower productivity and higher solvent consumption of batch processes, they are advantageous in other respects, such as in their simplicity (less design effort is necessary and the scale-up is easier), flexibility (changes in conditions can be easily handled), and versatility (multi- and center-cut separations from multi-component mixtures are easy to implement).

Somewhere in between, and in an attempt to work with the best of “two worlds”, we have developed a compact, two-column open-loop SMB process for enantiomeric separation, which is particularly effective when the resolution is limited.

On the other hand, product quality control is mandatory for chiral separations, which requires robust on-line monitoring of the individual enantiomer concentrations. We have successfully implemented an on-line monitoring system, comprising an HPLC set-up with two UV detectors, to measure the individual enantiomeric concentration profiles in the SMB unit. Our monitoring scheme does not use a polarimeter.

The chiral separation considered in this work is the separation of Reboxetine enantiomers on Chiralpak AD, using a mixture of Hexane-Ethanol-DEA as solvent. This system is one in which there is clearly a lack of resolution between the two components. It is thus difficult to obtain both products with high purity and yield by HPLC for the chosen chromatographic conditions. Reboxetine, (RS)-2-[(RS)-α-(2-ethoxyphenoxy)benzyl]-morpholine is an antidepressive NRI drug. Only the (R,R)-, (S,S)-pair is present as a racemic mixture in the active principle and the commercial formulations. Recent studies support the hypothesis that the (S,S)-enantiomer is a more potent inhibitor than the (R,R)- and that it is responsible for the vasomotor and cardiac side effects of Reboxetine.

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Automated iterative refinement of adsorption isotherm models for simulated moving-bed operation based on cyclic steady state data

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Keywords: Isotherm determination, Inverse method, SMB, Chiral separation, Enantiomers

Enantiomeric separations by simulated moving-bed (SMB) chromatography are usually carried out under strongly nonlinear conditions. The accurate determination of the competitive adsorption equilibrium of the enantiomeric species is thus of fundamental importance to allow computer-assisted optimization or process scale-up. This is especially true for the SMB, because its operating conditions cannot be designed conveniently without knowledge of the adsorption isotherms of the feed components.

The inverse method of isotherm determination is currently becoming very popular as a quick procedure for estimating the adsorption equilibrium data necessary for designing SMB separations. It derives the isotherm from overloaded band profiles of individual solutes or of their mixture. In recent studies, reporting comparisons of frontal analysis and inverse methods, it is concluded that the inverse method gives accurate estimates of the competitive isotherm parameters up to the maximum elution concentration of the overloaded bands. However, it is only moderately accurate from the maximum elution concentration up to the injected concentration.

In this work we describe and validate experimentally a procedure in which the inverse method is applied directly to the cyclic steady-state (CSS) concentration profiles of the running SMB process to update the parameters of the prescribed adsorption isotherm model. The operating conditions are then optimized for the newly determined isotherm parameters and applied to the running SMB process. This process is iterated and automated in our monitoring and control software.

As a proof of concept of the proposed methodology, it has been successfully applied to the chiral separation of Reboxetine enantiomers on Chiralpak AD, using a mixture of Hexane-Ethanol-DEA as solvent. The system was operated near the solubility limit of the the racemic mixture. Reboxetine is an antidepressive NRI drug. Only the (R,R)- and (S,S)-pair is present as a racemic mixture in the active principle and commercial formulations. Recent studies support the hypothesis that the (S,S)-enantiomer is a more potent inhibitor than the (R,R)- and that it is responsible for the vasomotor and cardiac side effects of Reboxetine.

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Risk estimation of compost application in agriculture: evaluating metal accumulation and biotransfer

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Keywords: Compost; Metals; Accumulation; Exposure; Risk assessment.

Composting is an attractive process for diverting solid organic waste from landfill disposal. It can be defined as a humification process of the organic matter under controlled conditions and in absence of soil resulting in compost. It offers a number of advantages in comparison with other mechanisms of elimination of residues. Compost acts as a bearer of nutrients to the soil, improving its structure and helping to reduce the erosion and the absorption of water and nutrients by plants. Nevertheless, the mechanisms of transfer, accumulation and exposure (soil - plant - humans), take great importance due to the possible presence of potentially toxic elements (PTEs) for the human health and the environment, like persistent organic pollutants (POPs) or heavy metals (Rojas, 2004). Compost for the production of agricultural crops must be controlled by certain procedures since some toxic metals contained in it may become of concern due to the high concentrations and the possible chemical and physical associations occurring in natural systems (Greenway and Song, 2002). Nowadays, there are diverse types of compost, which are characterized by the origin of its raw materials: urban residues, grass and forest residues, wastewater treatment sludge, other organic residues, and mixtures of them. Depending on the raw material, the presence of heavy metals may became important in a long-term scenario of application of compost for crops production as some variations in the concentrations of some of them (Cd, Cr, Pb, Ni, Zn, Cu) have been found, even with existing thresholds limit values. Thus, accumulation of heavy metals in soil may pose a risk as they can be transferred to the food web. Estimation of human exposure to heavy metals transferred from the applied compost could help on establishing safety conditions for compost application for agricultural crops production in a long term scenario.

In this work, an environmental risk assessment of compost application for agricultural crop production was carried out. Data of compost, soil and crops (heavy metal content and physico-chemical characteristics), taken from literature (Greenway and Song, 2002; Rosal et al., 2007) has been used. Four types of applied compost were evaluated: (C1) composting green (pasture and leaves), (C2) composting green mixed with muds of a filter system, (C3) composting of plant residues, and finally (C4) compost of wood residues. The comparison of average concentrations for metals (chromium, copper, cadmium, lead, zinc, cobalt) contained in different compost, have been investigated in European soils during several months. It was found an increase in the content of Cr, Co, Cd, Cu, Pb, Zn in soils with compost type (C1) and (C2) (Greenway and Song, 2002), this evidencing metal transference and accumulation.

The aim of this work is to estimate the potential risk from accumulation and biotransfer of these metals to the food chain and the final human exposure by different pathways. For obtaining these results, a multicompartment model developed from a previous one (Franco et al., 2006) was used for estimation of risk indexes. Information provided by international organisms (USEPA) and other studies (Efroymson et al., 2001; Sauvé et al., 2000) was used for model development. Thus, concentration of heavy metals in the plant, as well as animal exposure and human exposure was determined. Incremental risk was estimated considering a temporal horizon of 50 years of continuous compost application. Published data on metal concentration in compost, soil and vegetation (after compost application) was employed to develop and validate a metal bioaccumulation model in vegetation (which might be linked to a soil model), taking into consideration the main mechanisms involved, as explained in Figure 1. With these estimations an environmental way to approach an improvement would be obtained, leading to a decrease of the potential risk to human beings and the reduction of solid residues by the application of the compost under safe and appropriate conditions.

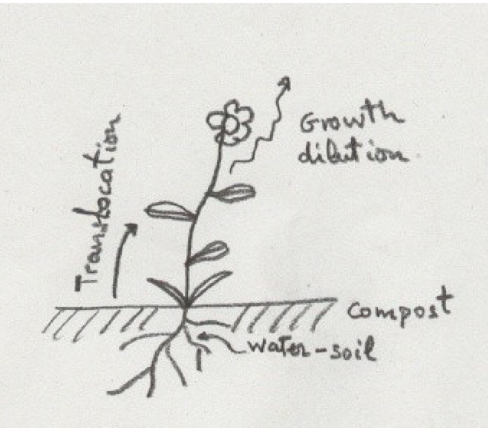

Figure 1. General scheme of metal biotransfer to vegetation.

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In what way may recent laws on chemical substances promote scientific research

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Keywords: REACH, Chemical risk, Safety, QSAR's

The European Parliament approved on 16th of December 2006 the Regulation (EC) No. 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals commonly known as the REACH regulation. As an UE regulation and contrary to a directive it entered immediately in force the date of its approval without the need to be transposed to the national body of laws. It will start requiring some action from enterprises the 1st June 2007.

The REACH text took about 6 years before its final shape, all of them full of intense discussions and polemic. Today, it represents a dramatic change in the way the risk from chemical substances to the human health and the environment is approached by the authorities and the enterprises in the European Union, having replaced more than 40 directives.

But besides its strict regulamentary purpose, REACH has the objectives stated in its Article 1(1) of promoting "alternative methods of assessment of hazards of substances" and "enhancing competitiveness and innovation".

For these objectives to be attained it is useful to know REACH if not in detail at least in its general structure and processes, understanding in which directions the legislation points needing new developments and better knowledge.

In this communication we will explain in what ways REACH will promote those goals and what are the concerned areas of scientific research, among them the use of QSARs, the development of new testing methods, the development of new tools for establishing exposure scenarios and the replacement of substances of very high concern by safer new ones.

REACH besides contributing to the collection of an huge amount of data for later exploitation, will provide through its dissemination of information mechanism an early alert that can be useful to focus research efforts on specific substances, on its characteristics and uses.

Particularly QSARS will be focused, as they promise to be an useful alternative to animal testing on vertebrates and to expensive methods. The need for regulatory acceptance of QSARS has led to the development of more stringent validation methodologies involving an unambiguous algorithm, the definition of a domain of applicability, and a statistical validation. Particular attention should be taken in the validation of a QSAR checking the reliability of the available data.

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Corrigendum to Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the REACH, establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC, *Official Journal of the European Union,* 29th May 2007.

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Revitalization of chemical processes – one way to make possible the competitiveness

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Keywords: Optimization, Planning Design and Chemical Processes.

Chemical industry constitutes an important economic field, which provides a remarkable contribution to the Brazilian gross product. The offer of products, and consequently the competitivity of companies, has increased with the Globalization. To increase the competitivity of its products in the international market the country must focus its efforts in the development of fine chemistry and biotechnology because theses areas add value to the products. In order to search for gains in competitivity this kind of industry has been forced to invest in research and development, trying to minimize costs and to increase the productivity, as well as the quality of its products (Rodrigues and Minceva, 2005).

Even though, the industry of fine chemistry is still incipient in our country and faces increasing difficulties to compete in the national market and eventually, in international markets. The implementation of articulation procedures between the knowledge generation system, the university, and the potential users of this same knowledge, the industry of fine chemistry, can help in the mentioned increasing process. The use of process optimization methodologies together with high level academic groups in organic synthesis can generate information that can enable new processes and products, with competitive values and quality, or even can propose changes in synthetic routes or reactional conditions for products that are already known, making them industrially viable.

The present work is an attempt in that sense, aiming to imagine a methodology that could enable the compatibility between the academic results in projects of syntheses with industrial focus. In order to do so, the association of experiment planning and the knowledge of the chemistry involved in the selected process can guarantee remarkable improvements, turning processes that are presently abandoned into viable ones, or making possible the industrialization of new products, tested only in the batch, and that present some inconvenient as low efficiency, difficult separation, among others.

A great number of chemical processes result in not desirable substances apart from the main product of the reaction, causing a waste of raw material and adding the necessity not only of separating and purifying the product but also of discarding the chemical garbage.

If we direct our efforts to the study of the chemical reaction itself, searching for the best conditions to attend the aimed objectives, we could believe that any improvement, such as, in the increase of efficiency, would bring advantages to the process (Steele and Zettler, 1995; Guervenou et al., 2002). The first one could be that with the efficiency increase of the desired product we would have an increase in productivity; secondly the amount of chemical garbage generation and its polluting charge could be reduced, “better than cleaning it is not to make the environment dirty”; thirdly, but not less important, it is possible to achieve a lower production cost as soon as lower expenses will be necessary in the product purification and separation steps, which could possibly even become not necessary anymore.

The literature shows that the most common problems in synthesis are solved through the adjustment in the reactional conditions, through the optimization of the efficiency of the desired reaction and/or through the development of a new method, more selective or with more specific reagents (Gottfries, 2006). The use of experiment planning, through multivariable strategies, enables the observation of the interactions between the variables in a simultaneous way and with better results. The usage of experiment planning in industrial processes can, among other things, enable revitalization in processes that are presently abandoned due to problems of low efficiencies and selectivity, as well as help in many other areas that require an approximation to the reality, a increase on the knowledge of a process and a selective direction for a determined product.

The factorial analysis and the response surface method can be applied in chemical processes, describing a model, which can be used in industries and laboratories, with influences and possible correlations among the process variations as close as possible to reality. It was tested on selected electrophilic aromatic substitutions reported in the literature resulting in increases of six times in the selectivity of isomers' ratio and yields near 99% for nitration of substituted aromatic rings. In some cases, optimization also led to elimination of purification steps or to lower reaction temperatures.

Such results can enable the reactivation of processes presently abandoned, the revitalization of processes in process, as it can be performed in operation, and make viable new processes, still in batch level, improving the operational conditions and making them industrially advantageous.

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Simulation optimizes feedback control on the temperature of a heterogeneous batch reactor

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Keywords: Dynamic simulation, Feedback control, Heterogeneous batch reactor, MATLAB, SIMULINK

Many dynamic models can be compactly represented in vector form by a pair of algebraic equations and differential equations: $(1)\ \underline{0} = \underline{g}(t, \underline{x}, \underline{a}, \underline{c})$ and $(2)\ \underline{x}' = \underline{f}(t, \underline{x}, \underline{a}, \underline{c})$, respectively. Here, t denotes the independent variable time; $\underline{x}, \underline{a}$, and $\underline{c}$ are sets of state variables, algebraic variables, and constants. Further, the prime (′) indicates differentiation with respect to t.

Simulation almost always means a chain of small steps of numerical integration because analytical solution is either unavailable or intractable. Simulation for design and optimization may require several runs each one starting from $t = 0$ and advancing to a pre-specified time or state, producing output at regular intervals. Boundary conditions and/or some constants may vary from one run to the next.

MATLAB® is a convenient simulation environment with many different driver routines for variable-step numerical integration, e.g. ode23, ode45, and ode15s functions. The latter is a driver for stiff models. Triggered after appropriate construction and data initialization, each driver routine supervises the run handling the chores and calling upon the nominated model function as often as necessary expecting it to solve (1) for $\underline{a}$ at the current time and state and then calculate and return fresh derivatives.

Simulators may be classified as equation-oriented or modular. When the model partly or wholely partitions (1) and (2) between sequential modules as in famous DYFLO (Franks, 1972) care must be taken to preserve simultaneity. ACES (Koçak, 1980) pioneered true simultaneous modularization by twice-round execution of modules. DYFLO and ACES are both Fortran-based simulators with chemical engineering modules.

SIMULINK® is far useful than the standard MATLAB® package for control system simulation. SIMULINK® enables the rapid construction and simulation of control block diagrams (Bequette, 2003). Its library offers many linear and nonlinear blocks as icons which can be dragged and dropped into a model workspace. Block parameters and coefficients may be entered here by double-clicking on the icon. Its easy to connect the blocks by arrows. Standart input functions such as step and ramp are supplied. The simulation parameters can be changed by going to the Simulation pull-down menu and modifying the stop time or the integration solver method.

A block titled "sfun" can be obtained from the nonlinear library in SIMULINK® and placed in the model workspace. When simulations are performed, the equations in the sfun m-file are solved simultaneously with other blocks in the block diagram. This work is a SIMULINK® application using sfun to solve a feedback control problem suggested by Franks (1972). The exercise involves adding a temperature control loop to a batch autoclave reactor and determining the optimum controller settings. The aim is to raise the reactor temperature to 120° C as rapidly as possible, with minimum overshoot, and maintain it at this level for the remainder of the reaction. The reactor temperature is measured with a thermal system having a first-order time constant of 0.4 minutes. The coolant temperature of through the coils is obtained by mixing two fluids with temperatures of 130° C and 70° C. The mixing is assumed to be linear with valve

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position. The valve dynamics can be simulated with first-order time constant of 0.05 minutes.

In the heterogenous reactor there are three phases, where several phenomena occur simultaneously, making manual calculations virtually intractable. An organic compund A is hydrogenated to compound C via an intermediate B, i.e. $A + H_2 \rightarrow B, B + H_2 \rightarrow C$. This reaction occurs on the surface of a metal catalyst in the form of loose particles, the reagents diffusing from the liquid phase to the catalyst surface, reacting and returning to the liquid phase. A degree of control over the internal temperature can be achieved by a series of cooling coils contacting the liquid phase. The reaction is highly exothermic, which will cause a significant rise in the temperature and pressure during the course of the reaction, monitored by suitable instrumentation. A significant exchange of material between the liquid and vapor phases occurs as the reaction proceeds with the simultaneous division of heat energy. However, all phases will have the same temperature since the agitation has sufficient violence to maintain equilibrium. The reaction system loses heat to the coils and the vessel.

For this reactor, Franks (1972) developed a dynamic model with five components by writing material and heat balances considering the reaction system as whole. A subsequent flash calculation involving trial-and-error established the vapour-liquid split as well as the equilibrium temperature and pressure. Open-loop simulation was then carried out harnessing DYFLO.

As stated above, this work is a SIMULINK® application using a nonlinear sfun block to solve the given feedback control problem. Firstly, the model was enriched with pertinent equations for the suggested loop, including a controller (P or PI) and two first-order lags. Secondly, open-loop simulation verified the harmony with the original model. The final step was the closed-loop optimization. The results will be presented at the conference.

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Study of volumetric oxygen transfer coefficient in a Taylor vortex flow bioreactor

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Key Words: Taylor vortex flow, mass transfer, volumetric oxygen transfer coefficient (K_La), animal cell culture, bioreactor

Introduction

In the last 20 years there have been rapid increase and progress in the development and use of large-scale animal cell cultivations for the commercial production of therapeutic and diagnostic biochemicals as viral vaccines, monoclonal antibodies and recombinants proteins (Ma *et al*., 2002). Nowadays, for commercial production, cells are often cultured in large scale stirred bioreactors. Within of that equipment, the fluid dynamic environment may be used to enhance delivery of oxygen and other chemical species (as nutrients) to cells through forced convective transport (Curran, Black, 2005). The supply of oxygen to production cultures during scale-up is an important part of bioprocess design (Butler, 2004). At high cell densities (> 10^6 cells/mL) or at high volume cultures (> 10L) gas diffusion is not adequate to meet the metabolic demands of the cells. A constant oxygen supply is required because of its low solubility and under these conditions, oxygen is often supplied by aeration through gas sparging (Konz *et al*., 1998). Nevertheless, the conventional methods the agitation and aeration used in bioreactors must be used carefully to animal cells, due to their more susceptibility to stress damage through these systems.

As it can be observed in the literature, the oxygen transfer is a limiting factor on the bioreactor design. Therefore, the main objective of this work is to assess the potential use of a Taylor vortex flow bioreactor (TVFB) designed and built at the Department of Chemical Engineering of São Carlos Federal University, for cultivation of animal cells. The overall volumetric oxygen mass transfer coefficient (K_la) and mass transfer correlation was estimated under Taylor vortex flow regimes, including laminar, wavy and turbulent. TVFB has a non-conventional configuration, when compared to other bioreactors like stirred tank and *airlift*, it consists of two concentric cylinders, one internal rotating cylinder and the other external stationary. Taylor vortices are secondary flow patterns that appear above a critical rotation, determined by the critical Taylor number, in the gap between inner (rotating) and outer (rotating or stationary) cylinder (Taylor, 1923). The vortex is capable of fulfilling the gap between the two cylinders in a stable and reproductive way that provides an efficient mixture of the medium, ensuring favorable conditions of pH, temperature, nutrients and a less aggressive agitation of the medium for the cells inside the bioreactor (Moore, 1994, Giordano *et al*., 1998, 2000a and b; Resende, 2002). When working with anchorage dependent animal cells, in a heterogeneous system, the rotation of the inner cylinder is an additional operating variable that may facilitate particle fluidization.

Materials and methods

The measurement of the K_La is based on the dynamic gassing-out method using an oxygen electrode. The method consists to provoke abrupt changes of dissolved oxygen concentration between two known steady states. It is possible by gassing it with nitrogen within of bioreactor, eliminating all dissolved oxygen until the indicating of zero (or near of it) by the oxygen sensor. After the elimination of oxygen inside the bioreactor, the aeration is turned on at the conditions desired for that experiment (agitation and air flow rate), which is equivalent to the application of a step function of aeration in the system. The experiments were carried out in the TVFB of 1L (0.8L working volume), operated in the absence of cells, at 37°C, containing culture medium DMEM (*Dulbeco's Modified Eagle's Medium* - Cultilab). The experimental conditions were inner cylinder rotation rate varying from 25 to 300 rpm ($244<Re<2928$) with aeration varying from 80 $mL.min^{-1}$ to 550 $mL.min^{-1}$. The dissolved oxygen concentration was measured by a sterilizable electrode (Mettler-Toledo, model INPRO 6100/120/S/N) bearing a Teflon membrane (Mettler Toledo, model InPro T-96). The data acquisition and control equipment and sensor transmitters are manufactured by Autsens Industry, model SIMAD. All data was collected from the process was stored in its internal memory and, after the experiment, was transferred to personal computer by a RS232 interface.

Discussions of results

The results of K_La in the TVFB ranged from 3.0 to 9.5 h^{-1}, which can be considered high when compared to other rotating wall and others conventional bioreactors for animal cell culture as stirred tank. Mass transfer characterization typically employed dimensional analysis to correlates a mass transfer parameter (Sherwood number) to experimental data.

Conclusions

These results suggest that the TVFB has been identified as a competitive solution for animal cell culture in bioreactors, once it provides an effective means for oxygen transport.

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Studies on synthetic medium composition for continuous production of clavulanic acid by *Streptomyces clavuligerus*

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Keywords: clavulanic acid, synthetic media, Streptomyces clavuligerus, continuous culture, ornithyne

Clavulanic acid (CA) is commercially produced by *Streptomyces clavuligerus* in rich media containing glycerol as the main C and energy source, and complex nitrogen sources, such as soybean flour, that yield high CA production. These complex nitrogen sources, however, normally contains also undesirable components that interfere with microbial metabolism. In this way, amino acids and soluble components such as protein extracts and hydrolizates produce broths less viscous and more homogeneous, enhancing mass and heat transfer, facilitating monitoring and control of process variables, as well as it can make simpler the separation and purification steps (Elander, 2003). Despite that the great majority of amino acids are well metabolized by the microorganism, only some of them are propicious to CA biosynthesis. Ives and Bushell (1997), fed a continuous culture with different media containing one aminoacid, and they observed that some of the amino-acids led to higher clavulanic acid production rate. In other recent work Bushell *et al.*, 2006, evaluated the C flux in media limited by P, N or C utilizing Metabolic Flux Analysis techniques. Among the amino-acids studied, they observed that ornithyne and arginine, CA precursors (Romero *et al*, 1986), promoted CA synthesis remarkably.

The aim of this work was to optimize the molar ratio between glycerol and ornithyne in the feed medium making use of Factorial Experimental Design (Box *et al*, 1978) in order to achieve higher CA concentration.

Streptomyces clavuligerus ATCC 27064 used throughout this work was stored in the form of vegetative cells (8.0 g.L^{-1} dry weight) at −70°C in cryotubes, utilizing glycerol 20% (w/v). Inoculum medium was composed of (in g/L) glycerol (15.0), soybean protein isolate (Soytone) (15.5), yeast extract (1.0), Malt extract (10.0), K_2HPO_4 (0.8), $MgSO_4.7H_2O$ (0.75), 1.0 mL/L of salt solution containing: (in g/L) $MnCl_2.4H_2O$ (1.0), $FeSO_4.7H_2O$ (1.0) e $ZnSO_4.7H_2O$ (1.0). Production medium had the same composition except that malt extract was not added. The media were supplied with 21 g/L of MOPS, and initial pH was adjusted to 6.8.

Fermentation was carried out in shake flasks (250 rpm, 28ºC). After 48 h cultivation, broth withdrawal and medium feeding were accomplished at D = 0.005 h^{-1}. The feed medium was composed of tap water 5%,, MOPS (21 g/L) and several combination of glycerol and ornithyne concentrations. Same medium and dilution rate were utilized for a 5 L bioreactor run (DO controlled above 40% saturation). CA was determined by the method described by Bird *et al.* (1982), cell mass was determined as dry weight and glycerol by the method described by Lambert and Neish (1950).

Concentration range for the independent variables, glycerol (x_1) and ornithyne (x_2) were defined based on the literature, setting the response variable as CA concentration (mg/L), in 144 h cultivation. Romero *et al.* (1986) observed that glycerol concentration of 165 mM inhibited CA synthesis while ornithyne and arginine (ca. 5 mM) exerted a positive effect. In all experiments, biomass was almost constant, around 8 g/L and the

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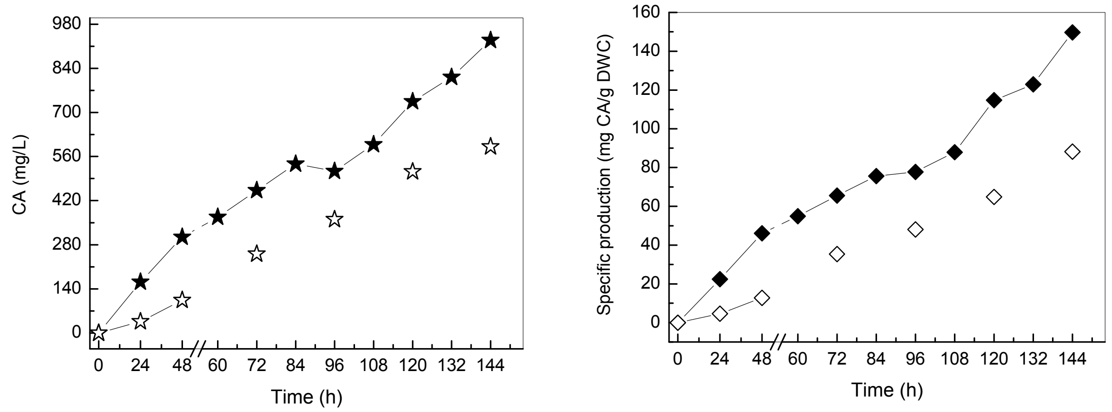
residual glycerol concentration was practically zero. Statistical analysis resulted in a response surface adjusted at 95% confidence level and 0.91, determination coefficient This surface equation predicted maximum CA concentration at a molar ratio glycerol to ornithyne of 40.

The optimized molar ratio was then utilized in a 5 L bioreactor provided with agitation and aeration, at the same dilution rate as that utilized in shake flasks, D = 0,005 h^{-1}. The process behaviour is illustrated in Figure 1. As it can be observed, in bioreactor the CA concentration achieved a value 60% higher than that in shake flasks which in turn, with the optimized ratio, reached a value 25% higher than that the control run.



(A) (B)

Figure 1 – Time course of (A) CA concentration and (B) specific yield in shake flasks (open symbol) and in agitated and aerated bioreactor (filled symbols), constant volume, D = 0,005 h□$^{-1}$, glycerol:ornithyne molar ratio = 40, with 70.7 mM glycerol and 1.72 mM ornithyne.

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Effect of physicochemical properties of solvents on clavulanic acid extraction from fermentation broth

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Keywords: clavulanic acid, extraction, organic solvent, dissociation constant, purification

The isolation of clavulanic acid (CA) from fermentative broth requires a series of steps. At the end of fermentation, the broth is clarified by filtration or centrifugation, after which it is subjected to the primary extraction, an operation that is based on several two-phase separation methods. One of these methods is the direct extraction from clarified broth with organic solvent, producing an organic phase containing CA, which is subsequently isolated (Butterworth, 1984, Nabais and Cardoso, 1995). Several patents describe the extraction of CA onto organic solvents. These patents explain that CA can be extracted from fermentation broth by different procedures, and that CA is isolated when its corresponding potassium salt is formed (Cole *et al* 1978 and Cardoso 1998). This salt is produced by reacting CA with potassium 2-ethylhexanoate in the solvent-rich phase, i.e., after cell removal, the resulting clarified broth is acidified to reach pH values between 2 and 3. Care should be taken, since CA is highly unstable depending on the pH of the medium (pH lower than 6.2 and higher than 7.0), the presence of ammonium compounds, and the temperature (Bersanetti *et al* 1995) On the other hand, CA forms unstable hygroscopic oil which cannot be used as a pharmaceutical compound, therefore, this purification step requires special attention to ensure an efficient process. The extraction of CA onto organic solvents is strongly affected by physicochemical properties such as solubility, polarity and dielectric constant.

This paper discusses the conditions, procedure and results obtained in the extraction of CA from fermented broth using different organic solvents. The effect of physicochemical properties on the CA distribution coefficient, D, in the aqueous media-solvent system was determined with different organic solvents, namely, butyl acetate, ethyl acetate, methyl-isobutyl-ketone, 2-butanol and n-butanol. In addition, the effect of each solvent's physicochemical properties on the values of D was examined.

All tests were carried out on broths fermented with *Streptomyces clavuligerus* ATCC 27064 according to Maranesi, *et al.* (2005). Before the extraction process, the broth was centrifuged to obtain a clear cell-free solution, whose pH was then lowered (3.5 - 4.0) to precipitate part of the soluble solids and proteins. Finally, the solution containing the product was centrifuged again and filtered through analytical filter papers to eliminate suspended impurities. The CA was determined by HPLC (Foulstone and Reading, 1982). The contaminants was determined by spectrophotometric analysis at 280 nm, disregarding the influence of CA at that wavelength.

Assuming that no CA molecule is charged during the extraction and that the extraction process takes place in a perfectly stirred tank, the distribution coefficient in equilibrium can be given by: $D=C_{OP}1/C_{AP1,}$ where C_{OP1} is the solute concentration in the light phase, OP, and C_{AP} is the solute concentration in the heavy phase, AP. The performance of the process can be analyzed in terms of such important parameters as yield Y, purification factor PF and concentration factor, CF.

The first influence observed, in the extraction, was on the organic solvent molecular weights. D is inversely related to the solvent's molecular weight, i.e., the higher the MW the lower the distribution coefficient. The extraction of CA with 2-butanol and n-butanol, representing the low MW solvents, was considerably higher than with the other solvents. As for the effect of the dielectric constant on D, there is no simple or

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theoretical function able to relate these two variables. However, if the data are divided into two regions, two directly proportional relationships are observed. The first region encompasses the results obtained with butyl and ethyl acetate, while the second region involves the other three solvents, with a dielectric constant higher than 10. The discontinuity of the tendency for the linear increase of D with the dielectric constant is intrinsically related to the molecular structure of each solvent, particularly with regard to the functional ketone group, and with the solubility of these solvents in water. CA contains two hydroxyl groups in its structure, allowing for interactions between molecules through hydrogen bonds. The dissolution of substances like CA is favored in polar solvents in which hydrogen bonds are formed, such as alcohols. Solvents like ethyl acetate and other esters present weaker intermolecular interaction (dipole-dipole) when compared with hydrogen bonds, leading to a low dielectric constant and a weak interaction between solute (CA) and solvent, despite their higher polarity.

The extraction of CA with n-butanol is better than with ethyl acetate, but the higher solubility of water in n-butanol impairs the final precipitation of CA. The values of the CF were found to decrease when ethyl acetate was used to extract CA, due to its low solubility in water. Although n-butanol presents a higher distribution coefficient for CA, the fact that it is more water-soluble actually hinders the purpose of the purification process, which is to trigger the precipitation reaction in this solvent. This is a decisive factor in choosing the most adequate solvent for the process. Therefore, ethyl acetate is potentially the solvent of choice to purify CA from fermentation broth. During the extraction of CA by organic solvents such as ethyl acetate and other esters, dipole-dipole type interactions occur. These are weak interactions, e.g., hydrogen bonding. Despite the higher polarity of some organic solvents, they usually have a low dielectric constant, which affects the CA extraction, and a weak solute-solvent interaction. These interactions may be stronger or weaker, depending on the components in the solution which are able to promote changes in the thermodynamic properties of the mixture, affecting the FP and Y.

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Kinetic production of the antioxidant ellagic acid by solid state culture

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Keywords: solid state culture, polyphenols, ellagic acid, ellagitannins, *Punica granatum*

Ellagic acid (EA) is a potent antioxidant with important physiological and functional properties. Our group, previously reported the first findings on fungal EA production through SSF (Aguilera-Carbó et al., 2008, Robledo et al., 2008) demonstrating that the pomegranate husk residue is an excellent alternative for EA production. Also, a biodegradation process of ET's for EA production has been proposed (Aguilera-Carbo et al., 2007). SSF is one of the most attractive alternative to management of agro industrial by-products, in this case the residues of pomegranate husk contain an interesting profile of nutrients such as large amounts of insoluble carbohydrates, small amount of protein, minerals and some remaining juice and other soluble substances favoring a rapid microbial growth.

These properties can be approached for the production of high value-added metabolites. In this study EA was produced by solid state fermentation (SSF) using pomegranate husk as support and nutrient source. *Aspergillus niger* GH1 was used to release ellagic acid from ellagitannins present into pomegranate residues. SSF was kinetically monitored during 168 h. Polyphenolic content was evaluated during the fermentation, EA released was measured by HPLC. Kinetic parameters associated to ellagic acid production, biomass consumption and substrate uptake were also calculated.

Obtained results demonstrated high rates of biodegradation of the hexahydroxydiphenic group from the ellagitannins. The highest EA accumulation was reached at the 96 h of culture (12.3 mg per gram of substrate). A yield of 0.3 g EA per g of substrate was obtained. EA formation specific rate was of 1.92×10^{-4} g product per g biomass per hour and the productivity of 1.29×10^{-4} g EA per g support per hour. SSF is an excellent alternative bioprocess for bio treatment of pomegranate residues to produce ellagic acid.

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Biorefinary of pulp and paper hardwood spent sulfite liquor

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CAIMA pulp mill produces bleached pulp by acidic sulphite pulping of *Eucalyptus globulus* wood. The Hardwood Spent Sulfite Liquor (HSSL) is the waste by-product, which is burned for energy recovering. HSSL contains essentially sulphonated lignin (lignosulphonates) and sugars from degraded hemicelluloses, mainly pentoses.

There are different applications for Single Cell Protein such as human and animal consume contributing for reduction the gap in demand and supply of food in the world (Anupama and Ravindra, 2000). Products from SCP made by food processing technology used as meat substitutes, texture providing agents and flavour enhancers are increasing their utilization day by day (Ugalde and Castrillo, 2002).

Many unpleasement environmental consequences due to effluents discharge can be reduced processing industrial by-products.

Cycling and recycling of residues trough fermentation will not only reduce pollution but also will serve as a potential source of carbon and energy of single cell protein very cheaply (Rajoka et all, 2006).

The objective of this work is the biomass production from HSSL using the fungus *Paecilomyces variotii* or one of the yeasts *Kluyveromyces fragilis* and *Candida utilis* in order to obtain single cell protein.

Hardwood Spent Sulfite Liquor (HSSL) has been analysed and its composition is shown in Table 1.

Component	Concentration (gL^{-1})
Lignosulphonates	159 ± 1
Acetic acid	8,2 ± 0,3
Furfural	Traces
Ash	15,1 ± 0,2
pH	3,40 ± 0,05
Reducing sugars	42,8 ± 0,4
D-xylose	20,6 ± 0,5
D-mannose	8,5 ± 0,9
L-arabinose	7,8 ± 0,3
D-galactose	4,5 ± 0,1
D-glucose	2,3 ± 0,1
L-rahmnose	1,6 ± 0,3

Each concentration was measured three times

After pulping process a high concentration of reducing sugars mainly xylose is still available and that can be used as substrate for growth of biomass in order to increase industrial profit.

Batch tests to biomass production have been carried out with the three different species. As it can be seen in figure 1 *Paecilomyces variotii* grows consuming sugars as well as acetic acid and presenting a 4 fold biomass increase.

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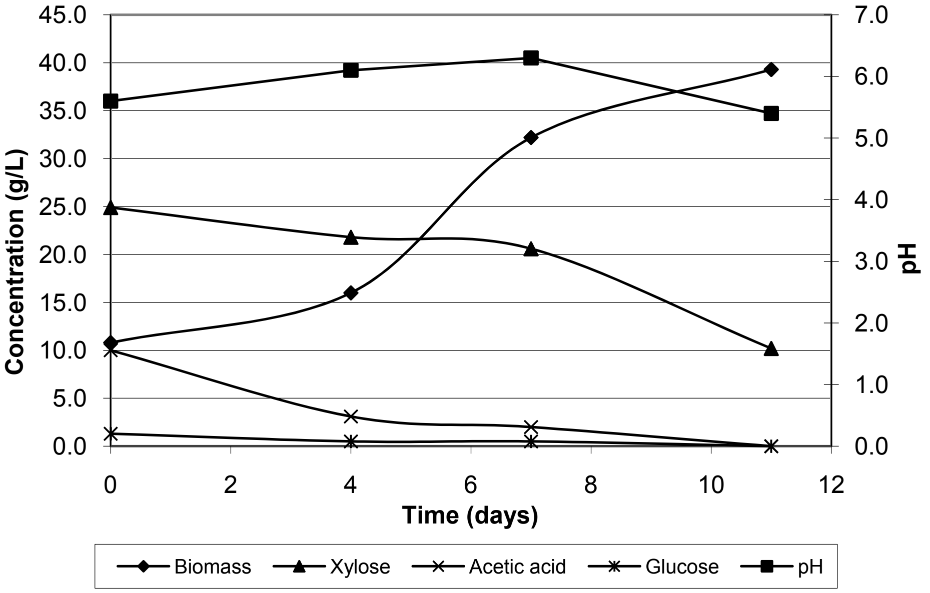

Figure 1 - *Paecilomyces variotii* growth time course.

After biomass optimization protein will be analysed in order to evaluate protein constitution and susceptibility to food and feed apliccations and industrial viability will be considered.

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Enzymatic interesterification of milkfat with soybean oil using lipases of different sources immobilized in polysiloxane–polyvinyl alcohol hybrid matrix

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Keywords: lipases, enzymatic interesterification, milkfat modification, enzyme immobilization, polysiloxane–polyvinyl alcohol hybrid matrix

Milkfat is a very complex food, constituted by a broad range of long and short chain fatty acids in the form of triglycerides. This complexity is responsible for its unique physical and organoleptical characteristics. Nevertheless, the consumption of milkfat has been declined due to its high degree of saturation, price and high percentage of hypercholesterolemic fatty acids (Balcão and Malcata, 1998). An alternative to overcome such drawbacks was to replace milkfat by partial hydrogenated vegetable oils, in the form of margarines and spreads. However, vegetal oil hydrogenation process generates also high amount of trans fatty acids as by-product. The ingestion of these fatty acids has been found to both increase the low density lipoprotein cholesterol (LDL-cholesterol), known as "bad" cholesterol, and decrease the high density lipoprotein cholesterol (HDL-cholesterol), known as "good" cholesterol. This has driven the food industry to reformulate products that could take advantageous of the beneficial effects of milkfat as conjugated linoleic acids (Haug et al., 2007) and avoid the presence of compounds which are related with some physiological disorders.

An interesting approach is to blend milkfat with vegetable oils either in the presence (interesterification reaction) or without catalyst (physical mixture). The former yielded a product with better spreadability properties and can be catalyzed by chemical or enzymatic route. Enzymatic interesterification is advantageous in comparison to chemical interesterification because enzymes (lipases) allow to carry out processes under mild reaction conditions (temperature and pressure) that results better preservation of the milkfat flavor (Rodrigues and Gioielli, 2003).

To fully exploit the technical and economical advantages of lipases, it is recommended to use them in an immobilized state to reduce the cost and the poor stability of the soluble form (Osório et al., 2006). There are many methods available for biocatalyst immobilization that span from binding on prefabricated carrier materials to incorporation into in situ prepared carriers. Recently, the potential of using a hybrid matrix of Polysiloxane-Polyvinyl Alcohol (POS-PVA) obtained by sol-gel process for lipase immobilization has been demonstrated in our laboratory (Paula et al., 2007).

Thus, in this work, two food grade commercial preparations of lipases produced from different microbial sources (*Rhizopus oryzae* and *Candida rugosa*) both manufactured by Biocatalysts (Cardiff, England) under, respectively, the trade name of Lipomod L036 and Lipomod L034, were evaluated with relation to their potential to perform milkfat interesterification with vegetable oil. These lipases were immobilized on POS-PVA activated with sodium metaperiodate and used to catalyze the interesterification of a solvent free medium containg milkfat (80%) and soybean oil (20%). Reactions were carried out at 45° C on a rotary shaker at 170rpm for a maximum period of 72h. Reactions were monitored by quantifying the formation of free fatty acids using acid-base titration and the profile of triglycerides (TAG) analyzed by gas chromatography using a packed column with 3% OV-1 as stationary phase. Starting materials and

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interesterified products were subjected to analysis of consistency determined by penetration tests using a 45° acrylic cone fitted to constant speed texture analyzer QT-25 (Brookfield, Middleboro, USA). Saponification, peroxide and iodine values were determined by standard methods (American Oil Chemists' Society, 2004).

The formation of free fatty acids during the reaction detected by an increase on the acid value was caused by the partial hydrolysis of the triglycerides. However, the initial humidity in the system was low and the hydrolysis degree was lower than 6% in the reactions. Results also showed that the product exhibited higher peroxide value than the raw materials mixture, suggesting that oxidation reactions took place. The interesterification occurred and was confirmed because both lipases resulted in changes on the triglycerides profile, with a decrease on the concentration of TAGs having C38 and C54 acyl-carbon numbers and an increase on the concentration of TAGs C26–C32 and C46–C50. It was also observed that the decrease in the concentration of the TAG C38, characteristic of milkfat, was more pronounced using *Candida rugosa* lipase. On the other hand, the use of *Rhizopus oryzae* lipase resulted in higher reduction in the concentration of the TAG C54, characteristic of soybean oil. The consistency analyzed after conditioning the samples at 10°C and expressed by the yield value, decreased 27.67 and 21.12% after the interesterification catalyzed by *R. oyzae* and *C. rugosa* lipases, respectively. By comparing the yield values for the interesterified product with the value correspondent to the milkfat, the decrease in consistency was about 3 times.

These results showed the potential of the selected immobilized lipases to change the TAGs profile of the milk fat-soybean oil mixture, with a correspondent decrease in the consistence similar for both lipases, meaning a better spreadability of the product after the reaction.

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Enzymatic hydrolysis of Buriti (*Mauritia vinifera*) oil for obtainment of beta-carotene

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Keywords: Lipase, Beta-Carotene, Buriti, Enzymatic hydrolysis, Vegetable Oil

β-carotene is the main source of provitamin A and is widely used as a food colorant and nutritional supplier. The global market for carotenoids, which was of US$ 887 millions in 2005 and has been growing at an yearly rate of 3%, is estimated to surpass US$ 1 billion in 2009, being β-carotene responsible for almost 30% of this market (Fraser & Bramley, 2004).

Most of the β-carotene sold in the world is produced by chemical synthesis from β-ionone, but a small amount is manufactured using biotechnological processes, based on different microorganisms, such as fungi (*Blaskelea trispora* and *Phycomyces blaskeleeanus*), yeasts (*Rhodotorula glutinis*), bacteria (*Flavobacterium multivorum*) and microalgae (*Dunaliella salina* and *D. bardawil*) (Bhosale & Bernstein, 2004; Dufossé *et al*, 2005; Kuzina & Cerdá-Olmedo, 2007; Maldonade *et al*, 2008).

Some oleaginous fruits, such as palm (*Elaeis guineensis*) and buriti (*Mauritia vinifera*), present high concentrations of carotenoids, specially β-carotene. Buriti is a palm tree that grows wild in different areas of Brazil, and has been traditionally used for the preparation of beverages and candies in the Amazon area. The recent interest about other natural sources of β-carotene stimulated the development of processes to extract the carotenoid-rich oil of the buriti fruit. Most processes, however, are still based on the conventional technologies for pulps, including drying and pressing the oil off the pulp (Rodriguez-Amaya, 2001; Santos, 2005).

The present work involves enzymatic hydrolysis of crude buriti oil, for further extraction and concentration of β-carotene. In the hydrolysis process, the performance of commercial lipase Lipozyme TL IM was evaluated. The analysed parameters in the hydrolysis process were temperature, enzyme quantity (enzymatic activity) and ratio of buriti oil / water. The experimental conditions were established based on an experimental design in order to set the maximum free fatty acids contents and the minimum loss of carotenoids.

The results were analysed for the free fatty acids content using titration; total carotenoids using spectrophotometry and its composition by HPLC, employing a YMC ODS-A column with a mobile phase of acetonitrile/methanol/THF (50/45/5). The optimized conditions of the enzymatic hydrolysis were 31.2°C (temperature), 25 U (enzymatic activity in 8 mL of mixture volume), 2.33 (ratio oil/water), at 300 rpm, during 4 hours of reaction. After the hydrolysis of the oil, some methods were tested to separate the fatty acids formed from the carotenoids, as partition with ethanol and winterization, increasing the β-carotene concentration in the final product.

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Enzymatic inulin hydrolysis using PVA-base matrices

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Keywords: Hydrogels, polyvinyl alcohol, inulin hydrolysis, inulinase, encapsulation

Inulin is a linear-linked fructose polymer that occurs as reserve substrate in chicory roots or dahlia. This polymer is an acknowledged source for the production of fructose syrups, in alternative to the multi-enzymatic starch hydrolysis or the less favored inverted sugar production. Inulin hydrolysis can be performed chemically, but this approach leads to unwanted by-products and colored compounds, shortcomings that can be overcome if the enzymatic route is used. Inulin hydrolysis is thus preferably performed by and exoinulinase acting synergistically with an endoinulinase. The enzymatic approach is further favored if a suitable immobilization method can be used. In the food industry this requires for a readily available support and simple immobilization procedures, to comply with the need to cut down costs and complexity. Polyvinyl alcohol is a synthetic polymer that can be used to form hydrogels, which furthermore combines the biocompatibility of natural polymers with high mechanical strength. Several methods have been developed to produce PVA capsules. One of the most recent relies on the controlled drying of a PVA based hydrogel (LentiKats® liquid), and subsequent chemical stabilization, leading to lens-shaped particles (Vorlop and Jekkel, 1999). This methodology has been applied to the hydrolysis of di- and oligosaccharides (Rebros et al., 2006, 2007) but not to polysaccharides such as inulin. In the present work two procedures for inulinase immobilization for inulin hydrolysis, based in the use of LentiKats® liquid are evaluated: Gelation of PVA by controlled drying, yielding lenticular shaped particles, and extrusion of PVA to polyethylene glycol (PEG), where gelification occurs instantaneously, in the form of spherical-like capsules. PEG could be recycled several times with noticeable effects on capsule formation.
Characterization of the immobilized systems as related to temperature and pH was performed, and matched to the free form. No significant differences were observed, although temperature runs were limited to an upper limit of 60ºC for the spherical like particles and 55ºC for the lens-shaped particles at 55ºC, the latter also reported by Rebros and co-workers (2007). On the other hand diffusion resistances were more noticeable in the spherical-lice particles, given the roughly 3-fold increase in the apparent K_M constant observed when these particles were matched to the free enzyme, whereas the increase in K_M for thin lens-shaped particles was below 2-fold. Lens-shaped particles were a little more sensitive to mechanical stress due to magnetic stirring, with some lenses disintegrating occasionally. Both enzyme preparations exhibited considerable operational stability, for no decay in productivity was observed following 10 repeated batches. Storage stability was also high, since the preparation retained 90% of the initial activity after 2 months of storage.
The use of LentiKats® provided a mild method for the production of immobilized forms of inulinase. The new methodology for capsule formation proved a suitable alternative to the well-known method of lens formations and given its further simplicity is also promising for large scale application.

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Fatty acid esters enzymatic production for food applications

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Keywords: Biosurfactant, Biodegradable, Organic reactions, Lipase.

Biosurfactants have several advantages over the chemical surfactants, such as lower toxicity, higher biodegradability (Zajic et al., 1977), better environmental compatibility (Georgiou et al., 1992), higher foaming (Razafindralambo et al., 1996), high selectivity and specific activity at extreme temperatures, pH, and salinity (Velikonja and Kosaric, 1993), and the ability to be synthesized from renewable feedstock (Desai and Banat, 1997). Several experiments were done aiming at the study on the enzymatic esterification of the oleic acid with fructose in ethanolic medium, focusing the synthesis of biodegradable biosurfactants. Many chemical surfactant molecules contain ester or amide linkages and are amenable to chemoenzymatic synthesis using microbial and other lipases or proteases. These enzymes typically act in nature as biodegradative hydrolases but can be exploited in bioorganic synthesis reactions by implementing the biocatalysis in low water environments which shift reaction equilibria to favour synthesis rather than hydrolysis (Singh et al., 2007). For that purpose, it was utilized the enzyme, *Candida antartica* B., at temperature of 55 ºC, in reacting time of 48, 72, 96 e 120 hours. Accordingly to the obtained results it was verified that the related enzyme catalyzed primarily the ethanol present in the reactional medium to form the ester: ethyl oleate. This fact was confirmed through magnetic nuclear resonance spectra (^{1}H and ^{13}C) as well as, through infrared spectrum, by the presence of absorption peak at 1738,4 cm^{-1}, characteristic of that ester. The results of the reaction of ethyl oleate production indicate that the highest yield was observed in about 96 hours time, and for the 120 hours time it was observed also an inferior yield. The experiments accomplished for the obtention of fructose esters from oleic acids in ethanolic medium were not successful like in other solvents not recommended for food use. The ethyl oleate shows a lipophilic character and in the food industry it finds application in the osmotic dehydration of tomatoes and peppers “dedo de moça”, improving water release, sugar yield and solar brightness. The use of ethyl oleate in the dehydration process decreases the drying time and increases its efficiency without leaving any traces of chemical residues.

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Kinetic study of enzymatic biodiesel production from macauba acid oil using *Candida antarctica* B lipase immobilized in coconut fiber

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Keywords: biodiesel, macauba acid oil, green coconut fiber, *Candida antarctica* B lipase, immobilized enzyme

Biodiesel production from acid oils is considered as a step up of recycling waste oil and has a positive environmental impact. The conventional technology for biodiesel production employs homogeneous acid or basic catalysts for the transesterification reaction requiring downstream purification equipment to neutralize the catalyst and to purify the biodiesel as well as the glycerol. On the other hand, recently, enzymatic transesterification has attracted much attention for biodiesel production. Enzymatic production of biodiesel is possible using free or immobilized lipases. As reported in the literature, immobilized enzymes reach higher conversion as compared to free enzymes. Use of immobilized lipases for biodiesel production eliminates the disadvantages of the homogeneous process by producing product of very high purity with less or no downstream operations (Fukuda *et al*., 2001). So, immobilized lipases are the key to reduce the biosynthesis cost and increase the performance of lipase-catalyzed transesterification.

Lipases can be immobilized in different supports; however, considering the high cost of some available commercial matrixes to enzyme immobilization, studies have been intensified in order to evaluate cheaper raw materials. In Brazil, the green coconut fibers stand out because it is an agroindustrial waste originated from high consumption of “in nature” and industrial coconut water. This material can be used to immobilize lipases by adsorption (Brígida *et al*., 2007a) or by covalent attachment (Brígida *et al*., 2007b). *Candida antarctica* B lipase (CALB), for example, has showed high operational thermal stability in organic media when immobilized in green coconut fiber by adsorption, showing 80% of residual activities after 6 reuses (Brígida *et al*., 2007a). This result is an indication of potential of CALB immobilized in green coconut fiber for the application of synthesis reactions. Therefore, the aim of this study was to investigate the kinetic of enzymatic esterification and transesterification reaction of macauba high acid oil for biodiesel production using *C. antarctica* B lipase immobilized in green coconut fiber.

Macauba acid oil utilized in this study was purchased from rural producers at Minas Gerais State, Brazil Free fatty acids in the oil were determined by AOCS Official Method. The sample was diluted in a neutralized solvent, followed by titration with a standard NaOH solution (0.1N), in the presence of phenolphthalein as indicator (AOCS, 2004). And, for obtaining the biocatalyst, CALB was immobilized by adsorption on green coconut fiber, at room temperature, through of contacting enzyme and support for 2 h. For each gram of dry support, 10 mL of lipase solution (1000 U/L), in 25 mM sodium phosphate buffer, were used. After immobilization, the biocatalyst was separated by filtration, rinsed with phosphate buffer (10 mL) and dried at vacuum for 10

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min. A batch small-scale reaction was performed in a thermostatized bath from a mixture of macauba acid oil and ethanol, for biodiesel synthesis. 5 to 10 (%, w/oil w) of biocatalyst was added to the system and the alcoholysis was carried at 30 rpm during 96h. For optimization of conversion, the rate of macauba acid oil and ethanol and temperature varied from 3:1 to 6:1 (w/w) and 35 to 50ºC, respectively. The conversion was accomplished at intervals of 24 h. To compare the coconut immobilized lipase with commercial immobilized enzyme, new experiments using Novozyme 435 (Novozyme Latin America Ltd) were conducted at the same experimental conditions.

Macauba acid oil characterization revealed a 27 to 30% free fatty acid content, expressed as oleic acid. Preliminar results of biodiesel production at 35ºC, with 10% of biocatalyst and rate of macauba acid oil and ethanol at 3:1 (w/w), showed that conversion of the acid oils was 53 % after 24.7 h, when CALB immobilized in coconut fiber was used as biocatalyst, and the maximum conversion was reached after 72 h (Figure 1). No significant differences were observed between coconut immobilized lipase and commercial lipase conversion (Figure 2). Thus, to obtain optimized conversion values, the influence of parameters as temperature and lipases loaded were analyzed.

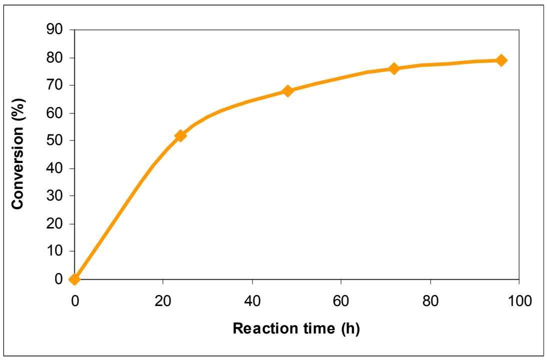

Figure 1. Kinetic of conversion of macauba oil in fatty acids ethyl esters using CALB immobilized in coconut fiber.

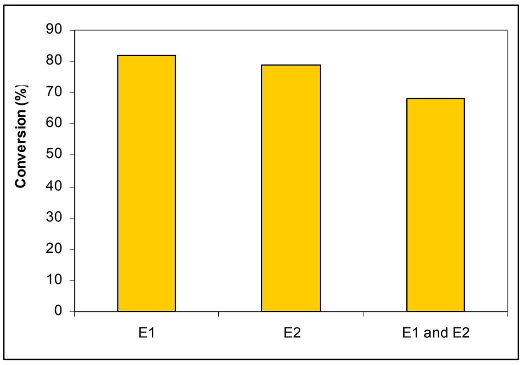

Figure 2. Maximum conversion of macauba oil in fatty acids ethyl esters using: E1 (CALB immobilized in coconut fiber); E2 (Novo 435).

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Enzymatic resolution and separation of *sec*-alcohols based on sustainable acylating agents

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Keywords: Secondary alcohols, lipases, enzymatic kinetic resolution, green chemistry

The pharmaceutical industry is relying increasingly more on the use of single enantiomer form of the active pharmacological ingredient to treat a number of common diseases.[1] Enantiomerically pure alcohols are one of the important intermediates for the production of these ingredients.

Many efficient and stereoselective strategies to access such compounds have been developed over the last decades, namely the enzymatic kinectic resolution.[2]

Enzymatic kinetic resolution of racemic alcohols is a well-established method and often the unique and/or most practical route for the preparation of enantiomerically enriched precursors. [3,4]

Despite of the cost and practicability, this method has some limitations in what concern to the separation step. The chromatographic techniques usually used on the separation of enantiomers are a major drawback on industry scale.

On course of previously work developed in this laboratory, where ionic liquids and ionic acylating agents were applied, it was reported a simple, robust, reliable, reusable and efficient preparative methodology for the one pot resolution-separation of *sec*-alcohols based on the enzymatic resolution with CAL B as biocatalyst. It was also demonstrated by this strategy the possibility of separate both enantiomers only by enzymatic resolution and extraction, without need or chromatographic separations techniques.

Herein we present the progresses achieved using a method for enzymatic kinetic resolution of secondary alcohols with out organic solvents and application of distillation as a green separation technique.

When one alcohol enantiomer reacts with acylating agent, one stays in medium, leaving the other enantiomer free to be distilled. The anchored enantiomer can be removed by enzymatic catalyzed reversible reaction (Scheme 1). With this methodology is possible to obtain both free enantiomers using only the biocatalyst and a sustainable acylating agent.

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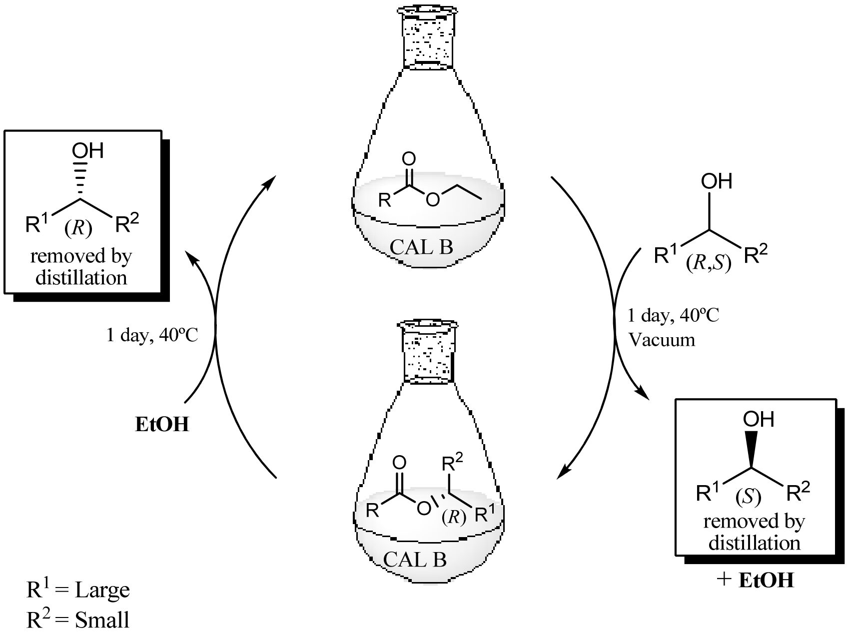

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A graphical approach for rationalization of bi-enzymatic reactions based on a kinetic model

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Keywords: Reaction Engineering; Kinetic Modelling; Cellobiose dehydrogenase; Laccase; ABTS

A model and a graphical method have been developed to describe and visualize the interaction between two enzymes with a redox mediator. In this bi-enzymatic process, the enzyme cellobiose dehydrogenase (CDH; EC 1.1.99.18) oxidizes lactose at the C-1 position of the reducing sugar moiety to lactobionolactone, which spontaneously hydrolyzes to lactobionic acid. 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) is used as electron acceptor and is continuously regenerated by laccase (EC 1.10.3.2) (Baminger et al. 2001; Ludwig et al. 2004). Oxygen is the terminal electron acceptor and is fully reduced to water by laccase, a copper containing oxidase. Oxygen is added to the system by means of bubble-free oxygenation (Fig. 1). The general aim is to assist in understanding the process and to provide a tool for rational design of similar biocatalytic processes leading to intensification and/or a more efficient use of the biocatalysts and redox mediator. This can be accomplished by describing the reaction system with differential equations and integrating them over a desired time interval. Hence, the simulation of reactions of much greater complexity can be attained in comparison with addressing a Michaelis–Menten, steady-state approach.

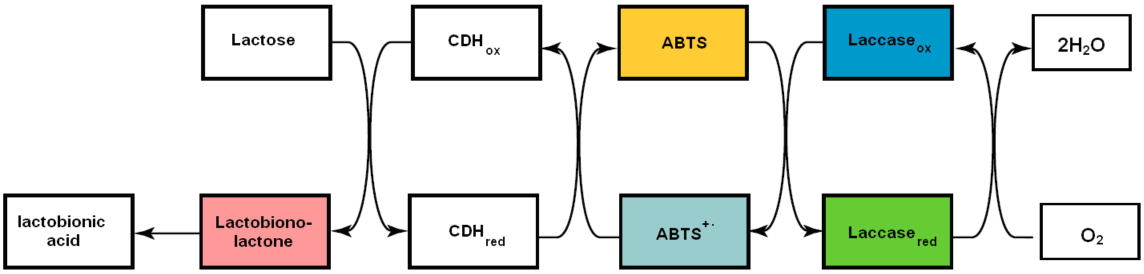

Figure 1: Continuous laccase-catalyzed regeneration of ABTS (redox mediators) used by CDH for the oxidation of lactose to lactobionolactone

We focused on elaborating a methodology to determine the influence of the limiting rate of cellobiose dehydrogenase and laccase and the initial ABTS concentration on the productivity for a given oxygen mass transfer coefficient. This way, the use of the enzymes and the redox mediator ABTS can be rationalized.

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Using the model, the productivity of the process is investigated by simultaneous solution of the rate equations for varying enzyme quantities and redox mediator concentrations, solved with the aid of a numerical solution.

The isocharts developed in this work, provide a novel and easy-to-use graphical tool to determine optimal process conditions. The used methodology can be extended for similar and other enzymatic conversions with cofactor regeneration.

The accuracy of the model has been assessed and is in good agreement with experimental data.

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Enhanced production of recombinant staphylokinase: An experimental design approach

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Keywords: Staphylokinase, Response surface methodology, Central composite design, Optimization, *Escherichia coli*

Thromboembolic disorders have emerged to be one main cause of mortality around the globe. Activation of plasminogen to plasmin is needed to dissolve the fibrin clots (Collen and Lijnen, 1991). The 15.5 kDa bacterial protein staphylokinase (SAK) isolated from *Staphylococcus aureus*, a non-enzymatic cofactor like streptokinase (Brockway and Castellino, 1974), forms a stochiometric protein-protein complex with plasmin(ogen). Cleavage of the peptide bond Lys10-Lys11 of SAK in this complex is the trigger for the formation of an enzyme species capable of activating plasminogen (Schlott *et al*. 1997). The SAK-plasmin(ogen) complex shows a high degree of specificity for cleavage of blood clots with reduced side effects because, in contrast to streptokinase activation, the SAK-plasmin(ogen) complex is strongly inhibited by α_2-antiplasmin circulating in the blood (Collen,1998; Lijnen *et al*. 1991; Silence *et al*. 1993). The protein staphylokinase is currently undergoing clinical trials for the therapy of myocardial infarction and peripheral thrombosis because of its profibrinolytic properties. One of the major contributor to cost reduction of a biological product is its production at high concentration (yield). Owing to the market potential, we aimed for development of a large-scale process for production of staphylokinase, especially with a significantly increased product titer.

Since expression level and physiological properties depend on the *Escherichia coli* mutant host, process parameters can not be generalized for every protein and every expression system. The requirements for maximum growth and productivity are highly case specific and are unique in terms of promoter systems, host-vector interaction, amino acid sequences and the characteristics of the recombinant protein (Shin *et al*. 1997). The effect of parameters such as medium composition, nutrient concentration and availability, dissolved oxygen concentration, specific growth rate, composition and rate of feeding, concentration of inducer and antibiotic (selection pressure), plasmid stability and formation of inhibitory by-products, on productivity, varies from case to case and have to be studied in detail for each process and expression hosts (Lee, 1996).

We formulated strategies for large-scale production of staphylokinase, using *Escherichia coli* cells transformed with plasmid pET-9b containing the gene for staphylokinase. The protein was expressed intracellularly in soluble form. Initial optimization studies, carried out for increasing SAK expression using defined medium resulted in a cell concentration of 1.15 g DCW/L and staphylokinase production of 84.5 mg SAK/L. Further optimization was carried out by using statistical methods, namely Response Surface Methodology (RSM). RSM was employed with 4 variables at 5 levels of concentrations using Central Composite Design (CCD), with glucose, KH_2PO_4, $(NH_4)_2HPO_4$ and various organic nitrogen sources in separate experiments and also in combination by D-optimal design. Cell growth in the optimized medium reached to 1.97 g DCW/L and the optimized staphylokinase expression level went up to 83.0 mg SAK/g DCW corresponding to 163.86 mg SAK/L. The results obtained were validated at fermentation of scale of 5 L, with final cell concentration of 5.8 g DCW/L and staphylokinase production exceeding 415 mg SAK/L in batch mode. In order to further increase cell concentration and staphylokinase production, fed-batch fermentation strategy with feeding of glucose and organic nitrogen sources, resulted in a cell

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concentration of 24.4 g DCW/L and 1290 mg SAK/L. The production of SAK was further scaled up to 20 L fermenter scale and the process was validated. Over all a stepwise optimization strategy using an experimental design allowed development of an improved process for production of recombinant SAK and the results will be discussed at the meeting.

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Biocataysis in organic media by using calixarene-myoglobin complex with pseudoactivity of peroxidase

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Keywords: Calixarenes; Liquid-liquid protein extraction; myoglobin; biocatalysis in organic media; pseudoactivity of peroxidase

During the last two decades, calixarenes have attracted a great attention both in academic and applied research due to their molecular recognition of biological molecules such as amino acids, proteins and carbohydrates (Ludwig, 2005 and Oshima *et al.*, 2005). Calix[*n*]arenes consist of cup-shaped molecules which can form host-guest complex with a wide range of guest molecules by introducing several functional groups either at the upper or lower rim (Oshima *et al.*, 2005).

The present work involves the use of lower rim substituted acid derivatives of *p-tert*-butylcalix[4,6,8]arene (CAL[4,6,8]-ACID) for selective extraction of myoglobin. All three calixarene compounds were found to extract this haemoprotein to organic phase, exhibiting extraction parameters higher than 0.90. Myoglobin-CAL[6]-ACID complex revealed pseudoactivity of peroxidase which catalysed the oxidation of seryngaldazine in the presence of hydrogen peroxide in organic medium containing chloroform. The effect of pH, protein and substrate concentrations was investigated in biocatalysis by using myoglobin-CAL[6]-ACID complex. The highest specific activity of myoglobin was 1.37×10^{-1} U. mg protein $^{-1}$ at initial pH of 6.5 in organic medium. Apparent kinetic parameters ($V'_{máx}$, K'_m, k'_{cat} e k'_{cat}/K'_m) for the pseudoactivity of peroxidase were determined in organic media for different pH values both by Michaelis-Menten and Lineaweaver-Burk plots.

Furthermore, the stability of the protein-calixarene complex was investigated for different initial pH values and $t_{1/2}$ values were obtained in the range of 3.5 – 5.2 days. Myoglobin-calixarene complex present in organic medium was recovered in fresh aqueous solutions at alkaline pH, with a recovery of pseudoactivity of peroxidase over 100 % (Semedo, 2008).

The results strongly suggest that the use of calixarene derivatives is an alternative technique for protein extraction and solubilization in organic media for biocatalysis.

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Immobilization of Lecitase ® Ultra in XAD-2 and XAD-4 under ultrasound irradiation

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Introduction

Lecitase® ultra, a new enzyme preparation with phospholipase A1 activity, was patented [1] and made commercially available. This enzyme is a preparation obtained from the fusion of the genes from lipase *Thermomyces lanuginosus* and phospholipase *Fusarium oxysporum*. Lecitase® ultra was developed principally for degumming process. However, it can be used as enantioselective biocatalyst in fine chemistry [2]. In this work, Lecitase® ultra was immobilized on amberlite XAD-2 and XAD-4 resin to improve the enzyme thermal stability, activity and recyclability.

Experimental

1.1 Enzyme activity assay

Lipase activity was checked by hydrolyzing a stabilized olive oil emulsion as proposed by Freire et al. [3] The methodology was modified to determine the activity of the solid biocatalyst. 0.1g immobilized enzyme was used in each reactor, which contained 19.0mL olive oil emulsion (5.0% w/v), a buffer (sodium phosphate 0.1M pH 7.0) and Arabic gum solution (10.0 % w/v). The reaction was stopped by adding 20mL acetone:ethanol solution (1:1) after the specified time (15 min). Control reactions were performed without the addition of the enzyme. Titration with NaOH 0.05N was performed until pH 11.

1.2 Protein assay

Protein concentration was determined by the Bradford method [4].

1.3. *Immobilization of lipase*

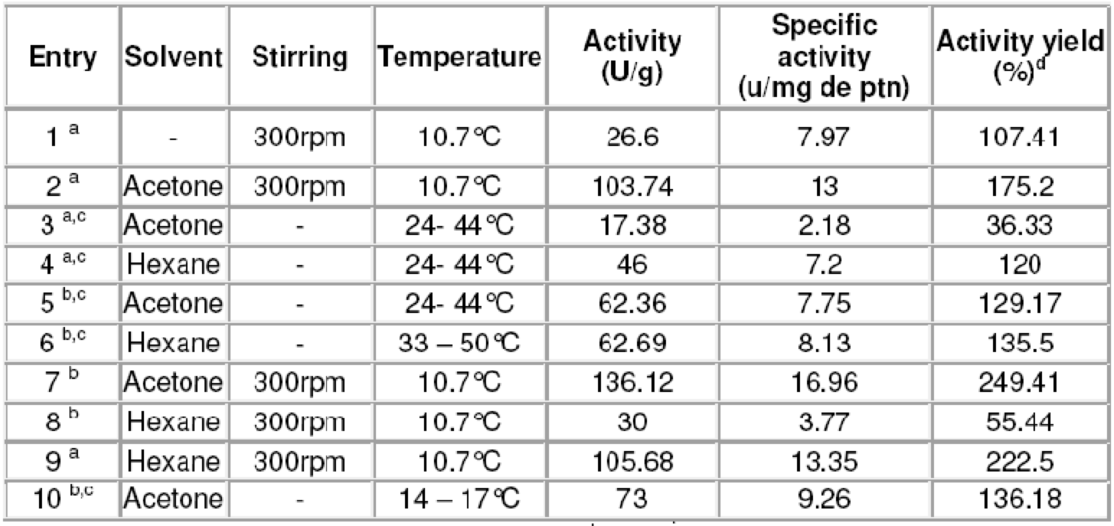
Crude lipase Lecitase® ultra (1g) was dissolved in 6 ml of buffer solution (pH 7.0) and mixed with 2g of amberlite XAD-2 or XAD-4. 30 ml of cold acetone or hexane was added, and mixture was stirred (300 rpm) for 1h at the immobilization temperature of 10.7°C. The immobilized lipase was collected by filtration, washed twice with n-hexane and the solvent evaporated under reduced pressure. The immobilized lipase was then lyophilized [5] and the effects of ultrasonic irradiation were studied.

Results

As shown in Table 1, the use of acetone and pre-treated XAD2 (Table 1, Entry 7), shows better results than the same procedure without previous treatment of XAD2 (Table 1, Entries 2). The use of ultrasound as an activating source does not lead to any improvement on lipase activity as shown in entries 5 and 10 (Table 1). The use of n-hexane as solvent for the Lecitase ® Ultra immobilization gave interesting results (Table 1, Entry 9) for the reaction with XAD 2 without treatment, leading to a specific activity close to the best result obtained with acetone (Table 1, Entry 7). Again, ultrasound does not enhanced the lipase activity under the conditions used in this work.

The best conditions obtained were tested for XAD-4 resin and the results are summarized in Table 2. As shown in Table 2, conversely to the results obtained for XAD-2, the use of ultrasound improved the specific lipase activity when compared with the same reaction profile with only mechanical stirring (Table2, Entries 1, 2 and 3).

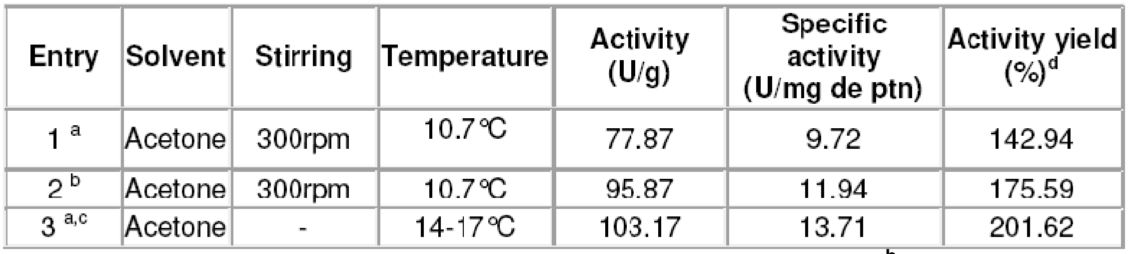
Table 1: Results obtained for the Lecitase ® Ultra immobilization in XAD2:



Entry	Solvent	Stirring	Temperature	Activity (U/g)	Specific activity (u/mg de ptn)	Activity yield (%)[d]
1 [a]	-	300rpm	10.7°C	26.6	7.97	107.41
2 [a]	Acetone	300rpm	10.7°C	103.74	13	175.2
3 [a,c]	Acetone	-	24- 44°C	17.38	2.18	36.33
4 [a,c]	Hexane	-	24- 44°C	46	7.2	120
5 [b,c]	Acetone	-	24- 44°C	62.36	7.75	129.17
6 [b,c]	Hexane	-	33 – 50°C	62.69	8.13	135.5
7 [b]	Acetone	300rpm	10.7°C	136.12	16.96	249.41
8 [b]	Hexane	300rpm	10.7°C	30	3.77	55.44
9 [a]	Hexane	300rpm	10.7°C	105.68	13.35	222.5
10 [b,c]	Acetone	-	14 – 17°C	73	9.26	136.18

[a]XAD 2 was used without previous treatment [b] XAD 2 was washed with methanol and water before immobilization c Ultrasound was used [d] Activity yield (%) = Specific activity of immobilized lipase x 100 / Specific activity of free lipase

Table 2: Results obtained for the Lecitase ® Ultra immobilization in XAD4



Entry	Solvent	Stirring	Temperature	Activity (U/g)	Specific activity (U/mg de ptn)	Activity yield (%)[d]
1 [a]	Acetone	300rpm	10.7°C	77.87	9.72	142.94
2 [b]	Acetone	300rpm	10.7°C	95.87	11.94	175.59
3 [a,c]	Acetone	-	14-17°C	103.17	13.71	201.62

[a] XAD 4 was washed with methanol and water before immobilization; [b] XAD 4 was used without previous treatment c Ultrasound was used; [d] Activity yield **(%) =** Specific activity of immobilized lípase **x** 100/Specific activity of free lipase

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Immobilization of Lipozyme TL 100L on Eupergit®

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Introduction

Lipozyme TL 100 L is extracted from *Thermomyces lanuginosus* and it's available from Novozyme®. Eupergit®, an attractive support for enzyme immobilization, is made by copolymerization of *N*,*N*-methylene-bis-methacrylamide, glycidyl methacrylate, allyl glycidyl ether and methacrylamide. The present work compare the result of two covalent immobilization methods employed for immobilization of Lipozyme TL100L on Eupergit C and Eupergit C 250L. In the first method, the enzymes bind Eupergit via its oxirane group, which react with amino groups of protein to form covalent bonds. The second method used, the support was pretreated and the immobilization carried in the presence of glutaraldehyde.

Lipase immobilization via oxirane groups

The conventional method for enzyme immobilization on Eupergit® involves the direct enzyme binding on polymers via oxirane groups. Unmodified Eupergit® C or C 250L was incubated with native lipase solution in 0,1 M sodium phosphate buffer pH 7.0 at 4 ºC. After incubation for 20 h, the beads were collected by vacuum filtration using a glass filter (Whatman), washed three times with 1M NaCl, afterwards with sodium phosphate buffer, pH 7.0 and stored in it at 4ºC until use.

Lipase immobi1ization by glutaraldehyde

The immobilization procedure consisted of three main steps: polymers pre-treatment with 1,2-diaminoethane, glutaraldehyde activation of polymers, and enzyme coupling to polymers. Glutaraldehyde reacts with the amino group of the amino-Eupergit C or C 250L, and the amino group of the enzyme is then coupled to the carriers via free carbonyl group of the supports bound dialdehyde. Activation of acrylic particles was carried out with 1Maqueous 1,2 diaminoethane solution for 4 h at 60 ºC (pH 10) followed by reaction with 2.5% (w/v) glutaraldehyde solution buffered at pH 8 (sodium phosphate buffer 100 mM) for 2 h at room temperature with occasional stirring. After that, the polymers were washed several times with water and phosphate buffer. Activated polymers were then incubated with native lipase solution in 100mM sodium phosphate buffer at pH 7.0 and 4 ºC for 20 h under slow shaking. After binding, any unbound enzyme was removed by washing the supports three times with 1M NaCl, afterwards with 100mM sodium phosphate buffer, pH 7 and stored in this buffer at 4 ºC until use.

Lipase Activity assays

Specific activities of enzyme preparations were determined in the hydrolysis of olive oil by previously described modified method [1]. The amount of produced fatty acid was determined by titration with 0,05 M NaOH. The activity was expressed as international units (U). One unit of activity is defined as the amount of enzyme needed to liberate 1 µmol of fatty acid per minute.

Protein assay

Protein concentration was determined by Bradford (1976). The protein concentration was determined spectrophotometrically at 595 nm using the calibration curve of BSA.

Results

As shown in Table 1, the immobilization of Lipozyme TL 100L on Eupergit ® without pretreatment with ethylenediamine and glutaraldehyde (Table 1, Entries 1 and 2) shows better results than the immobilization with pretreated support (Table 1, Entries 3 and 4).

Table 1: Results of Lipozyme TL 100L immobilization on Eupergit ®.

Entry	Support	Hidrolitic Activity [a]	Yield(%)	A_{rec}(%)[b]
1	C	148,2	60	11,55
2	C250 L	125,4	80	8,93
3	C modified	91,2	54,5	6,9
4	C250L modified	114	33,3	10,2

[a] U/ g of support [b] *A*Rec. = *A*support/AtSup (disappeared on supernatant).

Conclusion

Lipase immobilized on Eupergit via oxirane group shows higher hidrolitic activity than lipase immobilized with glutaldehyde. The first method was considered to be the most natural procedure, with less negative effects to the enzyme. Further studies on thermal stability as well a deeper evaluation on the influence of immobilization time are needed.

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Use of molecular biology techniques to compare the bacterial community diversity of a lab-scale biofilter and an industrial biofilter treating high loads of H_2S

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Current studies in the field of biological waste air treatment in biotrickling filters have addressed fundamental key issues, such as biofilm architecture, microbiology composition of the process and means to control accumulation of biomass. In the near future, these and new advances should allow a better design of reactor controls and the improvement of pollutant removal in these gas phase bioreactors.

In this study, 16S rDNA-based libraries were used to analyze and compare the microbial communities in lab-scale and industrial scale biotrickling filter (BTF) removing high-loads of hydrogen sulphide (H_2S). The main purpose was to assess the effect of different scale and operational conditions of two BTFs on the microbial community diversity.

Both BTFs were set up at 180 seconds of empty bed residence time (ERBT) for biogas treatment with inlet concentrations of 2000 ppm_v of H_2S. Even if both BTF were performing similarly, i.e. consistent removal efficiencies above 90%, the operating conditions differ between the two BTFs considered. Details of the lab-scale BTF can be found elsewhere (1). It was designed with a single compartment and packed with 3 L of polypropylene HD Q-PAC (Lantec) with a 4 x 4 mm grid opening. The pH was controlled between 6.5 and 7.5 and the working temperature was depending on room temperature. On the other hand, the industrial biotrickling filter was designed as 4 separated modules with a bed volume of 5.2 m^3 packed with 1" Pall rings. The pH of the industrial BTF was controlled between 2 and 3, and the temperature was allowed to fluctuate according to environmental conditions. As a consequence of these differences, we expected specific microbial communities developing in each BTF. Thus, the aim of the present work was to characterize and compare the main bacteria in the reactors, especially those involved in h2s removal to get a better knowledge about the active microorganisms developing in a similar process, depending on the operational conditions and the bioreactor design.

A 16S rDNA clone library was generated for each BTF, and one hundred clones were generated and screened for library. The library construction starts with the DNA extraction from samples of the packing material of the BTFs, followed by a PCR amplification of 16S rDNA of the purified chromosomal DNA using two universal bacterial primers. The products of the PCR reactions were cloned into the pCR® 4-TOPO vector following the instructions given by the manufacturer (Invitrogen TOPO TA Cloning Kits). After plasmid extraction from the positive clones selected, a hundred clones were analyzed by sequencing analysis. To determine the phylogenetic affiliation, similarity search was performed using BLAST (2). Nucleotide sequences were submitted to the database of Ribosomal Database Project II (RDP II) for creating the phylogenetic trees. A weighted joining algorithm (RDP II, Michigan State University) was used.

The results obtained show how critical it is to characterize the microbial community in bioreactors to increase the knowledge of the process. In this comparative study, different predominant microbial populations were found in the two BTFs. Among others details, the full paper will discuss the differences encountered in the microbial community which were mainly related to the operation pH of each reactor. Overall, acidophilic species were found in the industrial biofilter while neutrophilic species were found in the lab-scale bioreactor. Furthermore, the complete microbial populations, their relative abundance and its filogenetical trees will be presented. As an example, Figure 1 shows the phylogenetic tree of some of the species currently found in for the lab-scale BTF.

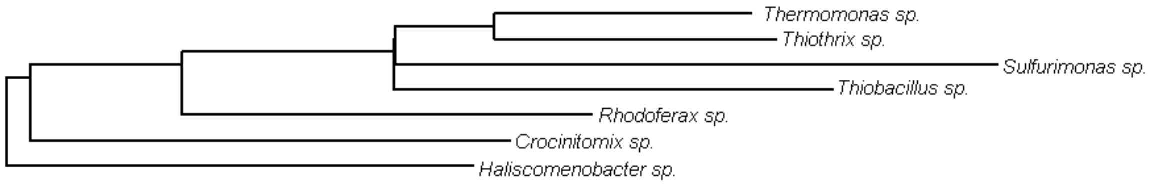

Figure 1. Phylogenetic tree including sequences from the main species from the two clone libraries for the lab-scale biotrickling filter.

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Immobilization of Lipozyme TL 100L in Activated Coal and PANI Under Ultrasound Irradiation

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Introduction

Lipases are enzymes that catalyze the hydrolysis of triglycerides and a wide range of soluble or insoluble esters and amides. Because of this versatility, lipases can be used by industry for a variety of purposes [1]. *Thermomyces lanuginosus* lipase (TIL).presents its three-dimensional structure stabilized by three disulfide bonds and contains four tryptophans, one of which, located in the lid, has been shown to be important for efficient hydrolysis. TIL has a preference for hydrolyzing medium chain (C10-C12) triglycerides and has a high thermal stability. Because of these properties, TIL has found important applications in the detergent industry as a facilitator of fat stain removal in washing powder [2]. In this work, we describe a method for immobilization of Lipozyme TL 100L in both activated coal and polianiline (PANI).

Experimental

1.1. Enzyme activity assay

Lipase activity was checked by hydrolyzing a stabilized olive oil emulsion as proposed by Freire et al.[3] The methodology was modified to determine the activity of the solid biocatalyst. 0.1 g immobilized enzyme was used in each reactor, which contained 19.0mL olive oil emulsion (5.0% w/v), a buffer (sodium phosphate 0.1M pH 7.0) and arabic gum solution (10.0 % w/v). The reaction was stopped by adding 20mL acetone:ethanol solution (1:1) after the specified time (15 min). Control reactions were performed without the addition of the enzyme. Titration with NaOH 0.05N was performed until pH 11.

1.2 Protein assay

Protein concentration was measured according to Bradford using bovine serum albumin (BSA) as a standard [4].

1.2. Imobilization of lípase in activated coal

The enzyme (10% crude lipase Lipozyme TL100L) was placed in contact with 1.0g of activated coal and 30 ml of phosphate buffer solution (25 mM, pH 7,0). The mixture was shaken (150 rpm) for 150 min at room temperature (25ºC). The resulting mixture was filtered and the residue was washed with phosphate buffer solution. The activity of the immobilized enzyme and the supernatants were checked.

1.3. Imobilization of lipase in polianiline

The enzyme (0.0830g crude lipase Lipozyme TL100L) was placed in contact with 100 ml of phosphate buffer solution (0.1 M, pH 6.0). After that, 50 mL of this solution and 1.0 g of polianiline was shaken for 2 hours at 4oC. The resulting mixture was centrifuged and the residue was washed with phosphate buffer solution. The activity of the immobilized enzyme and the supernatants were checked.

Results

The results on Table 1 show that the best conditions for immobilization of Lipozyme TL 100L on both activeted coal and PANI are in abscence of ultrasound irradiation (Table 1, Entries 1 and 3).

Table 1: Results of Lipozyme TL 100L immobilization on activated coal and PANI.

Entry	Support	pH	At_0 (U/mL)	At_f (U/mL)	At_d (U/g)
1	Activated Coal	7	41,8	33,17	94,96
2	Activated Coal	7 [a]	47,87	25,36	63,61
3	PANI	6	11,85	7,53	73,00
4	PANI	6 [a]	13,34	7,73	61,93

[a] immobilization under ultrasound irradiation; At_0= initial activity of the enzyme solution; At_f = supernatant activity; At_d = immobilized activity

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Production and chromatographic behaviour of polygalacturonase from *Pleurotus ostreatus* on immobilized metal chelates

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Keywords: Tomato pomace; Immobilized Metal Affinity Chromatography; polygalacturonase; lignocellulosic enzymes; *Pleurotus ostreatus*.

A strain of *Pleurotus ostreatus* was grown in submerged culture in tomato pomace as sole carbon source for production of polygalacturonase. The culture of *P. ostreatus* revealed a peak of polygalacturonase activity (2181 U/L) on 4th day with specific activity of 42.8 U/mg protein. Differential chromatographic behaviour of polygalacturonase, xylanase and laccase from *P. ostreatus* was investigated on immobilized metal chelates. The effect of ligand concentration, pH, the length of spacer arm and the nature of metal ion was studied for enzyme adsorption on immobilized metal affinity chromatography (IMAC). The presence of imidazole in the equilibration buffer abolished the adsorption of the enzymes to immobilized metal chelates. A one-step purification of polygalacturonase from *P. ostreatus* was devised by using a column of Sepharose 6B-EPI 30-IDA-Cu(II). Purified enzyme exhibited a specific activity of about 1600 U/mg protein, final recovery of enzyme activity of 80% and a purification factor of about 65. The purified enzyme preparation was analysed by SDS-PAGE as well as by in situ detection of enzyme activity. Purified preparation of polygalacturonase exhibited a pH and temperature optima of activity at 7.0 and at 50ºC, respectively. The kinetic parameters (Vmax, Km, Kcat, and Kcat/Km) of purified enzyme were found to be 5530.8 ± 260.7 U/mg of protein, 13.23 ±2.79 mg/ml of polygalacturonic acid, 5553.01 ± 261.7 $s^{-1}$and 419.72 $s^{-1}.mg^{-1}$, respectively. Purified enzyme exhibited a half life (t ½) of 60 ± 7.45 min and 35 ± 0.37 min at 50ºC and at pH 6.0 and 7.0, respectively.

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Release of ibuprofen, naproxen and flurbiprofen from ethylcellulose microparticles

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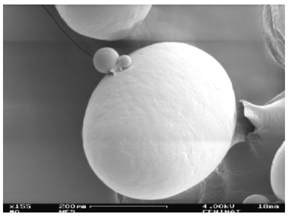
Keywords: NSAID, oral drug delivery, ethylcellulose microparticles, controlled release, polymeric drug delivery systems, diffusion coefficients

Purpose: This work reports the preparation of non-degradable microparticles made of ethylcellulose containing non-steroid anti-inflammatory drugs (NSAID), that have a common chemical group and different side chains, using the solvent evaporation method. For each drug, in vitro drug release studies were performed under different pH. Based on Fick´s second law of diffusion, the release concentration of drugs was described and the drug diffusion coefficient within the polymer matrix was determined. This work evaluates how the structure of the drug molecule affects the rate of drug release.The obtained particles were characterised for size and size distribution, morphology, thermogram properties and encapsulation efficiency and drug loading.

Methods: Empty and drug-loaded microparticles were prepared by the solvent evaporation method by dissolving ethylcellulose (EC) in methylene chloride. When preparing drug-loaded microparticles, the drug was added to this solution. This solution was slowly poured into a 2.5 % w/v methylcellulose aqueous solution and stirred with a two-baffled rotator for 1 hour at a controlled stirring speed. The formed oil-in-water (o/w) emulsion was than gently stirred at room temperature until complete evaporation of the solvent. Microparticles were then collected by centrifugation, washed, filtered and dried in a desiccator under vacuum at room.

The drug entrapped in the microparticles was determined by HPLC (Dubernet et al., 1991).The obtained microspheres were characterized in terms of shape and external morphology by scanning electron microscopy (SEM), particle size and size distribution by Laser light scattering (LLS) and cristalinity propreties by differential scanning calorimetry (DSC). In vitro drug release studies were performed in gastric (KCl 1.5 M; pH 1.5) and intestine (PBS; pH 6.8) simulating conditions at 37ºC (in sink conditions).



Results: All the drugs under study were successfully incorporated achieving encapsulation efficiencies between 80 % and 100%.

Morphology: Figure 1 shows the shape and morphology of ethylcellulose microparticles obtained by SEM. As can be seen, microparticles present a spherical shape and a smooth surface.

Size analysis: The mean diameter for all the microparticles obtained was found in the range from 100 to 800 μm. By increasing the stirring speed from 350 rpm to 1200 rpm the particle size decreases from 667 μm to 100 μm. However, a stirring speed of 1200 rpm causes great drug losses.

DSC analysis: The Tg of EC is barely shifted to 129.0ºC when EC is processed as microspheres. However, a significant fall of ethylcellulose Tg to 101.1 ºC, 97.47 ºC and 80.59 ºC is obtained for spheres loaded with ibuprofen, naproxen or flurbiprofen, respectively. These results show that the polymer chains are more flexible. Therefore, all the drugs under study act as plasticizers for ethylcellulose with flurbiprofen having the strongest plasticizing effect.

In vitro drug release kinetics:

The drug release from micropheres can be calculated using Fick´s second law of diffusion. Considering the spherical geometry of the investigated system:

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$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial r^2} + \frac{2D}{r}\frac{\partial C}{\partial r} \qquad (1)$$

where C represents the concentration of drug within the microparticles, being a function of time t and position r, and D represents the diffusion coefficient of drug within the polymeric microspheres.

The initial condition is: $t = 0 \qquad C = C_0$ for $0 \leq r \leq R$ (4)
where C_0 represents the initial drug concentration within the microparticles and R the micoparticle radius.

Assuming an infinite mass transfer coefficient in the boundary layer (this assumption was experimentally proved) and perfect sink conditions,
- the second boundary condition comes: $t > 0 \quad r = R \quad C = 0$ (2)

Using the method of Laplace transform (Crank, 1975) assuming constant drug diffusion coefficients, the following analytical solution of Fick´s second law to describe the drug release kinetics is therefore obtained.

$$\frac{M_t}{M_\infty} = 1 - \frac{6}{\pi^2}\sum_{n=1}^{\infty}\frac{1}{n^2}\exp(-\frac{Dn^2\pi^2 t}{R^2}) \qquad (3)$$

where M_∞ is the absolute cumulative amount of drug released at infinite time;

Table 1: Diffusion coefficients values for the model equation Eq3

Drug	pH	D (m^2s^{-1})	r^2
naproxen	1.2	1.23 (± 0.07) × 10^{-12}	0.999
	6.8	6.9 (± 0.5) × 10^{-12}	0.998
Flurbiprofen	1.2	2.2 (± 0.3) × 10^{-12}	0.974
ibuprofen	1.2	2.8 (± 0.70) × 10^{-12}	0.982

Therefore, fitting the experimental results with equation (3), the diffusion coefficient, D, for each drug in the polymeric matrix could be determined. The obtained values are presented in Table 1. The cumulative experimental drug release values versus time and the values given by the model equation for the experiments performed different release pH media and different drugs are presented in Figures 2 and 3, respectively. As can be seen in these Figures, a good agreement between the model profiles for the release process (solid curves) and the experimental values (symbols) was obtained.

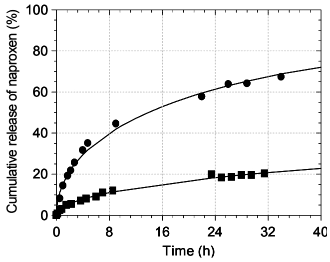

Figure 2: Cumulative amount of naproxen released, pH 1.2 (■) and pH 6.8 (●)

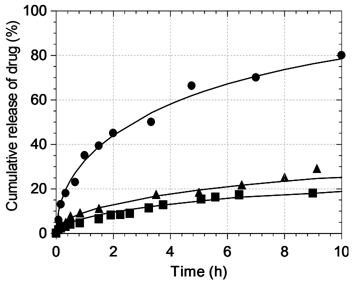

Figure 3: Cumulative amount of drug released pH 1.2: Ibuprofen (●), naproxen (■) and flurbiprofen (▲)

Conclusions: The speed of stirring is a key parameter that controls the particle size and drug encapsulation efficiency and consequently the drug release rate.The developed model describes satisfactorily the evolution of the cumulated amount of drug released in vitro with time. Using these model equations, it is possible to predict the effect of certain parameters on the drug release profile.

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Procyanidin Average Degree of Polymerization

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Keywords*:* Procyanidins, DPn, FT-IR spectroscopy, O-PLS, PLS1, grape seed.

Grape seeds are known as an abundant rich source of procyanidins formed by both (+)-catechin and (-)-epicatechin monomers (Escribano-Bailon et al., 1992), as well as by more polymerized fractions containing type-A and type-B procyanidin linkages (Passos et al., 2007) (Passos et al. 2007). Although the polymerized fractions represent more than 70% of all procyanidins, they have been only easily quantified up to tetramers (Monagas et al., 2003). The interest upon the knowledge of their degree of polymerization has influenced the application of conventional experimental methods to assess them. At the same time, growing interest is focused upon the development of faster, cheaper and cleaner methodologies. Nowadays, infrared spectroscopy appears as a very useful tool to accomplish a rapid evaluation of the procyanidin composition of a given sample, being a widespread technique in food components.

Chemometric methods allow the extraction of useful information from high complex samples, among which the multivariate regression methods are widely used to build calibration models. Partial Least Squares (PLS) regression (Wold et al. 1983) is one of the most adopted.

In this work, procyanidins were extracted with methanol and acetone/water from seeds of white and red grape varieties. A fractionation by graded methanol/chloroform precipitation allowed us to obtain a broad range of extracts with decreasing average degree of polymerization (DPn). Such extracts were characterized by thiolysis (pre-treatment) followed by HPLC-UV detection, from which the corresponding DPn values were finally determined. The same extracts have been also characterized by FT-IR spectroscopy within the region of 1300-800 cm^{-1}. Both results exhibit a good linear relationship as Figure 1 points out, where the relationship between measured and estimated DPn values is plotted.

In this essay, a data pre-treatment – called Orthogonal Projection to Latent Structures (O-PLS) (Trygg, 2002) – in tandem with PLS1 regression has been applied. Such approach clearly improved the model robustness by reducing the number of Latent Variables from 12 to 1, which facilitates the FT-IR features interpretation.

Summarily, a new simple and accurate model for the prediction of the DPn of procyanidins is presented.

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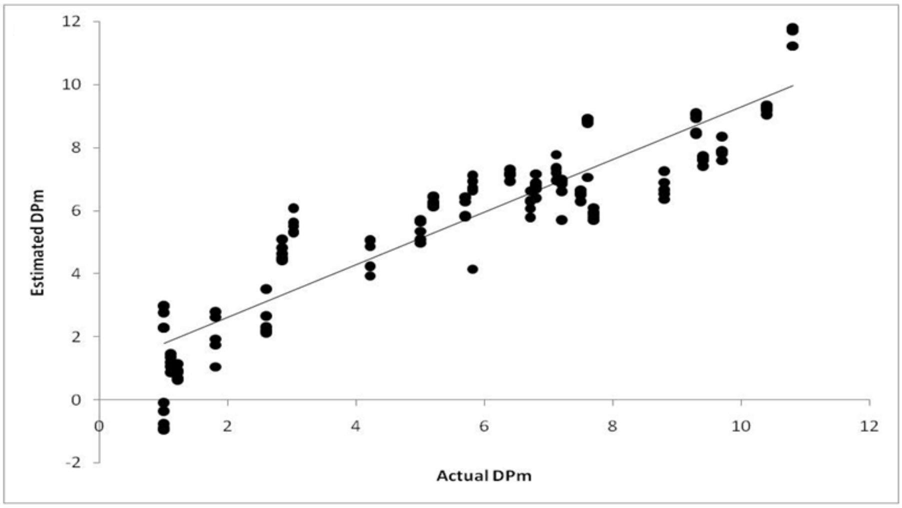

Fig1-: Relationship between measured *versus* estimated DPn values (Regression model using O-PLS).

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One-step purification of hemoglobin from human erythrocytes by using a novel chromatographic matrix based on polymer-bound calix[6]arenes

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Keywords: Chromatographic matrix; *p-tert*-butylcalix[6]arene; polymer; purification of hemoglobin; human erythrocytes; pseudoactivity of peroxidase.

Calixarenes are host molecules which exhibit molecular recognition towards a wide range of guest bioactive substances such as amino acids, proteins and carbohydrates (Ludwig, 2005 and Oshima *et al.*, 2002). The complexing abilities shown by these cyclic oligomeric compounds has stimulated their potential use as molecular and biomolecular receptors immobilized on an existing polymeric matrix, for which appropriate grafting methods have been developed (Barata *et al.*, 2004).

The present work involves the use of a novel chromatographic matrix based on a polymer-bound acid derivative of *p-tert*-butylcalix[6]arene for one-step isolation of haemoglobin from human erythrocytes. The red cell lysate was applied to a column packed with this matrix which was previously equilibrated in phosphate buffer. The adsorbed proteins were eluted from the column with a linear gradient of Na_2CO_3 pH 11.0 and the column fractions were analysed for protein and pseudo-activity of peroxidase. Human haemoglobin was purified in a one-step procedure with a recovery of pseudoactivity of about 35% and a purification factor of about 12. The purified preparation of haemoglobin was analysed both by SDS and native PAGE which exhibited single proteins bands with Mr of 16.0 and 61.0 KDa, respectively. Furthermore, the single protein band observed in native PAGE was coincident with the pseudoactivity of peroxidase band detected *in situ* on this gel. Protein adsorption on this novel chromatographic matrix involved several interactions such as electrostatic and hydrophobic and other parameters affected this process such as protein size, pI, pH, ionic strength and nature of buffer ion (Semedo, 2008).

The results strongly suggest that this novel chromatographic matrix presents some advantages over other matrices as far as selectivity is concerned for protein purification.

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Kinetic resolution of 1,2-epoxyhexane by whole cells of *Rhodotorula glutinis* in a two-liquid phase system

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Keywords: kinetic resolution, two-phase, whole cell, organic solvent

Introduction

Epoxides and diols are important intermediates for pharmaceutical and organic synthetic chemistry, because each enantiomer shows different biological activity. The preparation method for enantiopure epoxides is composed to asymmetric synthesis and kinetic resolution. For the production of enantiopure epoxides, some chemocatalytic and biocatalytic routes have successfully been developed. Recently enzymatic enantioselective degradation of racemic epoxides using epoxide-hydrolases, which are relatively cheap, has been recognized as an alternative to these synthetic routes. The productivity of this biocatalytic route is limited by the low solubility of hydrophobic epoxides to aqueous solution and inhibition of enzymatic reaction at high diol concentration. To resolve these problems, the organic solvent forming two-liquid phase has been used. Decane and octane were selected in resolution of 1,2-epoxyhexane (Choi *et al.*, 1999) and styrene oxide (Baldascini *et al.,* 2001), respectively. In this paper, we examined the kinetic resolution of racemic 1,2-epoxyhexane under the two-liquid phase system. We described the kinetic analysis of this system using decane and the effect of organic solvent on the initial reaction rate and enatiometric ratio, *E*.

Experimental

The yeast, *Rhodotorula glutinis* ATCC201718, was cultivated for 48 h in the medium described previously (Choi *et al.*, 1999) at 30ºC. The cells were harvested by centrifugation and washed with the buffer. Hydrolysis of 1,2-epoxyhexane was conducted in screwed capped bottles sealed with rubber septa. Ten ml cell suspension of *R. glutinis* (0.9 g-drycell/ml) and 10 ml organic solvent containing 1,2-epoxyhexane were added in the bottles. Samples were taken periodically from the organic and aqueous solutions. Organic samples were analyzed by chiral GC. Aqueous samples were extracted with an equal volume of ethylacetate and then analyzed by chiral GC.

Results and discussion

Figure 1 shows the time-courses of epoxides and diols in the presence of dodecane. It clearly appeared that *R. glutinis* prefentially hydrolyzed (R)-epoxide and produced (R)-diol.

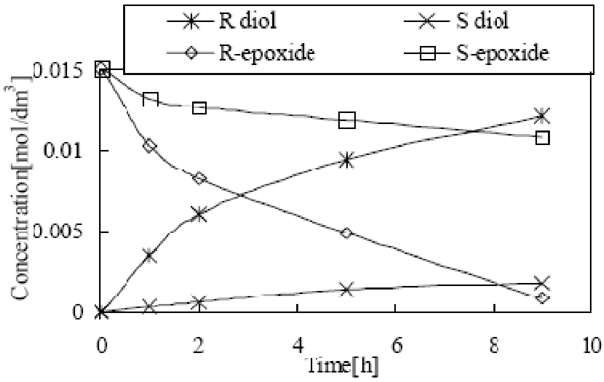

Figure 1 Kinetic resolution of *rac*-1,2-epoxyhexane by *Rhodotorula glutinis* using dodecane.

The enzymatic conversion of both enatiomers in the aqueous solution can be described by competitive Michaelis-Menten kinetics, competitive product inhibition and distribution equilibrium of epoxides.

$$r_{R_0} = \frac{V_{\max}^{R}[R]_0}{(1+D)K_m^{R} + \left(1+\frac{K_m^{R}}{K_m^{S}}\right)[R]_0 + (1+D)K_m^{R}\left(\frac{[D_S]_0}{K_I^{D_S}} + \frac{[D_R]_0}{K_I^{D_R}}\right)}$$

We determined the parameters included in above equation by kinetic experiment in the absence and presence of diols in the initial organic solution, and distribution experiment and showed in Table 1. Unlike in the case of hydrolysis of styrene oxide by *Agrobacterium radiobacter* AD1 (Rink and Janssen, 1998), preference of (R)-enantiomer was caused by the large difference in the maximum velocities of enatiomers.

Table 1 Kinetic parameters

Parameter	Value
V_{max}^{R}	3.41×10^{-2} mol dm^{-3}h^{-1}
V_{max}^{S}	1.20×10^{-3} mol dm^{-3}h^{-1}
K_m^{R}	6.90×10^{-3} mol dm^{-3}
K_m^{S}	4.25×10^{-3} mol dm^{-3}
$K_I^{D_R}$	1.80×10^{-2} mol dm^{-3}
$K_I^{D_S}$	7.33×10^{-1} mol dm^{-3}
D	36 -

We examined the effect of organic solvents on the initial velocities, r_{R0} and enantiometric ratio, *E* and tried to correlate the values of r_{R0} and *E* with the physical properties of the solvents. Figures 2 and 3 show the relationships between r_{R0} and log *P*, and *E* and solubility parameters, respectively. Good correlations were obtained.

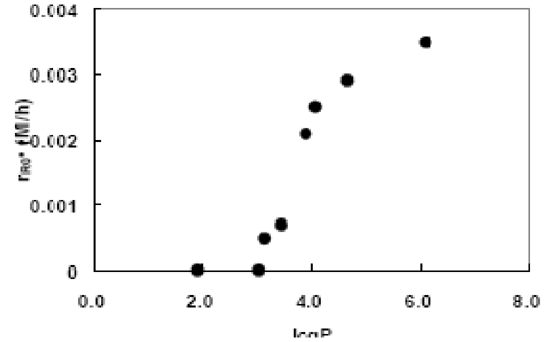

Figure 2 Relationship between r_{R0} and log *P*

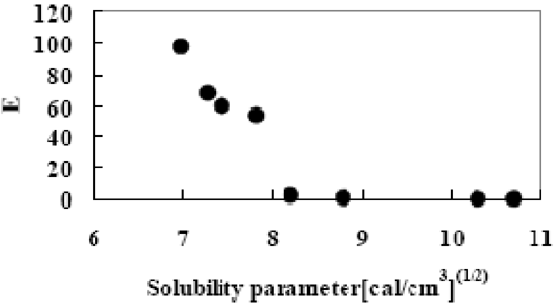

Figure 3 Relationship between *E* and solubility parameters

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A multipurpose hydrogel system for biocatalyst immobilization

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Keywords: Hydrogels, polyvinyl alcohol, immobilization of enzymes and cells, biotransformations, encapsulation

Hydrogels are suitable materials for biocatalyst immobilization and concomitant application in biotransformation systems of relevance in important industrial areas, such as food and feed, pharmaceutical, fine chemicals, renewable energy sources and bioremediation (Guisan, 2006). Hydrogels typically present biocompatibility, low coefficient of friction, and high water content. Still, most hydrogels, namely those from natural polymers, have low mechanical stability (Vorlop et al, 1996) and are furthermore prone to enzyme leakage (Cao, 2005). Polyvinyl alcohol-based matrices have been shown to lead to particles with considerable mechanical stability, and thus present a suitable alternative to overcome the drawbacks of natural polymers while retaining the benefits inherent to their use (Schlieker and Vorlop, 2006). Several methods have been developed to form hydrogels from polyvinyl alcohol (PVA). In the present work a modified procedure for the encapsulation of biocatalysts in PVA capsules from LentiKats® liquid (GeniaLab, Germany) is presented. In this method, the PVA based hydrogel is extruded to low molecular weight polyethylene glycol (PEG) or polypropylene glicol (PPG), where gelification occurs instantaneously, in the form of spherical-like capsules. The procedure is quite simple and the PEG or PPG can be recycled for several times. The feasibility of the method was evaluated through immobilization of enzymes (cutinase, penicillin acylase, inulinase) and whole cells (*Mycobacterium* spp. and *Rhodococcus* spp.), currently used in our laboratory, and the resulting biocatalysts were tested in their corresponding applications. These include inulin/sucrose hydrolysis (inulinase), penicillin G hydrolysis (penicillin acylase), and ester hydrolysis (cutinase), sitosterol side-chain cleavage (*Mycobacterium* spp.) and conversion of limonene-1,2-epoxide to limonene-1,2-diol (*Rhodococcus* spp.) No enzyme leakage was observed, neither during immobilization procedure, even for the low molecular weight cutinase (21 kDa), nor during bioconversion runs, provided temperature did not exceed 55ºC. Biocatalytic activity was maintained following immobilization, although diffusion limitations occurred, particularly when bulky inulin substrate was used. Nevertheless all immobilized biocatalysts were effectively used in aqueous or organic media, if the latter was more appropriate. PVA capsules maintained their integrity even in the presence of magnetic stirring and immobilized biocatalysts were used in consecutive batch runs without activity loss, particularly the multipurpose inulinase and penicillin acylase.

The method developed is quite easy to implement, is relatively cheap, can be easily scaled-up and applied to a wide array of biocatalysts, thus providing a suitable alternative to currently used approaches

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The effects of operation parameters on the electrodialysis recovery of citric acid from dilute solutions

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Citric acid is of paramount industrial importance because it is a useful product, which has been widely used in dairy, medicine and biochemical industries [1]. The production of citric acid is normally accomplished by fermentation whereby the separation from the fermenter broth is achieved by a chemical method. This traditional process causes a large amount of water effluent as well as solid residue. Electrodialysis is an environmentally friendly alternative to the technology which is currently in use. Electrodialysis has been developed not only for the recovery of useful components, but also for recycled use of water. In particular, there is an increasing use of sodium citrate in washing formulations. Sodium citrate is used as builder and buffering agent in liquid detergents to replace the tetrapotassium pyrophosphates, which has become a global concern for the environment [2].

The objective of this work was to investigate the effects of feed solute concentration, applied cell voltage, feed flow rate and pH of the feed solution on the overall performance of electrodialytic recovery of citric acid from dilute solutions.

The experiments have been performed in batch recycle mode at room temperature (24°C). The experimental set-up consisted of a three compartment perspex cell, a power supplier and three peristaltic pumps. The cathode and anode compartments of the cell were separated from the center compartment with Ionac MA-3475 anion exchange and MC-3470 cation-exchange membranes (Sybron Chemicals). The carbon cloth and stainless steel were used as an anode and cathode, respectively. The effective area of the membranes and electrodes inside the cell was 76 cm^2. The 0.05 M sodium chloride solution was circulated through the electrode compartments at a flow rate of 0.5 $mL.s^{-1}$ during the experiments. Citric acid solutions were prepared by dissolving solid citric acid in deionised water and the desired pH-values were obtained by adding NaOH. The variation of citric acid concentration was determined by titration method at every10 minutes.

The experiments can be summarized as follows:

i. To investigate the effect of cell voltage, the trials were carried out at constant feed solution concentration of 8.0 $g.L^{-1}$, feed flow rate of 2.60 $mL.s^{-1}$ and feed solution pH of 3.0, by applying the voltages of 7.5, 15, 30 and 45 V.
ii. To investigate the effect of feed flow rate, the trials were carried out at constant feed solution concentration of 8.0 $g.L^{-1}$, pH of 3 and cell voltage of 15 V, by applying 1.00, 2.60 and 6.80 $mL.s^{-1}$ flow rates.
iii. To investigate the effect of feed concentration, the trials were carried out at constant cell voltage of 15 V, flow rate of 2.60 mLs^{-1} and pH of 3, by using the concentrations of 8 $g.L^{-1}$ and 16 $g.L^{-1}$.
iv. To investigate the effect of pH, the trials were carried out at the constant feed solution concentration of 8.0 $g.L^{-1}$, feed flow rate of 2.60 $mL.s^{-1}$ and voltage of 15 V, by employing the pH values of the feed solutions as 3.0 and 4.0.

During the experimental studies, the effect of operation parameters on the recovery rate of citric acid, current efficiency and energy consumption were examined [3].

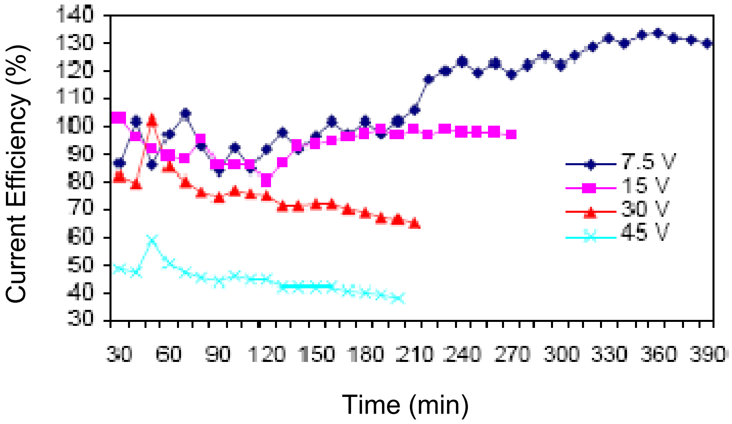

Figure 1. Variation of current efficiency with time for a range of applied of voltages (The concentration of the feed solution: 8.0 g.L^{-1}; pH=3 ; flow rate of solution=2.60 mL.s^{-1})

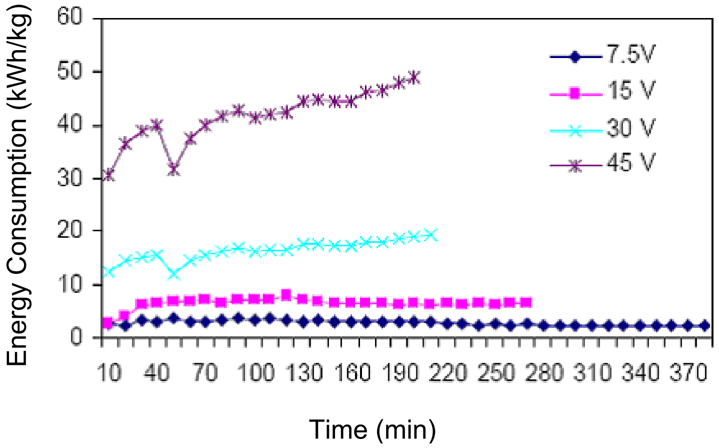

Figure 2. Variation of energy consumption with time for a range of applied of voltages (The concentration of the feed solution: 8.0 g.L^{-1}; pH=3 ; flow rate of solution=2.60 mL.s^{-1})

On the basis of the above mentioned experimental trials, electrodialysis appeared to be quite effective for the recovery of citric acid. As seen in figures 1 and 2, the results obtained by applying 7.5 and 15 V showed that the current efficiency and energy consumption values were nearly similar in both cases. However, since the recovery of citric acid took more time in the case of 7.5 V applied voltage, the 15 V applied voltage value was chosen as optimum by considering membrane fouling. On the other hand, the 30 V and 45 V applied voltages could not be considered as optimum values due to the lower current efficiencies and higher energy consumptions obtained when these voltages were applied. Regarding the experimental results, the optimum values applied voltage, feed solution concentration, feed flow rate and feed solution pH values were chosen as15 V, 8.0 g.L^{-1}, 2.60 mL.s^{-1} and 3.0, respectively, in view of the higher current efficiency (~96%) and the lower energy consumption (~6.0 kWh/kg).

The results of this work can also serve as a basis for the evaluation of electrodialysis by using both monopolar and bipolar membranes in demineralization of cheese whey or deacidification of fruit juice.

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Influence of operational fed-batch strategies on heterologous *Rhizopus oryzae* lipase production in *Pichia pastoris* system using mixed substrates

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The methylotrophic yeast *Pichia pastoris* has been widely reported as a suitable expression system for both basic research and industrial use. There are two main types of *P. pastoris* host strains with regard to their ability to assimilate methanol: Mut^+ and Mut^s (Cereghino and Cregg, 2000; Macauley-Patrick et al., 2005). Although Mut^s is not as sensitive to residual methanol in the cultivation media ad Mut^+ strains, the lower specific growth rate of Mut^s limits the productivity of the process.

To increase cell density and process productivity, as well as to reduce the induction time, a typical approach is the use of a multicarbon substrate in addition to methanol, generally glycerol or sorbitol (Cos et al., 2006). Recently, specific production rate of *Rhizopus oryzae* lipase (ROL) in *P. pastoris*, a protein that triggers the unfolded protein response in this host microorganism, has been improved by the co-feeding of sorbitol/methanol mixed substrates in batch and preliminary fed-batch cultures (Ramon et al., 2007).

In this work we present a systematic study of the influence of methanol set-point and sorbitol feeding rate in fed-batch operation with Mut^s phenotype. Four experiments were made at a constant methanol set-point of 0.5 $g \cdot l^{-1}$ and 2 $g \cdot l^{-1}$ controlling by a predictive control algorithm with two different sorbitol feeding rate to assure a constant specific rate of μ_{max} and $\mu_{max}/2$ by means of a pre-programmed exponential feeding rate strategy. Also the influence of ammonium addition in the induction phase has been tested. Ammonium addition has an important effect on protein production, and the best strategy was to feed jointly with sorbitol in its stoichiometric relation during the induction phase.

Lipolytic activity, yields, productivity and specific productivity, but also specific growth, consumption and production rates were analyzed showing that the best conditions were reached when the methanol set-point was 2 $g \cdot l^{-1}$ with a low influence of the constant specific growth tested.

Acknowledgements

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Ramón, R., Ferrer, P. and Valero, F. 2007. J. Biotechnol. 130:39-46,

Adaptive control of glycerol and methanol feeding in recombinant *Pichia pastoris* cultures: Impact on antibody titre

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Keywords: Aerobic cultivation processes, Dissolved oxygen control, Adaptive control, Recombinant *Pichia pastoris*.

Pichia pastoris is currently viewed as a very promising host for heterologous protein expression (Cereghino and Cregg, 2000). P. pastoris can be easily manipulated at molecular genetic level, it has the ability to express high levels of proteins, either intra or extracellularly and it has the capability to perform eukaryotic post-translational modifications such as glicosylation, disulfide bond formation and proteolytic processing (Cereghino and Cregg, 2000, Cereghino et al., 2002).

In recent studies, *P. pastoris* has been genetically engineered to secret human glycoproteins, such as rEPO, with homogeneous fully complex terminally sialylated N-glycans (Hamilton et al., 2003, 2006; Choi et al., 2003, Bobrowicz et al., 2004). These studies open new perspectives for the production of therapeutic proteins in yeast cell cultures with advantages such as reducing both fermentation and development (from gene to protein) times, lower operating costs, chemically defined media, higher protein titers and no viral contamination.

P. pastoris is normally cultivated in fed-batch mode in order to limit the amount of substrate in the reactor. This yeast (which can tolerate pH variations) does not seem to increase its cell productivity with pH changes, although achieving different protease activities (Shi et al., 2003).

This yeast is able to grow at very high cell densities with massive oxygen uptake requirements, being the oxygen transfer capacity, a critical limitation factor. Charoenrat et al. (2006) concluded that high cell productivity could be improved by increasing oxygen transfer rate by applying elevated air pressure or using oxygen limitation.

To apply oxygen limitation to the cultivation it is needed a feeding controller, which has been used by several authors. Oliveira et al. (2005) developed two indirect adaptive control algorithms, based on dissolved oxygen (for both algorithms) and oxygen transfer rate (for one of the algorithms) measurements. It was proved that off-gas measurements did not improve the controller performance since responses by estimator and controller were being caused by the measurement delays.

In this paper, a direct adaptive controller is derived and tested in *P. pastoris* cultivations. The culture is run in three phases: a batch phase, an exponential feeding fed-batch phase and a oxygen transfer limitation (OTL) phase, where the dissolved oxygen tension (DOT) is regulated at a constant value by the controller. An adaptive controller design was adopted, which is shown to be globally stable in the Lyapunov sense. The impact of the medium, starting biomass concentration and DOT set point (fin the OTL phase) was studied.

This work showed that the proposed controller can regulate the DOT at very low levels (5 %) with high stability, thereby enhancing the oxygen transfer at high cell density state, which finally leads to a higher antibody titre.

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Study of the influence of wetting and emulsifying agent in the activity of crude xylanase of *Bacillus pumilus* CBMAI 008 for use in bioscouring

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Keywords: stability, textile, cotton, bioscouring, xylanase

Enzymes application in textile processes for cotton fibre fabric biopreparation has increased significantly, mainly due to the advantages on the quality of the final product. Several enzymes can be used, however few of them have the ability to remove the lignin-containing seed-coat fragments (Csiszár et al., 2001; Csiszár et al., 2006; Hartzell and Hsieh, 1998). The use of enzymes in any industrial process requires strict operating conditions such as control of pH and temperature, because they affect significantly the activity, especially the enzyme stability. However, for application in industrial processes, particularly in the textile industries, it is verified that the chemicals used have a significant influence, maily if they are tensoactive agents (Mizutani et al., 2002; Calafell and Garriga, 2004; Paria et al., 2005). This study evaluated the thermostability of crude xylanase of B. Pumilus in the presence of different wetting concentrations and emulsifying agent, nonionic, through the remaining activity (Miller, 1959; Bailey et al, 1992). The results of these combinations showed that the emulsifying chemical products presented no effect on the enzyme activity, but the wetting chemical products showed significant effect, probably due to the aliphatic alcohols present in their chemical struture.

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Yarrowia lipolytica as biocatalyst for crude oil degradation

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Keywords: *Yarrowia lipolytica*, biodegradation, crude oil.

Oil spills pose a great hazard for terrestrial and marine ecosystems. The total annual influx of oil into the sea is estimated to be approximately 5.2 million tones, and in productive areas it is almost unavoidable (Leahy and Colwell, 1990). Therefore, a search for new technologies to clean up oil-polluted environments is always welcome.

Many microorganisms have the ability to utilize hydrocarbons as sole carbon sources of energy and carbon. *Yarrowia lipolytica* is an unique strictly aerobic yeast with the ability to degrade very efficiently hydrophobic substrates such as n-alkanes, fatty acids, fats and oils for which it has specific metabolic pathways (Fickers *et al.*, 2005). So, *Y. lipolytica* is emerging as a promising agent in crude oil degradation (Kim *et al.*, 1999; Zinjarde and Pant, 2002).

In the present work, the degrading potential of *Yarrowia lipolytica* IMUFRJ 50682, isolated from tropical estuarine water in Rio de Janeiro (Brazil), is evaluated. Amaral *et al.* (2006) demonstrated that the cell surface of this strain presents high hydrophobicity what justified its affinity for hydrophobic substrates.

An experimental design was performed varying the following input variables: initial cell concentration (0.1 and 1.0 mg/ml), initial petroleum concentration (0.1 and 1.0% (v/v)), agitation speed (70 and 160 rpm) and glucose concentration (0 and 1.0 g/l) with three central points on the following conditions: cell concentration 0.55 mg/ml, agitation speed 115 rpm, initial petroleum concentration 0.55 % (v/v) and glucose concentration 0.5 g/l. All experiments were performed in 250 ml erlermeyer flasks with 100 ml of inorganic medium.

Cell growth was followed by optical density measurements at 570 nm converted to mg/ml using a factor previously established. Extracellular glucose concentration was determined by glucose oxidase method (Enzimatic Colorimetric Glucose Assay Kit, HUMAN GmbH - Germany).

Initially, it was possible to observed that initial cell concentration can be considered as the variable that most influences the biodegradation process, followed by system agitation speed. By the statistical analysis, the initial petroleum and glucose concentration are the variables that have minor effect in cell growth.

The results obtained from the experimental design lead to the following conditions for crude oil degradation: 160 rpm agitation speed and 1.0 mg/ml initial cell concentration. Subsequent experiments in such conditions were performed for cell viability analysis through Colony forming units (CFU), and crude oil degradation analysis. To analyze crude oil degradation, an extraction of the oil phase was performed with chloroform in the beginning (control) and at the end of the crude oil degradation experiments. After that, the chloroform solution with extracted crude oil was analyzed in a spectrophotometer by a wavelength scan.

Such results demonstrate that crude oil is utilized as carbon source for cell growth (experiments without glucose). On the other hand, the presence of this second phase seems to have an inhibitory effect on cell growth when glucose is present, since the values obtained for number of cells (CFU/ml) in glucose presence, with and without crude oil, are not similar. This fact may indicate that the cell possibly needs a significant adaptation period to alternate between the consumption of an easily degraded carbon source (glucose) to the crude oil utilization.

Table 1 presents crude oil removal ($Abs_{control}$-Abs_{end}/$Abs_{control}$*100) for selected wavelengths. In the presence of higher crude oil concentration and absence of glucose it is possible to remove more than 50% of crude oil in all selected wavelengths (Table 1). Samples of crude oil in the beginning and after the biodegradation experiment are being analyzed through gas chromatography-mass spectrometry (GC/MS).

Table 1. Crude oil removal from initial (0 h) and end (120 h) of experiments at 160 rpm. Initial cell concentration: 1.0 mg/ml.

Conditions		Removal (%)		
Glucose (mg/ml)	Crude oil (%, v/v)	208 nm	266 nm	388 nm
0	0.1	5.6	8.2	45.4
1.0	0.1	5.1	7.5	38.0
0	1.0	54.5	59.7	89.0
1.0	1.0	36.8	29.7	80.4

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Hyperbaric bioreactors use with *Yarrowia lipolytica* cultures: cellular adaptation to hyperbaric conditions

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Keywords: hyperbaric bioreactor, pressure, cell concentration, *Yarrowia lipolytica*, oxidative stress

Laboratory research of microbial processes is usually performed at atmospheric pressure, but in industrial reactors, due to the large scale, the cells are distinctly exposed to total pressure conditions and to gas solubility, depending on the local position in the reactor, generally increasing 1 bar for every 10 m increase in depth (Onken et al., 1989). Due to the differences observed in the residence time distribution in large reactors, there will be cells growing at high pressure (at the bottom), whereas others (on top) will grow at low pressures. Many differences found between laboratory and industrial behaviour could be partially explained by these different environmental conditions. This is particularly important when gas solubility is a determinant factor in a process, as is the case of oxygen solubility in high-density aerobic cultures in large-scale fermentations. In many cases, the oxygen demand far exceeds the oxygen transfer capacity, meaning that the dissolved oxygen becomes limiting for microbial growth. Air pressure raise has been proven to be an effective way of oxygen transfer rate enhancement in yeast cultivation (Belo et al., 2003; Pinheiro et al, 2003) However, limits of pressure increase could be found due the oxidative stress caused to the cells by hyperbaric oxygen and, as a consequence, analysis of pressure effects in cell physiology and morphology must be considered (Belo et al, 2005).
For *Yarrowia lipolytica* cultures, the use of hyperbaric bioreactors presents particular interest since this is a strictly aerobic microorganism and oxygen has an important role controlling many enzymes activities of this yeast (Aguedo et al., 2005; Lopes et al., 2008).

The effects of hyperbaric conditions on the cellular behaviour of the strain *Yarrowia lipolytica* W29 were studied in batch cultures under pressures between 1 bar and 6 bar. Experiments were carried out in a 600 mL stainless steel stirred tank bioreactor (Parr 4563, Parr Instruments, USA), with 400 mL of YPD medium, at 27 ºC and 400 rpm. Compressed air was continuously sparged into the culture at a flow rate of 0.4 $L \cdot min^{-1}$
Figure1 shows the kinetic profile of *Y. lipolytica´s* growth during batch cultivations. Biomass productivity was improved by the use of air pressure raise. An increase of air pressure to 6 bar leads to a considerable increase (42 %) in the cellular growth rate. Also, a 5-fold cell dry weight increase was obtained at 6 bar compared with the experiments under 1 bar, corresponding to a biomass yield increase of 71 %.

In spite of the fact that the rise of the total air pressure from 1 to 6 bar led to a 58-fold induction of the superoxide dismutase specific activity at the end of fermentation, results demonstrate that *Yarrowia lipolytica* cells can cope well with increased air pressure without cellular growth inhibition.

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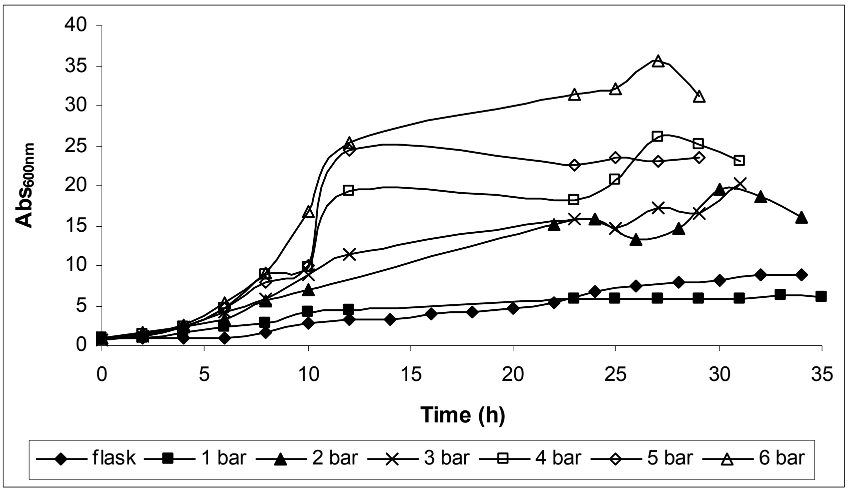

Figure 1 – Cellular growth profiles of *Y. lipolytica* in flask and in the pressurized bioreactor at different pressures.

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Development of bioprocesses based in biphasic media involving the yeast *Yarrowia lipolytica*

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Keywords: Biphasic media, *Yarrowia lipolytica*, lipase, castor oil, OMW

The ascomycetous yeast *Yarrowia lipolytica* is one of the more intensively studied non-conventional yeast species. This microorganism is not only of interest for fundamental research, but also for biotechnological applications. As it is considered as non-pathogenic and as GRAS by the American Food and Drug Administration, this yeast has been used in several industrial applications like production of single cell protein, peach flavour and citric acid. The range of substrates used by *Yarrowia lipolytica* include alkanes, fatty acids, organic acids, proteins and some sugars, which also contributes to the interest of this yeast.

Investigation in our laboratory has been made with this yeast in three main different fields: optimization of a peach-like aroma compound production; optimization of lipase production; and valorization of olive mill wastewater (OMW) by producing high-value compounds while degrading this waste.

Yarrowia lipolytica is able to carry out the biotransformation of ricinoleic acid into γ-decalactone, a peach-like aroma compound of industrial interest. Ricinoleic acid (12-hidroxioctadec-9-enoic acid) is a hydroxylated C_{18} fatty acid that in its esterified form is the major constituent (about 80%) of castor oil, which makes it an abundant compound, being the precursor most usually used in the production of this aroma.

Investigation has been done in our research group in order to optimize this production and to better understand the all process. Previous studies indicate that oxygen availability in the medium used for this biotransformation may be a determining factor in the process, intervening in the control of the peroxisomal β-oxidation pathway, which leads to γ-decalactone formation (Aguedo et al., 2005). Thus, work has focused in the optimization and modelization of oxygen mass transfer in this complex multi-phase system composed by an aqueous and an organic phase as well as a gaseous and a solid phase (Gomes et al., 2007).

The influence of using different substrates (castor oil and methyl ricinoleate) at different concentrations in the productivity of the process, as well as the role of extracellular enzymes, such as lipases and proteases, has also been aim of study.

Lipases, triacylglycerol hydrolases, are an important group of biotechnologically relevant enzymes finding vast applications in food, dairy, detergent and pharmaceutical industries. They are generally produced from a lipidic carbon source (oils, fatty acids, glycerol or tweens) in the presence of an organic nitrogen source. Due to the facility of single cell use and to the high secretion ability, *Yarrowia lipolytica* has revealed to be an interesting and alternative organism to be used in lipase production.

The extracellular lipase production is affected by different environmental factors such as pH, temperature, medium composition, agitation, aeration and proteases.

As previous works in the group have demonstrated that hyperbaric air can be successfully applied to yeast cultivation as a way of improving the oxygen transfer rate to aerobic cultures (Belo et al., 2003), the influence of moderate total air pressure on *Yarrowia lipolytica* cultures has been studied. It has been proven the enhancement of cellular growth and lipase production under hyperbaric conditions (Lopes et al., 2008).

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The ability of hydrophobic substrates use by *Yarrowia lipolytica* strains, has been used not only to develop industrial biporocesses but also to solve environmental problems, such as bioremediation and lipidic wastewaters treatment Fickers et al., 2005).Olive mill wastewater (OMW) is an example of those environmental problems resulting from the olive mill industry. OMW is a dark-colored stable emulsion effluent, characterized by a high polluting load.

The organic fraction of OMW includes sugars, tannins, polyphenols, polyalcohols, pectins and lipids, resulting in high values of chemical oxygen demand (COD). Thus, OMW can represent a possible renewal resource that might be a basis for fermentation processes.

Yeast species such as *Yarrowia lipolytica* can grow well in OMW media, consume the organic material and, simultaneously, produce biomass and other valuable products like enzymes and organic acids (D'Annibale et al., 2006), contributing for the valorization of OMW, which is the aim of the current investigation in our laboratory. Thus, in a first stage, work focused on the potential use of OMW as a culture medium for different *Yarrowia lipolytica* strains; i.e. their ability to grow and to produce high-value compounds, such as enzymes (e.g.: lipases), while degrading and detoxifying this waste was evaluated. For this purpose, different OMW from different regions of Portugal have been collected and characterized. Although all strains were able to grow in OMW based media without dilution, to consume almost all of the sugars present in the media and to significantly reduce COD values, the most adapted strains were selected.

Investigation now proceeds on the optimization of OMW fermentation process on a lab-scale bioreactor.

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Image analysis to investigate electrochemical stress in lipase production by *Yarrowia lipolytica*

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Keywords: stress, *Yarrowia lipolytica*, electric field, metabolism, yeast

Yarrowia lipolytica is a dimorphic and strictly aerobic organism, with the ability to provide a wide spectrum of important products into the medium, such as lipases, citric acid and unicellular proteins. Lipases are widely used enzymes that can be obtained from animals, plants and microorganisms. Microbial lipases are used in food, detergent, pharmaceutical, cosmetics and leather industries (Amaral et al., 2005). Biochemical processes are important to cell viability depend on the electric potential through cell membrane. The application of electric field to a living cell might promote metabolic responses and modification of cell membrane potential. The aim of this work is to investigate the effects of the application of electric field in the yeast *Yarrowia lipolytica* analyzing its physiology (growth cell, glucose consumption and lipase activity) and population morphological analysis through the digital image analysis.

Experimental conditions: A wild type strain of *Yarrowia lipolytica* (IMUFRJ 50682) was employed and kept at 4°C on YPD-agar medium. Cells were grown in YPD medium (1% yeast extract, 2% peptone and 2% glucose) along 48h at 28 ºC and 160 rpm. The cells were inoculated in a bioelectrochemical reactor containing modified YPD medium (0.64% peptone, 1% yeast extract, 2% glucose) with initial cell concentration of 1 mg d.w./mL. In cell growth experiments, the value of applied potential was 0.75 V_{SCE}, based on Araujo et al. (2004). Measurements of cell density were carried at 570nm. Glucose was measured by glucose oxidase method and the lipase activity using p-ntrophenylaurate. Cell morphology was observed using an Nikon optical microscope with 1,000·magnification in both control and stress conditions. Digital image analysis procedure following Kawasse et al. (2003) was performed in order to extract the cells' individual properties: hyphal length and hyphal width were determined as the maximum Feret diameter (FMax) and minimum Feret diameter (FMin), respectively. Elongation is given by the ratio between hyphal length and hyphal width.

Main Results and Discussions: Table 1 presents the results of maximum specific growth rate (μ) and glucose consumption rate (-dS/dt). The μ values are similar for both experiments (with and without electrochemical stress). However substrate consumption (-dS/dt) increases around 61% with application of electrochemical potential. Therefore, with the electrical potential, the metabolism increases, and the consumed glucose might have been diverted to another metabolite.

The Figure 1 presents lipase prodution by *Y. lipolytica* under eletrochemical stress conditions and the control (no stress). It is possible to observe that the peaks were very close, but in the cultive with stress lipase's activity peak was reached earlier. The productivity of the lipase is represented in the Table 2, where it is possible to observe that there is a 13% increase in lipase productivity with electrochemical potential application.

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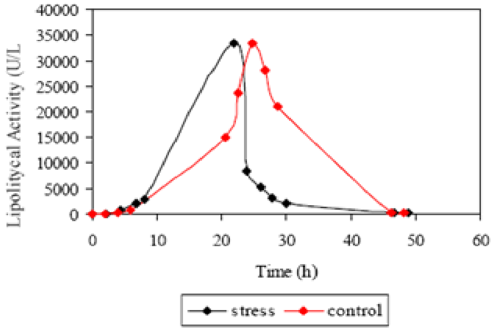
The result of the digital image analysis at the end of the experiments is shown in Figure 2, where it's clear that the application of electric potential changes the cellular morphology giving the cells a feature more elongated. Thus, it is possible to conclude that there is a relationship between the cell defense system and morphological changes.

Table 1: Specific growth rate (μ) and glucose consumption (-dS/dt) of *Y. lipolytica* cultivates with and without electrochemical potential

Stress	μ (h^{-1})	-dS/dt (g/l)
No	0,32±0,04	0,63±0,04
Yes	0,30±0,04	1,03±0,04

Table 2: lipase productivity by *Y. lipolytica* with and without electrochemical stress

Stress	Produtivity (U/l*h)
Não	1353,5
Sim	1526,4



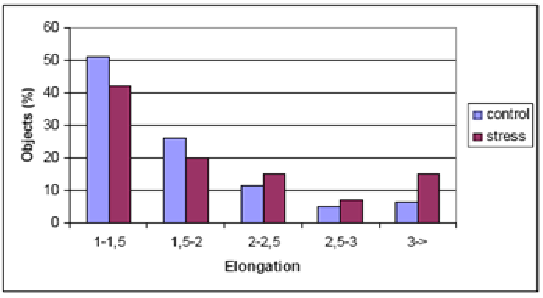


Figure 1. Lipase production with and without stress. Figure 2. Elongation factor distribution for control and stressed experiments

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Liquid-liquid extraction in the presence of electrolytes of nisin and green fluorescent protein (GFPuv)

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Keywords: Liquid-liquid extraction, green fluorescent protein, nisin

Abstract

In the biotechnology field, it has been suggested that extractions in two-phase aqueous complex-fluid systems can possibly be used instead of, or as complementary processes to, the more typical chromatographic operations, to reduce the cost of the downstream processing of many biological products (Lam et al., 2004; Mazzola et al., 2006). This method offer attractive conditions to be applied in this study, thereby two-phase systems can be exploited in separation science for the extraction/purification of desired biomolecules directly on the culture medium (Mazzola et al., 2008). This study aimed to evaluate the aqueous two phase system (ATPS) composed by a nonionic surfactant, Triton X-114 (TX), in presence or absence of electrolytes, to separate two interesting biomolecules: nisin and recombinant green fluorescent protein (GFP). Results indicated that nisin partitions preferentially to the micelle rich-phase, with significant antimicrobial activity increase (up to 10-fold). GFP partitioned evenly between the phases in TX system without electrolytes.

Production and purification of frutalin, a potential cancer marker, in *Escherichia coli*

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Keywords: *Escherichia coli*, Frutalin, Factorial experimental design, Size exclusion cromatography, Haemagglutination

Introduction

Frutalin, the α-D-galactose-binding lectin isolated from *Artocarpus incisa* seeds (breadfruit seeds), is a protein similar to jacalin, the well studied galactose-binding lectin isolated from the *Artocarpus integrifolia* seeds (jackfruit seeds). Like jacalin, frutalin binds specifically D-galactose but has a more marked biological activity, as it is shown by its higher haemagglutination activity (Campana *et al.* 2002). Active frutalin is a tetrameric protein which monomers are made of one α chain and one β chain bound by noncovalent linkages and contains a binding site for D-galactose (Moreira *et al.* 1998). This lectin is thought to be synthesized as a pre-pro-lectin, consisting of a signal sequence, a pro-peptide, a β-chain, a linker peptide and a α-chain. In mature frutalin, the signal sequence and the pro-peptide are moved through post- and/or co-translational modifications, and the linker is also excised to separate α and β chains.

Frutalin can be potentially use as a cancer diagnostic tool due to its ability to bind galactose complexes of cancer cell surfaces. This application will require large amounts of this lectin with the highest level of purity. The heterologous expression of frutalin in microorganisms may simply improve its availability. *Escherichia coli* was chosen as host strain for frutalin expression because it is a well studied expression system and offers a mean of rapid and economical production of recombinant proteins. These advantages coupled with a wealth of biochemical and genetic knowledge make *E. coli* one of the most popular systems for protein production (Jana and Deb, 2005; Kim *et al.* 2007). The aim of this work is to maximize the expression and purification of soluble biologically active recombinant frutalin in *E. coli*.

Material and methods

Frutalin codifying sequence was cloned into the multiple cloning site of the pET-25b(+) expression vector (Novagen). Recombinant frutalin was expressed with its native C-terminal in the bacterial strain *Escherichia coli* BL21-CodonPlus(DE3)-RIPL (Stratagene). LB medium, supplemented with 100 µg/ml of ampicillin and 50 µg/ml of chloramphenicol, was used for *E. coli* recombinant strains selection, maintenance and expression.

Frutalin expression was optimized after recombinant *E. coli* induction with different concentrations of IPTG (0.1 and 1 mM), incubated at different temperatures (22 and 37ºC) for different time periods (3 and 20 h). The *E. coli* cell-free extracts were obtained after cell lyses by sonication and clarification by centrifugation. Recombinant frutalin was detected in the *E. coli* soluble fractions by denaturing SDS-PAGE electrophoresis and Western blot analysis. Soluble recombinant frutalin was quantified on scanned gels using the Quantity One software (Bio-Rad). The effect of the experimented variables on the soluble expression of recombinant frutalin was assessed by implementing a factorial experimental design.

Size exclusion chromatography, with a Sephacryl® S-100 HR column and a FPLC system (Pharmacia Biotechnology), was performed as a preliminary step to purify recombinant frutalin from the *E. coli* cell-free extracts. The presence of recombinant

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lectin in the pooled fractions was made by checking its agglutination activity with rabbit erythrocytes. Fractions purity was evalueted by SDS-PAGE followed by silver nitrate staining.

Results and discussion

A single band of about 16-17 KDa, corresponding to recombinant frutalin, was detected in the soluble fractions of the *E. coli* extracts, and not detected in the extracts of the negative control (*E. coli* bearing the empty pET-25b(+) vector), by both SDS-PAGE and Western Blot analyses. This molecular weigth has a good agreement with the calculated molecular weight of the amino-acid residues of β and α chain conneted throught the linker of the cloned frutalin sequence (17.1 KDa). That fact may lead to the possibility that recombinant frutalin does not undergo any proteolytic cleavage in *E. coli*, as occurred with jacalin produced in *E. coli* (Sahasrabuddhe *et al.* 2004).

The factorial experimental design resulted in a third order interaction model demonstrating that the maximum soluble recombinant frutalin was obtained at 22ºC and 20 h of induction with 1 mM IPTG (12.6 mg/l). Temperature and concentration of IPTG were the main factors that most contribute to the soluble frutalin expression. The interaction between concentration of IPTG and induction length was the most significant interaction factor.

Haemagglutination activity in the *E. coli* extracts was only detected after a dilution factor of two or four times, suggesting the presence of agglutination inhibitory components. Size exclusion chromatography resulted in 50 ml of pooled fractions with haemagglutination activity per litre of *E. coli* culture. Several of these fractions presented a high level of purity, showing that most of the *E. coli* proteins contaminants were removed, nevertheless further purification and concentration steps will be needed, such as affinity or ion-exchange chromatography. The fact that recombinant frutalin exhibits haemagglutination activity, inhibited by D-galactose, in conjunction with the elution time in the Sephacryl column, which is higher than the BSA (66 KDa) elution time, suggests that it may have the tetrameric native structure (Sahasrabuddhe *et al.* 2004).

Conclusion

In this work, biologically active recombinant frutalin was successfully produced in *E. coli* and partially purified using size exclusion chromatography.

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Analysis of production of recombinant wild-type amidase from *Pseudomonas aeruginosa* in *Escherichia coli*

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Keywords: Expression of Recombinant protein; *Escherichia coli*; inclusion bodies; aliphatic amidase; Ethanol.

High level of expression of recombinant proteins often results in aggregation and accumulation in inclusion bodies which prevents the production of large amounts of soluble protein. *Escherichia coli* retains the dominant position as the first choice of host in recombinant DNA technology for reasons of speed and simplicity (Middelberg, 2002). However, researchers are often confronted with the formation of inclusion bodies when protein is over-expressed.

Inclusion bodies are dense aggregates of misfolded polypeptide chains which are formed intracellularly due to either protein aggregation characteristics or the inability of the cellular processes to ensure that the expressed protein is correctly folded and soluble (Lilie *et a*l, 1998). Although the formation of inclusion bodies could potentially facilitate protein purification due to the presence of homogeneous protein, additional steps of solubilization and renaturation are required which are difficult and increases the production costs for such recombinant proteins (Thomas and Baneyx, 1997). In order to overcome such additional unit operations for recombinant proteins is to avoid or at least reduce inclusion body formation. Thus, it is desirable to produce recombinant proteins in a soluble form *in vivo* and to purify the active polypeptide chains by conventional chromatographic techniques.

The reduction of the cultivation temperature for recombinant strains has been known to increase the amount of soluble protein due to a decrease in the rate of protein biosynthesis. Another possibility of increasing native and soluble protein is to control the induction of gene expression by promoters and these can be regulated by the inducer concentration. This strong correlation between cultivation temperature and inducer concentration such as isopropylthio-β-D-galactoside (IPTG) with the expression level and solubility of recombinant protein has been found in several studies (Garcia-Junceda *et al*, 1995). Furthermore, Thomas and Baneyx (1997) have reported that supplementing the grown medium with 3% (v/v) ethanol can be a powerful tool to improve the solubility of some recombinant proteins.

This work reports the optimization of the production of a recombinant amidase from *Pseudomonas aeruginosa* (E.C.3.5.1.4).overproduced in an *E. coli* strain. Microbial amidases, which in nature catalyse the hydrolysis of amides producing the corresponding acid and ammonium ions, have been used for several medical and industrial applications such as in neuro-biochemistry, plant physiology, medicine, environment and in the food and detergent industry (Cravatt *et al*, 1996; Fournaud *et al*, 1998).

The effect of several physico-chemical parameters such as temperature incubation, IPTG induction and ethanol shock on protein aggregation *in vivo* was investigated. The results obtained suggest the formation of inclusion bodies in all experimental conditions tested. Furthermore, the addition of ethanol in the culture medium at 25ºC resulted in amidase synthesis which exhibited a four-fold higher specific activity in the aggregated and biologically active form compared with the soluble form of the enzyme. The use of such active amidase aggregates with enzyme activity presents some advantages over

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inactive protein aggregates since solubilization and renaturation steps are not required which markedly reduces the total production costs of this recombinant enzyme.

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Mathematical modeling of recombinant *Escherichia coli* aerobic batch fermentations

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Keywords: Mathematical modelling, GFP production, acetate inhibition kinetics, recombinant *Escherichia coli*, parameter estimation

Introduction

Acetate, when present in *Escherichia coli* fermentations during aerobic growth on glucose is a major obstacle to cell growth and recombinant protein production. Many different mathematical models have been reported in the literature to describe the kinetics of primary metabolites and the acetate overflow metabolism in *E. coli* (Xu and Enfors, Varma and Palsson). However, there has been few studies focusing on models describing acetate inhibition kinetics and the effect of the substrate concentration on the culture growth kinetics in recombinant cultures.

In this work, three competing unstructured mathematical models representing the aerobic fermentation of recombinant *E. coli* with different acetate inhibition kinetic terms were evaluated. Different initial glucose concentrations (5g kg^{-1}, 10g kg^{-1} and 25g kg^{-1}) were used in a 5dm^3 bioreactor at constant temperature and pH.

Kinetic Model

The simulations considered the dynamics of biomass growth, acetate accumulation, glucose consumption and green fluorescence protein (GFP) production. Parameter estimation and model validation were carried out using the Systems Biology toolbox for Matlab (The *Mathworks*).

Four ordinary differential equations were obtained, capable of describing *E. coli* fermentation:

$$\frac{dX}{dt} = (\mu_1 + \mu_2 + \mu_3)X - \left(\frac{F}{W}\right)X \tag{1}$$

$$\frac{dS}{dt} = (-\mu_1 K_1 - \mu_2 K_2 - m_S)X - \frac{((\mu_1 + \mu_2 + \mu_3)K_5 + \beta)X}{K_6} - \left(\frac{F}{W}\right)(S_0 - S) \tag{2}$$

$$\frac{dA}{dt} = (\mu_2 K_3 - \mu_3 K_4)X - \left(\frac{F}{W}\right)A \tag{3}$$

$$\frac{dP}{dt} = (\mu_1 + \mu_2 + \mu_3)K_5 X + \beta X - \left(\frac{F}{W}\right)P \tag{4}$$

where the production of GFP includes a growth and non-growth associated term. The variables X, S, A and P represent biomass, substrate, acetate and product concentrations, respectively (g kg^{-1} and mg kg^{-1} for product); F and W are the mass flow feed rate and weight of the fermentation broth, respectively (for cultures operated in batch mode F is zero; the K_i are the yield coefficients and μ_i the specific growth rates (μ_1, oxidation of glucose; μ_2, fermentation of glucose and μ_3 oxidation of acetate); m_S is the maintenance coefficient.

Regarding acetate kinetics, Jerusalimsky (Roels, 1983) approach is an approximation to the non-competitive substrate inhibition. Cockshott (Cockshott and Bogle, 1999) approach describes the inhibition at high acetate levels and Levenspiel (Han and Levenspiel, 1988) considers a critical inhibitory acetate concentration that limits growth.

Results and Discussion

Model discrimination was based on the two model selection criteria (Akaike's information criterion and normalized quadratic difference between the simulated and experimental data criterion). According to this criteria, Jerusalimsky model was favored and selected for further analysis. Batch fermentations using the *E. coli* strain HMS174pacGFP were performed for the estimation of model parameters and model discrimination. Figure 1 compares the experimental data profiles and simulation results obtained after parameter estimation.

To validate the developed model it is necessary to use other experimental data than those applied to identify initial parameters. Therefore, the parameters obtained for an initial glucose concentration of 5 g kg^{-1} were also validated and tested using the experimental values obtained for 10 and 25 g kg^{-1} of initial glucose concentration.

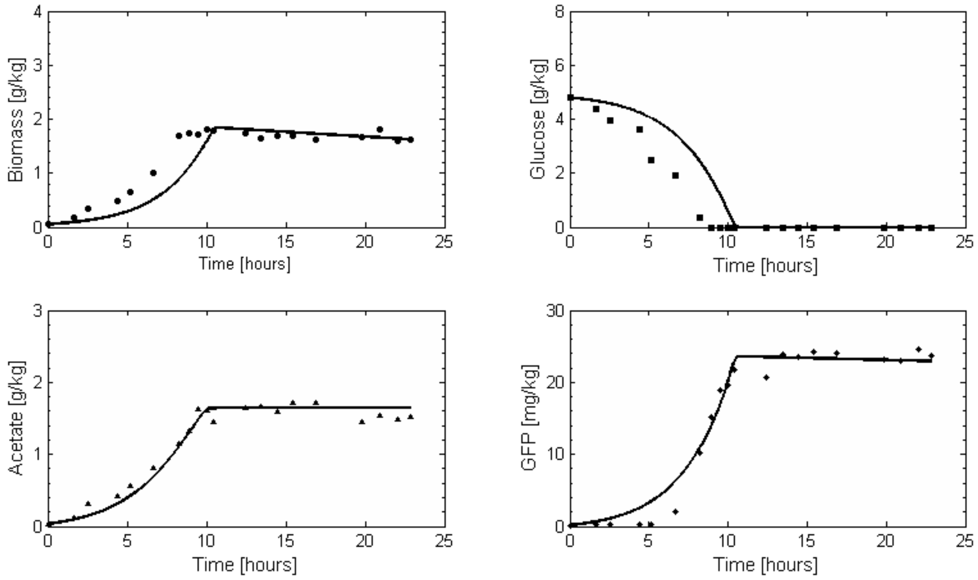

Figure 1. Comparison between experimental and simulated data for biomass, glucose, acetate and GFP concentrations in a batch fermentation with 5 g kg^{-1} of initial glucose. Solid lines represent the simulation data obtained with Jerusalimsky model using the fitted parameters obtained for all run fittings and the markers the experimental data.

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Overproduction of protein-bound polysaccharides from a *Coriolus versicolor* strain by submerged fermentation

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Keywords: Protein-bound polysaccharides, *Coriolus versicolor*, submerged fermentation, edible mushrooms.

Several species of mushrooms have been valued as edible and medicinal resources. Polysaccharides mushroom-derived have shown medicinal effects such as blood pressure and cholesterol lowering, liver protection, anti-fibrotic, anti-inflammatory, anti-diabetic and anti-viral activities. Additionally, some strains are able to synthesize new polysaccharopeptides with antitumoral and immunostimulating properties (Cui *et al*., 2007; Li *et al*., 2008). The protein-bound polysaccharides or polysaccharopeptides, produced by the white rot fungus, *Coriolus versicolor*, are effective immunopotentiators used to supplement the chemotherapy and radiotherapy of cancers and various infectious diseases (Cui and Chisti, 2003).

The aim of this study was to define experimental conditions to optimize the production of protein-bound polysaccharides by *C. versicolor*. Most of the reported studies referred polysaccharides isolated from mycelium, however a few studies on exopolysaccharides from *C. versicolor* have been reported (Kim *et al*. 2002; Tavares *et a*l. 2005). In fact, the polysaccharides represent the major constituent that establishes the rigidity and morphological properties of the fungal cell wall and, depending on the culture conditions, they can be excreted to the medium. In this work, endo and exo protein-bound polysaccharides, produced by *C. versicolor* will be investigated. In order to evaluate and to optimize the production of these compounds we performed submerse cultures with four different carbon sources: glucose, maltose, sucrose and manitol. Additionally, aiming for a clean process and to reduce the medium cost, two kinds of agro-industrial residues were used: tomato pomace and beet wastes.

C. versicolor was isolated from *Quercus suber* and kindly provided by ISEL. The culture was maintained on PDA, transferred to a fresh agar plate every month, and grown at 28 °C for approximately 7 days. The mycelia of *C. versicolor* on the media surface were cut and transferred to culture flasks. The cultures were prepared in shake-flask with a basal media comprised by the following ingredients per liter: yeast extract 2 g, KH_2PO_4 0.8 g, $MgSO_4.7H_2O$ 0.5 g, $Na_2HPO_4.2H_2O$ 0.25 g¸ NH_4NO_3 1 g, pH 6.0. The different carbon sources were added one by one to the basal medium. Flasks were incubated at 28°C, for 20 days using an orbital shaker at 150 rpm. Samples were collected periodically along the twenty days of the assays. The exopolysaccharides were extracted from the supernatant according to Lin and Sung (2006) and the polysaccharide concentration was determined by the phenol–sulphuric method (Dubois *et al*., 1956). Protein-bound polysaccharide concentration obtained in the batch cultures ranged between 150-900 $\mu g.mL^{-1}$. The highest polysaccharide concentration was achieved with tomato pomace as agro-industrial residues corresponding to the 14th day of culture. Based on these results *C. versicolor* was cultivated in the basal medium plus tomato pomace in a stirred bioreactor during 15 days, for the production of

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endocellular and exocellular protein-bound polysaccharides. The intracellular polysaccharides extraction was performed from the mycelia according to Lee *et al*. (2006) and Gern *et al*. (2008). Endocellular and exocellular protein-bound polysaccharides were quantified as described previously. The concentration of the polysaccharide compounds was 16 folds higher in the supernatant, than in mycelia. Absorption spectra of both polysaccharides have a maximum at 280 nm, however, supernatant complex has a protein concentration 40 folds higher than mycelia, showing the production of different protein-bound polysaccharides by this strain.

The comparison of the results obtained in this study with those reported in the literature, let us to confirm the interest of this selected culture medium in order to maximise the production of complexes protein-polysaccharides by *C. versicolor*. Besides, this clean process allows valuing the agro-industrial residues with a low cost and a higher production of these polysaccharides compounds. The different polysaccharide concentration in endocellular and exocellular complexes shows that this *C. versicolor* strain produces different protein-bound polysaccharides, so further studies are being done in order to isolate and identify the different *C. versicolor* complexes and investigate its bioactivity.

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Production, purification and partial characterization of protein – bound polysaccharides from *Pleurotus ostreatus* and *Lentinula edodes*

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Keywords: Protein – bound polysaccharides; *Pleurotus ostreatus* and *Lentinula edodes;* Batch and continuous cultures; HPLC and FTIR; gel filtration chromatography.

The medicinal power and the nutritional value of some mushrooms are widely known. However, only in the last decades of the last century that it was possible to isolate and partially characterize some biologically active anti-cancer substances [Parris *et al,* 2000].

The therapeutic properties of mushrooms are attributed mainly to their polysaccharide content which has exhibited immunomodulatory properties such as anti-cancer, anti-viral and anti-bacterial activities. Protein-bound polysaccharides are macromolecules which consist of a central core protein to which are attached a number of polysaccharide chains and PSP and PSK from *Coriolus versicolor* are the most widely studied protein-bound polysaccharides [Parris *et al,* 2000; Cui & Chisti, 2003].

Although protein–bound polysaccharides from mushrooms exhibit important medicinal properties, there is little information in the literature about their biological and physico-chemical properties [Cui & Chisti, 2003; Huie & Di, 2004; Lo *et el.*, 2007].

Therefore, the aim of this work consists of production, purification and partial characterization of these protein-bound polysaccharides from *Pleurotus ostreatus* and *Lentinula edodes*.

The fungal strains were grown in several culture media such as whey permeate supplemented with glucose, yeast extract and suitable salts. The cultures were grown in agitated and aerated bioreactors at pH 5.5 and 27ºC, with pH and temperature control for 10 days and suitable daily samples were removed from the reactor. These samples were centrifuged and intracellular and extracellular protein-bound polysaccharides were extracted from the biomass and culture supernatant, respectively. Subsequently, these polysaccharides were purified by gel filtration chromatography on a Sephacryl HR-100 column and fractions were analysed for protein, superoxide dismutase (SOD) activity and polysaccharides. Fractions containing protein-bound polysaccharides were characterized by HPLC which revealed UV and RI peaks with retention times of 6 and 13 min., respectively. On the other hand, FTIR analysis of these polysaccharide preparations revealed absorption peaks at 912, 1083, 1458, 1653, 2943 and 3409 cm^{-1} which are characteristics of such macromolecules. Moreover, purified polysaccharides exhibited SOD activity which is in agreement with the data published in the literature [Cui *et al.*, 2007; Wang *et al.*, 2007, Peng *et al.,* 2003; Kim *et al.*, 2003].

The results presented strongly suggest that these basidiomycete strains produce high levels of free and protein-bound polysaccharides which exhibit similar physico-chemical properties compared with the data in the literature as far as HPLC and FTIR analysis are concerned.

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Headspace - SPME as a screening system for yeast metabolic compounds - A case study

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Keywords: HS-SPME, *Candida* species, fusel alcohols

Microorganisms share similar metabolic networks regardless of different compartmentation. The fluxes and concentrations of metabolites depend on factors such as the regulation of metabolic enzymes or interactions with the cellular environment. But, in yeasts the catabolism of aminoacids such as leucine, valine, and isoleucine is entirely different from that found in other eukaryotes. Whereas in most eukaryotes the carbon skeletons derived from aminoacids enter the tricarboxylic acid cycle, in the yeast *Saccharomyces cerevisiae* the products of the aminoacid catabolism are long-chain and complex alcohols called “fusel” alcohols. Key issues in this field include the identification of: (1) the end-products of aminoacids catabolism and their routes of production, (2) the enzyme(s) involved in the metabolic process and (3) the physiological role(s) of the end - products.

This study aimed to identify the alcohols secreted by the opportunistic yeasts *Candida albicans* and *Candida dubliniensis* to further get insights into the aminoacid catabolism of those two organisms.

Fusel alcohols were screened in cell-free supernatants of yeasts cultivated in RPMI medium. For simultaneously identification and quantification of these alcohols a Headspace - Solid Phase Micro Extraction (HS - SPME) coupled with gas chromatography - mass spectrometry (GC - MS) methodology previously developed to analyse the volatile composition of fruits was followed (Coelho et al. 2006). The identification of *Candida* metabolites was achieved comparing the GC retention times and mass spectra, with those of the pure standard compounds. All mass spectra were also compared with the data system library (Wiley 275). A comparable analysis was done with growth medium and no interfering substances were found into or near the retention times of those compounds. The quantification was performed by preparation of growth medium solutions containing pure standards in the same conditions of the samples. For each compound, appropriate concentration ranges were chosen in order to include sample concentrations. Standard curves were generated for GC-MS peak areas *versus* concentration of each compound ($R^2>0.98$), with quantification relying above quantification limits.

Results obtained show that both C. *albicans* and *C. dubliniensis* produced isoamyl alcohol and 2-phenylethanol, end – products of the catabolism of the aminoacids leucine and phenylalanine, respectively.

The HS – SPME strengths include (i) the effective separation of small and polar molecules (with a molecular weight between 40 and 275), (ii) the simultaneous identification and quantification of fusel alcohols, (iii) small sample volume requirements, (iv) simplicity and (v) speed, in comparison with traditional techniques. This revealed to be a powerful tool for the screening system for yeast metabolic compounds.

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Modeling of CHO cell culture process

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Recombinant DNA technology has made possible the generation of high-valued proteins with therapeutic potentials. Sales of US$ 16 billion are expected for monoclonal antibodies alone in 2010. The complex structural nature of these therapeutic proteins and their requirement for post-translational modifications for function make mammalian cells a good host for complete expression of the proteins. Mammalian cell culture reproducibility and optimal operation depends on various environmental and physiological factors; fragile and susceptible to shear stresses, inhibition of growth rate by several products of cell metabolism, sensitivity to process parameter (pH, temperature and dissolved oxygen tension) changes on cell metabolism, apoptosis, and variable nutrient consumption and energy requirements. Heterogeneous nature of the culture system due to the apportioning of cell cycle phases, further worsen this problem. At this juncture, models of mammalian cell culture systems have a wide range of potential applications, such as analysis and prediction of experimental results, optimization of culture conditions, and perhaps most importantly, the investigation of fundamental metabolic processes and their subsequent elucidation, which will result in the design of more efficient mammalian cell culture systems both from a genetic engineering and process control perspective.

In this work, most of the predominant physiological phenomena that determines the intrinsic state of Chinese Hamster Ovary (CHO) cells will be modeled in an effort to unify them to estimate the onset of metabolism shift from available on-line and off-line measurements which may be noisy. Multi-level modeling will be adapted to characterize the intra-cellular and inter-cellular processes in mammalian cell cultures. A hybrid single cell model will be developed to quantify cell growth, death, lysis, nutrient uptake, metabolite and protein production and their dependency on various environmental and physiological factors. Population balance models will be combined with this hybrid model to characterize various phases in the cell cycle and in turn account for the heterogeneity in the cell populations.

For the cells in each cell cycle phase detailed flux distributions will be calculated using metabolic pathway models. The individualistic flux distribution information can be ultimately used in a closed loop strategy to identify the metabolism shifts online and take a corrective action immediately to drive the cell metabolism towards specified objective (ex: higher yield of therapeutic protein). The detailed model describing CHO cell culture process at various time scales and length scales is shown in Figure 1.

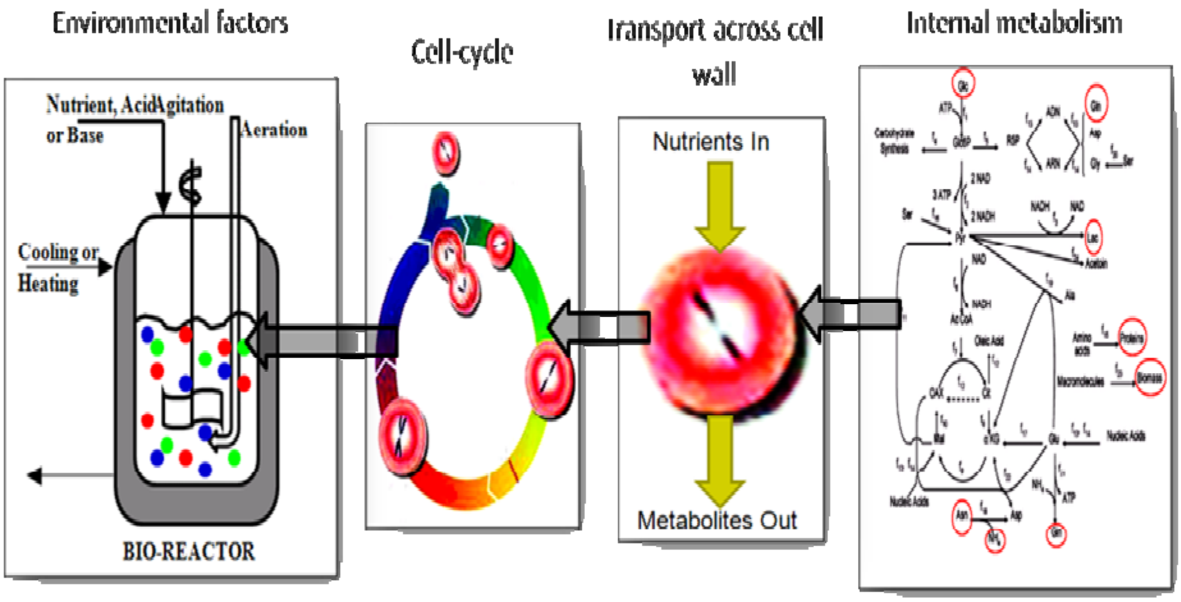

Figure 1: Schematic of detailed CHO cell culture model developed.

Model-based iterative design of dynamic experiments imodeling for optimization of a bioprocess for a recombinant protein production

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Keywords: Modeling, Optimization, Biotechnology, Dynamic Experiments.

Introduction

Today, many pharmaceuticals and high-value specialty products are obtained using genetically modified microorganisms. Since the first gene cloning in the beginning of the 1970s, it is possible to modify a microorganism to produce a desired substance, often a protein. To cope with a steadily increasing demand for biotech products along with fierce worldwide competition issues such as lowering downstream costs, shorten time to market of new products and improving productivity of installed capacity and culture broth should be addressed.

Bioprocess modeling and optimization is as a challenging task due to the complexity of metabolic regulation and organism's reactions to changes in culture environment as consequence of the implementation of an operating policy. As a result, several modeling strategies have been proposed which have different characteristics; for example, it can be found from purely statistical modeling methods to highly structured ones. Although those approaches are very valuables, many of them have shown inherent failures derived from basic assumptions done in model building and in some cases their development could be costly and very time consuming. Keeping above considerations in mind, a new point of view called Modeling for Optimization [1,2], which has been developed for batch processes in general, is being now explored for fast experimental optimization of bioreactor operation.

Modeling for Optimization is a systematic approach for biasing data gathering using one or several first-principles models of a given process with the explicit objective of improving its operating condition. Resorting to first-principles models reduces uncertainty in extrapolation when the process is driven to unknown operating regimes.

In this contribution, the issue of iterative design of dynamic experiments in Modeling for Optimization methods will be discussed and applied to the bioprocess development for a recombinant protein. For each iteration, a dynamic experiment is designed around the current estimation of an optimal policy and optimal sampling times along the experiment are calculated. The experiment is carried out and new data is collected. Before proceeding to (re)estimate model parameters, the sensitivity of the performance index with respect to model parameters is done using Global Sensitivity Analysis [3]. This will allow using new data more effectively to reduce parametric uncertainty in the subset of model(s) parameters which is the most relevant for policy improvement. A complete iteration of the proposed approach for integrating model development with dynamic optimization using a sequence of designed experiments is shown in the Figure below.

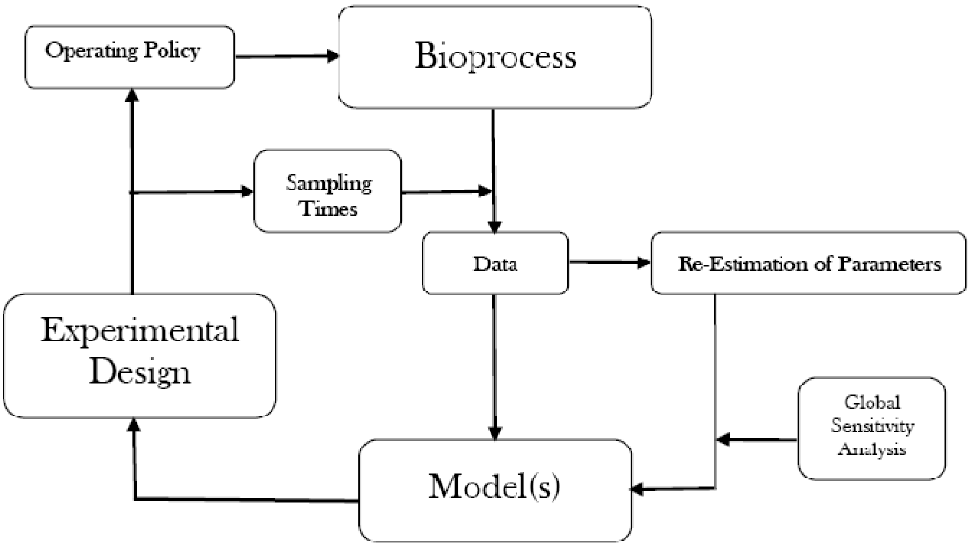

Fig. Iterative design of experiments in modeling for optimization

Case of study

Experimental optimization of an objective function defined as *"Recombinant Protein Amount at the Culture End"* is illustrated in a semi-continuous culture of a recombinant protein producer strain of *Escherichia coli* with dynamic experimental runs designed using the proposed approach. For that purpose, an unstructured kinetic model which describes state variables trajectories such as: biomass, glucose, acetate, recombinant protein concentration has been implemented and some structural modifications have been considered to account for the mismatch between "real" and "probe" *in silico* bioreactors. Also, the operation policy has been defined based on the substrate feeding rate and inductor final concentration as main component subject to optimization.

Significance

A novel method for design of dynamic experiments in bioreactor operation optimization will be presented and applied to a very important biotechnological case study. This novel iterative algorithm is model-based and use designed optimization runs to drive the process to a more profitable region near the optimum of the objective function so as to speed up bioprocess development. The proposed method has been shown to work very well in optimization of bioprocesses where uncertainty is located in kinetic parameters of the model adopted for optimization. In this work is proposed to explore robustness of the method in response also to structural uncertainty in model equations which will require to accommodate the issue of model discrimination in modeling for optimization.

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Application of a fuzzy controller in the baker's yeast production process

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Keywords: Automation, Bioprocess, *Saccharomyces cerevisiae*

Saccharomyces cerevisiae biomass is still one of the most important biotechnological products produced in the world, mainly in the form of baker´s yeast. According to Kasperski and Miskiewicz (2008) the costs of the substrate have a dominant position in the overall costs involved in the Baker´s yeast production. In this context, the maximization of the biomass yield at the highest achievable volumetric productivity has taken on a sense of importance to the control of the process. The yeast is the most prominent product for which fed-batch technique is used to reduce catabolite repression of sugar by incremental addition of carbon source molasses to the fermenter. Ethanol, which is not desired, is a by-product in the process. Ethanol formation takes places either by overfeeding the carbon source (Crabtree effect) or by oxygen limitation (Pasteur effect). Design efficient control strategies for this process is not an easy task due to lack of accurate models describing cell growth and product formation. This process presents a non-linear behavior and time-varying nature that makes difficult the design of reliable on-line sensors for the measurement and quantification of key state variables (Karakuzu et al., 2006). Due to these limitations, considerable attention has been focused on the development of on-line software sensors. Several estimation techniques have been proposed in the literature. The gas balance technique is widely used in studies of cellular activity and process control. The specific respiration rate, qO_2 (mmol/g/h) and the respiratory quotient (RQ), are the principal variables determined from the gas balance. Values of RQ for complete oxidation of some carbon sources to carbon dioxide and water are found in the literature. For the Baker's yeast production process a value of RQ close to the unit indicates the existence of the aerobic route.

This study implemented a fuzzy controller for using in Baker`s yeast biomass production process. The performance of the algorithm was compared to the On-off and PID control algorithms. Fuzzy set theory was proposed by Zadeh (1965). This approach can deals with the vagueness and uncertainty of the human knowledge and with noisy on-line data. It allows the representation of the system using linguistic terms. Fuzzy rules are more adequate and flexible than binary IF-THEN rules (Sousa Jr. and Almeida, 2001, Nucci et al., 2006). Further details about fuzzy logic system can be found in Zadeh (1965).

Experiments were carried out in a stirred tank bioreactor (5.0 liter working volume), filled with molasses diluted to 5 g/l of sucrose until completing 2.5 liter (pH adjusted to 5.0). The inoculum, 0.5 liter (a suspension of the commercial yeast - *S. cerevisiae* - culture in distilled water), was added and the cultivation process takes place. Stirrer speed was initially set to 300 rpm. The level of dissolved oxygen (DO) was maintained at 45% of saturation (Kasperski and Miskiewicz, 2008) by manipulating stirrer speed (PID control). Sterile air was supplied by a mass flowmeter controller at 2.0 SLPM. Temperature was kept at 31.0±0.5 ºC. The pH was not controlled. On line measurements of DO, pH, temperature, stirrer speed, air flow rate and carbon dioxide

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and oxygen (Sick|Maihak gas analyzer) in the exhausted gas were made throughout the runs. Experiments were performed in batch mode until the end of the carbon source (approximately two hours). Then the cultivations were conducted in fed-batch mode. Supplementary medium was fed into the bioreactor with a peristaltic pump driven by the controller that was implemented in the environment of programming LabVIEW. Samples were collected periodically in order to determine concentrations of biomass, sucrose and ethanol.

The following control strategies were tested: On-off, proportional-integral-derivative (PID) and a fuzzy logic. The gas balance technique was used to calculate the oxygen consumption rate, the carbon dioxide production rate and the respiratory quotient (RQ) in real time.

On-off and PID control strategies were implemented directly in the LabVIEW environment. The algorithm for fuzzy control was implemented in MatLab and then inserted in the LabVIEW environment. Data strings were exchanged in real time between the MatLAB program and the data acquisition system, which was coupled to the bioreactor.

As the RQ is the one of the main parameter for the process control, this variable was chosen to be fuzzyfied and became a figure language interpreted by the fuzzy controller. The same occurred with the derivative of the concentration of carbon dioxide in relation to time, which was another very important parameter used by the controller. The value of these linguistic variables fuzzyfied were then inserted into their sets of fuzzy inference, whose degrees of relevance determined the area whose center of gravity was a crisp value, which is the set-point for the peristaltic pump that would feed the reactor. First the parameters of the fuzzy algorithm were adjusted. In the next step the algorithm was inserted into a module in the LabVIEW environment. The derivative of carbon dioxide presents behavior similar to the derivative of RQ but with advantage to be much smoother. This fact avoids undesired noises that interfere in the control algorithm. It could be seen that the fuzzy control algorithm was gave a better results than On-off and PID control algorithms.

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Kinetic models evaluation for fed-batch alcoholic fermentation

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Keywords: Alcoholic Fermentation, Fed-Batch Process, Ethanol, Differential Evolution, Parametric Sensitivity.

Research to improve production of ethanol for use as an alternative to petroleum-based fuels has increased by ecological and economic reasons. Brazil is a major producer and exporter of alcohol and the country with the greatest potential for expansion of production of biofuels in the world, due to the privileged conditions of soil and climate for production on a large scale (Strapasson, 2006; Cabrini and Marjotta-Maistro, 2007).

The fed-batch processes have been shown efficiency and versatility in the majority of fermentation processes, presenting a great flexibility of operation, allowing the control of the feed flow and substrate concentration and the minimization of inhibitory effects on cell growth. The quantification of the dynamic behavior of the microorganism fermentation in conditions equivalent to those used industrially is essential for realistic modeling, simulation and optimization of dynamic processes and is dependent on the mode of operation used (Baltes *et al.*, 1994; Pertev *et al.*, 1997; Wang and Sheu, 2000). According to Shen (2006), the use of optimization methods for the purpose of parameters estimation of nonlinear models are related to the parameter identifiability and must be accomplished through the knowledge of the quality of estimative of model parameters considering the available observations, the uniqueness of the parameters and how much error is associated with the estimated parameters. These issues can be inferred by analysis of parametric sensitivity associated with the evaluation of the correlation between the parameters. The fact is that the parameter estimation or model calibration from experimental data still remains a bottleneck for a major breakthrough in biochemical processes (Rodriguez-Fernandez *et al.*, 2006).

This work presents an experimental and theoretical study of the dynamic behavior of the fed-batch alcoholic fermentation for ethanol production, utilizing the *S. cerevisiae* Y904 yeast. In spite of the recent studies on *Z. mobilis* (Lin and Tanaka, 2006; Bai *et al.*, 2008) on which its greater theoretical yield when compared with *S. cerevisiae* is emphasized, it presents constraints about the utilized substrates (Bai *et al.*, 2008) and is not economically competitive when compared with the *S. cerevisiae.* So, the possibility of its generalized application in the Brazilian industries is very remote.

The inoculum volume corresponded to 30% of the bioreactor volume with initial alcohol concentration equal to 4% typical of the industrial processes. The influences of feeding flow and substrate concentration were evaluated. The parameter estimation of the six parameter non-structured kinetic model proposed by Tosetto (2002), that represents the inhibition effects due to high ethanol and substrate concentrations, was obtained through the differential evolution algorithm of Storn and Price (1996). The evolution strategies are indicated as one of the most competitive algorithms for global optimization in spite of the requirement of too many evaluations of the objective function (Rodriguez-Fernandes *et al.*, 2006). The parametric sensitivity analysis and the estimate of correlation matrix were realized through the DDASPK code (Petzold *et al.*, 2000) and the methodology proposed by Sanz and Voss (2006), respectively. The output sensitivity functions (partial derivatives of the states with respect to the parameters) show that the maximal specific growth rate and the saturation constant have highest sensitivity among the others. On the other hand, most parameters exhibit strong interactions with one another. The correlations between the pair of parameters were consistent with the functional relations established by the kinetic model. The Tosetto model represented satisfactorily the fed-batch fermentation and according to the previous analysis a traditional Monod equation was also considered to represent the microorganism specific growth rate. Both models showed ability to predict *S. cerevisiae* fermentation courses in conditions equivalent to the utilized by Brazilian industries and due to their simplicity are adequate for the modeling, simulation and dynamic optimization of the process.

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Production costs of wheat straw-based ethanol in Mexico. A parametric analysis

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Keywords: Bioethanol production, lignocellulosic ethanol, conceptual design.

This work presents a parametric analysis of total production costs of wheat straw-based ethanol for a facility located in Mexico. Using the Net Present Value (NPV) method the ethanol price (USD/kg) was estimated as a function of plant size (raw material flow) and raw material cost, interest, investment period and equity percentage as financial parameters.

The analysis is based on a standard process flowsheet as shown in figure 1 [1] incorporating novel technology for pretreatment and saccharification operations [6]. Three main processing stages are considered (pretreatment, saccharification-fermentation and distillation) whilst water treatment is considered separately because its environmental relevance. Steady state mass balances were built for each stage and are discussed in detail in the paper. Mass balances calculations were made employing separation and reaction factors taken from the "state-of the-art" literature for pretreatment, saccharification and fermentation [1] [4] [6] as well as our own experience.

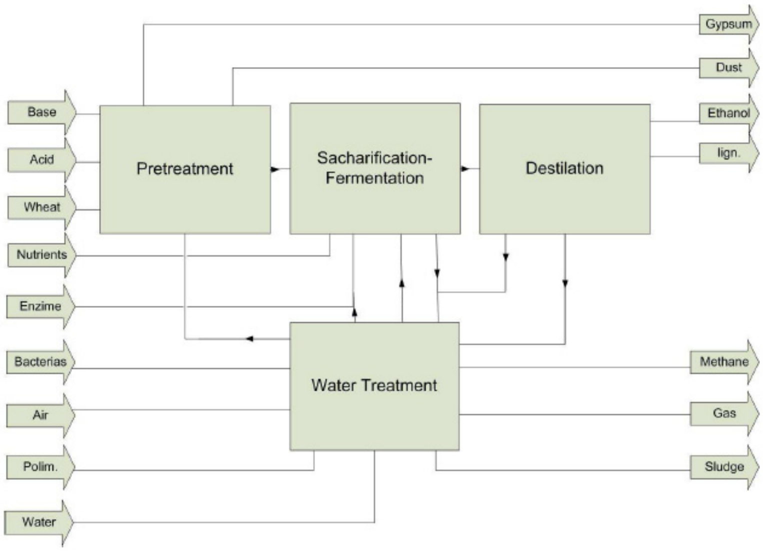

Figure 1: Flow Diagram for bioethanol production

The equipment costs for each stage were calculated using a standard conceptual design approach [5]. Raw material costs were obtained from domestic sources. Total capital investment and total product costs were calculated and adjusted to our location [2] considering 2.5 years for building the facilities.

The base case for calculating the return of investment (ROI) using Net Present Value (NPV) considered the following values: 10 years life period, 4% interest, zero equity and 200 ton/day of wheat straw. The paper discusses the sensitivity of the model and alternatives for improving the total cost of ethanol.

Figure 2 is an example of the results showing the ethanol price for different prices of wheat straw. For our base case, raw material (35%) and production costs (51%) are the main contributors. Equipment (10%), other capital (3%) and other non capital (10%) costs add up the rest. Therefore, improving process efficiency or exploring technologies for diminishing production costs must be considered as alternatives worth exploring. The paper discusses specific operations with the largest cost contributions.

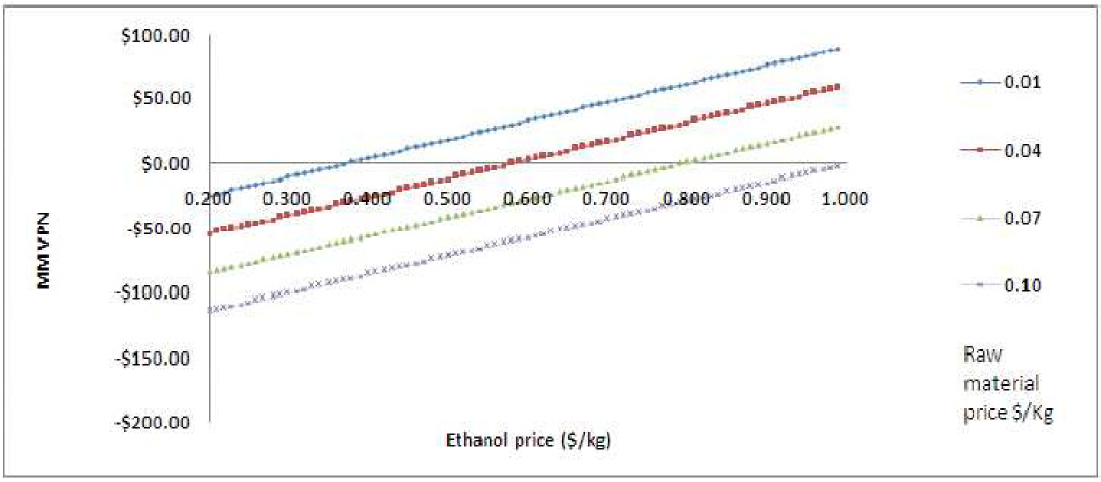

Figure 2: Net Present Value vs Ethanol Price with different raw material price

The ethanol production costs obtained in this work are in agreement with other published results [1]. However, prices are still high for commercialization in the short term. In the final part, the paper discusses Mexican policies on the use of bioresources [3] and, at the light of this results, the relevance of urgently exploring other alternative sources for bioethanol production.

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Optimization of process variables and kinetics of the xanthan gum biosynthesis using sugar cane broth

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Keywords: study kinetic, xanthan gum, optimization, sugar cane broth

Introduction

Biopolymers are produced commonly by submerged fermentation and among those with high commercial value is xanthan gum. Acting as thickening and stabilizing excellent, suspension agent and emulsifier this gum possesses many advantages due to its superior rheological characteristics. Studies of process variables optimization and kinetic profile using sugar cane broth in the xanthan gum biosynthesis have not been reported by the literature. Due the mentioned relevance, this work was developed with the purpose of optimizing agitation speed and aeration level in the xanthan production using sugar cane broth diluted, as well as to evaluate the kinetic behavior in the optimum condition.

Materials and methods

In previous studies, the nitrogen source was selected and a central composite design of sixteen experiments, it defined three quantitative variables to represent the optimum composition of the medium inside of researched range: sucrose concentration (27.0 g/L), yeast extract (1.8 g/L) and nitrate of ammonium (0.8 g/L). The fixed sources increased to the medium were Na_2HPO_4 (2.5 g/L) and KH_2PO_4 (2.5 g/L). In the described conditions, a factorial project 3^2 was idealized with the objective of discovering the influence of the agitation speed (500, 750 and 1,000 rpm) and the aeration (0.25; 0.5 and 0.75 vvm) in the process, have seen that their costs are considerable and they happen directly in the final price of the product. Concisely, the adopted procedure consisted in the accomplishment of two different stages: the fermentative process and the recovery of the gum. The fermentations were carried out in a Biostat-B bioreactor by *Xanthomonas campestris pv. campestris* NRRL B-1459 at constant temperature and pH of 28 ± 1^oC and 7.5, respectively, for 24 hours. The monitored responses were: substrate conversion to product ($Y_{P/S}$) and gum viscosity in solution 1% using 0.75 s^{-1}. The sucrose, after acidic hydrolysis, was assayed by the enzymatic method using glucose-oxidase and the viscosities were measured by the Brookfield RVDVIII rheometer. The measurements of dissolved oxygen for determination of the Oxygen Transfer Coefficient (K_La) were done in the absence of the inoculum. The recovery and the purification of the polymeric were done as suggested by the literature. The kinetic parameters ($\mu_{max.}$, $X_{max.}$, m, λ, α and β) of the Weiss & Ollis (1980) model, composed by equations of Verhulst-Pearl, Pirt and Luedeking-Piret, were obtained by non-linear regression using a multiresponse algorithm.

Results and discussion

The Table 1 presents the obtained results for the factorial project 3^2 executed. In this way, in agreement with the response surface methodology (RSM), the agitation speed and the aeration of 750 rpm and 0.35 vvm produced the most expressive responses for the conversion (0.607 $g.g^{-1}$) and the viscosity (23,002 cP) inside of the investigated optimization region. These results showing that the process costs are smaller when compared to the agitation speed (800 rpm) and aeration (0.5 vvm) fixed in the first

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central composite design for the sixteen assays. All of the experiments were accomplished in duplicate and the optimized point was confirmed.

Table 1 – Effect of the independent variables, agitation speed and aeration, in the responses $Y_{P/S}$ and viscosity.

Runs	Agitation speed [rpm]	Aeration [vvm]	K_La [h^{-1}]	$Y_{P/S}$ [$g.g^{-1}$]	Viscosity 0.75 s^{-1} [cP]
1	500	0.25	7.657	0.526	19,800
2	500	0.50	10.520	0.519	20,100
3	500	0.75	13.050	0.485	19,500
4	750	0.25	4.177	0.600	23,470
5	750	0.50	18.210	0.633	24,000
6	750	0.75	21.500	0.577	23,400
7	1,000	0.25	4.051	0.615	22,900
8	1,000	0.50	11.890	0.607	23,100
9	1,000	0.75	20.150	0.544	21,680
10	750	0.50	17.950	0.637	24,300
11	750	0.50	18.560	0.641	24,500

The Table 2 shows the kinetic parameters determined in the optimized conditions of aeration and agitation speed in the factorial project 3^2. The parameters values calculated in this study compared with the mentioned works presented variability, fact this that can be associated to the existent differences in the cultivation and in the strain of the microorganism, in the product formation, as well as in the diversity of raw material used.

Table 2 – Comparison of kinetic constant values from modeling and the literature.

Parameters	Assay in the optimized condition	Weiss & Ollis (1980)	Pinches & Pallent (1986)	García-Ochoa *et al.* (1990)	Serrano-Carreón *et al.* (1998)	Letisse *et al.* (2003)
μ_{max} [h^{-1}]	0.429 ± 0.037	0.152	0.29	0.110	0.25	0.38
X_{max} [g/L]	2.665 ± 0.094	2.450	2.15	1.790	4.42	3.30
α [gS/gX]□	0.334 ± 0.091	2.00	1.24	14.286	3.03	3.23
β□ [gS(gX h)$^{-1}$]□	0.529 ± 0.067	0.284	0.24	0.120	0.26	0.36
m [gP/gX]	0.120 ± 0.055	1.830	0.47	10.00	2.00	0.50
λ □[gP(gX h)$^{-1}$]□	0.342 ± 0.041	0.155	0.13	0.003	0.25	0.23

Conclusion

Therefore, in this context, the researched theme constitutes a differential to the literature publishing information concerning the optimization of the process variables in the xanthan gum biosynthesis from the sugar cane broth. Regarding the kinetic study, the Weiss & Ollis model adjusted the experimental data satisfactorily for the cellular growth, the substrate consumption and the product formation.

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A new strategy for xylanase production using wheat straw autohydrolysis liquor as substrate

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Keywords: Xylanase, Wheat straw, Wheat bran, Autohydrolysis liquor, Fermentation

In recent years, there has been an increasing trend towards a more efficient utilization of agro-industrial residues. Agro-industrial residues are lignocellulosic materials with a high content of cellulose, hemicellulose and lignin. Application of these residues in bioprocesses not only provides alternative substrates but also helps in solving pollution problems associated with their disposal. Besides, the chemical fractionation of these lignocellulosic materials into products derived from their structural components leads to an attractive possibility of their integral use in biotechnological processes. Treatments of lignocellulosic materials in autohydrolysis processes, under optimized conditions, lead to the solubilization of hemicelluloses (liquid phase, liquor) and formation of a solid phase enriched in both cellulose and lignin. These aqueous processes, when used in mild conditions, are an environmentally friendly technology for biomass utilization.

On the other hand, xylan is the major constituent of hemicellulose and the second most abundant renewable resource with a high potential for degradation to useful end-products. Microbial xylanases (1,4-β-D-xylan xylanohydrolase, EC 3.2.1.8) are the preferred catalysts for xylan hydrolysis due to their high specicity, negligible substrate loss and side product generation. During the last decades, xylanases have attracted considerable research interest because of their potential industrial applications.

The industrial production of xylanase preparations is based on microbial biosynthesis. Most often, mould strains of the species *Aspergillus* and *Trichoderma*, as well as bacterial strains of the species *Bacillus*, are used. Microbial enzyme producing strains can be cultivated in solid-state and submerged cultivation fermentations. Nowadays, submerged cultivation is the main process used as it allows for a higher degree of processes intensification and a better level of automation. Moreover, agricultural byproducts, as an alternative to synthetic medium, are a suitable and inexpensive culture medium for fungal industrial production processes.

In general, xylanase biosynthesis is induced by its substrate – xylan. The high xylan content of some wastes such as corn cobs and wheat bran makes them an accessible and cheap source of inducers. The inclusion of these components in the nutrient media composition is the main strategy in microbial xylanase biosynthesis.

In this work, the performance of wheat straw autohydrolysis liquor as adjunct in medium containing wheat bran for xylanase production in submerged cultivation of the strain *Aspergillus terricola* was evaluated.

Initially, a local wheat straw sample was milled and sieved through a 1 mm screen, air-dried, homogenized and stored. Water was added to the obtained wheat straw sample in a closed and pressurized vessel in order to obtain a liquid/solid ratio of 10:1 (w/w), taking into account the moisture content of the sample. The reactor was heated to the desired temperature (200 ºC) during 30 min. After each treatment, the liquid phase or liquor (hemicelluloses rich fraction) was separated from the solids by filtration. The hemicelluloses were then precipitated with three volumes of 95% ethanol (20 °C, 24 h)

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and dried for yield determination (4.9%), or used directly as liquid substrate. *Aspergillus terricola* strains were cultivated on Vogel medium (Vogel, 1964) using different carbon sources: 1% (w/v) birchwood xylan; 1% (w/v) wheat bran; 100% (v/v) wheat straw autohydrolysis liquor; combination of 1% (w/v) wheat bran and 2% (v/v) wheat straw autohydrolysis liquor; and combination of 1% (w/v) wheat bran and 10% (v/v) wheat straw autohydrolysis liquor. The cultivation conditions were 30ºC, 100 rpm, during a maximum of 7 days.

Xylanase was assayed by 3,5-dinitrosalicylic-acid (Miller, 1959) to quantify the products formed by the endo-action of this enzyme on xylan. The substrate used was 1% birchwood xylan in citrate-phosphate buffer, pH 6.0. One unit of enzymatic activity was defined as the amount that liberated 1 μmol of reducing sugar per minute on assay conditions.

The best conditions for xylanase production were observed when the microorganism was cultivated in birchwood xylan for 6 days; however, satisfactory results were obtained using a combination of 1% wheat bran and 2% or 10% autohydrolysis liquor, for 5 days fermentation, since the obtained productions were higher than observed using only 1% wheat bran. The obtained production using the liquor was very interesting once, after 7 days of cultivation, the xylanase production was higher than the ones obtained with wheat bran or by the combination of wheat bran and liquor. These results demonstrate that this can be an economical alternative way for xylanase production as low cost substrates are used.

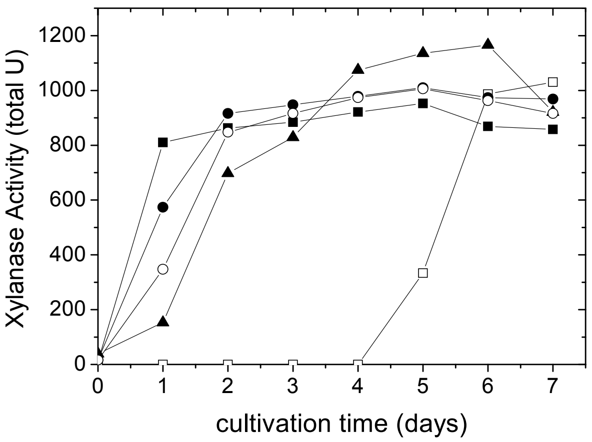

Figure 1. Fermentation performance for xylanase production using different carbon sources: 1% (w/v) birchwood xylan (-▲-), 1% (w/v) wheat bran (-■-), 100% (v/v) wheat straw autohydrolysis liquor (-□-), combination of 1% (w/v) wheat bran and 2% (v/v) wheat straw autohydrolysis liquor (-●-), and combination of 1% (w/v) wheat bran and 10% (v/v) wheat straw autohydrolysis liquor (-○-).

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Approach model for simulation of the starch hydrolysis by α- amylase and alcohol production from manioc root starch

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Key-words: starch, manioc roots, alcohol, kinetic model.

Being a renewable fuel and a lower pollutant when compared with petroleum fuels, ethylic alcohol is a combustible car fuel widely used in Brazil, and is desired by various countries. Recently, a search for new energetic sources was unleashed after the pronounced governing of various countries on a possible energetic crisis. At present, Brazil is in search of methods that will increase the ethanol offer by production of the combustible alcohol (Oliveira and Vasconcelos, 2006). Starch, the main reserve carbohydrate of several crops, is highly abundant in nature and can be easily extracted with high purity and at low cost (Cereda *et al.*, 2001). Recently, a search for new energetic sources was unleashed after the pronounced governing of various countries on a possible energetic crisis. Thus, it shows the optimal conditions to produce fermentable syrup from manioc starch and as a potential substitute to sugarcane in alcohol production. The food industry is using starch for a long time as ingredient because of its functional properties. This work shows optimal conditions of using the amylases from *A. niger* in starch hydrolysis and alcohol production from manioc root starch. Bioreactors contained between 7 and 23 g/L of Starch concentration, at pH 4.8 and temperatures varying between 28 and 61°C were made for evaluation of effects of starch concentration and temperature on hydrolysis yield. A new model for starch hydrolysis kinetic was fitted. Results showed that exponential models fitted more than other models. For substratum dependence [S] with the hydrolysis time (t), the fitting model was: $[S] = e^{-kt}$, while k is the kinetic constant of the product formation. For starch hydrolysis rate (d[S]/dt) was verified that the fitting model of type: $\frac{d[S]}{dt} = e^{\frac{V_{\max}}{K_M}[S]}$, while V_{max} and K_M are maxim velocity and Michaelis-Menten constant, respectively. These models possible to simulate the hydrolysis process by amylases and its scale-up of this process. On starch hydrolysis, it perceived that in optimal condition the hydrolysis yield got 80%. After fermentation an alcohol yield above of 45% was gotten (the theoretical is 51.1%). This work showed as to obtain alcohol of manioc root starch, as alternative to the substitution of the sugar-cane juice in alcohol production.

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RSM optimization of fractional precipitation and characterizing of amylase from maize malt

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Keywords: maize malt, purification, amylases, alcohol, acetone.

The enzymes α-amylase (EC 3.2.1.1, α-1,4 glucan 4-glucanhydroxylase) and β-amylase (EC 3.2.2.1 α-1,4 glucan 4-malthydrolase) with a molecular weight of about 50 kDa, are exoenzymes that simultaneously degrade starch to produce glucose, maltose and boundary dextrins, which are much utilized in food industries (Aguilar et al., 2000; Biazus et al., 2005). Their optimum pH is 4.8 and 5.4 and optimum temperature is 75 °C and 55 °C, respectively. According to Sigma (1996), the price of amylases enzymes is about U$ 1,500/gram of purified material. This work aimed to purify the amylases from maize (*Zea mays*) malt by solvent precipitation and it biochemistry characterization. A 2^3 factorial planning was making to study the effect of pH, temperature and solvent (alcohol or acetone) concentration on the enzymes purification index (Barros Neto et al., 2001). Malt was obtained by maize seed germination for five days (Biazus et al., 2005). Amylase purifications from maize malt by fractional precipitations using alcohol or acetone were assayed of according to Luccarine et al. (2005). The pH and meddle reaction temperature were varied to determine their effect on the amylases activity (Aguilar et al., 2000). Enzyme activity and total protein contents were measured by DNS (Biazus et al., 2005) and Bradford (1976) methods. Square model was the best fitting to description of precipitation process behavior and the large purification index were found for conditions of low temperature, and high pH and alcohol concentration, while a purification factor of 10 times was obtained. Optimal pH was obtained between 4.5 and 6.5 and optimal temperatures were of 50°C for β-amylase and 90°C for α-amylase and the values of K_M and V_{max} constants was of $7.69.10^{-2}$ g/L and $7.69.10^{2}$ g/L.min, respectively.

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Butanediol production from eucalyptus hemicellulosic hydrolysate by fermentative process with *Klebsiella pneumoniae*

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Key words: Butanediol, Hemicelullosic hydrolysate, Lignocelullosic residues, *K. pneumoniae*

Introduction

Lignocellulosic materials, like residues from agro industry and forests, are renewable and natural resources that have been hardly studied as carbohydrate source for fermentative processes. The character renewable makes these materials to be advantageous in relation to the fossil ones. The carbon dioxide produced from such renewable energy sources has no negative impact on environment like fossil fuels. In addition, it must be considered the frequent crises related to the energy sector around the world. 2,3-Butanediol, a di-alcohol, can be obtained by biotechnological process using *Klebsiella pneumoniae*, witch can metabolise the xylose and others sugars obtained by hydrolysis of agro industry or forest wastes, as eucalyptus waste. The calorific power of the diol (27.200 kJ/kg) is greater than that of methanol (22.100 kJ/kg) and similar to that of ethanol (29.100 kJ/kg), becoming a promising compound to be used as liquid fuel, representing environmental advantages. The low freezing point of the levo isomer (- 60 ºC) constitute the basis for the commercial interest in butanediol as an anti-freezing agent. Butanediol is also a biodegradable compound, and can be employed as solvent or linkage agent for inks and polymers manufacturing, witch are environmentally safe (Afshar et al.,1993). This work aimed to evaluate the production of 2,3-butanediol from eucalyptus hemicellulosic hydrolysate by fermentative process.

Material and Methods

The substrate source corresponded to the residues (branches, leaves and peels) remaining in the field after eucalyptus crop. The acid hydrolysis was evaluated by a factorial design and the following factors were studied: reaction time, temperature, acid solution concentration and liquid: dry material ratio. Treatments for inhibitors removal consisted in the precipitation (variation of pH) followed by adsorption with active charcoal and adsorption by ion exchange resins. For all experiments it was used the bacterium *Klebsiella pneumoniae* NRRL B-199.

For the batch phase of the fed-batch system the broth was composed by the nutrients used by Frazer, McCaskey (1989) and xylose P.A. (30 g/L). The feeding medium corresponded to the evaporated and treated hydrolysate, supplied with the same nutrients mentioned above.

The assays were carried out in fed-batch system, in an 1.35 L fermentor (Bioflow III). The conditions were: 37 ^{o}C, pH controlled at 5.8 and initial k_La value of 100 h^{-1}. Batch phase volume was 600 mL and 750 mL of feeding broth was added, at 72 mL/h.

The salting out technique, with K_2CO_3, was tested for product recovery from the fermented broth. These assays were accomplished according to a completed factorial design 2^2.

Sugars (glucose, xylose and arabinose) and butanediol concentrations were determined by (HPLC), in chromatograph WATERS. Cellular growth was measured by optical density and the concentrations were obtained by a calibration curve.

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Results and discussion

The best sugar extraction was obtained under the following conditions: 20 minutes reaction time, temperature of 160 °C, 0.8% acid solution and liquid to solid ratio of 3:1. Under such conditions the total sum of xylose, glucose and arabinose was 47.9 g/L, corresponding to 79.2% of sugars recovery. Although the obtained mathematical model predicted 95.8% of sugars recovery, the respective conditions promoted sugars degradation. Treatment with ion exchange resins showed to be more efficient to remove the major microbial inhibitors from the hydrolysate than activated charcoal. As can be verified in Table 1, a substrate accumulation up to the end of feeding was observed, and the maximum product concentration was 13.5 g/L, when charcoal was used for hydrolysate treatment. For ion exchange resins treated hydrolysate (Table 2) there was no substrate accumulation and the maximum product concentration was 32.0 g/L, with a negligible loss of substrate.

Table 1. Concentrations of cells, substrate and product, as a function of time, from the fermentation of hydrolysate treated with pH variation and activated charcoal.

Time (h)	Cells (g/L)	Substrate (g/L)	Butanediol (g/L)
0	4.8	17.4	3.4
3	5.5	20.5	8.8
6	3.2	27.4	10.9
9	3.9	35.3	11.9
10,75	3.6	37.8	11.7
16	3.6	33.0	13.5
18	3.6	31.2	13.5

Table 2. Concentrations of cells, substrate and product, as a function of time, from the fermentation of hydrolysate treated with ion exchange resins.

Time (h)	Cells (g/L)	Substrate (g/L)	Butanediol (g/L)
0	4.2	21.5	1.3
3	6.3	22.7	10.7
6	8.1	23.1	14.3
9	5.7	20.2	17.6
10,5	4.9	19.8	22.1
15	6.2	6.3	31.5
18	6.2	1.3	32.0

Using the salting out method it was possible to recover 92% of the product obtained during the fermentation. This result was obtained at 20 °C and using 70% of K_2CO_3 for the extraction. With these conditions, the obtained mathematical model predicted 95% of product recovery, witch was confirmed by another extraction run. The possibility of using this technique is advantageous, considering the high heat consumption by the distillation.

Conclusions

Acid hydrolysis is an adequate method for sugars recovering from eucalyptus residues, by witch a sugar syrup, for butanediol production by fermentation, can be obtained. Ion exchange resin is a promising treatment for inhibitors removal from the hydrolysate that will be fermented by *Klebsiella pneumoniae*. Fed-batch system showed to be proper for the present process development and the salting out technique provide more than 90% of product recovery from the fermentation broth.

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Hydrothermal treatments of corn cob and hemicelluloses extraction

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Keywords: Autohydrolysis, Extraction, Hydrothermal treatments, Hemicelluloses, Xylan

Agricultural wastes are of lignocellulosic nature (with cellulose, hemicelluloses and lignin as structural components). Chemically, about 35-55% of the dry material is the glucose polymer cellulose, much of which is in a crystalline structure; while another 25-35% is hemicellulose, an amorphous polymer. The remainder is mostly lignin plus less amounts of minerals, waxes, and other compounds (Sun and Cheng, 2002). Cellulose is formed by beta-(1- 4) glucosyl linkages in a linear backbone, whereas hemicelluloses are branched polymers composed of several monosaccharides (Fengel and Wegener, 1989).

Treatments of lignocellulosic materials in autohydrolysis processes (or hydrothermal treatments), under optimized conditions, lead to the solubilisation of hemicelluloses, leaving a solid phase enriched in both cellulose and lignin. These aqueous processes, when used in mild conditions, are an environmentally friendly technology for biomass utilization (Garrote et al., 1999). Autohydrolysis proceeds through hydronium-catalyzed reactions. Thus, in the initial reaction stages, the hydronium ions come from water autoionization, whereas the acetic acid generated in the media is the main source of catalyst in further reaction stages (Garrote et al., 2001). This work deals with the characterization of reaction products from corn cob autohydrolysis processes, an information for assessing further applications of the extracted fractions.

Local corn cob samples were milled to pass a 1 mm screen, air-dried, homogenized and stored. These corn cob samples and water were mixed in a closed and pressurized vessel in order to obtain a liquid/solid ratio of 10:1 w/w, taking into account the moisture content of the sample. The reactor was heated to the desired temperature (165-195 ºC) by periods ranging from 10 to 60 min. After each treatment, the liquid phase was separated from the solids by filtration. The hemicelluloses were then precipitated by pouring the concentrated supernatant fluid with three volumes of 95% ethanol (20 °C, 24 h). The precipitates were recovered by filtration, washed with 70 % ethanol and dried (30 ºC).

The compositional data of the corn cob samples used as raw material (average of three replicates) are shown in Table 1. The results are expressed as glucan, hemicelluloses and lignin. Thus, lignocellulosic materials can be defined by their contents of glucan (corresponding to cellulose), xylan (measured as xylose equivalent, which forms the main polymer backbone), xylan substituents (arabinose units, reported as arabinan expressed in sugar equivalent, and acetyl groups), and total lignin (measured as soluble lignin and Klason lignin).

As can be seen from Table 2, xylan and xylan substituents (hemicelluloses) accounted jointly for 34.7 % of corn cobs (in oven-dryed basis). Further, the treatment of corn cob released 0.28 - 9.68 % hemicelluloses (in respect to dry starting material), corresponding to the dissolution of 0.8 and 27.9 % of the original hemicelluloses, respectively. These results revealed that autohydrolysis of the corn cob was effective,

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and that the highest extraction yield was obtained with 30 min and 185 ºC (27.9% of the total available hemicelluloses).

Table 1. Chemical composition of corn cob

Chemical Components	Composition (%)
Glucan	31.8 ± 0.8
Hemicelluloses	34.7 ± 1.1
Xylan	29.4 ± 0.2
Arabinan	1.1 ± 0.2
Acetyl group	3.1 ± 0.4
Total lignin	27.9 ± 0.4
Soluble lignin	11.3 ± 0.2
Klason lignin	16.6 ± 0.4
Total Composition	93.3 ± 0.9

Table 2. Yield of hemicellulose (%)

	Yield of Hemicellulose (%)		
Time (min)	165 (ºC)	185 (ºC)	195 (ºC)
10	0.66	0.28	0.92
20	0.48	1.72	2.41
30	2.84	9.68	6.33
40	2.92	9.02	1.39
60	7.72	1.30	1.22

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Bioconversion of xylose from lignocellulose wastes in raw materials for biochemical syntheses

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Keywords: Bioconversion, xylose, fixed bed reactor, phosphoketolase

The great majority of the actual research studies dedicated to find new ways to valuate (ligno) cellulosic residues are directed to fermentative procedures. Less attention have been paid so far to the discovery of processes leading to production of high-value compounds. To overcome this shortcoming, setting up of an enzymatic reactor able to convert xylose to high value biosynthetic intermediates based on highly efficient enzymes was performed. In cellulosic wastes, xylose is encountered as xylan, which is a polysaccharide component of hemicelluloses.

In Romania, at present, the lignocellulosic wastes are only used as a cheap fuel. Although installations for processing these residues as well as their transformation in furfurol had been available, now they are deactivated. However, the large amount of lignocellulosic wastes produced would make reasonable the re-activation and/or building of new facilities towards superior valuation of this type of residues.

There have been numerous studies concerning xylose conversion to value-added materials. Some of them used fermentative processes based on different microorganisms (Kuyper et al., 2005; Taniguchi et al., 2004) sometimes genetically engineered (Nyyssola et al., 2005). However, xylose fermentation is much more problematic as compared to glucose fermentation and frequently, even recombinant microorganisms, cannot exclusively use xylose as a carbon source but also need a supplementary sugar (Krishnan et al., 1999).

Xylose metabolism in bacteria has been described for a wide array of microorganisms including *Escherichia coli* (Lawlis et al., 1984) *Lactobacillus* ssp (Bor et al., 1992), *Staphylococcus xylosus* (Sizemore et al., 1991) and *Lactococcus lactis* (Erlandson et al., 2000). Free xylose is initially transported via low-affinity transporter (Xyl E or Xyl T) or high-affinity binding-protein dependent systems (XylFGH). Xylose isomerase (*xylA* gene) then converts xylose to xylulose which is phosphorylated to xylulokinase (*xylB* gene). Xylulose-5 phosphate is a common intermediate for various pentose metabolisms. During heterofermentative degradation in lactic acid bacteria, xylulose-5 phosphate is further metabolized via the phosphoketolase pathway.

The xylose conversion to gliceraldehyde 3-phosphate was carried out by a dynamic reactor-cascade system, made up of three modules each of them containing a substrate conditioning element, a fixed-bed reactor and an adequate storage tank. The modular form permitted analysis of each of the enzymatic processes, assuring at the same time, separation of the obtained products.

In the first reaction, xylose was converted to xylulose in an equilibrium reaction by the immobilized xylose isomerase derived from *Streptomyces murinus*, having its optimal temperature at 60 °C. A conversion rate of 25% was obtained, although it raised the problem of the overleft xylose that inhibits phosphoketolase activity required in the last phase of the transformation; solved by separation of the resulted mixture by high-performance liquid chromatography.

Xylulose was phosphorilated by xylulose kinase in the presence of ATP in a subsequent reaction, in which a conversion rate of 90% was achieved. The enzyme D-xylulose-5-phosphokinase obtained by cloning of the *xylB* gene into pET28a(+) and heterologous expression in *E. coli* strain BL21(DE3)pLysS showed a 5,15 U/mg catalytic efficiency measured by a coupled activity assay

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Xylulose 5-phosphate phosphoketolase from *Leuconostoc mesenteroides*, obtained by cloning of the phosphoketolase gene into pHAT-2 plasmid and expressed into *E. coli* strain BL21(DE3)RIL was used to carry out the final step of the xylose conversion reaction. Characterization of catalyitic properties of the obtained phosphoketolase was carried out by determination of conversion of xylulose 5-phosphate to glyceraldehide 3-phosphate under optimized conditions showing a specific activity of 0.6-0.7 U/mg.

The final reaction performed by the obtained phosphoketolase was conducted at 37 °C, in the presence of inorganic phosphate as well as the cofactor Mg^{2+}. The above obtained xylulose 5-phosphate (Xu5P) was converted into acetyl phosphate (AcP) and glyceraldehyde 3-phosphate (G3P). The latter product represents a high-added value raw material, with different possibilities of application ranging from the cosmetic industry through fine organic syntheses to the pharmaceutical industry.

Immobilization of both xylulose kinase and phosphoketolase was carried out taking the advantage of the N-terminal 6XHis tags present as fusion partners in the obtained enzyme preparations. One step purification and separation of the obtained proteins was achieved by binding to chelating support Ni^{2+}- NTA- Sepharose, which was found to be the optimal matrix from the point of view of binding capacity and reduced cation leakage.

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High ethanol productivity by fermentation of concentrated industrial substrates using ethanol-tolerant *Saccharomyces cerevisiae* strains

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Keywords: bioethanol, ethanol tolerance, ethanol titre, industrial media, corn steep liquor.

Growing industrial development makes imperative to find environmentally sustainable energy sources. Biofuels have gained popularity in the last decades, especially due to the fossil fuels depletion and the increasing environmental concern. Of the many fossil fuels' alternatives, ethanol has been highly used and studied, since it can be added to fuels for combustion engines to levels of up to 20% without any modification to the engine (MDA, 2007). In order to make ethanol production more economical, a high productivity bioprocess is necessary. Ethanol productivity can be improved by using highly concentrated substrates. However, ethanol is well known as a toxic metabolite for yeast cells, which raises a serious problem. Thus, strains that can grow well under high ethanol stress condition are highly desirable. This work aims to select and characterize *Saccharomyces cerevisiae* strains with improved ethanol tolerance. Moreover, it aims to evaluate the feasibility of industrial residues as fermentation media and to optimize the composition of such media.

Screening for strains or mutants with high ethanol tolerance has been often performed by viability observation on ethanol-containing agar plates (Carrasco et al., 2001) and by growth rates determination on ethanol-containing medium (Hirasawa et al., 2007). Although these and other similar techniques are easy and fast, they screen for the ability to grow under high ethanol stress rather then for fermentation ability in these conditions. Thus, selection must be conducted under conditions similar to those faced by yeasts in industrial processes. In the present work, strains were selected for ethanol tolerance in batch fermentations of high-glucose media, which causes osmotic (sugar) stress in the beginning and increasingly ethanol stress towards the end of the fermentation.

Three *Saccharomyces cerevisae* strains were tested for ethanol production and tolerance, from which CA 116 and RL 11 are industrial strains isolated from Brazilian "cachaça" fermentation and BY 4741 is a laboratorial strain. The most ethanol-tolerant strain was able to ferment 300 g/L glucose, producing up to 17.4 % (v/v) of ethanol in trials carried out in anaerobic shake-flasks.

Aiming to develop an inexpensive and highly fermentable industrial based medium, corn steep liquor (CSL) has been added to high glucose solutions, as sole nutrients source in replacement of both peptone and yeast extract. CSL), a major by-product of corn starch processing, is a cheap and readily available source of proteins, amino acids, minerals, vitamins, reducing sugars (such as dextrose), organic acids (in particular, lactic acid), enzymes, and elemental nutrients (such as nitrogen) (Aktar et al., 1997; Amartley et al., 2000; Keller and Heckman, 2006). Although its primary uses are as feed supplement for ruminants and nutrient source for poultry (Akhtar et al. 1997; Keller and Heckman, 2006), CSL has been used in many works as a rich and effective nutrient supplement, including for ethanol production (Lawford, 1997; Amartley et al., 2000; Carvalheiro et al., 2006). Likewise, the results obtained have confirmed the feasibility of CSL as peptone and yeast extract substitute, since supplementation of 300 g/L glucose medium with CSL concentrations around 90 - 110

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g/L has resulted in fermentation performance similar to that observed in YP medium with the same glucose concentration.

Recent studies have reported ethanol titres as high as 19 % v/v (Alfenore et al., 2002; Cot et al., 2007). However, these values have been achieved in fed batch processes with exponential vitamins feeding, besides other controls such as pH regulation and constant glucose concentration. In the current work, fermentations were carried out in simple anaerobic shake-flasks without process control; thus, higher performance may be achieved by process improvement.

In order to design sustainable bioethanol production processes it is important to understand which factors contribute for a higher microbial ethanol tolerance, as well as to design fermentation media totally based in industrial by-products or other cheap resources.

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Biodegradability of polymer blends based on renewable resources

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Keywords: Polymeric blend, biodegradability test, reactive extrusion

The growing utilization of polimeric materials when compared to other materials (steel, aluminium) is mainly related to their low cost, good properties, aesthetic qualities and resistance to physical ageing and biological attack. However, the resistance to environmental degradation of synthetic polymers, especially in such applications where they are used for a short period of time before becoming waste is forcing the industries to seek for more ecologically friendly materials for their products. Most biodegradable polymers have relatively poor physical properties, such as high brittleness, moisture sensitivity, low heat distortion temperature, difficult their processability,that restrict their use in a wide-range of applications. Therefore, new approaches to develop biodegradable blends are a subject of increasing research interest.

The objective of this work was to prepare and evaluate the biodegradabitity of biobased polymeric blends using a standard method. Thus, blends of high density polyethylene (HDPE) and biodegradable polymers (poly(lactic acid) (PLA), poly(ε-caprolactone) (PCL) and Mater-Bi (thermoplastic starch with PLA or PCL)) were prepared in a co-rotating twin-screw extruder, together with polyethylene modified with maleic anhydride (PE-g-MA) used as compatibiliser. In this work, biodegradation tests were carried out using the standard ISO 14851 (1999) which specifies a method for the determination of biochemical oxygen demand (BOD) in a closed respirometer. Polymers were reduced to powder to create a suspension of the polymer in the test system. Thus, the surface area of the polymeric material available to microbial attack was increased. For PLA and PCL tests were also carried out with allylthiourea (ATU) which proved to be an effective inhibitor of the nitrification process (e.g. oxidation of ammonium to nitrate) which might contribute to the BOD of the polymeric blends. The biodegradation of the polymers and blends was followed by FTIR spectroscopy. Thin films of the initial materials and the residues after biodegradation were prepared by compression-moulding and analyzed directly using a solid film support. Based on the results from the biochemical oxygen demand test (Figure 1), it can be observed that the blend containing PCL is more biodegradable than the blend containing PLA. The biodegradability of the blend containing PLA increased with the addition of starch, in turn the same effect was not observed for PLC. The biodegradability test suggests that starch is more biodegradable than PLA but less than PCL. The BOD/ThOD ratio (experimental value divided by the thoretical one) ranged from 0.22 (for PLA60) to 1 (for PCL).

Respirometric experiments carried out in the presence of allyltheorea (ATU), an inhibitor of the nitrification process, are depicted in Figure 2. BOD results determined with ATU for PCL and PLA were lower than the ones obtained without. This result can be explained by the fact that oxygen was consumed both for the mineralization of polymeric carbon to carbon dioxide and water and for the oxidation of ammonium to nitrate. The BOD/ThOD ratio decreased in the presence of ATU. The reduction was 37 % for PCL and 20 % for PLA.

The qualitative results of FTIR spectroscopy of initial and biodegraded polymeric blends are in agreement with the ones obtained in the standard biodegradability tests.

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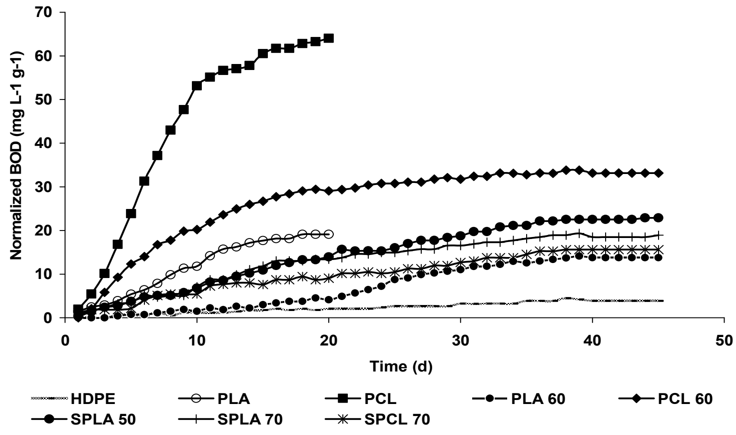

Figure 1 - Normalized biochemical oxygen demand values expressed as mg/L of O_2 divided by mg of polymeric C of each sample.

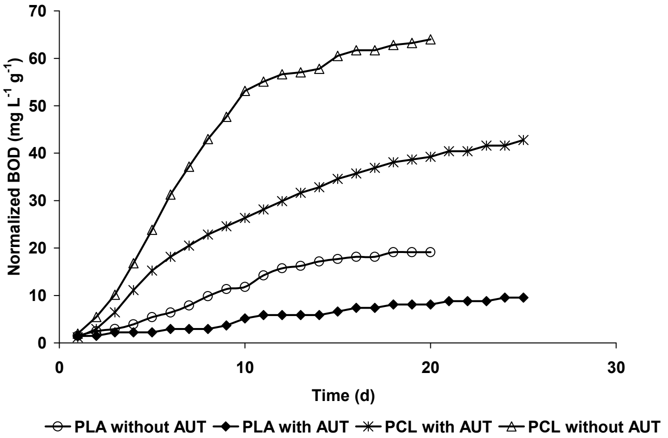

Figure 2 - Normalized biochemical oxygen demand values expressed as mg/L of O_2 divided by mg of polymeric C for PLA and PCL (with and without AUT).

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The effect of paper waste and food waste on biohydrogen production at mesophilic temperatures in batch reactors

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Keywords: Biohydrogen, Organic Waste, Volatile Fatty Acids, Anaerobic, Batch

Hydrogen appears to be an ideal candidate as an alternative to fossil fuels. It has the highest energy content per unit of weight for any known fuel and it can be used to generate electricity by fuel cell technology. Hydrogen can be obtained biologically via fermentation via anaerobic bacteria and photolysis via algae and cyanobactera. However, hydrogen is produced at higher rates with fermentation compared to photolysis and has the potential to combine organic waste management with simultaneous hydrogen production. Dark fermentation of hexoses has been extensively studied using a variety of anaerobic inocula under different growth and operational conditions. Few reports have demonstrated biohydrogen production directly from food waste or paper waste. In addition, the effect of different amounts of paper waste and food waste on biohydrogen production has not been studied. The objective of this work was to examine the effect of different amounts of paper waste and food waste on biohydrogen production in batch reactors.

Batch experiments were conducted in 125-mL serum bottles containing 20 mL total of inoculum and media. Food waste and paper waste were used as substrates for biohydrogen production. The amount of each substrate used in the batch reactors were blends of both substrates with 100% food waste and 100% paper waste serving as controls. Food waste and paper waste were obtained from the cafeteria at the University of Minho and a local paper manufacturer, respectively. The inoculum used in the batch experiments was obtained from an anaerobic digester from a municipal wastewater treatment plant. Methanogenesis was inhibited by the addition of bromoethanesulfonate (25 mM). The initial pH of the batch experiments was adjusted to 6.5 by flushing the headspace of each batch reactor with 100% CO_2 for several minutes. Batch cultures were incubated at 37 ºC (± 2 ºC). Hydrogen production was monitored using GC-TCD with nitrogen as the carrier gas. Soluble microbial products (formate, acetate, propionate, *n*- and *i*-butyrate, valerate, and ethanol) were determined using HPLC. VFA and hydrogen production were observed with both paper waste and food waste although the amount varied depending on the amount of food and paper initially fed to the batch reactors. The results of this work will help to optimize the amount of hydrogen that can be produced using a real world waste stream. In addition, this work will also assist in the development of optimal hydrogen production and yields from organic wastes in a continuous system.

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Optimization of ethanol production from cheese whey powder by Kluyveromyces fragilis using factorial design and response surface methodology

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Keywords: Cheese whey, Ethanol, *Kluyveromyces fragilis*, Response surface methodology.

The dairy industry represents an important part of the food processing industry and generates significant liquid wastes that can be used for the production of value-added products, such as galactooligosaccharides, biopolymers, vitamins organic acids and alcohols (Ghaly and El-Taweel, 1997, Yang and Silva, 1995). Cheese whey (CW), a by-product of the cheese manufacturing process, whose major components are lactose (44-52 g/L), proteins (6-8 g/L) and mineral salts (4-9 g/L), constitutes an inexpensive and nutritionally rich raw material for the production of ethanol by fermentation. However, the production of ethanol from non-concentrated CW is not economically feasible because the levels of ethanol obtained at the end of fermentation reach only about 2%, making the distillation process too expensive. Otherwise, costs are significantly reduced with the increase of lactose concentration up to 100-120 g/L (González Siso, 1996). Therefore, dry cheese whey powder (CWP) could be an attractive raw material for ethanol production. CWP is a dried and concentrated form of CW and contains lactose in addition to nitrogen, phosphate and other nutrients essential for the microorganisms (Kargi and Ozmihci, 2006). The use of CWP instead of CW for ethanol fermentations has significant advantages such as elimination of costly ultrafiltration processes to concentrate lactose before fermentation, compact volume, long-term stability and high concentrations of lactose and other nutrients yielding high ethanol concentrations by fermentation (Ozmihci and Kargi, 2007).

In the present work, ethanol was produced by CWP fermentation, and the influence of the process parameters lactose concentration (from 50 to 150 g/L), temperature (from 25 to 35ºC) and inoculum concentration (from 1 to 3 g/L) was evaluated through a 2^3 statistical experimental design.

Batch experiments were carried out in 500 mL Erlenmeyer flasks containing 100 mL of medium composed by CWP solution with initial pH adjusted to 5 by citric acid addition. The media were inoculated with a strain of the yeast *Kluyveromyces fragilis* (Kf 1), which was taken from the culture collection of the Centre of Biological Engineering, University of Minho (Portugal). The flasks were maintained in an orbital shaker at 150 rpm for 44 h. At the end of fermentation, samples were withdrawn for analysis of biomass, lactose and ethanol.

The results proved the feasibility of using cheese whey powder as substrate to produce ethanol concentrations higher than those obtained by direct fermentation of non-concentrated cheese whey.

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Influence of extraction conditions on flavonoid yields from different Portuguese forest residues

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Keywords: Flavonoids, Solvent extraction, Forestry residues; Shrubs, Maritime pine

Introduction

The Portugal forest area represents around 38% of the territory, which could represent a large quantity of forestry residues (pruning) and shrub species that can be used as raw material for biofuel production, like bioethanol. This subject is currently under investigation in our R&D Unit and attending to the biorefinery concept all the plants used in the project will be investigated for biologically active products. Among the bioactive compounds present in plants, polyphenols and particularly flavonoids are widely apreciated for their potential beneficial health effects, like antioxidant and anticarcinogenic activities (Ren et al., 2003). The present work is a preliminary study about the characterization of solvent extracts of some plant species studied in this project, namelly *Pterospartum tridentatum* (known as *carqueja* in Portugal), *Cistus ladanifer* (rock-rose), *Arbutus unedo* (strawberry tree) and a wood species, *Pinus pinaster* (maritime pine). So, a study of the influence of some critical extraction variables (type of solvent, time of contact, solvent renovation and solid/liquid ratio) on the total and flavonoids yield of resultant extracts was carried out.

Material and Methods

The arbustive species and pine residues were collected in May 2007 and stored at room temperature during six months. All plants consisted of wood/stalks, bark and leaves and were milled in a Retsch cutting mill to a particle size between 0,180 mm and 0,500 mm. The solvent extractions were carried out by maceration using methanol and ethanol 95% as solvents. Time contact and solid/liquid ratios were varied and the influence of solvent renovation was observed according to the following schema:

$$\begin{array}{r}\text{Ethanol maceration}\\ 5 \times 4\text{h}\end{array}\begin{cases}1/10\\1/20\\1/50\end{cases} 2 \times 10\text{h}\begin{cases}1/10\\1/20\\1/50\end{cases} 20\text{h}\begin{cases}1/10\\1/20\\1/50\end{cases} \quad \begin{array}{r}\text{Methanol maceration}\\ 2 \times 24\text{h}\end{array}\begin{cases}1/10\\1/20\\1/50\end{cases} 1 \times 48\text{h}\begin{cases}1/10\\1/20\\1/50\end{cases}$$

Methanol was also used for an extraction with Soxhlet apparatus until the solvent became colourless. Flavonoids were determined by aluminium chloride colorimetric method using quercetin for the preparation of calibration curve (Pourmorad et al., 2006). All determinations were carried out in duplicate.

Results and Discussion

All the studied lignocellulosic materials were found to be important sources of flavonoids, although the efficiency of the extraction varied with experimental conditions and plant species (Fig. 1 and 2). Among the two solvents used (ethanol and methanol), methanol was the most favourable for total extractables, but by using longer extraction times. For extracting flavonoids, the selectivity of both solvents was dependent on the plant species.

For some species, the flavonoids extraction was highly influenced by solvent renovation (e.g. *A. unedo*), while some others were negatively affected (*P. pinaster*).

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The 1/10 ratio was found to give the best results for the flavonoids ethanol extraction from *A. unedo*, while from *P. pinaster* the ratio was 1/50. The extraction of flavonoids from *C. ladanifer* seemed to be the lesser influenced by the extraction conditions and solvent used.

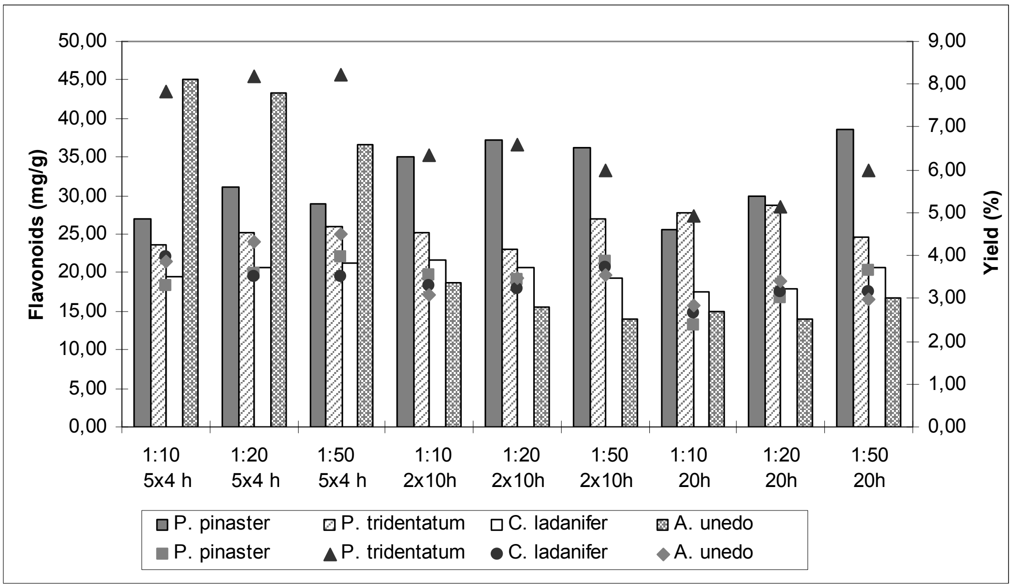

Fig. 1: Flavonoids in mg/g of oven dry material (columns) and total extract yields (markers) of plant extracts for different extraction conditions with ethanol 95%.

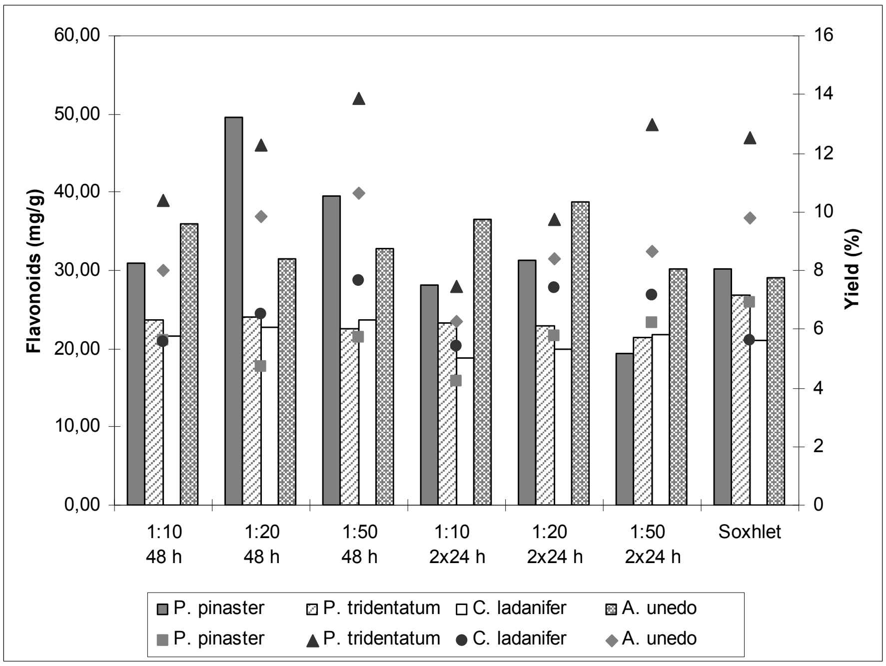

Fig. 2: Flavonoids in mg/g of oven dry material (columns) and total extract yields (markers) of plant extracts for different extraction conditions with methanol.

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Application of normalized Weibull model for describing the air drying of mango slices at different maturity stages

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Keywords: Moisture content, water diffusion coefficient, simulation

Objectives

The objectives of this study was the determination of the applicability of normalized Weibull model in describing the air drying of green and half-ripe mango slices, and determining the water effective diffusion coefficient of mango slices.

Materials and methods

Green and half-ripe mangoes were washed, peeled and cut into slices with an average thickness of 3.0 x10^{-3} m and the moisture content was determined. The mango slices were uniformly spread in a drying tray as thin layer and the drying experiments were carried out at air velocity of 1.76, 1.80 and 1.91 m/s and temperature of 50, 60, 70 and 80 ºC. Changes in weight of slices were monitored at 10 min intervals until there is no large variation in the weight. Drying experiments were done in triplicate. The moisture content of the dried samples was calculated depending on the basis of weight changes of the samples. The moisture content data at the different drying conditions were fitted to normalized Weibull model (Marabi et al., 2003):

$$\frac{X_w - X_{we}}{X_{w0} - X_{we}} = \exp\left[-\left[\frac{t}{\alpha_n}\right]^{\beta}\right] = \exp\left[-\left[\frac{t\,D_e\,R_g}{L^2}\right]^{\beta}\right] \qquad (1)$$

where X_w is the moisture content at any time t, X_{we} and X_{w0} are equilibrium and initial moisture contents, α_n is the normalized scale parameter, D_e is the calculate water diffusion coefficient, R_g is the geometric factor, L is the half-thickness of the slices for drying from both top and bottom sides and t is the drying time.

To establish the adequate parameter (R_g) to be utilized for the implementation of the normalized Weibull model, Fick's model for difussion has been used (Crank, 1975). This model was utilized to simulate the conditions prevailing under theoretical processes assuming D_e values. In order to simulate realistic conditions, a set of random errors taken from a population with normal distribution with zero mean was introduced:

$$\left[\frac{X_w - X_{we}}{X_{w0} - X_{we}}\right]_{noise} = \left[\frac{X_w - X_{we}}{X_{w0} - X_{we}}\right]_{theoretical}\left[1 + SD\ x\ Zn\right] \qquad (3)$$

where SD is the standard deviation (%) and Zn is the random number with standard normal distribution, $Zn \sim (0.1)$. Then, the normalized Weibull model was fitting to data derived from simulation and R_g was determined. Non linear regression was used for fitting the normalized Weibull model to experimental moisture content data at different drying conditions.

Results and discussion

Results of fitting normalized Weibull model to data derived from simulation are shown in Table 1. The high coefficients of determination ($R^2 > 0.98$) and the low asymptotic standard errors (ASE) for derive parameters (β) indicated the goodness of fit. Table 1 shows that the derived normalized Weibull parameters (β and R_g) values were maintained practically constant even when the diffusion coefficient varied by 3 orders of

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magnitude. Similar results were found for rehydration of food particulates (Marabi *et.*, 2003).

Table 1. Effect of theoretical D_e on derived parameter (β) and geometric factor (R_g) of normalized Weibull model.

D_e (m^2/s)	β	ASE	R_g	R^2
1.0×10^{-10}	0.83	0.01	13.1	0.999
1.0×10^{-11}	0.80	0.01	13.2	0.997
1.0×10^{-12}	0.76	0.01	13.2	0.994

The high coefficients of determination ($R^2 > 0.98$), the low asymptotic standard errors for D_e and β, the low chi-squared (χ^2) values (Table 2) and no pattern evident with the residuals across the range of diffusion coefficients (non shown) indicated the goodness of fit of experimental data to Eq. (1).

Table 2. Water effective diffusion coefficient (D_e) and shape parameter (β) of green and half-ripe mango during air drying at different air velocities (V) and temperatures (T).

T	V	Green mango				Half-ripe mango			
(ºC)	(m/s)	D_e x10^{10} (m^2/s)	β	R^2	χ^2	D_e x10^{10} (m^2/s)	β	R^2	χ^2
50	1.80	1.76 ± 0.29	0.76	0.993	0.00032	2.30 ± 0.14	0.79	0.994	0.00029
	1.91	2.23 ± 0.01	0.78	0.993	0.00035	2.51 ± 0.54	0.79	0.992	0.00035
60	1.80	2.08 ± 0.44	0.78	0.994	0.00038	2.46 ± 0.15	0.79	0.996	0.00038
	1.91	2.71 ± 0.36	0.80	0.994	0.00033	2.90 ± 0.49	0.80	0.994	0.00031
70	1.80	2.49 ± 0.01	0.79	0.994	0.00034	2.79 ± 0.71	0.80	0.994	0.00035
	1.91	3.06 ± 0.01	0.80	0.994	0.00032	3.07 ± 0.34	0.81	0.995	0.00032
80	1.80	2.99 ± 0.01	0.80	0.994	0.00036	3.11 ± 0.53	0.80	0.995	0.00036
	1.91	3.14 ± 0.08	0.80	0.994	0.00039	3.25 ± 0.23	0.81	0.996	0.00032

Analysis of variance showed that water diffusion coefficient increased ($p < 0.05$) with increasing air velocity and temperature.

Conclusions

The normalized Weibull model describes the air drying of green and half-ripe mango slices at different air temperatures and velocities and allows determining their water effective diffusion coefficient.

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Determination of suitable thin layer model for air drying of mango slices

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Keywords: Midilli and Kucuk model, green and half-ripe mango slices.

Objectives

The objectives of this study were to determining the suitable model for predicting the thin layer drying kinetics of green and half-ripe mango slices at different drying air temperature and velocity conditions, and analyze the effects of temperature and velocity on the constants of the suitable model.

Materials and methods

Green and half-ripe mangoes were washed, peeled and cut into slices with an average large of 45.2 x10^{-3} m, average width of 34.4 x10^{-3} m, and average thickness of 3.0 x10^{-3} m, The moisture content was determined by drying under vacuum (1.93 Pa) at 60 °C until constant weight. The mango slices were spread in a tray as thin layer and the drying experiments were carried out at air velocity of 1.76, 1.80 and 1.91 m/s and temperature of 50, 60, 70 and 80 ºC. Changes in weight of slices were monitored at 10 min intervals by a digital balance of 0.01 g accuracy. The drying was continued until there is no large variation in the weight. Drying experiments were done in triplicate. The moisture content of the samples was calculated depending on the basis of weight changes of the samples. The experimental moisture content data at the different drying conditions were converted to moisture ratio (MR) and fitted to following models by non linear regression:

Exponential or Newton model. $MR = \exp\,(-k\,t) \quad (1)$

Henderson and Pabis model. $MR = a\exp\,(-k\,t) \quad (2)$

Modified Henderson and Pabis model $MR = a\exp\,(-k\,t^{n}) + b\,t \quad (3)$

Page model. $MR = \exp\,(-k\,t^{n}) \quad (4)$

Two term model. $MR = a\exp\,(-k_0\,t) + b\exp(-k_1\,t) \quad (5)$

Two term exponential model. $MR = a\exp\,(-k\,t) + (1-a)\exp(-k\,a\,t) \quad (6)$

Logarithmic model. $MR = a\exp\,(-k\,t) + c \quad (7)$

Verma et al. model. $MR = a\exp\,(-k\,t) + (1-a)\exp(-g\,t) \quad (8)$

Midilli and Kucuk model. $MR = a\exp\,(-k\,t^{n}) + b\,t \quad (9)$

Wang and Singh model. $MR = 1 + a\,t + b\,t^{2} \quad (10)$

Coefficient of determination (R^2) and reduced chi-square (χ^2) were used for the determination of the best suitable model.

Results and discussion

As expected, MR decreased considerably with increasing drying time. The increase in the drying air temperature decreased ($p<0.05$) MR and consequently increased drying rate and decreased drying time. Moisture ratio decreased ($p<0.05$) with increasing air velocity between 1.76 and 1.91 m/s. Similar results were found for figs (Xanthopoulos *et al.*, 2007) and for plums (Goyal *et al.*, 2007).

Among the mathematical models investigated, the Midilli and Kucuk model satisfactorily described the drying behavior of mango slices at different maturity stages with highest R^2 (0.999) values and lowest chi-square. Similar results were obtained by some authors on drying of fruits and vegetables (Akpinar, 2006; Menges and Ertekin,

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2006). Values of constant of Midilli and Kucuk model for green and half-ripe mango at different air temperature (T) and velocity (V) are shown in Table 1.

Table 1. Values of constant of Midilli and Kucuk model for green and half-ripe mango at different air temperature (T) and velocity (V).

V	T	Green mango					Half-ripe mango				
(m/s)	(°C)	a	k x10^{2}	n	bx10^{4}	R^2	a	k x10^{2}	n	bx10^{4}	R^2
1,76	50	1.00	1.17	1.17	-1.3	0.999	1.00	0.72	1.24	-0.5	0.997
	60	1.01	1.53	1.15	-2.1	0.998	1.00	0.98	1.23	-4.0	0.998
	70	1.00	2.04	0.96	-1.2	0.997	0.99	1.21	1.22	-7.9	0.986
	80	0.99	2.05	0.89	-0.5	0.999	0.99	1.37	1.19	-0.5	0.997
1,80	50	1.00	1.01	1.18	-0.5	0.998	0.99	0.53	1.29	-0.5	0.998
	60	1.01	1.13	1.15	-1.2	0.997	0.99	0.68	1.26	-3.5	0.986
	70	1.00	1.24	1.13	-1.8	0.999	0.99	0.78	1.23	-3.1	0.997
	80	1.00	0.71	1.10	-4.1	0.998	0.99	1.15	1.19	-0.5	0.998
1,91	50	1.01	1.08	1.11	-0.5	0.997	0.99	0.53	1.22	-6.5	0.986
	60	1.01	1.47	0.99	-0.5	0.999	1.00	0.65	1.21	-0.5	0.996
	70	1.00	1.75	0.94	-8.0	0.998	1.00	1.06	1.18	-1.3	0.998
	80	0.99	2.05	0.87	-12.6	0.997	0.99	1.25	117	-0.5	0.986

The values of constant *a* for both maturity stages are approximately equal 1. Analysis of variance (ANOVA) showed that constants *k*, *n* and *b* for Midilli and Kucuk model were significant different ($p<0.05$) for both maturity stages and affected by air velocity and temperature and its interactions. Constant *k* increased ($p<0.005$) with increasing air temperature while constant *n* decreased ($p<0.05$). There are not trends in constants *k* and *n* with increasing velocity. There are not trends in constant *b* with increasing temperature and velocity. The *k* values are higher for half-ripe mango while the *n* an *b* values are higher for green mango. The constants *k* and *n* as a function of air temperature followed an Arrhenius relationship for all the air velocities.

Conclusions

Midilli and Kucuk and logarithmic models could be used to predict the moisture content of the product at any time of drying process with high ability between drying air temperatures of 50 and 80 ºC and velocities of 1.76 and 1.91 m/s.

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Analysis of cellular tissue of chestnuts during drying and rehydration process

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Keywords: Microstructure, Convective drying, Shrinkage, Swelling, size distribution

Food processing involves different operations such as dehydration and rehydration that produce changes in the structural characteristics of the cell tissue. These changes are directly related to heat and mass transfer gradients that take place during these processes (Prothon *et al*., 2001). The type and magnitude of these gradients depend on the process conditions and dehydration technique (convective, osmo-convective, freeze drying, etc). Microscopy techniques (optical, scanning electron, transmission electron) are important visualization tools of the cell structure and therefore, are used to interpret the dehydration and rehydration process (Aguilera y Stanley, 1999). Optical microscopy is a simple technique that allows the observation of the absence or presence of damages on the cell tissue.

Microscopic follow-up has been carried out on cell tissue of chestnuts prisms (10 x 10 x15 mm) at different moisture contents: fresh, dried and rehydrated. Experimental conditions during convective drying were at 65 ºC and 30 % of relative humidity for different times. After drying, samples of chestnuts were rehydrated employing distilled water at 25 ºC during different times. To visualize cells of chestnuts by microscopy, thin slabs from lateral and superior (top) surfaces were cut using a razor blade. These samples were put on a thin glass plate under the objective (10x). One side of the slab was stained with toluidine solution (0.1 % during 15 s). In this way, samples were ready for observation under the light microscope (Zeizz Axioskop 40). A digital camera (Nikon D70) was attached to the microscope to take images of cells structures and transfer them to a computer. Images were calibrated with a micrometer of 10 mm length and divisions of 0.1 mm interval (Zeiss). The images were analysed using "Image Tool" (free software available from Health Science Centre, University of Texas). Several geometrical parameters (area, perimeter of the contour, Feret diameter, roundness, elongation and compactness) of cells were determined. These parameters were previously considered by other authors for other food materials (Reeve, 1953; Lewicki and Pawlak, 2003; Mayor et al., 2005).

The characterization of the size and shape parameters for cells of fresh chestnuts indicated that values vary in a wide range. Average area value (876.1 μm^2) was less than values obtained for other products, such as apple and pumpkin (Lewicki y Porzecka-Pawlak, 2005; Mayor *et al*., 2005). The size distribution of fresh chestnuts cells showed the highest quantity (74 %) was in the range from 500 to 1050 μm^2. By other hand, in the case of shape parameters the range was similar to pumpkin cells.

The obtained results indicated that size distribution of chestnuts cell during drying process showed a high increase (from 0 to 62 %) in the minor range size (from 100 to 400 μm^2) and the cells population corresponding to the larger sizes (from 750 to 1350 μm^2) decreased (from 35 to 18 %). The changes were more important during first hours, period in which drying rates are also higher. Cellular shape changed during drying. The cells lost roundness and compactness and became more elongated (typicalbehaviour during drying of food materials).

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The analysis of surface cells during rehydration indicated a considerable increase of size in a short period of time. The relative cell population of smaller cells (from 100 to 750 μm^2) decreased and population (from 1050 to 2350 μm^2) corresponding to the largest size increased as rehydration process. A great number of cells achieved size values higher than the corresponding cells of fresh chestnut. The size parameters (area, perimeter and Feret diameter) after 30 minutes at 25 ºC were similar to fresh chestnut, but the shape parameters (elongation, roundness and compactness) were slightly lower.

The found results indicated that analysis by microscopy is an interesting tool to link macroscopic phenomena with microscopic processes and to improve the knowledge ofmass transfer processes in cellular tissues.

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Study of the influence of structured water on the drying of Baker's yeast

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Keywords: Yeast, drying, structured water, thermogravimetry.

Interactions of water molecules with food materials are of major importance for the study of product processing and viability. The classical practical approach separates free "bulk like" water from bound water whose properties are affected by the presence of the complex food structure (Fennema (1996)). A recent study for water types identification of rehydrated *Saccharomyces cerevisiae* has been realized by Turov *et al.* (2005). While this offers a new comprehension of water types in baker's yeast, little new information arise on the importance of water interactions on the industrial step of drying.

Quality of the dry product is a major concern of industrial yeast production but complete drying mechanism including viability aspects is still challenging. The classical approach attributes the falling rate period to the evaporation of bounded water (Bayrock and Ingledew (1997a)). However, this period can be caused by many different phenomena (Bear and Buchlin (1991)). Recent modeling for the drying of granulated yeast insists on the influence of transport phenomena inside the granules (Türker *et al.* (2006);Debaste *et al.* (2008)). Still, the impact of bounded water is not well established.

In this work, attention is paid to the influence of bounded water on drying kinetics. A classical approach links reduction of the evaporation rate to the apparition of an extra amount of energy needed to evaporate bounded water. This energy is known as net isosteric heat of sorption (Rückold *et al.* (2003)). It is here calculated using desorption isotherms (Mulet *et al.* (1999)) from literature and newly made experiments.

Figure 1 presents the calculated evolution of net isosteric heat of sorption as a function of solid water content. Net isosteric heat of sorption is important only for low humidity, an important decrease is observed with increasing humidity. For the smallest humidity values a decrease with decreasing humidity is again observed. However in this region the error on the calculated value is too high to assess the existence of a decrease.

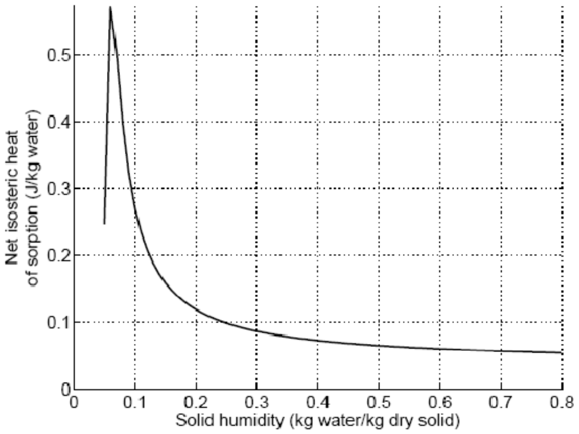

Figure 1: Evolution of net isosteric heat with solid water content

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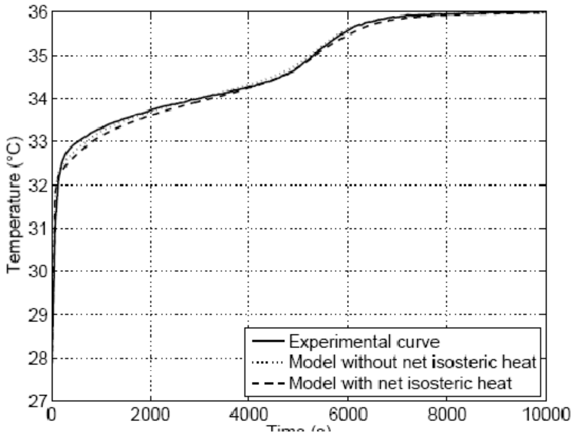

Figure 2: Temperature evolution of the sample during a TGA experiment: comparison of experimental and modeled values with and without net isosteric heat.

To evaluate of the impact of net isosteric heat on the drying, thermogravimetric analysis (TGA) of samples of yeast at constant temperature with dry air are realized. These experiments correspond to a well controlled drying process for small amount of solid matter. A simple model of heat transfer in the sample is compared to the experiment. Simulations are made including and excluding the calculated net isosteric heat of sorption. Figure 2 presents the temperature evolution of the sample in a typical TGA experiment. Continuous line corresponds to the experimental values while the two models with and without net isosteric heat of sorption are presented respectively in dashed and point lines. Both models reproduce well the temperature evolution. Although differences between the curves are presents, they are not significative.

This analysis shows that structured water types have negligible effects on the drying of yeast, at least through their impact on vaporisation energy. However, neither does this study invalidate existence of those type of water, nor does it evaluate their impact on yeast survival.

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Molecular biomarkers to characterize *Amanita ponderosa* mushrooms

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Keywords: *Amanita ponderosa*, edible mushrooms, M13-PCR, RAPD.

Mushrooms are exhaustively known for its high nutritive value, gastronomic and medicinal proprieties. Consequently, the consumption of the eatable mushroom comes increasing. Most *Amanita* species are obligatorily ectomycorrhizal and play a significant role in forest ecosystems. Some *Amanita* species were edible, namely *A. rubescens*, *A. caesareae* and *A. ponderosa*. However, others *Amanita* species present toxic properties, namely *Amanita muscaria*, *A. phalloides* and *A. virosa*.

Amanita ponderosa strains are wild mushroom, very appreciated in gastronomy, with a high exportation potential. This specie grows spontaneously in some microclimates in Iberian Peninsula, due to its Mediterranean characteristics, namely in Alentejo (Portugal) and Andaluzia (Spain) (Moreno-Rojas *et al.,* 2004).

Due to the great diversity of wild mushrooms, it is essential be able to differentiate and identify the several mushrooms species. Genetic profiles and fingerprinting of polymorphic sequences can constitute important tools for a fast and effective characterization. Therefore, is important to improve a molecular approach to characterize the different Amanita strains.

The method of RAPD was shown to be a valuable tool to distinguish different genotypes in edible mushrooms such as *Ganoderma lucidum* (Hseu *et al.*, 1996), *Lentinula edodes* (Zhang and Molina, 1995), *Agaricus bisporus* (Khush et *al.*, 1992) and *Pleurotus ostreatus* (Lee *et al.*, 2000) and to evaluate the genetic similarities (Andrade *et al.*, 2006). However, it's a new approach, for *Amanita ponderosa* strains. A second fingerprinting approach, using the minisatelite M13 (MSP-PCR) were also used to characterize the molecular profiles. The MSP-PCR technique makes use of the occurrence of high copy numbers of interspersed repeats motifs in most genomes, and has been used successfully to amplify hypervariable repetitive DNA sequences with high reprodutibility (Martin *et al.*, 1998).

To characterize the genetic profile of *Amanita ponderosa* mushrooms, fruiting bodies were collected, since February to April 2007, from several sites of Alentejo region (Portugal), namely Beja, Évora, Mina de São Domingos, Santo Aleixo da Restauração and Vila Nova de São Bento and one from Cabeza Rubia (Andalusia region, Spain).

In the present study the six *Amanita ponderosa* strains, and some others eatable mushrooms were characterised with molecular biomarkers in order to establish different genetic fingerpritings, between strains. Eight Basidiomycetes (*Amanita ponderosa*, *Pleurotus ostreatus*, *Lactarius deliciosus*) and one Ascomycete strains (Tuber sp.) were used:

The molecular profiles of selected mushrooms were analyzed by RAPD-PCR using four micosatelites primers and by MSP-PCR using the M13 primer. Similarity evaluation of the different strains was performed by a phylogenetic tree (UPGMA). In addition, the ability of the above methods to discriminate *Amanita ponderosa* at strain level was also tested.

After isolation, DNA was quantified and diluted to a final value of 100 ng/ml for PCR assays. The RAPD-PCR was performed with the microsatellite primers OPF-1 (5'-ACGGATCCTG-3'); OPF-6 (5'-GGGAATTCGG-3'); OPF-9 (5'-CCAAGCTTCC-3');

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OPF-13 (5'-GGCTGCAGAA-3'); OPG-2 (5'-GGCACTGAGG-3'); OPG-3 (5'-GAGCCCTCCA-3'). The M13 primer (5'- GAGGGTGGCGGTTCT-3') were used for MSP-PCR. Amplification products were separated by agarose gel electrophoresis.

Results showed that different patterns were generated for each nine strains tested. RAPD assays produced polymorphism in all the studded strains, showing 3-14 bands ranging from 0.2 to 2.8 kb. The amplification using M13 primer has generated a profile with 9-19 bands ranging from 0.2 to 1.2 kb. Among all RAPD primers tested, the OPG2 and OPF6 allow to distinguish the Basidiomycetes than Ascomycetes strains, presenting a cluster for all Basidiomycetes with a similarity of 23% and 30% respectively. Both fingerprintings obtained with OPG2 and OPF6 primers showed a cluster of *A. ponderosa* strains with a similarity of 65%. The fingerprinting profile obtained with M13 primer also allowed differentiate *Tuber* sp. from the studded Basiodiomycetes where *Amanita ponderosa* strains were grouped in a cluster with a similarity of 67%.

RAPD analysis with microsatellite primers enable to differentiate at species and strain level. However, the degree of discrimination by means of RAPD-PCR depends highly on the primers used. The MSP-PCR is a fast method to amplified DNA polymorphic sequences, with high reproducibility and similarity for the same specie that allow to characterise the genetic profile of the edible mushrooms *Tuber* sp., *Lactarius deliciosus, Pleurotus ostreatus* and *Amanita ponderosa.* Moreover, constitute a valuable tool to distinguish the different genotypes of *Amanita ponderosa* strains, allowing to identify at specie level and to differentiate *A. ponderosa* strains from each origin sites.

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Effect of micronized benzoic acid against *Alicyclobacillus acidoterrestris* in orange juice

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Keywords: benzoic acid, micronization, *Alicyclobacillus acidoterrestris*

Introduction

Alicyclobacillus acidoterrestris is a sporeformer bacteria, thermo and acid tolerant, able to spoil pasteurized fruit juices (CERNY *et al.*, 1984). The benzoic acid is largely used as preservative in acid drinks. The micronization process represents an alternative to reduce the concentration of the preservative, as a consequence of improving solubility in aqueous media, and increasing particles bioavailability (GIESE, 1994). The rapid expansion of supercritical solutions is a promising new technology for particle formation. On such technique, the rapid expansion of supercritical fluid solution (RESS) process has been used to produce ultra fine powders (MATSON *et al.*, 1989). The aim of this study was to evaluate the performance of micronized benzoic acid as compared to commercial preservatives sodium benzoate and benzoic acid against *A. acidoterrestris* in orange juice.

Material and methods

The benzoic acid was placed inside in a pressurized extractor vessel (ROTH model) with CO_2. The experiment was conducted in supercritical conditions at 40°C and 160bar. Sample was taken after the opening of the valve, and collected in a container after the sudden change of solubility. The characterization of the micronized material was done by the Scanning Electron Microscopy (SEM) and Optic Microscopy. Sodium benzoate and benzoic acid at concentrations of 0.005 and 0.01g/100mL and micronized benzoic acid at 0.0025 and 0.005g/100mL were added to flasks of orange juice, treated by fluent steam, cooled and inoculated with an *A. acidoterrestris* CCT 4384 spore suspension to final concentration of 10^3spores/mL. The flasks were kept at 45°C for up to 28 days, and samples were collected at each 7 days for bacterial growth evaluation.

Results and discussion

The diameter of micronized benzoic acid was 10μm, determined by SEM (Figure 1a). The elongated crystals and their stick shape can be seen in Figure 1b, obtained by optical microscopy. The length of some particles reached 200μm. However the untreated particles had irregular length and width of up to 500 μm. Samples containing 0.005g/100mL of micronized benzoic acid differed significantly ($P \leq 0.05$) as compared to the initial inoculated number, with reduction of more than 1 log cycle throughout the 28 day period, acting as bactericidal (Figure 2). The effect of micronized preservative was significantly different ($P \leq 0.05$) as compared to other preservatives at the 28th day. The untreated preservatives showed only bacteriostatic effect. The micronization process increases the contact surface area with a consequent increase in solubility and bioavailability of the benzoic acid in orange juice. This information may explain the bactericidal effect of 0.005g/100mL micronized benzoic acid for up to 4 weeks.

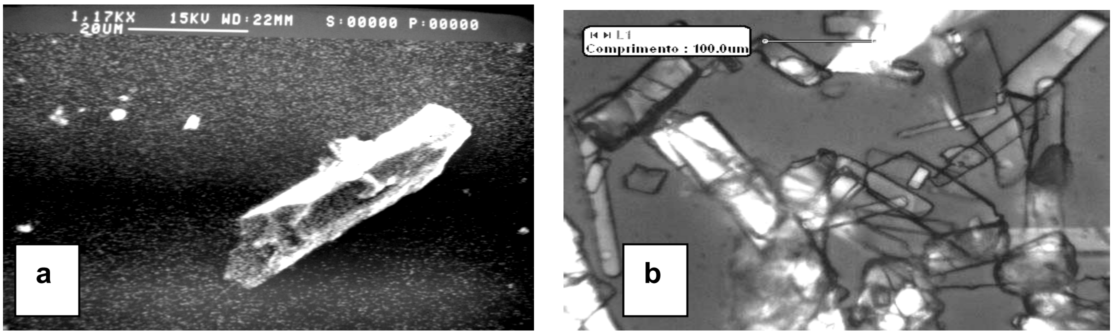

Figure 1 – Fotomicrographs of micronized benzoic acid. 1a - SEM; 1b – optical microscopy

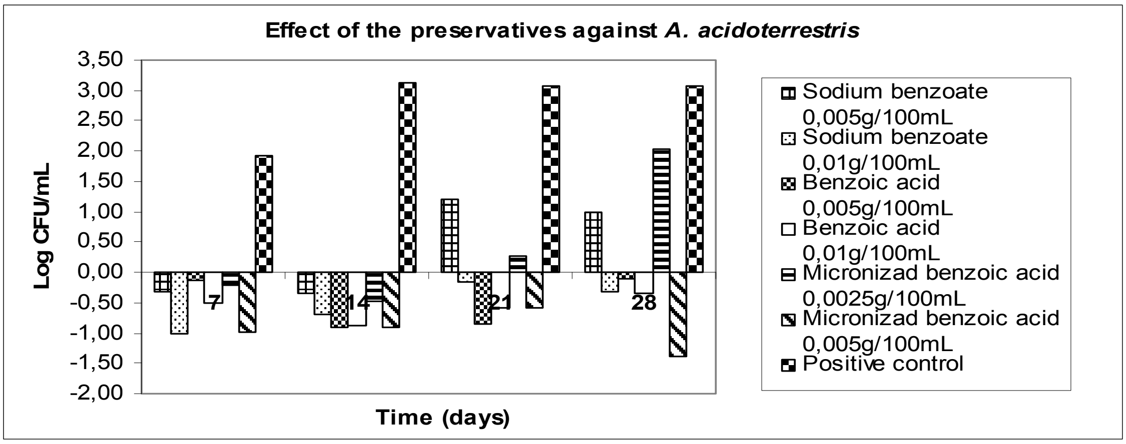

Figure 2 - Behavior of *A. acidoterrestris* in reconstituted orange juice added of preservatives and maintained at the 45°C for up to 28 days. Counts were deduced of the number of cells initially inoculated.

Conclusion

It is concluded that benzoic acid micronization proved to be an effective alternative to reduce the preservative concentration as the effect was maximized. Even at lower concentrations (0.005g/100mL) the growth of *A. acidoterrestris* was hindered for up to 28 days at an abusive temperature of 45°C, as compared to commercial preservatives sodium benzoate and benzoic acid. Moreover, the micronized preservative reduced the *A. acidoterrestris* population acting as sporicide.

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Identification of coalho cheese microbiot of districts of Pernambuco, Brazil

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Key words: chesse, identification, bacteria, DNA, molecular biology

The coalho cheese is manufactured with mass semi cooked and traditionally consumed fresh or ripened. It is produced there is more than 150 years, in several States of the Northeast Area of Brazil starting from milk of cow raw and/or pasteurized milk. Formerly it was used for coagulation of the milk the coagulum of the dry and salted stomach of wild animals or calves. Now this practice was substituted by the use of coagulum industrial (Lima, 1996). Last years, they have been increasing the interest in the studies genotipics and fenotipics wild type isolated starting from handmade cheeses produced without the addition of cultures starters (Coppola et al., 2001). Molecular techniques supply appropriate tools for the typification, taxonomies and accompaniment of the evolution of the microorganisms involved in the processing of the victuals (Germond et al., 2003; Rossetti and Giraffa, 2005). Several species-specific primers have been drawn and used for identification of acid-lactic bacteria through PCR or reactions of multiple PCR that are faster and convenient than hybridization techniques (Fortina et al., 2001).

The present work sought the identification of the goods of bacteria found in the coagulum cheese "A" and "B" produced in the small ones, averages and Micro Industries in some municipal districts of the rural area of the Pernambuco, Brazil Batches of coalho cheese produced with raw and pasteurized milk obtained starting from local producers previously registered. Batches of coalho cheese, two ripened in the autumn and winter, and the other seven in the spring and summer, were manufactured by two cheese-makers in accordance with traditional methods. These cheese samples were taken to the laboratory under refrigeration, being kept below 5ºC, and were analysed on arrival. The stumps were cultivated in ldr10% (skimmed milk reconstituted to 10% of solids non greasy), for 24 at 48 hours, in greenhouse 30 ºC and 37 ºC (1 tube to 30 ºC and other to 37 ºC). After the proven growth for the coagulation of the milk, it was proceeded the plated and the purification of the microorganisms in the means Agar MRS, Agar M17 to 37ºC and 30ºC and Agar Miller-Hinton to 37ºC for 18 at 24 hours. With the isolated bacteria, they were made the test of Gram and catalase test. With the species-specific primers, it was made the identification of the stumps through the technique of PCR (polimerase chain reaction), for a final volume of 15 ml. The amplification was accomplished for through the genic sequencing. Of ownership of the results, any difference was not verified in the growth of the isolated ones in half MRS, Agar M17 to 37ºC and 30ºC and Agar Miller-Hinton to 37ºC.

The bacterial isolated presents in the cheese coalho were mainly *Staphylococcus* spp., *Lactobacillus* spp, *Lactococcus* spp. *Streptococcus* spp. (Guedes Neto et al, 2005, what demonstrates the contamination for other groups of microorganisms.

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Biosurfactants: Microbiology and Biotechnology

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Keywords: Biosurfactant, Biodegradable, Organic reactions, Lipase.

Surfactants, due to their surface activity properties, are considered one of the most important and versatile class of chemical products, used for a variety of applications, such as household, environment, industry and agriculture (Deleu and Paquot, 2004). Biosurfactants are molecules that exhibit pronounced surface and emulsifying activities, produced by a variety of microorganisms. A wide range of chemical structures can be found among these compounds, such as glycolipids, lipopeptides, polysaccharide protein complexes, phospholipids, fatty acids and neutral lipids (Ahimou et al., 2001; Angelova and Schmauder, 1999; Desai and Banat, 1997; Lin, 1996; Rodrigues et al., 2006).

In the beginning, biosurfactants were noticed as good hydrocarbon solubilization agents, but the interest in these molecules has increased considerably in the past five decades as an alternative to chemical surfactants (carboxylates, sulphonates and sulphate acid esters) specially in food, pharmaceutical and oil industry (Banat et al., 2000; Desai and Banat, 1997). The main reasons for the spreading interest in biosurfactants are their environmental friendly nature, since they are easily biodegradable (Mohan et al., 2006) and have low toxicity (Flasz et al., 1998), in addition to their unique structures which provide new interesting features that their chemical counterparts may lack (Nitschke and Costa, 2007).

Several organic reactions (esterifications, interesterifications, transesterifications and hydrolysis) can be accomplished via chemical or biochemical catalytic synthesis. The use of biocatalysts can largely improve a process, namely reducing the operational costs as a consequence of smaller reaction times, lower energetic needs and less time consuming procedures. Therefore, it is increasing the interest on the application of biochemical catalysis for organic synthesis (Aires-Barros, 2002; Faber, 1997).

An interface is any boundary between two different phases and microbial life may be more common at interfaces as evidenced by microbial biofilms, surface films, and aggregates. Given that, all microbial life is impacted by interfacial phenomena, and biosurfactants are a common mechanism by which microorganisms deal with interfacial challenges (Van Hamme et al., 2006).

Additionally, these lipases have been used both for industrial (food industry, cosmetics and perfumes, biomedical, pesticides, detergents, among others) and research purposes. Lipase is the generic name given to a group of enzymes belonging to the class of hydrolases, and acts on ester bonds. These enzymes are not only able of catalyzing hydrolysis reactions, but also synthesis reactions in aqueous-restricted media, such as esterification reactions, interesterification, transesterification, alcoholysis and aminolysis, and by not acting on natural substrates (Jaeger and Eggert, 2002; Pandey et al.,1999).

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Isolation of thermophilic strains and investigation of their lipolytic activity

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Keywords: Thermophiles, Lipase, *Bacillus*, Enzyme, Stability

In recent years, considerable attention has been paid to extremophilic microorganisms as potential sources of novel enzymes that could be used in industrial processes carried out under harsh conditions, at which conventional proteins are completely denatured. Enzymes from thermophilic and hyperthermophilic organisms are particularly relevant, due to their inherent thermostability and resistance to denaturation by proteolysis, extreme pH values or chemical agents. A number of commercially interesting thermophilic enzymes (i.e. glycosidases, proteases, esterases, DNA polymerases) have been identified, and in some cases thoroughly studied (Hough and Danson, 1999; Daniel and Cowan, 2000; Gomes and Steiner, 2004).

Triacylglycerol hydrolases or lipases (EC 3.1.1.3) are enzymes able to catalyse ester bond hydrolysis in acylglycerol with the release of fatty acids and glycerol. The enormous potential of microbial lipases arises from the facts that they are quite stable and active in organic solvents, they do not require cofactors, they exhibit a high degree of enantio- and regioselectivity, and possess a wide range of substrate specificity for the conversion of various substrates. Also, they are able to catalyse both hydrolysis and synthesis reactions, which provides a wide range of potential applications. The occurrence of lipolytic activity in several thermophilic strains from the genus *Thermus* has been ascertained, and some relevant information related to the adequate conditions for enzyme production, both in flask and bioreactor scale, enzyme properties and purification procedures is available (Dominguez et al., 2004, 2005, 2007; Fuciños et al., 2005a, b).

In this context, the isolation of new thermophilic strains and the investigation of applications for their enzymes could be very interesting for the further development of biocatalysis at high temperatures. In the present work, several hot springs in Northwest Spain have been investigated as potential sources of lipolytic enzyme-producing thermophilic microorganisms. After isolating twelve esterase producing-strains on tributyrin-agar plates, nine of them were assured to be true lipase-producers by spreading on Rhodamine B-olive oil agar plates. Then, the nine selected strains were grown in flasks at 55ºC on a complex liquid medium, obtaining remarkable extracellular activities by two of them (around 200 and 500 U/L, respectively), as well as adequate production rates (maximum enzyme production in 2 days). Genetic identification by 16S rRNA sequencing was carried out for these two strains, and they were found to belong to the genus *Bacillus*, and tentatively denominated *Bacillus* sp. CH6A and *Bacillus thermoamylovorans* CH6B.

Additionally, some relevant properties of the produced enzymes, such as pH and temperature-activity profiles, and thermal stability, were investigated. Most of the assayed enzyme preparations showed maximum activity at alkaline pH and temperatures between 55 and 95ºC. As for thermostability, which was assessed at 65 and 95ºC, depending on the crude enzyme, significant residual activities were detected in all cases. The effect of additives like salts and polyalcohols, that are proven stabilizers in many investigations (Obon et al, 1996) was analysed. It was concluded that the stability was greatly improved when glycerol, $CaCl_2$ or xilitol was added.

Finally, lipase production by *Bacillus thermoamylovorans* CHB6 in submerged culture was further investigated. The most adequate pH and temperature for the production

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process were determined in shake flask cultures, and found to be 7.0 and 55ºC, respectively. Then, the culture was carried out in a stirred tank bioreactor, using the optimized conditions. A preliminary study of the influence of agitation and aeration rates on enzyme production was undertaken, and the importance of these variables was confirmed. The best results were obtained when operating at 300 rpm agitation rate and 0.33 vvm aeration. The biomass and extracellular enzyme experimental data were fitted to logistic kinetic models, and the suitable parameters determined.

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Fractionation of fructo-oligosaccharides by gel filtration

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Keywords: FOS, Gel filtration, Sugar fractionation

Nowadays, production of fructo-oligosaccharides (FOS) has gained large commercial interest due to its beneficial properties in the human health as prebiotics. Fermentative processes appear to be a good technique for large scale production of FOS, namely kestose (GF2), nistose (GF3) and fructo-furanosilnistose (GF4). However, the sugars mixture that results from the fermentations contains high levels of fructose (F), glucose (G) and sucrose (GF) which have to be eliminated. Thus, the aim of the present study was to separate FOS from those components. The separation was performed by gel filtration chromatography using Bio-Gel P2 polyacrylamide. The elution of sugars from the column was based on the molecular weight with the largest sugars eluting first and the smallest later. The molecular weights of sugars present in the fermentative broth are respectively: 180 (F, G), 342 (GF), 504 (GF2), 666 (GF3) and 825 g/mol (GF4). Bio-gel P2 appears to be an efficient gel for the purification of these sugars since it allows the fractionation of molecules with molecular weight between 100 and 1800 g/mol. Two different samples were used in this study; one from the end of fermentation (Sample 1) consisting of 16.1 (F), 47.0 (G), 64.84 (GF), 84.37 (GF2), 13.18 (GF3) and 0 g/L (GF4); and the other (Sample 2) previously purified in a charcoal column to concentrate FOS consisting of 0.92 (F), 1.64 (G), 2.68 (GF), 18.06 (GF2), 46.02 (GF3) and 7.96 g/L (GF4).

Two hundred microliters of sample were loaded onto the Bio-Gel P2 column (100 X 1.6 cm) and eluted with distilled water at a flow rate of 0.3 mL/min. Fractions were collected and analysed by ELSD (*Evaporative Light Scattering Detector*) and HPLC. Results achieved from the fractionation of sample 1 showed that it is possible to obtain pure kestose, and that sucrose and monosaccharides (G/F) are also well separated. Nevertheless, and although its presence was detected by ELSD (elution volume: 104-112 mL) it was not possible to quantify nistose by HPLC as it was much diluted. Kestose and nistose were obtained from the fractionation of sample 2. As the concentrations of fructo-furanosilnistose, sucrose and monosaccharides in the initial sample were very low it was not possible to quantify them by HPLC. However, the presence of GF4 (elution volume: 92 - 102 mL) and sucrose (elution volume: 125-132 mL) was detected by ELSD.

In sum, although gel filtration with Bio-Gel P2 provides a good fractionation of FOS from fermentative broths, the process is time consuming (8h) and the amounts of final product achieved are very small. Therefore, unless the final product has a high commercial value in its fractionated form, the implementation of this procedure at an industrial scale is not economically viable. However, the achieving of pure sugars makes the chemical, clinical and nutritional characterization of each single sugar possible.

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Structural and kinetics effects of amidase from *Pseudomonas aeruginosa* encapsulated in reversed micelles

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Keywords: Reversed micelles, amidase from *Pseudomonas aeruginosa* ; Hydroxamic acids; Fourier Transform -Infrared Spectroscopy

Aliphatic amidase (E.C. 3.5.1.4) catalyzes the hydrolysis of amide bonds in small aliphatic amides as well as the transfer of acyl groups to amines which enlarges the possibility of synthesizing different compounds containing amide bonds (Pacheco *et al*, 2005).

The reaction catalysed by amidase involves a ping-pong bi-bi mechanism with the formation of an acyl-enzyme complex that is able to transfer the acyl group either to an amine or to the water present in the system, with formation of the transamidation or the hydrolytic product, respectively. Hydroxamic acid derivatives, with the general formula R–CO–NHOH, are products of transamidation reaction when the acyl acceptor is hydroxylamine.

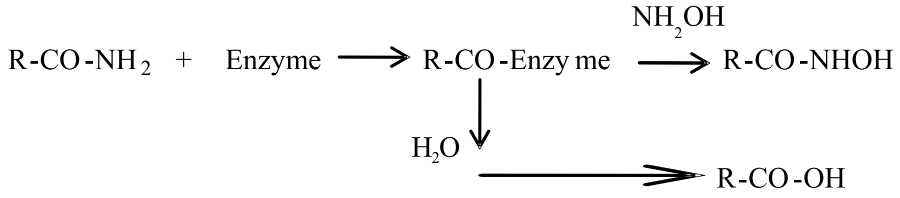

Figure 7-Aliphatic amidase catalyses the hydrolysis and transamidation reactions of amides.

Hydroxamic acids are chelating agents which are known to inhibit metalloproteinases often associated to several human pathologies. These compounds can be used in a number of interesting applications, such as in medicine and neuro-biochemistry in several drugs since they are constituents of growth factors, antibiotics, antibiotic antagonists, tumor inhibitors and antifungal agents. On the other hand, hydroxamic acids can also be applied in food industry and waste-water treatment (Fournand *et al* 1997a).

Regarding the above reaction scheme, such transamidation reaction can be successfully performed in an organic media where the water content is low (Fournaud *et al* 1997b). Reversed micellar systems are one such example of the use of nonconventional media for synthesis of hydroxamic acids by using amidase (Pacheco *et al*, 2005)

Reverse micelles are spherical waterdroplets surrounded by a monolayer of closely packed surfactant molecules dispersed in a solvent of low polarity. In reverse micelles, the polar heads of the surfactant molecules are in contact and only hydrated by few water molecules that lie in the interior of the micelle; the hydrophobic tails of the surfactant molecules are oriented and in contact with the surrounding organic solvent (Gómez –Puyou and Gómez –Puyou, 2007).

The present work involves the entrapment of either purified amidase from *Pseudomonas aeruginosa* amidase (E.C.3.5.1.4), cell-free extract or whole cells in a reversed micellar system composed of cationic surfactant tetradecyltrimethyl ammonium bromide (TTAB) in heptane/octanol (80/20% V/V). Our work reports the effect of the enzyme entrapment on both biocatalysis of synthesis of acetohydroxamic acid and amidase structure. The results obtained revealed that there was an increase in enzyme activity for the synthesis of acetohydroxamic acid in reverse micelles compared with the conventional aqueous system.

Reverse micelles are optically transparent and when enzymes are transferred to the interior of reverse micelles, the system continues to be transparent, and, thus the entrapped enzyme is susceptible to spectroscopic studies (Gómez-Puyou and Gómez-Puyou, 2007). Fourier Transform Infrared Spectroscopy (FTIR) was used to analyze structural differences on amidase by using medium engineering since it is a useful technique for analysis of structural changes of biomolecules in a wide range of environments (Haris and Severcan, 1999).

FTIR analysis revealed modifications on secondary structure of encapsulated amidase since amidase structure exhibited mainly α-helices (1650–1655 cm^{-1}) as opposed to the enzyme structure in aqueous solution which shows a predominant β-sheet structure (1620-1630 cm^{-1}). The results obtained from FTIR spectra in conjunction with biocatalysis of amidase strongly suggests that the synthesis of acetohydroxamic acids is higher with α-helical structure compared with β–structure. Our findings clearly demonstrated some advantages in the use of encapsulated amidase in reverse micelles for synthesis of acetohydroxamic acids compared with aqueous system as well as the importance of structural modifications on amidase molecule for high yield of transamidation products.

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Thermosensitive nanogel for controlled pimaricin release

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Keywords: nanogel, PNIPA, pimaricin, antibiotic, drug release.

Introduction and objectives

Pimaricin (natamycin) is a polyene macrolide antibiotic produced by submerged aerobic fermentation of *Streptomyces natalensis* and related species. It is used as a food additive to control the growth of yeasts and moulds on the surface of cheese and other non-sterile products, such as meat and sausages (Vanden Bossche et al., 2003).

In order to desing a suitable delivery which are able to protect the pimaricin from hostile environment as well as to manipulate the pimaricin release utilizing environmental stimuli we propose the use of thermosensitive nanogels.

The thermoresponsive polymers or gels, such as poly(N-isopropylacylamide), have been widely investigated for controlled drug delivery. This type of nanogel has a mechanism which allows drugs loaded into PNIPAAm gels to be squeezed out of the polymer when the temperature is raised above the lower critical solution temperatures (LCSTs) (Wu et al., 2005).

The objective of this work was to study the pimaricin loading and release kinetics in PNIPAAm hydrogels. Pimaricin was loaded into the gel and the release was investigated at different release temperatures.

Materials and methods

Pimaricin was loaded into the PNIPA hydrogel at room temperature (25ºC) overnight. Then the pimaricin-loaded swollen gel samples were placed in a dialysis tubing and was dialyzed in milliQ water at two different temperatures, 15 and 37ºC. Pimaricin concentrations in the release medium were analyzed by spectrophotometry at 319 nm (Recio et al., 2006).

Results and Conclusions

The fractional release of pimaricin from dialysis tubing without nanogel showed that practically all the pimaricin was released, 89% of the initial sample, at 37ºC. While the release at 15ºC was lower than 37ºC, 65% of the initial sample, because of the diffusion goes up with temperature (figure 1). Data were fitted to the following equation to calculate the final amount released:

$$F = F_{\max} + \left[(F_0 - F_{\max}) \cdot e^{-m \cdot t}\right] \qquad \text{eq 1.}$$

In this eq F, F_0 and F_{max} are the model fractional release, the fractional release at initial time and the maximum fractional release of pimaricin, respectively.

In presence of nanogel the results were consistent with the positive squeezing mechanism reported in literature (Wu et al., 2005; Shin et al., 2001), when the temperatura is increased, the release rate peaks rapidly, followed by a very show release process (figure 2.). The release rate was manteined in first and second cycle and then the release riches the equilibrium (figure 1).

Fitting data release at 37ºC to the eq 1 we could obtain the final release ratios, which were F_1 = 0,10 and F_2 = 0,11.

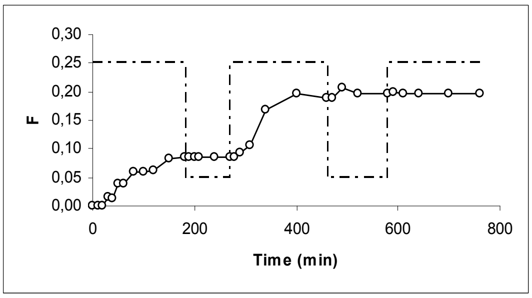

Figure 1: Controlled release of pimaricin from PNIPA nanogel. The dashed line shows the temperature change from 37ºC to 15ºC.

In order to determine whether a particular device is diffusion-controlled, the early-time release data can be fit to the following empirical relationship (Huang and Lowe, 2005).

$$\frac{Ct}{C_\infty} = kt^n \qquad \text{eq 2.}$$

The constants *k* and *n* are caracteristics of the drug polymer-system. The diffusional exponent, n, is dependent on the geometry of the device as well as the physical mechanism for release. C_t and C_∞ are the amounts of pimaricin released at time t and the maximum amount of pimaricin released, respectively.

For Fickian diffusion and Case II transport from a swellable sphere the exponent takes the value n = 0,43 and n = 0,85, repectively. Fickian diffusion (n = 0,43) and Case II transport (n = 0,85) are often obtained when drugs are released from diffusion-controlled and swelling-controlled systems, respectively. A system controlled by both diffusion and swelling usually generates 0,43 < n < 0,85, named like Non-Fickian or anomalous transport (Huang and Lowe, 2005).

In our system, after data fitting, we obtained n = 0,60 for both F_1 and F_2 ratios release. Therefore, the pimaricin release from nanogel follows a Non-Fickian diffusion, that is the release is controlled by both diffusion and swelling mechanisms.

Whereas the pimaricin release in absence of nanogel through the diálisis tubbing shows n = 0,42, corresponding with a Fickian diffusion. In this case release is controlled only by difusión of the piamrin into the relese medium.

Therefore, the mechanism proponed was successful to use in controlled release of pimaricin from nanohydrogels. The mechanism allows a uniform release rate when the temperature is manteined above the LCST.

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Identification and evaluation of fungal strains with fucoidan degradation potential

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Keywords: Fungal strains, Fucoidan, Fucoidanases.

Fucoidan is a sulfated fucose heteropolysaccharide of brown algae with important medicinal application as anticoagulant, antithrombotic, antitumoral activity (Berteau and Mulloy, 2003; Ellouali et al., 1993) and as inhibitor of the human immunodeficiency virus (McClure et al., 1992). Although, scientific and practical interest of studying the relation between their structure and biological activity have carried out, only a partial elucidation n of their structure have been reported.

Specific enzymes capable to tailored fucoidan sulfated matrix are minimums. Fucoidanases have found in marine organism, principally marine bacterium as *Vibrio sp.* (Furucawa et al., 1992), *Pseudoalteromonas citrea* (Bakunina et al, 2002) *Pseudomonas sp*., *Alteromonas sp*. (Sakai et al., 2004), *Flavobacteriaceae sp*., (Urvantseva et al., 2006). There are any reports of terrestrial and neither fungal microorganism that hydrolyzes fucoidan. For that reason, the aim of the present work was to identify fungal strains that can growth over fucoidan medium and to evaluate enzymes excretion over minimal conditions.

Ten strains, genus *Aspergillus*, *Penicilium* and *Mucor*, isolated from Northeast Mexicandesert were used. Screening methodology was carried out preparing agar gels with fucoidan-peptone, fucoidan-urea and fucoidan-sodium nitrate (5 and 2 gL^{-1}, carbon and nitrogen source, respectively), pH 4.5, dispensed in 6 mm petri dishes. Microorganisms were inoculated in the center and growth was measured per 10 days. Fermentation experiments were realized in 250 mL Erlenmeyer flask with fucoidan and urea as minimal medium (10 and 5 gL^{-1}, respectively). Conditions followed were: 1x106 spores mL^{-1}, 30°C and 140 rpm. Enzymatic activity was measured by reduced sugars (DNS method). One unity is definied as the amount of enzyme that realeases 1 µmol of sugar reduced per minute.

Aspergillus niger PSH, *Mucor sp*., and *Penicillum purpurogenum* GH2 showed growth over fucoidan-urea medium (Fig. 1) 24 h after inoculation. These microorganisms are capable to excrete metabolites that degrade the heteropolysaccharide matrix. On the contrary, any strain growth in fucoidán-peptone and fucoidan-sodium nitrate complex.

Submerged fermentations were realized only with the microorganism that hydrolyzed fucoidan gels. Higher biomass production was 0.84 gL^{-1} with *Mucor* and 0.49 gL^{-1} with

Aspergillus PSH, after 72 h. Despite of enzyme activity were lower, with values of 0.0138, 0.005 and 0.004 UL^{-1} for *A*. PSH, *P.* GH2 and Mucor, respectively, there are any reports of fungal fucoidanases by induction procedure.

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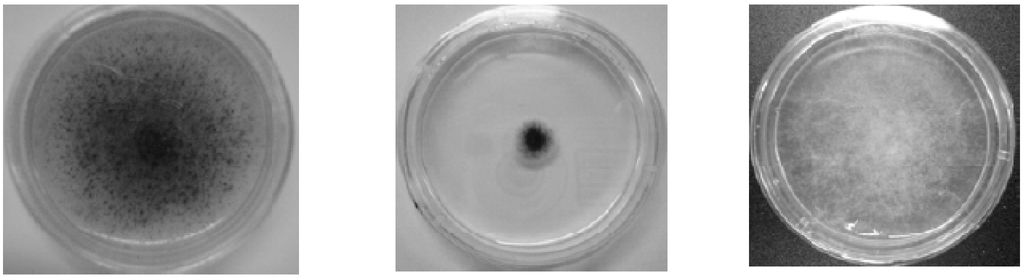

Aspergillus niger PSH *Penicillum purpurogem* GH2 *Mucor sp*

Figure 1. Fungal growth over fucoidán-urea gels.

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Modeling and experimental evaluation of poly(3-hydroxybutyrate) production in *Bacillus megaterium*

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Keywords: Biopolymers, Poly(3-hydroxybutyrate), *Bacillus megaterium*, modeling, optimization.

Plastics have been regarded as ideal materials for the production of various consumer products because of their durability and inherent resistance to degradation. However, these same qualities are sources of environmental and waste management problems. These problems have created much interest in development of biopolymer. In addition, biopolymers can be obtained from agriculture or from biotechnological processes and are therefore, in principle, available from renewable resources (Luengo et al., 2003; Reddy et al., 2003).

Polyhydroxyalkanoates (PHAs) biopolymers are polyesters synthesized by numerous bacteria and are accumulated on inclusion bodies in the cytoplasm of the cells as energy storage; they are produced from renewable carbon sources and, generally, with limiting nutrient; and their main characteristic is the biodegradability (Lee, 1996b; Madison and Huisman, 1999). Poly(3-hydroxybutyrate) (P(3HB)) is the most characterized PHA, partially crystalline polymer, and with material properties similar to polypropylene (Lee, 1996a; Sudesh et al., 2000).

In this work the production of P(3HB) in submerse cultures of bacteria *Bacillus megaterium* in the mineral medium, using sucrose as carbon source and ammonium sulphate as nitrogen source was carried out. The mineral medium used is the same medium used in Wang and Lee (1997). This bacterium shows different behavior from bacteria commonly used on biopolymer production, like *R. eutropha*, and it is able to produce large amounts of P(3HB) without nitrogen limitation. Same results are found in Omar et al. (2001) and Maccol et al. (1996).

Shaker experiments were carried out, according to a central composite design and three repetitions in the central point, in order to evaluate the best initial sucrose concentration and carbon-to-nitrogen ratio. In these experiments, P(3HB) was accumulated in the cell about 75%. In most of the cases, there was no limitation in the sucrose or nitrogen, and a strong correlation between accumulated P(3HB) and pH of the medium was observed.

Bench-scale bioreactor experiments were carried out with controlled dissolved oxygen and pH. The substrate initial concentration was chosen according to the optimal point of the surface response curve from the experiment design. In this operating condition, P(3HB) accumulation in bioreactor, about 30%, was much lower than in shaker. Experiments without pH control showed the importance of using a programmed control strategy using this variable. Then another experiment using a new range control strategy for pH was accomplished in order to keep the pH above 5 and below 7, resulting in the desired production as obtained in shakers.

Different mathematical models for microbial growth, polymer production, and substrate consumption were analyzed and improvements were proposed in their structure. The proposed model includes a variable polymer production specific rate according to the biomass growing phases. The models were implemented in the EMSO process simulator (Soares and Secchi, 2003) and their parameters were estimated using this tool and the experimental data from the bioreactor. The proposed model showed a better performance than the available models found in literature, with a smaller number of parameters.

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Statistical analysis of oxygen effect on the culture of *Bacillus thuringiensis*

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Keywords: Biological fight; *Bacillus thuringiensis*; Dissolved oxygen; Statistical analysis; Sporulation

Abstract

Oxygen supply is known to be an important constraint on the growth of the aerobic sporeforming bacteria while the effects of oxygen on sporulation of these bacteria have been limited studied. In *Bacillus thuringiensis* (Bt), sporulation is associated with production of an entomocidal parasporal crystalline toxin, also known as δ-endotoxin, which is generally the most important feature of this bacterium. Thus the effect of oxygen in relation to toxicity must be considered too. The actual disagreements between reports show that oxygen role is far from to being completely understood. Statistical analysis could be a very powerful tool to interpret the diverse results at this situation. In this work, attempts are made to study the effect of dissolved oxygen (DO) on sporulation development of Bt. Iinvestigations have been carried out involving fed-batch cultivation of *Bacillus thuringiensis* H14 followed by a batch culture after 24 h at different DO concentration. The sporulation in three different levels of DO (0, 50 and 100%) were followed by using microscopic methods and on-line measurements. The viability decreasing due to sporulation was also monitored. Statistical analysis of the effect of oxygen in sporulation was performed using a regression method and with the help of Minitab as the available software. The results showed a significant effect ($P < 0.05$) of oxygen not only on sporangium formation but also on mature spore liberation. The statistical models were proposed to show the relations between sporulation development and oxygen levels. These statistical models were able to predict satisfactory the sporangium and mature spore formation at different levels of DO.

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Characterization of phenolic compounds of OMW: toxicity and degrability by yeasts

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Keywords: Lipolytic yeasts, phenol compounds, lipase, OMW

The olive oil extraction, performed by the 3-phases process, results on a large amount of an effluent, usually known as Olive Mill Wastewater (OMW). It is mainly produced in the Mediterranean area, with the exception of Spain that is one of the major producers but mostly use the 2-phases process. OMW represents a major environmental problem due to its high organic content, being characterized by a strong acidic smell and an intensive brown to dark color due to the presence of biodegradable, recalcitrant and biostatic compounds. OMW phytotoxicity can be attributed to the phenolic compounds (Lanciotti et al, 2005).

Owing to their instability, OMW phenols tend to polymerise during storage into condensed high-molecular-weight polymers that are difficult to degrade (Crognale et al, 2006). Thus, uncontrolled OMW disposal can create severe risks to water and soil quality. OMW is currently concentrated by evaporation in open pools, but this method is not satisfactory because a black foul-smelling sludge, difficult to remove, is produced. Instead of disposal solutions an approach of using this waste as a resource to be valorized is of greater interest. In fact, OMW contains sugars, lipids, mineral elements and phenolic compounds (10 % of the organic matter) that could be either directly recovered by chemical extraction and subsequent purification, or utilized as a basis for fermentative processes.

The research on OMW valorization is focused on the recovery or on the degradation of the phenolic compounds since its presence is considered to be the limiting step in the biotreatment of OMW (Tsioulpas et al, 2002).

One of the aims of the present investigation was the characterization of OMW, focusing the phenolic compounds.

As a first approach to characterize the phenolic compounds of OMW, two distinct extraction methods were used: (1) a liquid-liquid extraction by acidified ethyl acetate, according to the procedure of De Marco et al. (2007) and (2) a solid-liquid extraction with acidified methanol. The analysis of these extracts by reversed phase liquid chromatography showed that hydroxytyrosol was the most abundant phenolic compound in OMW, and that, this compound was more efficiently recovered by the solid-liquid extraction technique.

Hydroxytyrosol recovery from OMW is of obvious industrial interest due its well recognized nutraceutical properties as antioxidant (Leonardis et al, 2007).

Subsequently to the identification of the major components of the phenolic compounds on OMW, it was also a goal of this work to study its toxicity to yeast strains that are under investigation for potential use of OMW as culture media (Araujo et al, 2005).

For this purpose, strains of *Yarrowia lipolytica*, *Candida rugosa* and *Candida cilyndracea* where cultivated in YPD medium in the presence of different phenolic compounds commonly found in OMW. For the range of phenolic concentrations used (up to 1 g/L), no cell growth inhibition was observed. However, the phenols degradation was quite difficult, particularly when more easily degradable organic matter is still present in the medium.

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Two strategies for biodegradation of high concentration phenol: consecutive adaptations vs. adapting to other compounds

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Key words: phenol, biodegradation rate, adaptation, catechol 1,2-dioxygenase

Phenol is widely used as an intermediate or solvent in pharmaceuticals, dyes, pesticide, semiconductor industry etc. However, because it is suspected to be neurotoxin and can produce offensive odors, its release to the environment is strictly controlled (Polymenakou and Stephanou, 2005). Phenol was discharged accidentally twice from electronic and phenolic resin factories to Nakdong river, water reservoir for drinking water in 2001 and 2008 and this caused nationwide shock in Korea. Among phenol treatment technologies, biological treatment using microorganisms receives the most attention due to its environmentally friendly approach and its ability to completely mineralize toxic organic compounds (Prpich and Daugulis, 2005). However, since biological treatment uses living microorganisms, it is fragile to high concentration of toxic compound (Prpich and Daugulis, 2005). In this study, two methods were suggested to treat high concentration of phenol.

(1) Consecutive Adaptations: The newly isolated *Pseudomonas fluorescence* KNUJ417 degraded 100, 300, 500 and 700 mg/L of phenol in 24, 36, 42 and 65 hours, respectively but it was not able to degrade phenol over 1,000 mg/L. When cells were pre-adapted to 100 mg/L of phenol, the degradation time was remarkably shortened by 20 ~ 43 hours with phenol concentration. In addition, 1,000 mg/L of phenol was degraded completely in 42 hours. Interestingly, the cells pre-adapted to 700 mg/L of phenol degraded 1,300 mg/L of phenol but the cells pre-adapted to 100 ~ 500 mg/L did not. Repeated adaptations to the same phenol concentration did not increase the limit of degradable phenol concentration. It was found that higher concentration of phenol induced higher catechol 1,2-dioxygenase (C12DO), a key enzyme cleaving aromatic ring, and that its activity was highest while phenol was being actively degraded. As stated in a previous study, aromatic ring cleavage may be a rate limiting step in phenol metabolism and higher induction of C12DO increase the degradation rate of phenol (Yeom and Yoo, 2002). In order to degrade high concentration of phenol as fast as possible, stepwise adaptations were applied and optimum adaptation routes for the various target phenol concentrations were suggested. For example, 1,000 mg/L of phenol was the most quickly degraded by the consecutive adaptations of 100, 300 and 1,000 mg/L of phenol and the best route for degrading 1,300 mg/L of phenol was adapting the cells to 100 mg/L first, then to 700mg/L and finally to 1,300 mg/L of phenol sequentially.

(2) Adapting to other compounds: As mentioned above, aromatic ring cleavage is believed to be a rate limiting step in phenol metabolism. Accordingly, if cells are previously adapted to a compound inducing higher level of C12DO, phenol degradation rate may be enhanced. In this study, various aromatic compounds were tested as a pre-adapting agent and they were benzene, benzyl alcohol, benzaldehyde, toluene, benzoic acid, catechol, phenol and 3-methyl-catechol. The result showed that phenol- and benzene-adapted cells degraded 1,000 mg/L of phenol in 75 and 50 hours, respectively. The data indicated that the degradation rate of 1,000 mg/L phenol by benzene-adapted cells was as high as that by optimum consecutive adaptations. It was found that benzene induced the highest C12DO activity. However, the effect of benzene-adaptation did not last long and additional benzene was required to maintain high phenol degradation rate.

(3) Comparison of two methods: In a batch mode, second method is more effective because adaptation time to benzene was only 10 hours and the adaptation was enough to degrade as high as 1,000 mg/L. However, in a continuous mode, second method requires continual feeding of benzene with some interval, while first method requires only one consecutive adaptation. Consequently, consecutive adaptation is suitable for continuous phenol degradation and adapting to benzene is for batch mode.

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Changes in the wastewater bacterial community of oil refinery during phenol shock loadings

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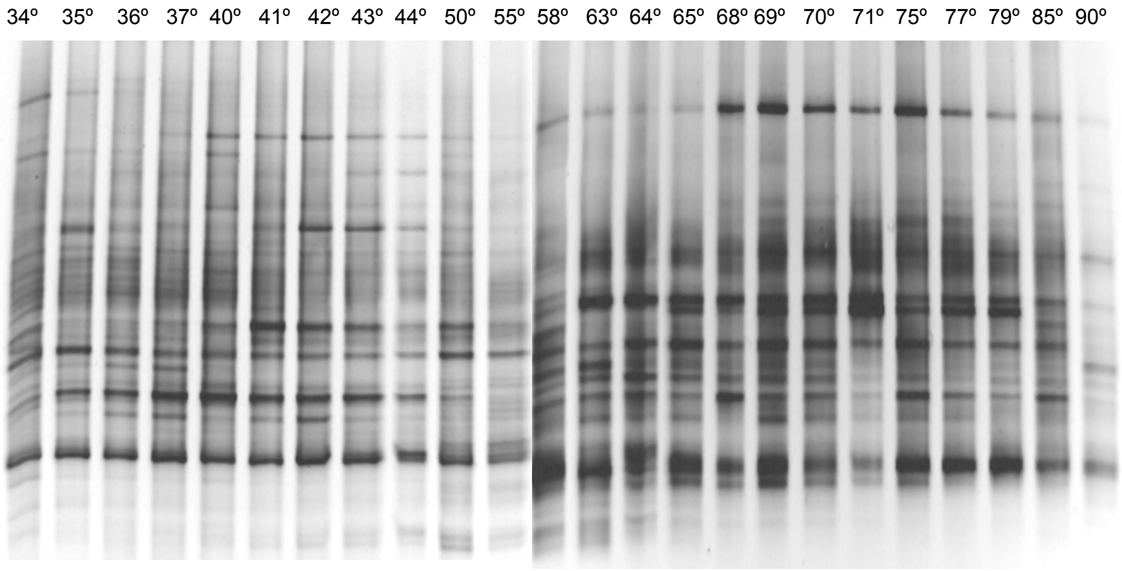
Keywords: oil refinery wastewater, bacterial community, 16S *rRNA* gene, PCR-DGGE

Petroleum refinery wastewaters have a wide range of chemical compounds that are toxic to the environment. Phenolic compounds have remarkable importance as pollutants in refinery wastewater, since they are highly toxic for human beings, fish and also impair several biochemical functions (Barrios-Martinez et al. 2006). Effluent from oil refineries aggregate and carry wide type substances, such as phenolic compounds and polyaromatic hydrocarbons (PAHs). Increasing in the stringency of environmental laws for disposal the wastewater has been stimulate the industries to search forms of reducing the presence of these compounds in industrial effluent (Marrot et al*.,* 2006). Studies of biodiversity are important for macro- and micro ecology due to the potencial correlation to ecosystem function (Schwartz et al*.* 2000). Increasingly microbial biodiversity has become a research theme for understanding engineered ecosystems such as bioreactors. (Liu et al., 2007). The objective of this work was to study the bacterial community of the wastewater treatment microbiologic for seeking the toxic compounds present in wastewater oil refinery. Sludge diversity microbial was monitored by amplification of a segment from *16S rRNA* gene followed by denaturing gradient gel electrophoresis (DGGE) analysis of the resultant PCR products during the process of phenol shock loading in submerse membrane bioreactor (SMBR). The bacterial population found in a wastewater treatment in an oil refinery during phenol shock loadings was monitored over the course of 34 days. Phenol shock loading was divided into two stages: a period of 18 days with high phenolic load (mean value of 85 mg L^{-1}) and a subsequent period of 16 days with low phenolic load (mean value of 15 mg L^{-1}). It was used a submerged membrane bioreactor (SMBR) operating with constant flux. The quality of those effluents was consistently high considering phenol (≤ 85 mg L^{-1}) and total ammonia (≤ 158 mg L^{-1}), since an efficient removal of 95–75%, respectively, was obtained. Changes in the bacterial population during phenol shocks were monitored by PCR-DGGE amplification of the V3 region of the*16S rRNA* gene (Figure 1). The analysis by PCR-DGGE indicated that the microbial community has been able to adapt to changes in environmental conditions and showed a relative stability, despite changes during the process, in its microbial diversity with coefficient of similarity exceeding 57% throughout the process. The efficiency of the removal of phenol in SMBR (over 90%) associate to the stability of microbial population, mainly in the ultimate phase of the process, was decisive for confirmation that the microbial community of sludge in SMBR was able to adjust to such variations in the effluent. These results indicate that functionally stable wastewater treatment bioreactors have stable microbial community structures under normal operating conditions but are able to adapt, in response to perturbations, sustaining the high effluent quality.



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Comparison of the operation of a membrane bioreactor for municipal wastewater treatment with different membrane modules

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Keywords: flat sheet membrane, membrane bioreactor; municipal wastewater, fouling

Introduction

In the last years, membrane bioreactors (MBR) have represented an important leap forward in the treatment of municipal waste water. Compared to other treatment techniques, it requires a smaller surface area and produces a better effluent quality which allows a reuse of the treated water. Membrane filtration replaces the conventional sedimentation unit for separation of the treated water from the sludge and also serves as an advanced treatment unit for coliform bacteria and suspended solids (Judd, 2006). This technique needs an exhaustive pre-treatment, because membranes are sensitive to fouling and damage, so it is important to remove sand, fats and similar from the wastewater to reduce the operational costs related to cleaning and to increase the lifetime of the membranes. In this study the operation of the same pilot scale MBR plant with two different membranes was compared: filtration hollow fibber membranes and flat sheet membranes, during the treatment of a municipal wastewater.

Materials and methods

During this study, a pilot scale wastewater treatment plant introduced in a lorry container was used. The main units of this plant were: a 2 m^3 buffer tank, two 2.0 and 0.5 mm screens, a 0.5 m^3 MBR, permeate tanks and a control panel connected to a PC by using a PLC (Figure 1). The MBR was composed by three chambers: an anoxic chamber of 0.2 m^3 volume; an aerobic chamber 0.2 m^3 and a second aerobic chamber of 0.1 m^3 connected in series. Sludge from the second aerobic chamber was recycled to the anoxic chamber in order to reduce nitrate concentration in the permeate (effluent).This pilot plant was located in the municipal wastewater treatment plant of Bertamiráns (Galicia, Spain). The characteristics of the municipal water were those typically found in for moderate or diluted urban waters: COD concentration typically ranged from 200 to 1000 mg/L, Ammonia was around 20-30 mg N/L and orthophosphate concentration was 5-8 mg P/L. HRT was gradually diminished during the two stages from 50-100 h to around 16-20 h.

The operation of the MBR was divided in two different experimental stages: Stage I: operation with 2 different hollow fiber membrane modules during 140 experimental days: a Microfiltration (MF) and a Ultrafiltration (UF) modules. The surface of the two modules was 1.8 m^2; and Stage II, operation with a 2 m^2 flat sheet membrane module during around 120 experimental days. During stage I the reactor was operated as an hybrid MBR with both suspended biomass and biofilms growing on it. A granular plastic product was used as carrier for biofilm development in the first aerobic chamber. This carrier was made of small polyethylene rough particles with a density of 900 kg m^{-3} and was removed before operating stage II.

Figure 1. Image of the pilot Hybrid Membrane Bioreactor plant: 1.- equalization tank; 2. Membrane Bioreactor; 3.- Permeate tanks, 4.- Control panel; and 5 Fine screen

Dissolved Oxygen concentration (DO), temperature, permeate flow and transmembrane pressure (TMP) were continuously monitored in the pilot plant by using PLCs and a computer acquisition program. By sampling twice a week, chemical analyses of ammonia, nitrate, nitrite, phosphate, SS, COD as well as permeability tests, D.O and pH measurements have been done during operation of the MBR to monitor the plant.

Results and discussion

During the two experimental stages HRT was gradually diminished, from 50 to 16 h and from 100 h to 20 h during stages I and II, respectively. During stage I, Total COD concentration in the influent varied between 150 and 1400 mg COD/l. The COD concentration in the permeate of the two hollow fiber membranes was similar and generally lower than 100 mg COD/l. The average COD concentration obtained with these membranes was 42 mg COD/L and the average COD efficiency was 93%. Similar results were observed during stage II with the flat sheet membrane. COD concentration was similar, between 20 and 50 mg/L. However the evolution of ammonia in the effluent was different between the two experimental stages. Ammonia concentration in the permeate decreased rapidly during the first 10 operating days of the stage I, afterwards it was around 3 mg $N\text{-}NH_4^+/l$. This parameter was not affected either by temperature that varied between 10 and 20 ºC (Average Temperature 13 ºC) or by the low pH that ranged between 5.8 and 7. On the contrary during the stage II, Ammonia concentration in the permeate was more affected by the observed operating conditions of the reactor. The better behavior observed during stage I, was a result of the presence of support with biofilms (Artiga 2007).

During stage I, The MF module fouled easily, in fact its surface tended to be covered with a brownish sludge layer. Nevertheless, the UF membrane maintained its whitish appearance during the experiments. Fibers of this module seemed to be clean. The maintenance of the MF module was more problematic that that of the UF module. In fact, the UF was not subjected to any chemical or physical cleaning during the 140 experimental days. On the other hand, during the first operating days of stage II, the low HRT was a consequence of the very low permeability of the membrane observed during the first experimental days, of only 20-30 $L/m^2 \cdot h \cdot bar$. In order to increase the permeability of the membrane this was chemical cleaned and hydrophilised at the operating day 18. After that, permeability increased till 200-500 $L/m^2 \cdot h \cdot bar$.

Acknowledgements

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Degradation of acid, basic and reactive dye mixture in a continuous bioreactor

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Keywords: Fungal, decolorization and textile dyes

The increasing global scarcity of water it is imperative to develop technologies that permit to treat wastewater for its subsequent reutilization. The treatment of coloured wastewater from some manufacturing processes is a challenge due to the complex contaminants involved. White-rot filamentous fungi have shown their capacity to degrade molecules with high molecular weight that are analogous to their natural substrate, i.e., lignin, which are present in the effluents from wood pulp and paper mills, sugar refineries and olive oil plants, among others. The degradation mechanism is based on the action of extra-cellular enzymes that are synthesized and secreted by fungi during the secondary metabolism stage under limiting nutrient conditions. This ligninolytic system has also been successfully used in the degradation of synthetic dyes (1,2).

The purpose of this study is to quantify the action of the extracellular oxidation system of *Phanerochaete chrysosporium* over multi-component coloured solutions in order to establish the potential degrading capacity in the effluent from a textile plant. Previous studies Urra et al., 2006 (3) determined, by means of screening, that the ligninolytic system of this fungus has a greater affinity for those azoic dyes containing naphthalene rings in their molecules. Based on these results, mixtures were prepared by selecting dyes for which the enzymatic system has shown a different affinity This strategy would also lead to the prospect of evaluating possible synergic effects between substrates. For this purpose, fixed-bed bioreactors were used with immobilized biomass and fed with bi-component mixtures of textile dyes: acid (Acid Black 1 and Acid Orange 6), basic (Basic Violet 4 and Basic Blue 24) and reactive (Reactive Blue 19 and Reactive Orange 16). The biological unit was fed in increasing steps with equimass mixtures at varied concentration.

Materials

Microorganism: *Phanerochaete chrysosporium* BKM-F-1767 (ATCC 24725) was kept at 4° C in malt extract plates (4). *Dyes*: All are of analytical grade (SIGMA – ALDRICH CO)

Methods

Fungal immobilization and bioreactor operation: The *fungal immobilization* and subsequent inoculation of the bioreactor took place following the protocol described by Urra et al., 2006. The medium feed to the bioreactor consisted of: $5.75 g\cdot l_{-1}$ glucose, $0.0144 g\cdot l_{-1}$ ammonium tartrate, $16.4 g\cdot l_{-1}$ sodium acetate as buffer (pH 4.5), $0.0056 g\cdot l_{-1}$ Mn_{+2} as $MnSO_4 \cdot H_2O$, $100 ml\cdot l_{-1}$ BIII and $5 ml\cdot l_{-1}$ Tween 80 (5,6). This culture media was supplemented by the previously sterilized mixture of dyes, with the global concentration varying from 20 to 160; 20 to 240 and 10 to $60 mg\cdot l_{-1}$ for acid, reactive and basic dyes, respectively. *The bioreactor operated* with a hydraulic retention time of 24h (7) and $8.4 ml\cdot h_{-1}$ of flow. The system was oxygenated by a pulsed flow of gaseous O_2 applied with a frequency of $0.0625 s_{-1}$. The temperature was kept at 37°C by circulating hot water between the walls of the bioreactor. Two samples of the culture media were taken every 24h, the first at the bottom and another at the top of the column during the whole operation period. Enzyme activity and analytical assays: *MnP activity* was measured by the oxidation of 2.6-dimethoxyphenol at 468nm (8). *LiP activity* was assayed against veratryl alcohol as described by Tien and Kirk (1988). One unit was defined as 1 μmol of product produced per min. *Dye decolorization* was monitored by the reduction absorbance spectrum of the mixture between 380 and 750nm. The

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spectrum was adjusted to a polynomial equation using the Curve Expert v1.3 program. Integration of this function permitted to estimate the decolorization degree reached day by day. The absorbance spectra of each of the three formulated dye mixtures are shown in Figure 1 below.

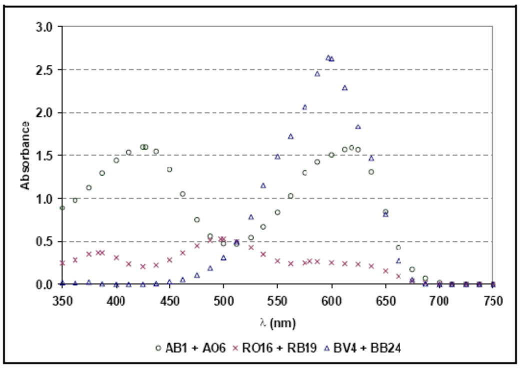
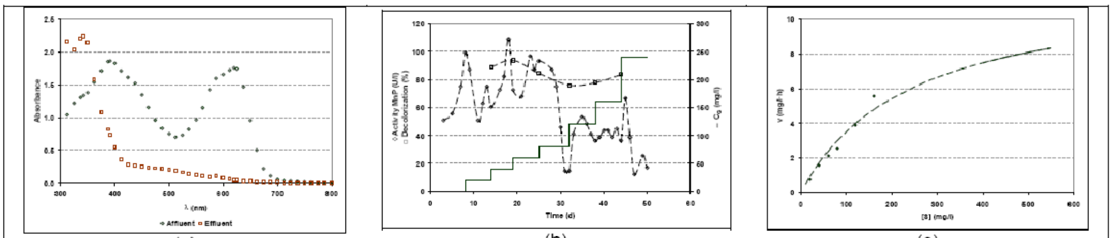


Figure 1. Absorbance Spectra of Mixture Dyes.

Results

Figure 2 shows, by way of example, the results obtained from the mixture of acidic dyes. Figure 2 (a) shows a sustained reduction of the typical absorbance peak of each dye in the mixture. The continuous system response to the increase of dye concentration in the feeding media suggests the possibility of operating with bi-component mixtures, resulting in a global decolorization greater than 90% (Figure 2 (b)). It also shows that the MnP activity dropped 50% in dye concentrations greater than 50 mg·l-1, however decolorization reduction was only 10%.

The process kinetics was satisfactorily interpreted by the classic Michaelis-Menten model (Figure 2 (c)), resulting in a half-saturation constant of 12 mg·l^{-1}, and a maximum velocity of 124.8 mg·l^{-1}·h^{-1} (R^2=0.935). The mixture of reactive dyes had a similar kinetic behaviour, while basic dyes showed an inhibition effect.



(a) (b) (c)

Figure 2. Mixture of Acid Dyes. (a) Bioreactor affluent and effluent absorption spectrum (60mg·l^{-1}), (b) Outline of decolorization and enzymatic activity in bioreactor, (c) Michaelis-Menten decolorization kinetics

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Optimization of industrial textile dye colour removal by *Pseudomonas oleovorans*

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Keywords: Azo dye; *Pseudomonas*; colour removal; factorial design; optimization

Azo dyes comprise a diverse group of synthetic chemicals that are widely used by textile, leather, food, cosmetics, and paper product industries (Khalid et al., 2008). The annual world production of azo dyes is estimated to be around one million tons (Pandey et al., 2007; Stolz, 2001), with 26,500 tons in Brazil alone (Ulson de Souza et al., 2007).

Many of these dyes are visible in water at concentrations as low as 1 mg L^{-1}. Textile processing wastewaters with dye contents in the range of 10-200 mg L^{-1} are highly coloured (Pandey et al., 2007). Some of the dyes and their degradation products are carcinogenic in nature. Three fourths of industrial textile effluents in metropolitan area of São Paulo have positive mutagenic responses, and azo dyes contribute to mutagenic activity of ground and surface waters (Umbuzeiro et al., 2005).

This work describes the optimization of industrial textile dye colour removal environmental conditions by *Pseudomonas oleovorans*.

Materials and Methods: The dye used was a kindly gift from Clariant of Brazil (São Paulo, Brazil), since it is from commercial use, the *Colour Index*'s name and number, and also its molecular structure will be omitted further in this work.

A pre-culture in late exponential phase or early stationary phase was inoculated in tubes containing Nutrient Broth with dye where a factorial experimental design was performed. The variables used in the factorial design (2^{n-2}) were dye concentration (30, 50, 70 mg L^{-1}); agitation (0, 75, 150 RPM); initial pH (6.0, 7.0, 8.0); temperature (27, 32, 37 °C); time (36, 48, 60 hours); tube volume (25, 50, 75%); and initial culture size (0.5, 1.0, 1.5 D.O.).

After the proper culture time, samples were collected and analyzed. Decolourization was quantified by the colour removal (%) = $(A_{\lambda,inicial} - A_{\lambda,final}) / A_{\lambda,final} \times 100$, at the end of the process.

Results and Discussion: The main results are showed in figure 1. It was observed that the tube volume was the most influence variable with a negative response. The azo dyes reduction in anaerobic and anoxic conditions is an extracellular and non-specific process (Kalme et al., 2007). This suggests that the oxygen presented in the medium has a negative limiting role in the colour removal early stages.

The dye concentration also presented a negative response on the decolourization process. The increase of dye concentration inhibits the cellular growth which should be a result of dye toxicity through inhibition of metabolic activity.

The interaction of dye concentration and tube volume shows that decolourization increases by the decrease of volume and decrease of dye concentration. Although, the reduction of tube volume alone give a colour removal of over 90% and so, it is viable to decolourize effluents with dye concentration higher of 50 mg L^{-1}.

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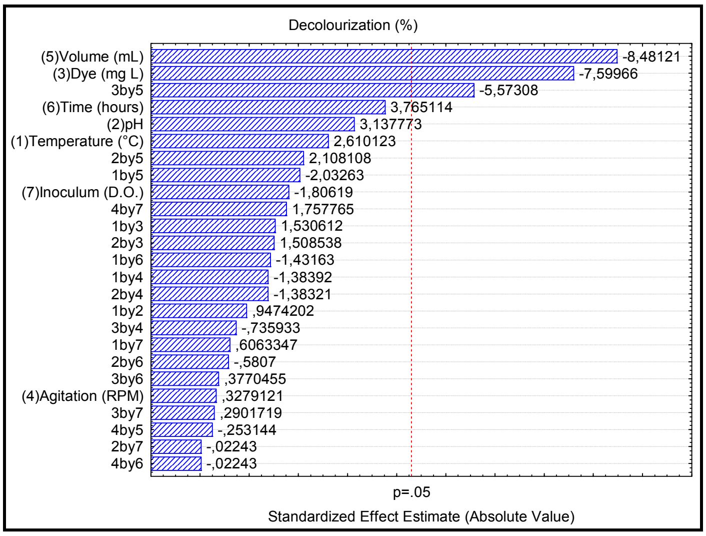

Figure 1: Pareto Chart of Standard Effects

Even though agitation has a positive effect on decolourization, this effect has little influence over the decolourization process. The inoculum size has a negative effect, and as the agitation it has little influence. Since this study aims at an industrial application of colour removal, the lack of agitation is of extreme vantage.

The increase of pH, from an acid pH to a slight basic pH, showed an increase of decolourization, even though it was not significant. Pearce et al. (2003) described that bacterial cultures usually have maximum decolourization rates at neutral pH or low basic.

Conclusion: The best conditions to dye decolourization by *Pseudomonas oleovorans* based on the experimental design and also economic factors were agitation 0 RPM, pH 8.0, temperature 32 °C, 25% of tube volume, initial culture size of 0.500 abs, dye concentration of 50 mg L-1 and culture time of 36 hours.

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Phosphorus removal from water by polyolefins activated with Al_2O_3

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Keywords: eutrophization, phosphorus, polyolefins, aluminium oxide (Al_2O_3)

Eutrophization of aquatic ecosystems due to high phosphorus concentrations is associated with algal blooms which lead to poor water quality (Ribeiro et al, 2008; Martins et al, 2008). Processes used in remediation of water bodies include removal of P from the water column by precipitation and P inactivation in sediments (sediment capping). Remediation solutions have been tested for a long time but with limited success. Chemical precipitation of P with aluminium, iron, and calcium salts added to the water column has several disadvantages, namely is inadequate to remove low concentrations of phosphorus, contaminates water with high concentrations of sulphate and chloride ions, and generates large quantities of flocs that settle to the sediment (Ramm and Scheps, 1997; Lijklema et al, 1993; Jin et al, 2006). A sediment capping layer would remain in place indefinitely, unless disturbed by ebullition of sediment gases or some major seismic or climatic disturbance. Potential problems with sediment capping are bioturbation and wave action (Hano et al,1997; Donnert and Salecker 1999). For this reasons, we propose a new method for phosphorus removal based in polyolefins activated with a sequestering agent (Al_2O_3).

Polyethylene (PE) and polyethylene grafted with two different concentrations of maleic anhydride (PE-g-MA and PE-g-MA$^+$) activated with Al_2O_3, were tested for phosphorus removal from an aqueous solution with an initial phosphorus concentration of 100 μg/L. Kinetic experiments were carried out in glass beakers under static conditions using polymeric plates with similar areas, 10.5 cm^2 (4.0 cm × 1.0 cm × 0.2 cm), at room temperature for 3 months (Figure 1). Samples were taken regularly and phosphorus content was measured. Experiments were carried out in triplicate. The effect of the polymeric matrix in the adsorption process was analysed carring out experiments with granulated PE, PE-g-MA$^+$ and Al_2O_3. In this trial, the solutions were continously shaken in an orbital shaker at 100 cicles per min at 22 ºC for 7 d. Samples were taken at the begining and at the end.

A very high percentage of phosphorus removal was obtained for the three tested materials as well as a very low remaining phosphorus concentration in solution (below 10 μg/L), as depicted in Figure 1. The kinetics of P uptake was faster for PE-g-MA$^+$ (polyethylene enriched with MA), more than 50 % of P was removed in the first two days. This result seems to indicate that polymers with higher polarities have more affinity to P. Kinetic essays carried out with granulated PE, PE-g-MA$^+$ and Al_2O_3 indicated that PE and PE-g-MA$^+$ remove about 40 % and Al_2O_3 about 100 % of P from a 100 μg/L aqueous solution. Granulated Al_2O_3 reached the maximum uptake only in 3 days. The adsorption of phosphorus into activated polyolefins plates was influenced by the pH of the solution, the amount removed was higher at pH 3 for PE, pH 4 for PE-g-MA and pH 6 for PE-g-MA$^+$. Activated polyolefins effectively removed phosphorus from an aqueous solution which indicates that this material can be used for remediation of eutrophic water. The possible recovery of phosphorus for reused and absence of precipitation make this a very interesting process for water remediation.

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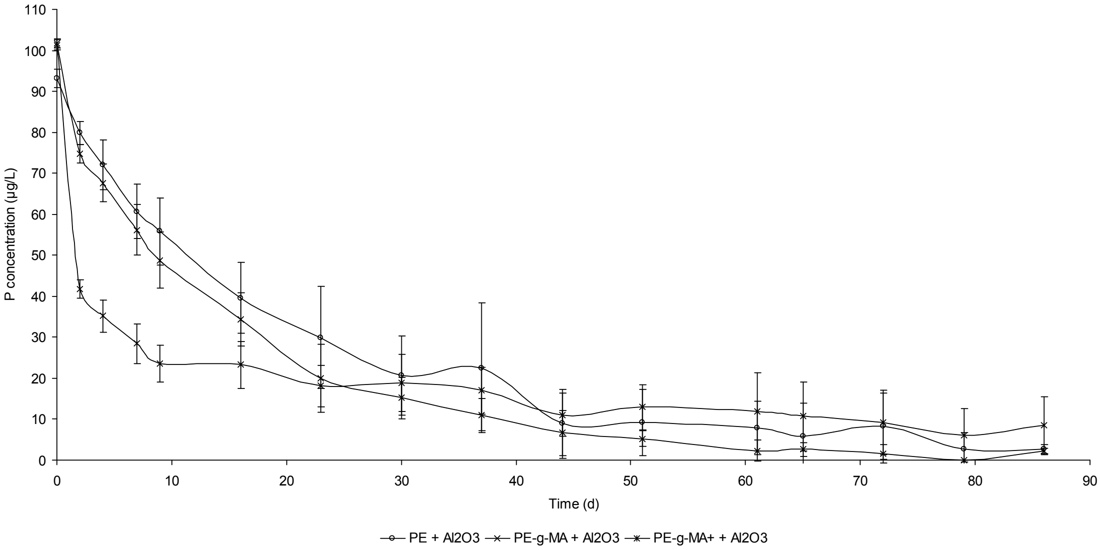

Figure 1 – Phosphorus concentration along time.

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Biodegradation of humic substances under denitrifying conditions

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Keywords: Humic substances, biodegradation, alkaline denitrification

Humic substances (HSs) are recalcitrant molecules and represent about 75 % of the dissolved organic carbon (DOC) in rivers (Barreto et al., 2003). HSs encompass a variety of fractions (fulvic acids, humic acids and humine) identified on the basis of their solubility in alkaline and acidic solutions. The presence of HSs in water treatment plants is undesirable because they increase coagulant and disinfectant demands, and in the presence of chlorine may provoke the formation of dangerous disinfection by-products (DBPs) such as the carcinogenic trihalomethanes (THMs). Because of their role as DBP-precursors, it is crucial for the drinking water sector to improve environmental technologies increasing water quality prior to treatment. From a limited number of studies it is known that HSs support both biofilm and suspended growth under aerobic, anaerobic and denitrifying conditions (Lovley et al. 1999; Rodrigues et al., 2007). In this context, the knowledge about the kinetics of complex organic matter biodegradation needs more consistent studies. In the present work, biodegradation of HSs was assessed in batch tests by measuring the rate of N_2 production during denitrifying conditions.

Batch tests were carried out according to Buys et al. (2000). A commercial humic acid (HA) was used to simulate HSs. Four essays were performed in duplicate: **E1** was the control and run without N-NO_3 addition and with 214.7 mg L^{-1} HA; **E2** run with initial concentrations of HA and N-NO_3 of 214.7 mg L^{-1} and 100 mg L^{-1}, respectively, and with a viable inoculum; **E3** was similar to E2 but HA was substituted by NaAc with an initial concentration of 365.9 mg L^{-1}, and **E4** run with both carbon sources, HA 214.7 mg L^{-1} and NaAc 365.9 mg L^{-1}, and 200 mg L^{-1} N-NO_3. Biomass concentration was 0.91 g L^{-1} as volatile suspended solids and pH was adjusted to 8.5. The rate of gas production during denitrification was measured by tracking both the pressure and the gas composition in the headspace of a sealed vial. The pressure in the headspace was measured daily for the first week and then every three days. N_2 production was calculated from the headspace volume, gas composition and pressure data by applying the ideal gas law. Gas composition was determined by gas chromatography. Nitrogen ions (nitrate, nitrite and ammonium) were measured by spectrophotometric methods and total and volatile solids were determined by a gravimetric method according to Standard Methods (APHA, 1998). All parameters were analyzed in the beginning and at the end of the experiment.

N_2 production in the batch tests during 55 d is depicted in Figure 1. Based on this data it is evident that the essay performed without nitrate (E1) presented a significant lower N_2 production than the other essays (E2 to E4). Comparing essays E2 and E3, carried out with HA and with acetate as carbon sources, it was found that N_2 production was faster in the essay performed with acetate (E3) than in the one carried out with HA (E2). One reasonable explanation for this result is that HA is less biodegradable than

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acetate due to their lower solubility in water, higher molecular weight, and higher complexity because of the presence of several chemical functional groups. The highest N_2 production was observed in essay E4, carried out with both carbon sources, as expected.

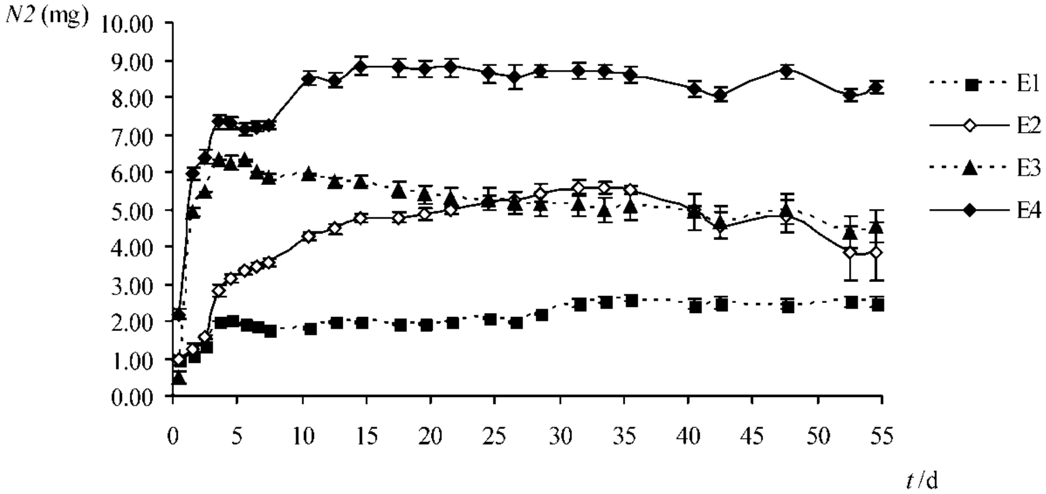

Figure 1 N_2 production during 55 d: E1 - without nitrate; E2 - HA and nitrate; E3 - acetate and nitrate; E4 - HA, acetate and nitrate.

Nitrate and nitrite concentrations measured at the end of the essays were approximately zero in all cases. On the other hand, final ammonium concentrations (107.6 mg L^{-1} and 118.8 mg L^{-1}) were higher than the initial ones (77.8 mg L^{-1} - 92.0 mg L^{-1}) in all essays. The later observation might be explained by cellular lysis. This result was corroborated by an observed decrease in volatile suspended solids during the time life of the essays.

The present work provides evidences that humic acids are degraded under denitrifying conditions.

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Characterization of PAH degrading bacteria isolated from hydrocarbon slurries

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Keywords: polycyclic aromatic hydrocarbons; hydrocarbon slurry; Bacillus pumilus; Alcaligenes faecalis; biofilm

Polycyclic aromatic hydrocarbons (PAHs) are environmental contaminants frequently present in soil and water ecosystems and represent a risk to human health due to their carcinogenic, mutagenic and toxic characteristics. Microbial degradation of PAHs is considered to be the major decomposition process of these contaminants in nature despite their low solubility in water and high adsorption to soil particles that leads to slow biodegradation rates and consequently environmental persistence. The PAHs degrading strains that have been characterized so far in the literature are taxonomically diverse and belong mainly to the genera Pseudomonas, Alcaligenes, Sphingomonas, Bacillus and Mycobacterium. The genera Mycobacterium have been studied extensively, yet little attention has been paid to the remaining ones.The aim of this work was to evaluate the growth of *Alcaligenes faecalis* and *Bacillus pumilus* isolated from hydrocarbon slurries on solid PAHs and to assees their emulsifying and biosurfactant activities. In this study were used five PAHs namely anthracene, fluoranthene, fluorene, phenanthrene and pyrene. The bacteria were isolated from hydrocarbon slurries from a car maintenance facility on media supplemented with PAHs crystals as described in Bastiaens et al. (2000). 16S rRNA sequence analyses of the isolates have shown that they were affiliated with *Alcaligenes faecalis* and *Bacillus pumilus* (Figure 1).

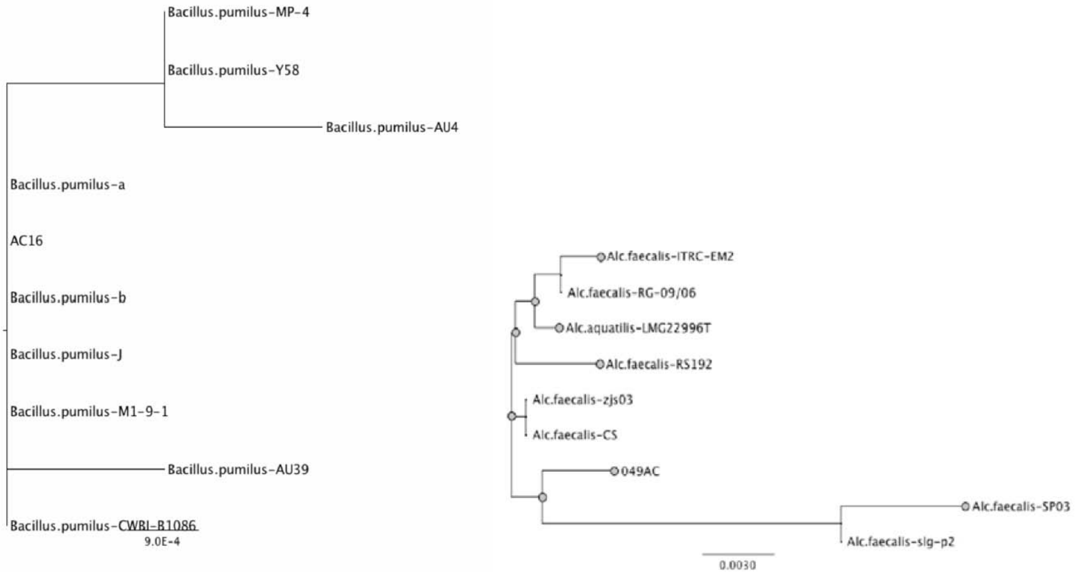

Figure 1. Neighbors-joining phylogenetic tree based on the nearly full length of the 16S rDNA gene of the two strains: (A) *B. pumilus* AC16, and (B) *A. faecalis* 049AC.

Growth experiments were performed in batch using PAHs as sole carbon and energy sources at a concentration of 30 g L^{-1}. Suspended cell growth was routinely measured as total protein. The growth curves are depicted in Figure 2. Both bacterial isolates have shown a maximum growth on pyrene followed by anthracene in the case of *A. faecalis* and phenanthrene in the case of *B. pumilus*.

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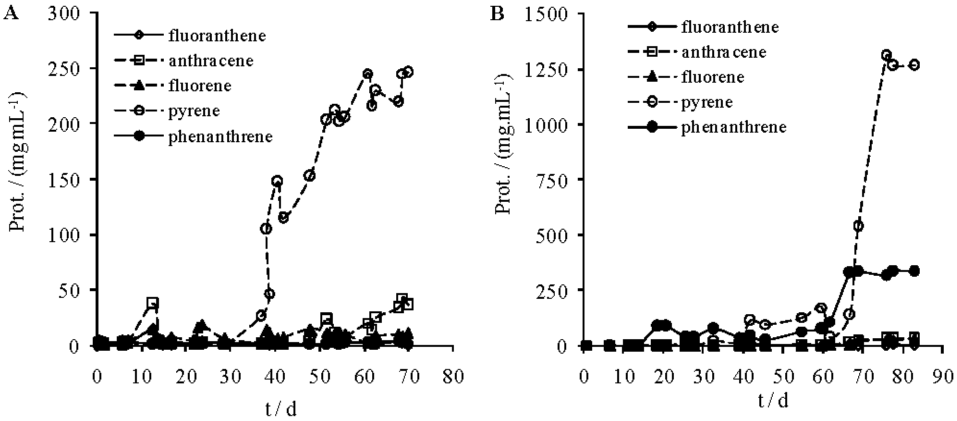

Figure 2. Batch growth curves of (A) A. faecalis and (B) B. pumilus on 30 g.L^{-1} of PAH's crystals. Growth was determined as cell protein content by the method of Lowry.

It is well known that microorganisms growing on hydrocarbons frequently produce biopolymers with emulsifying or surfactant activity. These properties are considered as a biological strategy to facilitate the availability of hydrophobic substrates. In this study, assays were carried out to evaluate the capacity of *A. faecalis* and *B. pumilus* to produce extracellular biopolymers with biosurfactant or bioemulsifier activities (Table 1). The emulsifying activity of the biopolymers produced by the selected strains was detected by a modified version of the method described by Cooper and Goldenberg (1987). The surface tension of the produced biopolymers was measured with a Krüss K6 tensiometer, using a ring method. The experimental results have shown that the selected microorganisms were able to produce extracellular polymers with emulsifying activity. However, none of the isolated biopolymers showed biosurfactant activity. These results are supported by the studies conducted by Toledo *et al.* (2006). Further experiments will be aimed at detailed investigation of extracellular biopolymers characterization. Scanning Electron Micrograph (SEM) showed that biofilm growth occurred on the crystal surfaces. In order to monitor biofilm activity measurements of oxygen consumption using microelectrodes are being performed.

Table 1. Results of the emulsification and surfactant activity tests

Strain	Emulsifying activity[a]		Surface tension
	Mineral oil	Gasoline	(nNm^{-1})
B. pumilus AC16	45	65	60
A. faecalis 049AC	30	70	63

[a] results are expressed as percentages of the total volume occupied by the emulsion, values are means of three determinations.

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Biodegradation of fuel oil hydrocarbons by a bacterial consortium and strategies to enhance process efficiency

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Keywords: bioaugmentation, biodegradation, bioremediation, biostimulation, fuel-oil

The accidental release of fuel oils into the environment happens frequently, not only as a consequence of spectacular oil tanker spills, but also on a smaller scale along pipelines, or during filling of containers and tanks. Treatment of contaminated soils can be carried out by physicochemical and biological methods. One of the best approaches to restoring contaminated soil is to make use of microorganisms able to degrade those toxic compounds in a bioremediation process. Thus, bioremediation is an attractive approach of cleaning up petroleum hydrocarbons because it is simple to maintain, applicable over large areas, cost-effective and leads to complete destruction of the contaminant. Some of the recently used strategies to enhance bioremediation process efficiency, include bioaugmentation, biostimulation, rhizoremediation, the use of chemotaxins, the biomimetic catalytic system approach, and integrated techniques (Cunningham et al. 2004). Among them, in this work, the application of strategies of bioagumentation and biostimulation has been assayed.

Biostimulation requires adjustments to the contaminated site in order to provide bacterial communities with a favourable environment in which they can effectively degrade contaminants. In cases where natural communities of degrading bacteria are present in low numbers, or even absent, bioaugmentation by the addition of contaminant-degrading microorganisms can speed up the degradation process (Seklemova et al., 2001; Barathi and Vasudevan, 2001).

Bioremediation process of contaminated soil from sites near a fuel oil station in Orense (Spain) was carried out in three treatments to evaluate the efficiency of fuel oil degradation in the contaminated soils, using sterilized soils as controls. The treatments were: (a) natural attenuation (soil's natural ability to degrade the contaminant); (b) biostimulation (adding nutrients to improve the natural biodegradation rate); and (c) bioaugmentation (addition of a microbial consortium from selected species isolated from a contaminated soil plus nutrients).

Preliminary selection of the best oil-degrading microorganisms was done. In this study fuel oil contaminated soils (34,210 mg total fuel oil hydrocarbon/Kg dry soil) was used as inoculum for isolating fuel oil-degrading bacteria. For culture enrichment, 10 g contaminated soil and 2 ml fuel oil were added to 200 ml Bushnell Haas (BH) medium. Samples were cultivated at 30ºC and 180 rev/min for 7 days, and the inoculum culture (5% v/v) was transferred into 1% (v/v) fuel oil-supplemented BH medium. Twenty one microbial strains were distinguished based on morphological differences among the colonies on LB plates. Selection of the best oil degraders was done following the method described by Hanson et al. (1993). In this method, the time taken for decolourization of the blue (oxidized) 2,6-dichlorophenol indophenol (DCPIP) to the colourless (reduced) form was representative of the ability of these strains to degrade oil. The strain M1A, which decolourized the 82% of DCPIP in 4 days, was chosen as the best oil degrader.

In the next stage a comparative study of this strain with the initial consortium was carried out in submerged cultures containing fuel oil. The hydrocarbons were analysed by GC (Hewlett Packard Series II 5890) with a flame ionization detector, using an HP-5 capillary column (30 m length, 0.23 mm internal diameter and 2.5 μm film thickness).

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In order to determinate the influence of several factors such as nutrients source and concentration and fuel oil concentration, several submerged cultures were carried out. The results obtained operating at an initial fuel oil concentration of 7% (v/v) are shown in table 1. In all cases, the microbial consortium, which had originally been isolated from fuel oil contaminated soil, led to the best results. It was capable of degrading over a time period of 7 days to about 98% of the original concentration when the medium was supplemented with BH medium. Moreover, higher levels of inorganic nitrogen appeared to decrease the growth of the microorganims and their oil degradation ability. According with these results the consortium was used for the bioremediation of fuel oil-contaminated soil. Among the strategies tested, the bioaugmentation permits to obtain the best results with a total fuel oil hydrocarbon degradation of 35% in 30 days.

Table 1.- Initial fuel oil hydrocarbons concentration and their degradation after 27 days, operating in submerged cultures inoculated with isolated strain M1A and soil consortium.

		BH+Chaîneau *		BH		BH+Commercial Fertilizer**	
	Initial concentration (ppm)	M1A degradation (%)	Consortium degradation (%)	M1A degradation (%)	Consortium degradation (%)	M1A degradation (%)	Consortium degradation (%)
Ethylbenzene	27,99	0,00	0,00	0,00	40,69	0,00	47,86
m, *p*-Xylene	18,11	0,00	0,00	0,00	59,77	0,00	64,17
n-Decane	58,49	0,00	8,12	25,11	92,18	9,71	79,99
n-Dodecane	329,02	0,00	12,06	31,33	100,00	2,68	86,31
n-Hexadecane	358,49	0,00	5,70	28,46	93,93	0,43	92,25
n-Heneicosane	229,08	0,00	3,28	24,41	100,00	8,77	95,06
Naphthalene	162,00	12,65	2,16	9,09	100,00	5,00	4,06
Phenanthrene	100,00	0,00	0,00	18,63	100,00	0,00	25,74

* Chaîneau, et al. (2003) (N:P = 10:1)

** Commercial fertilizer provided by COMPO (N:P = 20:5)

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Fate of aniline and sulfanilic acid under denitrifying conditions

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Keywords: Aromatic amines, Biodegradation, Denitrification, Deamination, Toxicity, Bioreactors

A logical concept for the removal of azo dyes in biological wastewater treatment systems is based on anaerobic treatment, for the reductive cleavage of the azo linkages, in combination with aerobic treatment, for the degradation of the products from azo dyes cleavage, aromatic amines (Van der Zee and Villaverde, 2005). A drawback of aerobic treatment is that many aromatic amines from azo dye cleavage are prone to autoxidation, which mostly increases the size of the molecules, thereby reducing their biodegradability. Alternatively, one may consider using nitrate, instead of oxygen, as electron acceptor. Nitrate is a powerful electron acceptor, and it has been reported that at least some aromatic amines can be degraded coupled to nitrate reduction (Kuhn and Suflita, 1989). Moreover, the presence of nitrate does not lead to autoxidation of reduced azo dyes. A further interesting feature of using nitrate is that the first step of denitrification yields nitrite, a compound that has been found to react with aromatic amines, resulting in deamination, thereby yielding aromatics with a higher biodegradation potential than aromatic amines (A.J.M. Stams, personal communication). Although it can be stated that anaerobic-anoxic treatment holds promise as a strategy for azo dye degradation, its potential has never been explored.

With the objective to investigate the fate of aromatic amines under denitrifying conditions, two upflow anoxic bioreactors were operated. The reactors were fed with synthetic wastewaters containing aniline and/or sulfanilic acid (SA) and a mixture of volatile fatty acids (VFA) as the primary electron donors. The terminal electron acceptors were stoichiometric amounts of either nitrate (R1) or a mixture of nitrate and nitrite (R2). Supporting batch denitrification experiments were conducted to monitor toxicity and ultimate biodegradability.

The results demonstrated biodegradation of a substantial fraction of the influent anilineconcentration in R1 (Fig. 1). In contrast, SA was not degraded (data not shown). The presence of nitrite at low pH (influent R2) caused a chemical reaction that led to immediate disappearance of the aromatic amines, resulting in the formation of phenol and two other main compounds (compound 1 and 3). Phenol and compound 1 appeared associated with nitrite-reacted aniline and nitrate-reacted SA, respectively, whereas compound 3 was only formed when both amines were present. Bioreactor treatment removed about half of compound 3, most of the phenol, and mostly all of compound 1, whereas a new metabolite (compound 2) was formed. The overall COD removal was always higher in R1 than in R2, suggesting toxicity of nitrite and/or the products of the chemical reaction between aromatic amines and nitrite.

Whereas a replacement of amino-groups by hydroxyl-groups (as in the observed formation of phenol from aniline) holds promise for biodegradability, the results indicate that the chemical reaction is more complex, resulting in the formation of compounds that were not mineralized during the course of the experiment.

It is the authors' intention to identify compounds 1-3 using LC-MS.

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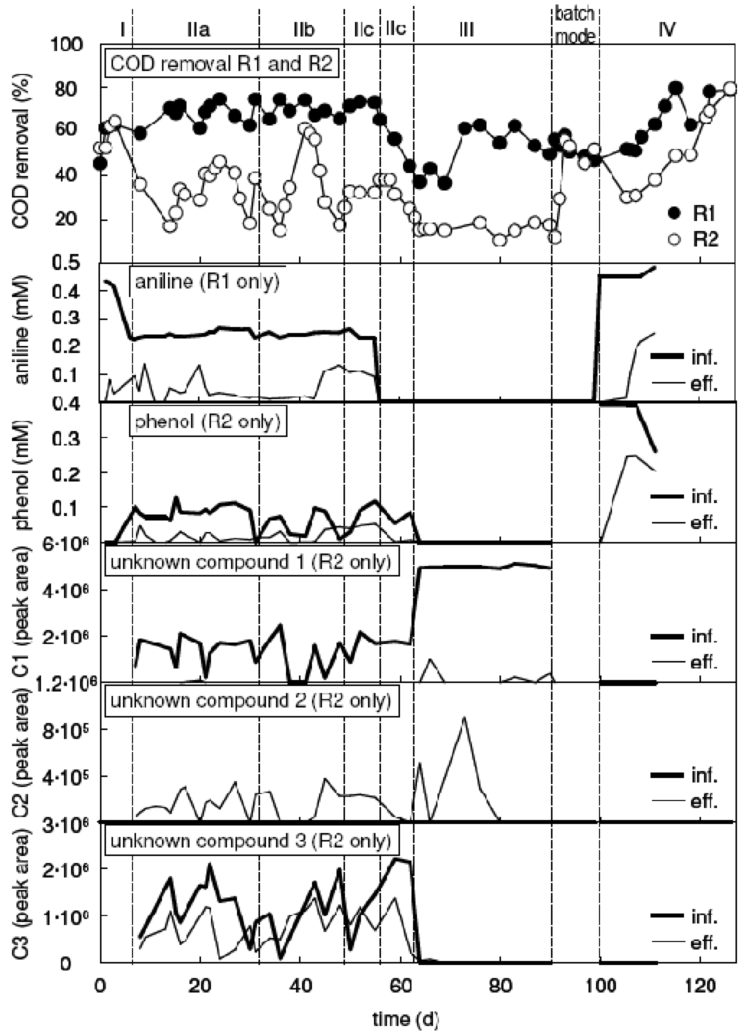

Fig. 1: COD removal and concentrations of aromatic amines and derivatives in influent and effluent of R1 (nitrate) and R2 (nitrate+nitrite). The Roman figures refer to operational phases I: Aniline + SA + 200 mg L^{-1} VFA-COD, inf.-pH 7; IIabc: Aniline + SA + (a)200, (b)300 and (c)400 mg L^{-1} VFA-COD, inf.-pH 4.8; III: SA + 300 mg L^{-1} VFA-COD, inf.-pH 4.8; IV: Aniline + 300 mg L^{-1} VFA-COD, inf.-pH 4.8.

Acknowledgements

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Application of an *E. coli* biofilm supported on kaolin to the removal of Cd(II), Cr(VI), Fe(III) and Ni(II) from aqueous solutions

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Keywords: biofilm, biosorption, clay, cadmium, chromium, iron, níquel

Aim

The pollution caused by heavy metals is one the major environmental problems that is imperative to be solved. New technologies, easy to implement and to adapt to any system, deserve special attention and are the focus of this presentation.

This work aims to investigate the biosorption behaviour of a biofilm of *E. Coli* supported on kaolin clay for the treatment of cadmium, iron, nickel and chromium aqueous solutions. The effect of heavy metals initial concentrations was studied and the relationship between pH and removal efficiency was analysed. Adsorption characteristics of a biosorbent can be depicted by an adsorption isotherm. Langmuir, Redlich-Peterson and Sips are commonly used for the description of adsorption data and were applied to the experimental results described by this report.

Materials and Methods

The bacterium *Escherichia coli* was obtained from the Spanish Type Culture Collection of the University of Valência. Heavy metals solutions were prepared by diluting $K_2Cr_2O_7$, $FeCl_3.(6H_2O)$, $CdSO_4.(8/3H_2O)$ and $NiCl_2(6H_2O)$, in distilled water. Atomic absorption spectrometric standards were prepared from 1000 mg.l-1 metal solutions. The kaolin was obtained from Minas de Barqueiros, S.A. (Apúlia, Portugal).

A medium with 5 g/l of beef extract, 10 g/l of peptone and 5 g/l of NaCl (pH 7.2) was used for the microorganism growth. The medium was sterilized at 121 ºC for 20 min, cooled to room temperature, inoculated with bacteria and kept at 37 ºC for 24 h with moderate stirring in a incubator. Then, batch experiments were conducted using 1 g of kaolin clay with 15 mL of *E. coli* culture media and 150 mL of the different heavy metal solutions (10, 25, 50, 70, 80, 100) mgL^{-1} in 250 mL Erlenmeyer flasks. All experimental work was conducted in duplicate. The Erlenmeyer flasks were kept at 37 ºC, with moderate stirring for about 10 days. Samples of 1 mL were taken, centrifuged and analyzed for metals using atomic absorption spectrophotometry, Varian Spectra AA-400.

Results and Discussion

The results obtained for the batch biosorption studies showed differences on the biosorption performance between the four heavy metals studied (Table 1). Kaolin surface in water had a net negative surface charge (Turan *et al.*, 2007) and the bacteria have also a net negative charge (Tavares *et al.*, 2006). These negative surfaces obviously favour the biosorption of cations. In addition to the valence state, the electronegativity, atomic weight and ionic radius are also characteristics that influence the biosorption capacity.

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Table 1- Uptake values (mg/g) and removal percentage values for Cr (VI), Cd (II), Fe (III) and Ni (II), obtained for the higher initial concentration of heavy metal (37 ºC, 150 rpm), for the biofilm of *Escherichia coli* supported on Kaolin

Cr (VI)			Cd (II)			Fe (III)			Ni (II)		
C_0 (mg/l)	Up. (mg/g)	Rp (%)	C_0 (mg/l)	Up. (mg/g)	Rp (%)	C_0 (mg/l)	Up. (mg/g)	Rp (%)	C_0 (mg/l)	Up. (mg/g)	Rp (%)
116	4,6	26,2	97	10,3	71,3	110	16,5	100	101	6,9	45,3

The order in terms of uptake values by the *E. coli* biofilm supported on kaolin was Fe > Cd > Ni > Cr. The worst biosorption behaviour obtained for chromium is justified by the anionic state. Comparing the results obtained for the cationic metals, the better results were obtained for iron and can be justified with the higher electronegativity and reduced ionic radius which promote the penetration in the polymeric net of the biofilm. Considering the ions Cd and Ni, the nickel ion have higher electronegativity, lower atomic weight and ionic radius and these characteristics allowed good qualities for metal entrapment. However, better results were obtained for cadmium. A possible explanation for these unexpected results might be the xenobiotic effect of the metal, more accentuated in the nickel case, on the biofilm. In a system biofilm-kaolin both materials contribute to the biosorption performance and if the biofilm is affect by the toxic nickel ion, the biosorption performance is affect too.

It is possible to characterize biosorbents under various operational conditions through the modelling of equilibrium data and this point is essential for future industrial applications. Adsorption isotherms were experimentally determined for the biosorbent used (biofilm + kaolin), and results are shown in Figure 1. Three different models - Langmuir, Sips and Redlich-Peterson- were fitted to the experimental data. For iron and for the range of initial concentrations tested, the whole metal was immediately and totally biosorbed so the concentration at equilibrium was always zero and consequently it is not possible to represent Ce (mg/l) vs Qe (mg/g). The best fit for chromium and for nickel was obtained with the Redlich-Peterson model isotherm and for cadmium the best fit was reached with the Sips model.

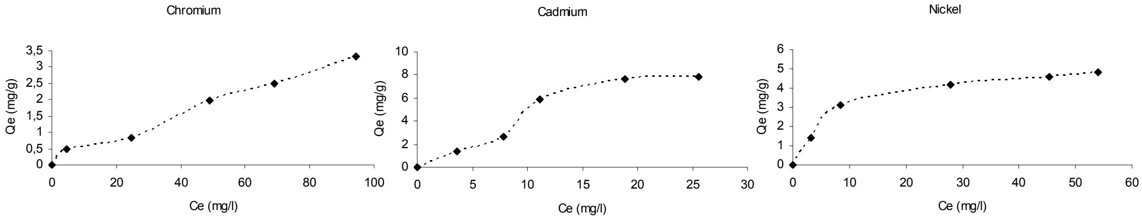

Figure 1- Adsorption isotherms for chromium, cadmium and nickel, at 37 ºC, using a biofilm of *Escherichia coli* supported on Kaolin.

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Reactive dyestuff removal by cork residues

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Keywords: cork, adsorption, dyestuff

The need of treating textile wastewaters is nowadays well known, not only concerning primary and secondary treatments for organic matter and suspended solids removal, but also a tertiary treatment, mostly for residual colour removal.

Considerable work has been carried out on the removal of colour from textile wastes through chemical coagulation, chemical oxidation and biological techniques and by adsorption. Adsorption appears to offer the best prospects over all the other treatment techniques.

Activated carbon is one of the most widely used adsorbents in wastewater treatment. However, this treatment needs a high investment and leads to high operation costs, due to the high price of the activated carbon and to the high wastewater flow rate always involved, which can be greatly increased when there are no regeneration units nearby, as in the case of Portugal This work intends to explore the adsorption capacity of cork (*Quercus suber L.)* granules to remove dye (Rouge Maxilon), from contaminated waters. The cork adsorption capacity has been studied for other pollutants (Domingues *et al.* 2007).

Adsorption batch assays with dye, using activated carbon and granulated cork with several different sizes, several pH values and different temperatures, were performed, either to determine the equilibrium time or the efficiency of the removal.

The adsorption of dye in fixed beds columns with activated carbon and granulated cork were investigated.

The results obtained have demonstrated that the cork is a cheap, efficient natural removing agent for Rouge Maxilon that may be explored in future to act as a treatment of wastewaters in textile industry.

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Influence of airflow rate on soil remediation using soil vapour extraction

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Keywords: Soil Vapour Extraction, Airflow rate, Soil, Volatile Organic compounds

The release of contaminants into the soil matrix produces a negative impact on soil quality, creating, in some cases, situations of risk to public health. Halogenated volatile organic compounds and the group constituted by benzene, toluene, ethylbenzene and xylene are common compounds found in contaminated sites. To remediate these sites several techniques can be used: biological processes (such as bioremediation or phytoremediation), thermal processes (such as incineration or thermal desorption) and physico-chemical processes (such as chemical oxidation or soil vapour extraction) (Khan *et al*, 2004).

Soil Vapour Extraction (SVE) is one of the most used technologies that can be applied to soils contaminated with volatile or semi-volatile organic compounds in the unsaturated zone. It applies vacuum to the soil matrix, creating an airflow that transports the contaminants to extraction wells. The air circulation through the soil matrix produces mass transfer into the gas phase, due to free pollutant volatilization, desorption from the soil and dissolution from the aqueous phase. Before reaching the atmosphere, these vapours are usually treated to guarantee legal limits and air quality. The success of a soil vapour extraction project depends on several parameters including contaminant characteristics such as vapour pressure and solubility, soil properties such as natural porosity, permeability, organic matter or water contents and operational conditions like temperature or airflow rate (Chai and Miura, 2004).

Experiments previously carried out with two soils contaminated with cyclohexane allowed concluding that for air flow rates lower than 1.5 $cm^3\ s^{-1}$ STP, the remediation efficiency did not depend on the volume of gas passed through the soil, because the equilibrium between the pollutant and the different phases present in the soil was reached and the diffusion effects were avoided (Albergaria et al, 2006).

A laboratorial installation was developed and used to simulate soil remediation using SVE. The remediation process was monitored and the contaminant quantification performed by gas chromatography using a flame ionisation detector.

In this work, six typical petroleum contaminants were studied, allowing the establishment of operational SVE conditions for the remediation of frequently found contaminated soils. The influence of airflow rate on soil vapour extractions was evaluated for sandy soils contaminated with benzene, toluene, ethylbenzene, xylene, trichloroethylene and perchloroethylene. The main objective was to select the best airflow rate to remediate soil contaminated with the studied contaminants and under the contaminations range experimented.

The results obtained for SVE performed with airflow rates within 0.50 and 5.3 $cm^3\ s^{-1}$ (STP) in sandy soils contaminated with benzene, toluene, ethylbenzene, xylene, trichloroethylene and perchloroethylene allowed concluding that:

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a) the remediation efficiencies were always higher than 90%,

b) depending on the contaminant, the airflow rates should be lower than 1.2 - 1.9 $cm^3\ s^{-1}$ (STP).

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Anthracene and lead adsorption on a Portuguese soil – competitive studies

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Key words: Anthracene, lead, soils, sorption isotherm

Abstract

The environmental contamination by organic and inorganic chemicals has been, for a long time, one of the most common problems in soils and sediments.

Many organic chemicals, such as polycyclic aromatic hydrocarbons (HPAs) can persistent in soil environment for a long time (Sabljic, 2001). Due to the possibility of ring fusion at different positions, there are more than 100 HPAs that are recognized by IUPAC (Jacques et al., 2008).

Some inorganic elements like heavy metals are natural constituents of soils. Due to anthropogenic activities, significant changes occurred in the global account of these metals at the earth's surface (Serrano et al., 2005).

Anthracene and lead were selected as they can be found as co-contaminants in storm water (Lau and Stenstrom, 2005) or in petrochemical industries or oil refining products and waste. They can also result from incomplete combustion of organic substances as coal, oil, gas, wood and garbage (Jacques et al., 2007).

Sorption is a key process controlling mobility, bioavailability, toxicity and fate of pollutants in soil (Chen, 2007). As a consequence, this study was conducted in order to assess the partitioning of anthracene and lead on a Portuguese soil, in single and competitive systems.

A sample of a loamy sand soil was collected in Oporto, Portugal (41º25'15.58"N and 8º45'58.27"O) (Fonseca et al., 2008). Batch tests were performed in order to evaluate de adsorption kinetics and equilibria. For the equilibrium tests, single or multi solutions containing anthracene and/or lead with different concentrations, were prepared in 0.01 M $CaCl_2$. Then they were added to 2 g of soil, and the systems were shaken at 100 rpm for 72 h, at room temperature. Kinetics was evaluated for contact times up to 72 h, after soil sample saturation and addition of stock solution.

Lead concentration in the liquid phase was quantified by atomic absorption spectrometry (Spectra AA-400). After the addition of deuterated phenanthrene to the samples, anthracene was determined by gas chromatography – mass spectrometry (Varian 4000). The quantification of anthracene was performed using external standard calibration (Domine, 2007).

Kinetics and isotherm equations were fitted to experimental data in order to describe the adsorption process of the anthracene and lead, in competitive and non-competitive systems.

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Relative hydrophobicity of (PEG or Ucon)-salt ATPSs

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Keywords: aqueous two-phase systems, partition coefficient, hydrophobicity, amino acids

Introduction

Aqueous Two-Phase Systems (ATPSs) are obtained when two aqueous solutions of certain polymers or a polymer and a salt are mixed above certain critical conditions (e.g. concentration, temperature). These systems are regarded as a powerful extraction technique because they are mainly composed of water (> 80%) and provide a gentle environment for the partitioning of sensitive biomaterials, such as proteins. However, the wide use of this technology is partially limited by the lack of a clear comprehension of the factors and mechanisms that govern the distribution of solutes in ATPSs. Therefore, attempts to obtain more information that allows understanding and predicting the behaviour of a solute are extremely important.

Among the studied solvent properties of ATPSs, the free energy of transfer of a methylene group between the coexisting phases, $\Delta G(CH_2)$, has been shown (Zaslavsky, 1985; Willauer et al., 2002) to be useful for the characterization of ATPSs. $\Delta G(CH_2)$ represents the affinities of the phases for a CH_2 group and is considered as a measure of the relative hydrophobicity of the phases.

In this work, $\Delta G(CH_2)$ for five different polymer-salt ATPSs are obtained from the experimental partition coefficients (K) of a series of dinitrophenylated amino-acids (DNP-amino-acids) at 23 ºC.

Experimental

Preparation of ATPSs: Biphasic systems were prepared at 23 ºC mixing suitable amounts of water and stock solutions of the polymers (PEG or UCON) and the salts (Na_2SO_4, Li_2SO_4 or $(NH_4)_2SO_4$) in eppendorf tubes. The total weight of all systems was about 1.2 g.

Partition coefficients: The partition coefficients of the five DNP-amino-acids (*N*-(2,4-dinitrophenyl)glycine, *N*-(2,4-dinitrophenyl)-L-alanine, *N*-(2,4-dinitrophenyl)-DL-*n*-valine, *N*-(2,4-dinitrophenyl)-DL-*n*-leucine and *N*-(2,4-dinitrophenyl)-DL-α-amino-*n*-caprylic acid) were obtained adding different amounts of a given DNP-amino-acid stock solution (from 0 to 100 mg) to six replicates of the ATPSs with the same feed composition. The components were vortex mixed for 2 minutes and centrifuged at 10^4 r.p.m. for 15 minutes to achieve phase separation. Samples of each phase were withdrawn, conveniently diluted, and their absorbance at 362 nm was measured. Partition coefficients were calculated as the slope of the straight line obtained when comparing the concentration (mg/mL) in the polymer-rich phase against that in the salt-rich phase.

Results and Conclusions

The logarithms of the partition coefficients as a function of the number of equivalent methylene groups, $n(CH_2)$, in the aliphatic side chain of the homologous DNP-amino-acids are shown in figure 1.

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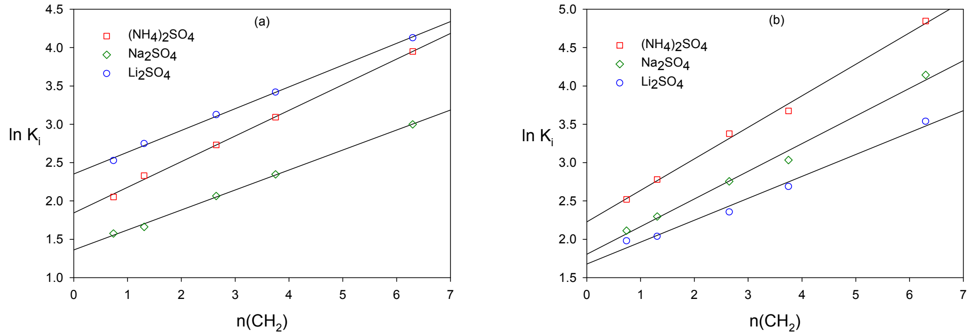

Figure 1: Logarithm of partition coefficients for a series of DNP-amino-acids in (a) PEG-salt and (b) Ucon-salt ATPSs at 23 ºC. Lines are linear regressions of experimental data.

$\Delta G(CH_2)$ values were calculated from the partition coefficients of the five DNP-amino-acids in the five polymer-salt ATPSs (Zaslavsky, 1985). Table 1 presents the $\Delta G(CH_2)$ values obtained together with values from literature (Rodríguez et al., 2007) for comparison purposes.

Ucon-salt ATPSs provide higher values of $\Delta G(CH_2)$ than the corresponding PEG-salt systems. This fact indicates that the Ucon-rich phase is more hydrophobic than the PEG-rich phase. The same conclusion can be obtained from the direct inspection of the PEG and Ucon chemical structure: PEG is an ethylene oxide homopolymer while Ucon is a random copolymer of ethylene oxide and propylene oxide monomers (larger alkyl chain).

The higher relative hydrophobicity of the Ucon-rich ATPSs suggests a more efficient biphasic separation system, with lower cross-contamination.

Table 1: Comparison of $\Delta G(CH_2)$ for six ATPSs at 23ºC

System	**-$\Delta G(CH_2)$ kcal/mol**
PEG-Na_2SO_4	0.153*
PEG-Li_2SO_4	0.167
PEG-$(NH_4)_2SO_4$	0.196
Ucon-Na_2SO_4	0.212
Ucon-Li_2SO_4	0.179
Ucon-$(NH_4)_2SO_4$	0.242

* from Rodríguez et al. (2007).

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Surface modification of thermoplastic polyurethane in order to enhance reactivity

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Keywords: Surface modification, Contact angle, Cell adhesion

The definition of coating procedures that could result in strong cell-repellent surfaces has been an extremely important issue, in the actual days.

Cells are unable to adhere if no cell accessible peptide sequences (e.g. RGD) are present at the material surface to which integrins can bind or other molecules are attached to which adhesion molecules like cadherins are able to interact. Several serum proteins and ECM exhibit these sequences. Therefore the capacity of cells to interact and spread on surfaces is directly defined by the ability of the surface to bind ECM [Vadgama, 2005] and/or serum proteins exhibiting these features. Latter capacity is defined by surface chemistry and structure. If cells are brought into contact with these materials no cell protrusions are seen. In culture, cell attachment and spreading is preceded by the adsorption of extracellular matrix molecules (ECM) onto the substrate surface. Cells are unable to attach and spread onto substrates that resist the adsorption of ECM proteins [Vadgama, 2005]. The biological response to materials and devices is, therefore, largely controlled by materials surface chemistry and structure. On this matter, the modification of polymeric surfaces is of outstanding importance aiming to develop materials that could maximize the materials performance in specific applications. Using such approach it is possible to join the bulk properties of certain polymers by modifying only its surface [Makamba, 2003]. Depending on the application, different properties may be desired.

In this research work reactivity of a thermoplastic polyurethane (TPU) material (Elastollan\ 1180A50) surface was improve and to turn its surface cell repellent.

Square samples of Elastollan® 1180A50 (30x20x2mm) processed by injection moulding, were oxidized with hydrogen peroxide (H_2O_2) in order to enhance the reactivity of the TPU surface and then impregnated with Polyethylene glycol (PEG 400). The oxidation time (2, 4 and 7 hours) were evaluated as well as the effects of the PEG impregnation.

Material's performance and properties were evaluated using different polymer characterization techniques: Determination of surface energy by contact angle measurement for surface analysis; and dynamical mechanical thermal analysis (DMTA) for thermal characterization.

Generally, the contact angle decreases when different modifications were carried out. This result can be ascribed to the presence of hydroxyl groups, which leads to an increase in hydrophylicity. This fact is also observed by the results obtained for the surface energy, which were determined according to Owens-Wendt- Rabel and Kaelbe method (OWRK) using the obtained contact angles. The increase of surface energy can be explained by the introduction of polar functional groups.

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The graft copolymerizations revealed small changes of glass transition temperature (Tg) compared to the unmodified TPU.

Cell spreading behavior on the different materials were analyzed. Half part of each sample was plasma treated prior inoculation with 3T3 mouse fibroblast cells in order to remove locally the coatings and at the same time increase hydrophillicity. The incubation period lasted two days. Then the cells were fixed and the actin cytoskeletons as well as the nucleus were stained with fluorescent dyes. The cell spreading was analysed by confocal microscopy.

Cells inoculated on non plasma and plasma treated H_2O_2 coated surfaces (2h, 4h and 7h) developed also a well established actin cytoskeleton.

A part of the inoculated 3T3 fibroblasts was able to adhere on the H_2O_2PEG coatings. This was true for the plasma as well as the non plasma treated samples. However, on non plasma treated surfaces less cells were able to adhere than on the plasma treated surfaces. On plasma treated and on non plasma treated coated surfaces cells developed an actin cytoskeleton, which was poorly established and often reduced to a small area around the nucleus.

From all the studied modifications, surface oxidation for 2 hours and then impregnated with PEG seems to be the most cell repellent surface.

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Adhesion to and viability of *Salmonella* Enteritidis on food contact surfaces

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Keywords: adhesion, food processing surfaces, *Salmonella* spp., viability

Many cases of food poisoning occur in the domestic environment and can be associated with improper food handling and ineffective cleaning by consumers. These practices could lead to the introduction and spread of bacterial contamination in the kitchen and if not subsequently removed could present an infection risk. When adhered to kitchen surfaces, cells are not easily removed by normal cleaning procedures. Therefore, they can be a source of contamination for other foods and objects. This phenomenon is known as cross-contamination. Many studies have investigated bacterial contamination in domestic kitchens and demonstrated that utensils/sites like sponges, clothes, sinks, taps, chopping boards, and hands of kettle and refrigerator can be contaminated (Gorman et al., 2002; Kusumaningrum et al., 2003; Haysom and Sharp, 2003). Like some other foodborne pathogens, *Salmonella* are able to adhere to inert food contact surfaces and to form biofilms. Nowadays, there are over 2500 known types, or serotypes, of *Salmonella*. However, *Salmonella* enterica serovars Enteritidis and Typhimurium are the *Salmonella* types most frequently associated with human illness. Recently, *S.* Enteritidis has emerged as a major serotype in human infections and in chicken contamination (Burr et al., 2005).

In order to modify the surface characteristics of materials to minimize bacterial adhesion it is important to investigate which properties are determinant in the adhesion process. The aim of this work was to investigate the influence of surface roughness and hydrophobicity in the adhesion ability of four isolates of *Salmonella* Enteritidis. The adhesion was performed on eight types of materials of different surface properties, commonly found in kitchens (stainless steel 304 (SS 304), marble, granite, glass, polypropylene from a basin (PPb), polypropylene from a cutting board (PPcb) and two kinds of silestone (white silestone (wST) and beige silestone (bST)).

Three clinical isolates (355, 357, CC) and one food strain (355) of *Salmonella* Enteritidis were used in this study. Adhesion assays were performed in a static mode, in which 2 cm^2 coupons were immersed in the bacterial suspension for 2 hours. For enumeration of adherent bacterial cells, the coupons were stained with DAPI 0.1 g/l and observed under epifluorescence microscopy. The coupons with adhered cells were also observed by scanning electronic microscopy (SEM). The viability of adhered cells was determined using the LIVE/DEAD Backlight kit (Molecular Probes, Eugene, OR, USA) through which cell membrane integrity is assessed. The hydrophobicity of the materials as well as of the bacterial cells was determined by contact angle measurements through the sessile drop technique. Material surfaces roughness and topography was assessed by atomic force microscopy (AFM).

Figure 1 demonstrates that *Salmonella* strains adhered in greater extent to stainless steel and in the lowest extent to both polymers, while no significant differences on the number of adhered cells were detected among the other materials assayed. It can also be observed that, *S.* Enteritidis 358 is the strain that displayed a higher amount of adhered cells, followed by strain CC, 355 and 357. No correlation was observed between the extent of adhesion and the hydrophobicity of the tested materials. Actually, stainless steel (hydrophobic material) presents the highest number of adhered cells while both polymers (also hydrophobic materials) present the lowest number. In addition, glass and beige silestone are hydrophilic materials and present also a great extent of colonization by the *Salmonella* strains.

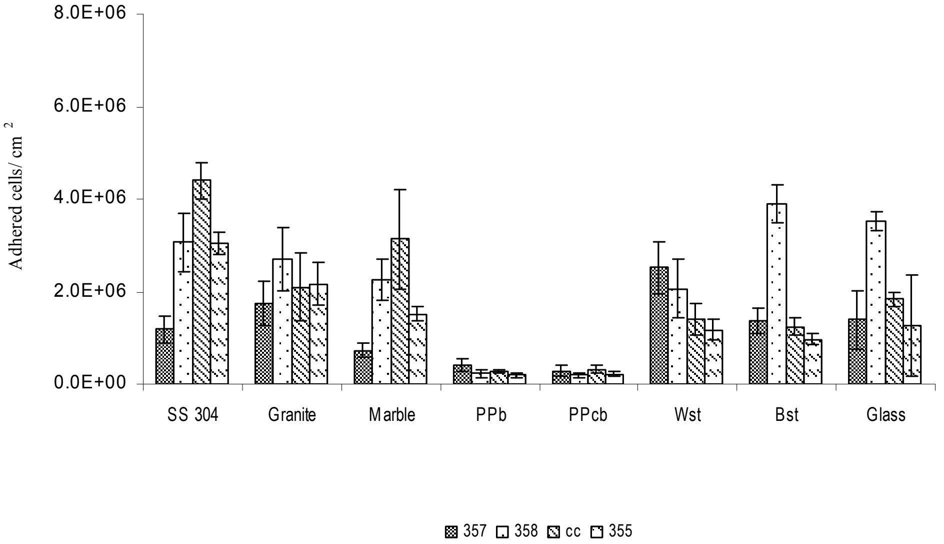

Figure 1. Number of adhered cells per cm^2 of the *Salmonella* strains to the materials.

Considering surface roughness, no correlation was also found between this property and the ability of adhesion of the *Salmonella* strains. In fact, glass is the smoothest material (average roughness of 1.6 nm) and presents a high number of adhered cells of all strains. On the contrary, white silestone is the material with the highest roughness (average roughness of 31.5 nm) and does not display the highest extent of adhesion.

It must be noted that, even though both silestones have incorporated an antimicrobial product (Microban), these materials present an extent of adhesion greater than both polymers. Thus, it seems that the incorporation of Microban is not totally effective against the adhesion of these pathogenic bacteria. On the other hand, this raised the question that adhered cells, especially to silestone, might not be viable. However, cell viability assays, revealed that the lowest percentage of *Salmonella* strains survival was found on white silestone (16 % cells with intact membrane) while, curiously, in silestone beige the percentage of viable cells was high (53.0 % cells with intact membrane). Stainless steel was the material displaying the highest number of viable cells (99.0%).

The results put to evidence that the physico-chemical properties of surface materials are not a determinant factor in the process of adhesion of the *Salmonella* strains studied. In fact, the main conclusion that was drawn is that *Salmonella* adhesion is strongly strain dependent and this can constitute a factor of virulence.

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Biofilms formation and biocorrosion in coupons exposed in marine environment

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Keywords: biocorrosion, biofilm, carbon steel AISI 1020, stainless steel AISI 304L

Abstract

The study of the biocorrosion showed the attention of numerous investigators due to necessity of developing technology that it aims to increase the time of useful life of the materials used in the diverse industries, or the development of new more resistant materials (Videla,2005). So that such fact occurs is necessary a deeper knowledge regarding the mechanisms that involve the formation of biofilms as well as the involved microorganisms in this process, considering itself it variety of microorganisms species of different regions (França et al. 2000). The existence of biofilmes is one of the most exemplary organized forms of microbial life capable of adaptation in extreme ambient conditions, as temperatures between -12°C and 110° C, values of pH between 0,5 and 13, pressures between 0 and 1400 bar (Flemming,1991). It is estimate that 90% of the microorganisms of the planet live under the form of biofilmes and that almost does not exist no surface that is not or it cannot come to be colonized for bacteria (Characklis and Marshall, 1990). This work had for objective to study the biocorrosion in a static system using sea water from a located Thermoelectric Plant in SUAPE-PE, Brazil

Materials and methods

The experiments had been lead in opened static system. The water samples always had been collected in the same place and analyzed under the microbiological aspect. Two types of coupons had been used: carbon steel AISI 1020 and stainless steel AISI 304L. The coupons had been collected and analyzed in the period of 45, 90 and 120 days. It was quantified the sessiles microorganisms: aerobic bacteria and anaerobic totals, iron-oxidants bacteria, sulfate-reducing bacteria (SRB), *Pseudomonas aeruginosa* as well as fungi filamentous of the water.

Results and Discussion

In carbon steel coupons aisi 1020 it was observed presence of different microbial groups in the surface of the metal in function of the exposition time. Important microorganisms responsible for the biocorrosion process are present. We have to stand out the iron-oxidants bacteria that had reached the maximum value in 30 days. These bacteria are capable to produce flocs and fouling deposits (inorganic and biological) in the industrial water systems and produce cloggings in the extractive industry of oil.

The biofilms formed in the surfaces of stainless steel coupons AISI 304L had also shown the presence of different microbial groups. It can be observed that the sessile bacteria had presented a reduction in its concentration with time, with exception of the BRS and fungi that had presented a small increase.

The concentrations of anaerobes and brs had increased with time; this should be certainly because of the decrease of oxygen in the system, in consequence of its consumption for the aerobic microorganisms and formation of biopolymers that hinder the access for the biofilm base. It was observed that after 45 days of exposition of coupons de carbon steel aisi 1020 to the sea water, presence of all the analyzed microorganisms and a moderate corrosive rate of 0,0882 mm/year. While that in stainless steel aisi 304l revealed more resistant presenting taxes of corrosion between 0,0007 and 0,0023 therefore considered low. These results already were waited, in view of the chemical composition of the material whose chromium content is superior to the one of the carbon steel aisi 1020.
The figure 1 shows the adopted static system in the experiments.

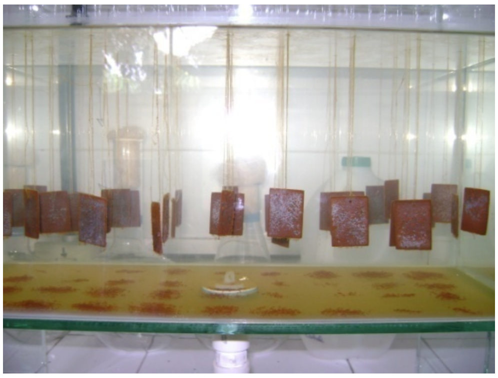

Figure 1- The static system used in the experiments.

We must to point out that the performance of the microorganisms in the corrosion process was proven through tests in sterile systems.

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Drinking water biofilm monitoring by Propella™ and Flow Cell bioreactors under different operating conditions

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Keywords: Drinking water; Biofilm monitoring; Flow rate; Support material

The dynamics of the microbial growth in drinking water networks is very complex, as a large number of interacting processes are involved. Many problems in drinking water distribution systems (DWDS) are microbial in nature, including biofilm growth, nitrification, microbial mediated corrosion, and the occurrence and persistence of pathogens (Camper 2004; Emtiazi et al. 2004). Biofilms are suspected to be the primary source of microorganisms in DWDS that are fed with treated water and have no pipeline breaches, and are of particular concern in older DWDS (Simões et al. 2006). Drinking water pipes inner-surfaces are invariably colonized by biofilms, regardless of the presence of a disinfectant residual. In addition to the possibility of causing corrosion, taste and odour problems, biofilms control the microbiological contents of the distributed water and are a potential source of pathogens (Szewzyk et al. 2000). In fact, biofilms formed within potable-water systems contain bacterial pathogens such as *Legionella pneumophila* and coliforms of intestinal and nonintestinal origin (World Health Organization 1993). Furthermore, protozoa are commonly found within water distribution systems and have been associated with the persistence and invasiveness of pathogens (Simões et al. 2007). Such findings implicate the importance of maintaining a continuous disinfectant residual in DWDS. Moreover, the examination of a DWDS reveals the complexity of such a technical system. There are not only many different materials used for the transportation and regulation of the water flow but also dramatic variations in the flow conditions between different locations. Obviously, microorganisms face a diversity of habitats with distinct physicochemical and nutritional conditions during treatment, storage, and distribution of drinking water.

In this study, monitoring of biofilm subjected to different conditions was performed using two distinct bioreactors, Propella™ and flow cell system. The drinking water source was from the public network in Braga (North of Portugal). Prior contact with the biofilm system, the residual chloride was removed from drinking water by means of granular activated carbons filter columns. Biofilms were grown on polyvinyl chloride (PVC) and stainless steel (SS) coupons, two materials commonly used on drinking water networks, under laminar (Reynolds number of 2000) and turbulent (Reynolds number of 11000) flow. The parameters analyzed were culturable cells, using R2A, and total bacteria, which were assessed using the 4,6-diamino-2-phenylindole (DAPI) stain coupled with epifluorescence microscopy. The impact of the different operating conditions in the studied parameters was established after the biofilms reached the steady state. It was found that the biofilm steady state was achieved 3 days after the starting of operating conditions for turbulent flow conditions and for both bioreactors and adhesion surfaces. Under laminar flow conditions, it was only achieved 6 days after. For those cases, the number of total bacteria was invariably higher than the culturable cells (differences always higher than 2 log). Total and culturable bacteria in turbulent flow-generated biofilms were similar in both bioreactors, regardless the adhesion surface ($p > 0.05$). Nevertheless, differences were encountered for laminar flow-generated biofilms. The Propella™ bioreactor had a higher number of total and culturable bacteria than those from the flow cell system ($p < 0.05$). Comparing the effect of the flow regime on biofilm accumulation, it was only found for the flow cell system, where turbulent flow-generated biofilms had a higher amount of total and culturable bacteria than those formed under laminar flow ($p < 0.05$). In terms of adhesion surface effects, in the flow cell system, a higher number of cells formed

biofilms on PVC surfaces comparatively to SS (*p* < 0.05), while biofilm formation on PVC and SS was similar (*p* > 0.05) in the Propella™ system, for both flow regimes.
This work demonstrates that distinct bioreactor configurations provide different biofilm data. In fact, the use of PVC or SS as adhesion surfaces lead to biofilm accumulation variability when using the Propella™ or the flow cell bioreactors. Moreover, this study highlights the need for a deeper understanding of how the large spectrum of conditions interact and affect biofilm formation potential and accumulation with the final purpose of predicting the total and culturable bacteria attached to real drinking water distribution pipes based on the system characteristics. Although the practical use of these conclusions by drinking water network companies is still limited, the information provided here might be used as a framework for future studies.

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Kinetics of *Pseudomonas fluorescens* inactivation with *ortho*-phtalaldehyde

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Keywords: Antimicrobial action, Kinetics of inactivation, Modelling of disinfection, *Ortho*-phtalaldehyde

Control of microbial growth is required in many microbiologically sensitive environments, particularly when wet surfaces provide favourable conditions for proliferation of microorganisms. An effective and wide spectrum disinfection strategy helps to overcome not only cross-resistance problems and existence of persister populations, but also the formation of recalcitrant and multi-resistant biofilms in disinfection dependent processes (Gilbert and McBain, 2003). According to the Directive 98/8/EC of the European Parliament and of the Council of 16 February 1998, concerning the placing of biocidal products on the market; biocides are active substances and preparations containing one or more active substances, put up in the form in which they are supplied to the user, intended to destroy, deter, render harmless, prevent the action of, or otherwise exert a controlling effect on any harmful organism by chemical or biological means. The biocide *ortho*-phtalaldehyde (OPA) is an aromatic compound with two aldehyde groups that has been claimed to have an effective bactericidal character with potential for high-level disinfection (Simões et al. 2007). OPA has excellent microbiocidal, mycobactericidal and sporicidal activity, acting on multiple biochemical targets (Simões et al. 2006).

In this study, the antimicrobial effects of OPA at varying concentrations, pH and exposure times were assessed against *P. fluorescens* using respirometry. The data were modelled using the classical mechanistic model of Chick-Watson (CW) formulated as the integrated form: $\log_{10}(A_0/A) = kC^nt$, where Ao is the bacterial respiratory activity without OPA application; A is the respiratory activity of the after exposure to different OPA concentrations; C is the OPA concentration; n is the concentration exponent; k is the specific coefficient of inactivation; t is the exposure time. The CW log-linear model is mechanistic in the sense that it follows the principles of mass action.

The effect of OPA on the respiratory activity and consequent *P. fluorescens* viability was invariably more significant with increasing biocide concentration. For every OPA contact time and pH value, the biocide application caused the respiratory activity decrease. However, such a decrease was more pronounced when cells were suspended at pH 5 and 9. Concerning OPA contact time, its influence was only noticeable when comparing the results 5 and 60 min after biocide exposure ($p < 0.05$). The comparison of the data obtained 60 and 180 min after OPA exposure revealed no significant differences ($p > 0.05$). The bacterial inactivation data modelling by the CW method gave good log-linear regression coefficients (R^2 always higher than 0.739 – range values: 0.739-0.966). A susceptibility coefficient (k) of 87.9 ± 9.69 and a concentration exponent of 1.04 ± 0.0476 were associated with OPA inactivation effects. The results demonstrated that OPA was efficient in the inactivation of *P. fluorescens* cultures. The mechanistic CW disinfection model accurately described the OPA antimicrobial action for the tested conditions. This collection of experimental data on biocide antimicrobial action with the application of mathematical models can facilitate the development of more reliable antimicrobial strategies and the prediction of the outcome of biocide potential on microbial behavior.

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Inocula selection for VOC removal in the non-clogging Biological Plate Tower

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Keywords: BPT, VOC, Clogging, Biofilm, Inocula

To obtain the best performance in biological reactors, an appropriate selection of microorganisms – the inoculum – is usually the first step. Three different sources of microorganisms were tested in batch and in a continuous reactor – the Biological Plate Tower, BPT – to assess the best conditions for biomass production due to the oxidation of volatile organic compounds – VOCs –, such as phenol, for batch, and toluene, for BPT experiments. Many researchers prefer to use a pure species that is known to have good performance degrading a particular one or a mixture of similar pollutants. In order to keep it from being contaminated by other microorganisms, special care must always be taken. In nature, the occurrence of pure species is very unusual. If natural conditions are to be simulated, then complex inocula should be preferred. The three inocula used were: i) *Pseudomonas putida* ATCC 17514. This bacterium had already proved its good properties in the biodegradation of toluene in several works, namely as the inoculum of a biotrickling filter (Peixoto and Mota, 1998); ii) A sample of activated sludge from a municipal wastewater treatment plant. Complex mixtures of microorganisms (e.g., bacteria, fungi and protozoa) are present in activated sludge. The great variety of compounds you can find in municipal wastewaters leads to the development of many different microorganisms. iii) An activated sludge sample from an industrial wastewater plant was the third source of microorganisms. Petrochemical industries produce wastewaters containing a great variety of compounds, where the aromatic group is included (e.g., benzene, toluene, phenol and xylenes). The ones with low molecular weights are all VOCs. Most inocula must go through an acclimatization period (that may last from minutes to months) before they are able to degrade the compounds at a desired rate. Inocula from environments where the microorganisms are already in contact with the pollutants have usually shorter times for acclimatization (enzyme induction).

For air pollution control, when VOCs and odors are the pollutants, there are three main classical biological solutions: biowashers (bioscrubbers), biotrickling filters and biofilters. The big mistake in the transposition from physical-chemical to biological reactors is to forget that the presence of biofilms completely changes the behavior and performance of the reactor. Biofilm growth is chaotic and never tridimensionally homogeneous on a random packing. Oriented packing has better results but not yet good enough. A good physical-chemical reactor does not have to be a good biological one and it is indeed a poor option in many situations (Sercu et al, 2006). Four phase reactors (liquid, gas, solid support and biofilm) always bring about hydrodynamic problems. Sloughing, channeling and clogging always occur. Good removal efficiency only makes it happen faster. Measures to deal with clogging are mostly oriented to limit the growth of the microorganisms, which are contradictory actions. Indeed, the best performance is achieved when the biofilm activity is the highest, which implies a maximum growth rate.

To solve these problems and make the process easy to operate steadily for a long time, a new concept of reactor – BPT – was designed and tested with air polluted with VOCs. The effluent simulation was achieved with the mixing chamber described in Peixoto and Mota (1997).

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The observation of the growth on the plane surfaces (top liquid distributor, base plate) of a biotrickling filter suggested a design based on horizontal surfaces. The BPT is a modular reactor. Each module contains a pile of parallel circular plates, with a single hole on the border. A new tower, with rectangular plates, is being built for future research. The plates are placed in such a way that the holes will alternate (180^o) from one to the next plate. In this way, a cascade of liquid will go downwards, changing direction from plate to plate. The gaseous stream follows the opposite direction, upwards. The bacteria attach to their top surface. The distance between plates is set according to the desired operating time. When the biomass growth causes the limiting pressure drop then a fresh one replaces the saturated module. If convenient, different modules may be seeded with different species. The performance is quite stable (the biofilm activity, surface-dependent, is kept approximately constant) and the constant surface contact area makes easy to model and scale up the process. The total surface area and the space between plates can be designed for the desired operating time and removal efficiency (Sercu et al, 2006).

The batch studies (phenol degradation) showed best results for the petrochemical plant inoculum: greater phenol concentration tolerance, and greater removal rate (up to about 90 % with a consumption rate of 0.23 h^{-1}). The specific growth rate of the microbial community was 0.14 h^{-1}, with 5 h acclimatization time. The global yield (biomass produced divided by phenol mass consumed) was 0.6 g/g.

Continuous assays with the BPT (toluene degradation) also presented best results for the petrochemical plant inoculum when biofilm activity, and both protein and polysaccharide quantities were considered.

In previous works using *Pseudomonas putida* ATCC 17514, the assays to quantify the VOC removal were done with toluene, benzene and xylene. Three contacting times were implemented (3.0 min, 1.8 min e 1.3 min). For the longer one, the average percent removals were 92 %, for toluene (with inlet concentrations up to 19 g/m^3), 96 %, for benzene (with inlet concentrations up to 16 g/m^3), and 85 %, for xylene (inlet concentrations up to 5 g/m^3). The simultaneous removal of the three VOCs, all together, was also checked. The bacteria tolerance to temperature (between 15 ℃ and 47 ℃) and to pH (from 2.2 to 10.6), one at a time, was also studied. Inside the biofilm, the bacteria proved to be very well adapted to those wide changes, and little effect on the removals was felt. The same happened when the liquid flow was severely reduced and finally stopped. No significant effect was detected in the reactor performance after some hours of operation without any liquid flow.

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Environmental conditions in *Nannochloropsis gaditana* cultivation for effective aquaculture live feed

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Keywords: *Nannochloropsis gaditana*, culture conditions, fatty acids, pigments, aquaculture

An important drawback in hatcheries is the need of large space facilities and associated costs to produce microalgae in large quantities. The use of new and versatile photobioreactors, as well as the optimization of culture conditions to produce enriched microalgal biomass, is the way to improve production yield on-site with lower costs (Tredici and Materassi 1992). Simultaneously, microalgae must have peculiar attributes for aquaculture purposes, such as high growth rate, and must be not very sensitive to fluctuations on temperature, light radiation or nutrient composition, that usually occur in open and outdoor cultivation systems. They must also have a good nutritional composition that might be transferred up to the food chain.

Aquaculture requires the effective production of microalgae as live feed, which are the primary producers for molluscs, larval stages of crustaceans and some fish species, as well as zooplankton used in food chains. Although several microalgae have been tested in aquaculture for feed, only a few of them have been successful and extensively used (Brown, 2002).

The eustigmatophyte *Nannochloropsis gaditana* is employed in many aquaculture hatcheries as the start up in the food chain and recognized as a good source of pigments and polyunsaturated fatty acids. Its high content in EPA (C20:5n3) is particularly attractive, from a dietary point of view. Rotifers which consume the microalgae carry the fatty acids to the fish larvae. However, the benefits of those compounds must be economical balanced. In fact, the environmental microalgae culture conditions, such as temperature, pH, availability of essential ions in the medium and light radiation intensity are the main factors that strongly affect both the microalgae growth and the pigments and PUFAs production yields. Finding the optima of those factors, in order to maximize the metabolite productivity, is not an easy task, mainly because they have crosslinked interferences.

In this work, we compared the influence of different media composition on the *Nannochloropsis gaditana* growth rate, pigments and fatty acid production, with the goal of selecting the most appropriate conditions for scale-up.

We observed that the use of natural resources, such as sun light radiation without any precautions, may not be the best way to achieve high productivities. The synergy (or cross effect) between the light intensity and the daily temperature variation has a stronger effect than have both factors dissociated, and it will be the main criterion on the selection of the culture system (either indoor or outdoor).

Increased concentrations of $MgSO_4$ (6 - 9 mM) in the culture medium favours the cell density and chlorophyll yield but not the fatty acids and chlorophyll cell content. pH control is essential for large scale cultivation, that must use sun light but avoiding large temperature variations, as well as light inhibition. The production of EPA, as a structural fatty acid, was shown to be associated to the cell growth and not directly obtained from reserve lipids.

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On-line monitoring of penicillin G acylase production using a fuzzy logic algorithm

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Keywords: Computational intelligence, Bioprocess automation, Fermentation process, Enzyme, *Bacillus megaterium*.

Bioprocess automation is an imposition of the highly competitive environmental in which industrial biotechnology processes are inserted. The ability to control bioprocesses at their optimal states is a necessity in modern fermentation industries. The aim is to reduce production costs and increase yield while at the same time maintaining the quality of the metabolic products (Assis and Maciel Filho, 2000). Classical inference and control techniques, however, often are not able to reach reasonable results due to the inherent complexity of these processes. The use of advanced non-conventional techniques is necessary to overcome these difficulties (Shügerl, 2001). Fuzzy systems and neural networks, for example, have attracted growing attention in the literature (Silva et al., 2001; Fernandes et al., 2007).

Fuzzy set theory, proposed by Zadeh (1965), can deal with the vagueness and uncertainty of human knowledge. It allows representing the system using linguistic terms. Fuzzy rules are more adequate and flexible than binary IF-THEN rules (Sousa Jr and Almeida, 2001, Nucci et al., 2006).

This work investigates the use of a fuzzy logic algorithm to infer the time for bioreactor harvesting in penicillin G acylase (PGA) cultivations. It was observed a relationship among the experimental enzyme activity and on-line variables. Several variables were tested to identify the better fuzzy set input. Good results were found when batch time, carbon dioxide concentration and its derivate were employed as input variables. All the rules include all the input variables, each one associated to three fuzzy sets: a) <u>antecedent</u>: input *batch time* – early (ET), excellent (XT) and late (LT); input *carbon dioxide concentration in the effluent gas (molar fraction)* – low (LC), medium (MC) and high (HC) and input *carbon dioxide concentration derivate* – negative (DCN) and zero or positive (DCZP); b) <u>consequent</u>: no-stop cultivation (NS), attention (AT) and stop cultivation (S). The proposed fuzzy logic algorithm had 13 rules. It was written in MatLAB® and implemented in LabVIEW®. Data strings were exchanged in real time between the MatLAB program and the data acquisition software, which was coupled to the bioreactor.

The microorganism employed was *B. megaterium* ATCC 14945, from Fundação Tropical (Campinas, SP, Brazil). The experiments were carried out in a 2-liters bioreactor (Applikon®) operated in batch and fed-batch mode. Dissolved oxygen concentration was controlled by manipulating stirrer speed. Temperature was kept at 30°C and the aeration rate at 2.0 L.min^{-1} (1 atm and 21°C). The bioreactor was coupled to a data acquisition system (National Instruments®) and on-line variables were stored each 10 seconds (pH, temperature, dissolved oxygen concentration, stirrer speed and molar fractions of carbon dioxide in the effluent gas). Further details about the composition, procedure and laboratory analyses can be found in Silva et al. (2006). A sumary of the most important results are shown in Table 1. The parameters of the fuzzy algorithm were tuned using a set of five assays (Runs 1 to 5). Runs 6 to 10 were used for validation. From the results (Table 1), it can be seen that the fuzzy algorithm could succesfully ascertain the region

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of maximum enzyme concentration, when harvesting should start. In all cases, values above eighty percent degree of certainty were found.

Table 1. Sumary of the most important resulsts obtained during penicillin G acylase production process by *Bacills megaterium*.

Assays	Cell Concentration (g.L^{-1})	Enzyme Activity (UI.L^{-1})	Degree of Fuzzy Certainty (%)	Optimal interval to stop the cultivation (h)
1	4.6±0.5	283± 5	81 % (12 h)	12 – 14
2	4.1±0.4	284± 4	82 % (16 h)	15 – 17
3	4.2±0.3	297± 4	80 % (14 h)	14 – 16
4	3.6±0.3	275±13	81 % (15 h)	16 – 18
5	4.5±0.3	532±13	80 % (16 h)	16 – 18
6	4.0±0.4	535±14	80 % (14 h)	15 – 16
7	4.6±0.3	275± 3	85 % (16 h)	18 – 20
8	4.9±0.3	346±10	81 % (12 h)	12 – 13
9	4.5±0.3	143±10	81 % (18 h)	17 – 20
10	4.1±0.2	240± 6	83 % (16 h)	16 – 18

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Monitoring by HPLC-PDA impurities generated by forced degradation conditions on an active pharmaceutical ingredient

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Keywords: Degradation Studies, FDA stability guidance

Abstract

The US Food & Drug Administration (FDA) stability guidance for active pharmaceutical ingredients (API) stipulates that an API should be subjected to a number of forced degradation and stress conditions to evaluate its stability. The current ICH guidances (Q2A and Q2B) do not specify how degradation studies are to be conducted; this was left to the discretion of the responsible companies.

Forced degradation is one of the activities that must be performed early in the development process to ensure that the quality control method is capable of discriminating the desired API from its degradation products, before a lot of time, effort and money are invested. The guidance documents do not indicate detailed conditions, so the conditions and interpretations are left up to the team who work in the field to be decide. Suggested forced degradative conditions are summarized in table 1.

Forced degradation	condition
Acid	HCl 0.01M, 30 minutes
Basic	NaOH 0.01M, 10 minutes
Oxidative	3% Hydrogen peroxide, 10 minutes
Light	Light chamber, 9 days
Temperature	5ºC, H=60%, 1 day
	25ºC, H=60%, 1 day
	60ºC, 5 days

Trial and error are needed to find the proper combination of stress agent concentration and time to effect degradation, preferably in the 20-30% range. Depending on the API, not every stress agent may effect degradation, but each agent has to be evaluated to determine whether degradation results. In this study we examine diferent alternatives for producing and then analysing with HPLC-PDA the stability of a class of industrially important APIs in late phase development.

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FT-IR spectroscopy and chemometrics in clinical microbiology

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Keywords: FT-IR Spectroscopy, Multivariate Analysis, Microbiology

Clinical microbiology deals with the laboratory diagnosis of infectious diseases trying to elucidate spreading routes of given bacterial strains and further characterise the microbial species and their resistance to antimicrobial agents. Most clinical laboratories rely on the phenotype-based or molecularly oriented methods for straightforward microbial typing at the different taxonomic levels. Although precise, these methods are expensive, time consuming, and they require a highly trained staff, which limits their applicability on the routine basis.

The Fourier transform infrared (FT-IR) spectroscopy has been by now successfully applied for rapid, accurate and cost-effective identification of pathogenic microorganisms. It is considered to be a powerful and versatile technique in microbiology that can assess the overall molecular composition (spectral fingerprints) of microbial cells in a non-destructive manner. Still a major concern in using FT-IR for pathogens discrimination are spectral preprocessing procedures and their impact on the increase of chemometrics models robustness. A hierarchical classification strategy together with unsupervised and supervised (Principal Component Analysis – PCA, Partial Least Squares - PLS) pattern recognition methods was used for the discrimination. Since selection of discriminative spectral regions is a critical step in calibration model development, different variable selection methods (genetic algorithm, PLS-bootstrap) have been tested as well.

In this presentation, the general contribution of FT-IR spectroscopy and chemometrics to pathogenic microorganisms' discrimination at different taxonomic levels will be discussed. Analysis of *Acinetobacter baumanni*, *Enterococcus faecium* and *Staphylococcus aureus* isolates showed clear separation within the genus level. Closely related *Salmonella enterica* serotypes (Enteritidis, Typhimurium, Bredeney, Goldcoast, Rissen, Hadar, Derby, Essen, Virchow, Mbandaka, Menden, Agona, Brikama, Saintpaul, Anatum, Infantis, Braenderup, Mikawasima) examined by FT-IR spectroscopy and chemometrics demonstrated the possibility of correct differentiation at the serotype level. Our latest results show as well that there is a considerable potential to effectively fulfil the current requirements for rapid and correct differentiation of *Salmonella* Enteritidis phage types.

Risk quantification of microbiological contamination in aseptic processes

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Abstract

Being the sterility assurance of products based on the conformity of all the manufacturing process parameters – Quality by Design, it's essential to have a way of measuring the impact of the different manufacturing scenarios on the global microbiological risk.

Here we report on a risk quantification tool that allows the rational integration of the available information regarding microbiological contamination of aseptic processes.

This tool is based on a mathematical model appropriate to quantify situations involving multiple criteria. The success of its implementation depends on the correct identification of the critical points and the cooperation of people with solid process understanding.

Although the work presented emphasizes the process characterization, like the enhancement of possible worst-case manufacturing scenarios, other applications can be considered, like the support of batch release decisions or as a PAT tool, since it's possible to use in it real time data.

A discussion of all these aspects will be made based on the experience obtained in developing and applying this toll in a sterile production facility.

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Novel method for accelerated analytical control of biological (bacteriological) agents

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Keywords: biological pollution, analytical control, hard nutrient media, solid inorganic material.

The present work is devoted to creation of the accelerated microbiological analysis for environment monitoring. The new approach to preparation of hard nutrient media is proposed, namely, – impregnation by solution of nutrient materials by a special way of the treated plates from solid inorganic materials. Applying such materials as a basis for hard microbiological nutrient media became possible after the capacity of different microorganisms for active growth and formation of colonies on a surface of such nutrient media was detected in which were used as a sealing basis specially prepared solid inorganic materials but not gel-forming matters.

The plates prepared by the given way, in sterile conditions seal in polyethylene packages where they can be stored a long time without loss of exploitative properties. The duration of preparation of such media directly to an inoculation makes 5-10 min. The specimens of different microorganisms groups, actinomyces, streptococci, yeasty and mouldy fungi grow well on such media.

The results of tests of hard microbiological nutrient media prepared by developed and known ways confirm that the developed way allows to prepare nutrient media with higher exploitative properties, provides essential reduction of duration and simplification of preparation process of media to an inoculation. It meets the requirements of express extraction pathogenic microorganisms from environment and their cultivating.

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Influence of phosphate and maltose concentrations in the dextransucrose production by *Leuconostoc m. B-512-f*

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Keywords: dextransucrose, dextran, *Leuconostoc mesenteroides*

1 Introduction

Dextran $(C_6H_{10}O_5)_n$ is a polysaccharide of D-glucose. The main applications are: blood-plasma substitute, chromatographic separations, or sweetener and thickening agent in the food products. The enzyme responsible for the synthesis of dextran is known as dextransucrose, synthesized by species from genera *Leuconostoc and Streptococcus*.

This research evaluate the influence of phosphate and maltose concentrations on the production of dextranosucrose by *Leuconostoc mesenteroides* B-512-f using sucrose as substrate. The presence of other enzymes different from dextransucrose was studied.

2 Material and Methods

Leuconostoc mesenteroides NRRL B512-f from DSMZ was used. The strain was stored in Petri dishes at 4° C and the inoculum was prepared in MRS broth and cultivated for 18 h at 30 ºC.

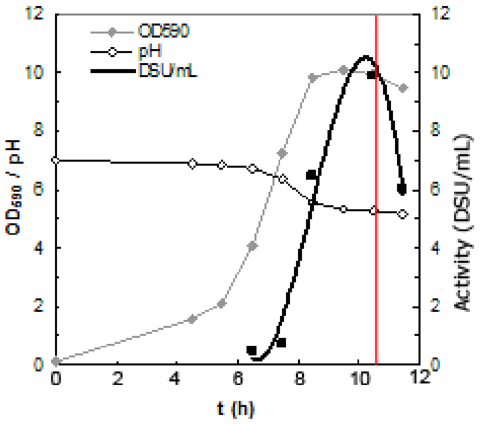
A batch reactor (1 L) perfectly mixed, 200 rpm, was employed. The fermentations were conducted at 23ºC and an initial value of pH of 7. The composition of the medium was: 40 g/L sucrose, 20 g/L extract yeast; NaCl: 0,01 g/L; $MnSO_4 \cdot H_2O$: 0,01 g/L; $FeSO_4 \cdot 7H_2O$: 0,01g/L; $MgSO_4 \cdot 2H2O$: 0,20 g/L; $CaCl_2$; 0,10g/L.; K_2HPO_4 concentrations ranged from 1 to 40g/L and Maltose concentrations from 2.5 to 10 g/L.

Analysis of pH, optical density, enzymatic activity of dextransucrose (measured as DSU) and carbohydrates were carried out.

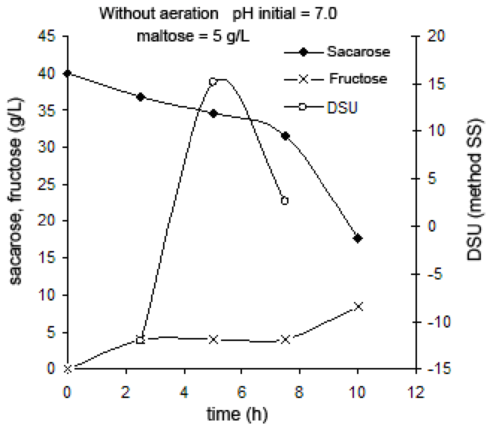
3 Results

Influence of the acceptor maltose

Figure show the results corresponding to fermentations, without maltose (a) and with a concentration of 5 g/L (b). As it can be seen the enzyme activity is higher when maltose is present in the fermentation medium.



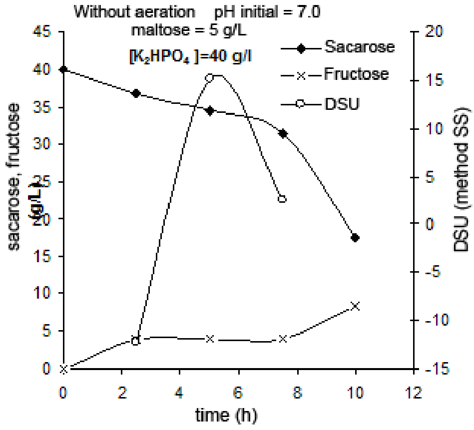
(a) maltose: 0 g/L

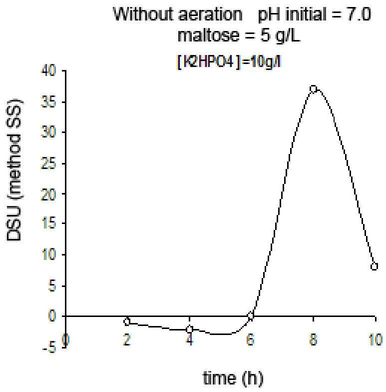


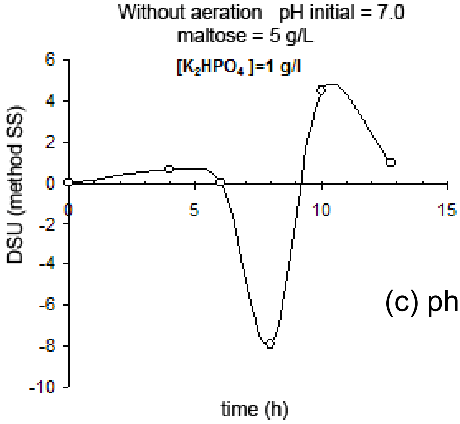
(b) maltose: 5 g/L

Influence of phosphate concentration

The results obtained in the essays are shown in next figure. Dextransucrose exhibited a greater activity (up to 35 DSU) for 10 g/L of phosphate and 5 g/L of maltose.







(c) phosphate = 1 g/L

Finally, electrophoresis in polyacrilamide gel with SDS was used for check enzymes. Results indicated a clear lane (molecular weight of 190 kDa) related to dextransucrose, and diffuse lanes, that would indicate the presence of other enzymes in very low concentrations, and that could interfere in the activity of dextransucrose.

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Acknowledgements

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A software tool for design of evaporator systems

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Keywords: Single and multiple effect evaporators, Forward feed, Backward feed, Software tool

The concentration of a solution of a non-volatile component is usually accomplished by evaporation (McCabe et al., 1993). This unit operation is used in different types of industries, ranging from the food to the chemical industry. Due to the fact that evaporation is an energy intensive unit operation, multiple-effect evaporators are usually used to decrease energy consumption. However, the increasing equipment cost limits the maximum number of evaporators to be used in a multiple-effect system. The evaluation of the optimal number of effects requires an exact calculation and cost evaluation. Evaporation calculations are usually iterative and tedious, a hard task to carry out manually. The calculations require the decision on the type of mass and heat balances to be used and data for enthalpy and boiling point raise, which is usually available in the form of graphics and tables. This usually requires numerous interpolations, and sometimes, even extrapolations.

The development of a software tool for the design of single and multiple effect evaporators appears to be a potentially useful tool for engineers. Thus, this paper presents a software tool, developed in Visual Basic for Applications, and available in Microsoft Excel for the design of single and multiple effect evaporators. This software is based on the Newton-Raphson method, and allows the design of evaporator systems with forward or backward feed, and having either negligible or significant boiling point elevations (Holland, 1975).

The calculations of single and multiple effect evaporators require a set of initial data, which include:

- Initial and final concentration of the solution being evaporated;
- Temperature of the diluted solution at the inlet of the evaporator;
- Temperature and/or pressure of the heating steam;
- Data on the properties of the solution undergoing evaporation: specific heat, enthalpy, heat conductivity and boiling point. And also, steam properties and type of flow.

Figure 1 shows, as an example, the data introduced for a system with 3 multiple-effect evaporators, working in backward mode, used for the concentration of a solution of sodium hydroxide.

The preliminary design of an evaporator system usually reduces to the calculation of the heat transfer area and steam consumption needed to achieve the desired concentration, for a specified set a steady-state operating conditions. Figure 2 shows the output of the application, giving the vapour flowrate needed, the area of each effect and the corresponding temperatures.

This application can be used as a training and/or design tool for single or multiple effect evaporators, and as a teaching aid for chemical and biological engineers.

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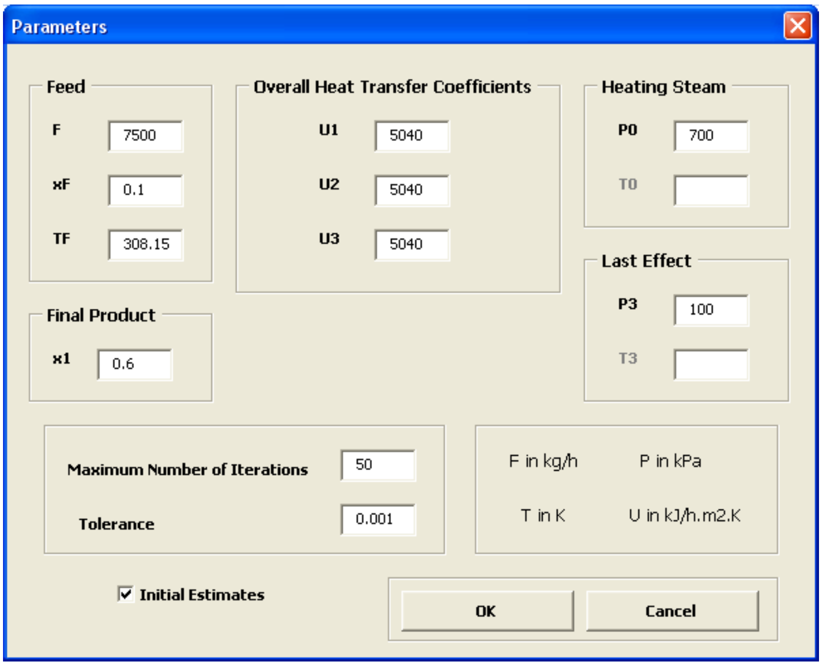


Feed

F: 7500

xF: 0.1

TF: 308.15

Final Product

x1: 0.6

Overall Heat Transfer Coefficients

U1: 5040

U2: 5040

U3: 5040

Heating Steam

P0: 700

T0:

Last Effect

P3: 100

T3:

Maximum Number of Iterations: 50

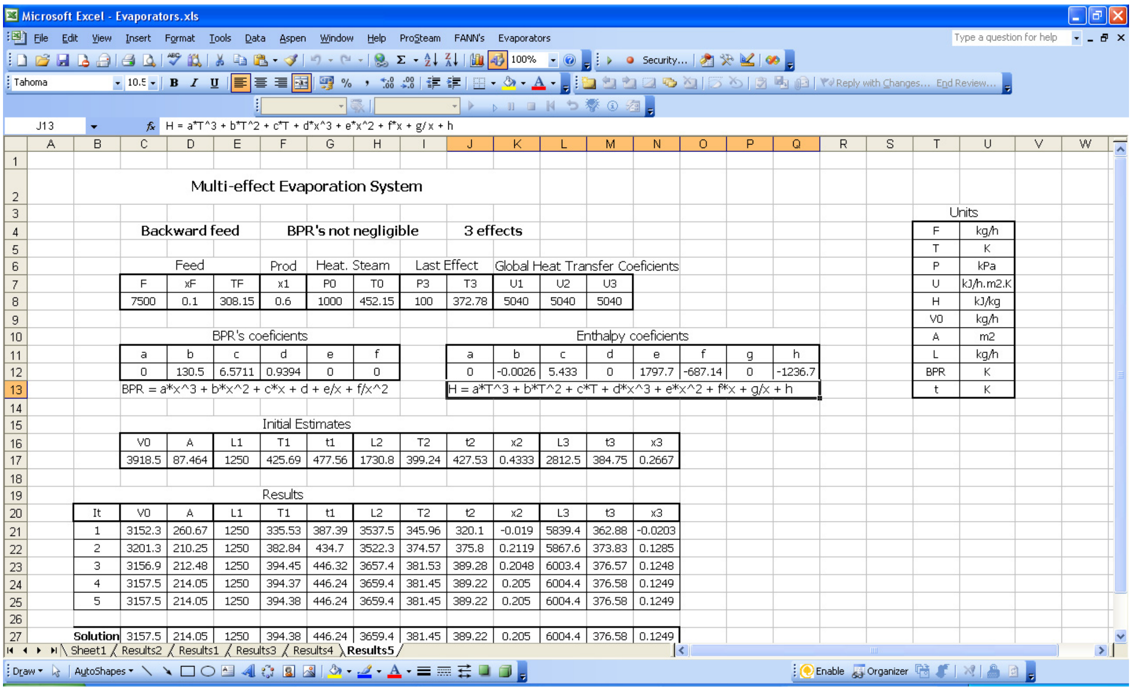
Tolerance: 0.001

F in kg/h P in kPa

T in K U in kJ/h.m2.K

☑ Initial Estimates

Figure 1 – Data introduced for a 3 multiple-effect evaporator system



J13 H = a*T^3 + b*T^2 + c*T + d*x^3 + e*x^2 + f*x + g/x + h

Multi-effect Evaporation System

Backward feed BPR's not negligible 3 effects

Feed			Prod	Heat. Steam		Last Effect		Global Heat Transfer Coeficients		
F	xF	TF	x1	P0	T0	P3	T3	U1	U2	U3
7500	0.1	308.15	0.6	1000	452.15	100	372.78	5040	5040	5040

BPR's coeficients

a	b	c	d	e	f
0	130.5	6.5711	0.9394	0	0

BPR = a*x^3 + b*x^2 + c*x + d + e/x + f/x^2

Enthalpy coeficients

a	b	c	d	e	f	g	h
0	-0.0026	5.433	0	1797.7	-687.14	0	-1236.7

H = a*T^3 + b*T^2 + c*T + d*x^3 + e*x^2 + f*x + g/x + h

Units

F	kg/h
T	K
P	kPa
U	kJ/h.m2.K
H	kJ/kg
V0	kg/h
A	m2
L	kg/h
BPR	K
t	K

Initial Estimates

V0	A	L1	T1	t1	L2	T2	t2	x2	L3	t3	x3
3918.5	87.464	1250	425.69	477.56	1730.8	399.24	427.53	0.4333	2812.5	384.75	0.2667

Results

It	V0	A	L1	T1	t1	L2	T2	t2	x2	L3	t3	x3
1	3152.3	260.67	1250	335.53	387.39	3537.5	345.96	320.1	-0.019	5839.4	362.88	-0.0203
2	3201.3	210.25	1250	382.84	434.7	3522.3	374.57	375.8	0.2119	5867.6	373.83	0.1285
3	3156.9	212.48	1250	394.45	446.32	3657.4	381.53	389.28	0.2048	6003.4	376.57	0.1248
4	3157.5	214.05	1250	394.37	446.24	3659.4	381.45	389.22	0.205	6004.4	376.58	0.1249
5	3157.5	214.05	1250	394.38	446.24	3659.4	381.45	389.22	0.205	6004.4	376.58	0.1249
Solution	3157.5	214.05	1250	394.38	446.24	3659.4	381.45	389.22	0.205	6004.4	376.58	0.1249

Figure 2 - Output of the application.

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Process synthesis for hydrogen production from glucose: bioreactor design and scale-up and downstream process

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Keywords: Reactor scale-up; hydrogen from biomass; separation; process simulation.

Introduction. Recognized as one of the most promising energy carrier of the future hydrogen can be produced using several sources, renewable or not. Amongst the renewable alternatives, some have been distinguished as the most promising, such as: electrolysis of water, electrolysis of steam, thermochemical decomposition of water, photoelectrochemical processes, biophotolysis of water, photodecomposition of organic compounds, ethanol reforming and fermentation of organics. The fermentation of organic compounds is considered one of the most promising sustainable alternatives in the future for hydrogen production from biomass. On this basis, this work aims to discuss the integration of the following subjects in new process design: experimental data published in literature; bioreactor design and scale up; synthesis of downstream process; process simulation; technical-economic viability analysis, and sustainability. Hydrogen production by fermentation route was chosen as a case study.

Methodology. The species from the genus *Clostridium* are known as acid producers and usually they can ferment glucose to butirate, acetate, carbon dioxide and molecular hydrogen. Lin et al. (2007) carried out a series of experiments with four species of *Clostridium* and proposed a Monod like kinetic model modified by an empirical law that takes into account pH inhibition. Amongst the studied microorganisms, *C. beijerinckii* presented the greatest productivity, whose parameters of reference had been used in this work.

Using the experimental and kinetic data presented by Lin et al. (2007), new kinetic parameters model were adjusted in this work, considering only glucose as limiting substrate. After that, bioreactor scale-up was accomplished (stirred batch reactor), using mathematical unsteady-state mass balances (Bailey and Ollis, 1986) and computer simulation. The model is a set of ordinary differential equations with initial value problem and it was solved using the free software Scilab (www.scilab.org).

The proposed downstream process is constituted by: (i) gas separation subsystem, with hydrogen recovery and compression; it was also considered the purification and compression of carbon dioxide as valuable byproduct; (ii) liquid separation, namely acetic and butyric acids, by means of liquid-liquid extraction and distillation. The proposed downstream process flowsheet was simulated using the free software and chemical processes simulator Sim42 (Assis and Oliveira-Lopes, 2005; Assis et al., 2006) with VMGSim interface (www.virtualmaterials.com). For CO_2 capture, it was considered absorption with MEA and it was considered a 99% of recovery, as reported in the literature. Peng-Robinson and Advanced Peng-Robinson were used as thermodynamic package in all simulations. The costs of the equipments were calculated using the methodology proposed by Peters and Timmerhaus (1991) and Henley and Seader (1981).

Results and discussion. The process flowsheet sinthesys was accomplished following the conceptual design methodology proposed by Douglas (1988) at level 4. Simulation shows that it is possible to obtain hydrogen with a high purity (0.999%), which increases the yield of fuel cells using hydrogen and oxygen as reactants for

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energy generation. The accuracy of a “preliminary design estimate” is typically -30% to +50% (Peters and Timmerhaus, 1991). At this level of accuracy, the resulting costs of production were 1.17 USD and a profit of 0.66 USD for kilogram of produced hydrogen. The cost of hydrogen production by fermentation reveals economically attractive, and more viable than the gasification of the biomass, that presents a cost of production of 2.2 USD for kilogram of H_2, approximately.

Conclusions. Although in initial steps of development, the technology of hydrogen production by fermentation reveals to have both technical and economical viability. In this work, it was presented a preliminary design flowsheet considering reaction and downstream. The profit showed to be attractive and detailed flowsheet and cost estimates should be done in further investigations. Based on the steps presented here, the student can see how to integrate basic and technical information in sustainable process design and simulation.

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Teaching and learning strategies to improve the development of student skills in Chemical Engineering degree

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Keywords: Teaching-learning strategies, Skills, Chemical Engineering

The organization of university education in Spain, according to the European Higher Education Area (EHEA) guidelines, will be organised in three cycles corresponding to university degrees known as Grado, Master and Doctorate. This new reorganization of university education involves relevant changes in teaching and learning methodologies. In this sense specific strategies will have to be implemented in order to help students to learn more successfully and to develop important skills and competences requested by the professional world. The subjects included in each level of education degree will be design in terms of learning outcomes and competences. Learning outcomes will be defined on basis of specific contents developed in each subject whereas the acquisition of skills requires a parallel programming of them. A summary of the main progress in competence training for chemical engineers of Valladolid University got up to now is presented in this work. Moreover, main transferable skills are identified, the possibilities and resources to develop them within our actual program of studies are analysed and a reflection of evaluation procedure is done.

The education of chemical engineers in the frame of Bologna process must be focused not only to develop learning outcomes directly connected with knowledge but also transferable skills. In this sense, the new European Higher Education Space involves and important modification of the actual teaching-learning system, based on the use of new methodologies to help student to acquire the knowledge and the skills and competences requested by the proffesional world.

The teaching and learning methods should also help to develop students' skills (Molzahn, 2006), to work both independently and in teams. Thus, to learn to function in teams, group work is necessary. To be able to communicate, communication tasks must be given and solved. To learn to learn and to take responsibility for their own learning, students must receive appropriate self-study and problem solving tasks during their study. To understand ethical, societal, environmental and professional issues, suitable examples for illustration or discussion must be included (Felder & Brent, 1999).

Recent studies have revealed that the majority of chemical engineering graduates find employment in a wide range of industries including oil, petrochemicals, pharmaceuticals, biotechnology, food and the environment. Chemical engineers are professionals able to tackling a wide variety of problems: turn laboratory bench-top phenomena into production processes, have an integrated approach to systems, work witin and often manage teams of theoretical and experimental chemists, mechanical and civil engineers, accountants and health and safety officials, apply fundamental scientific understanding and engineering principles to industrial, laboratory and molecular processes. The new working positions require the development of general and transferable skills besides chemical engineering knowledge. As a consequence universities should place more emphasis on developing students' transferable skills (Grant and Dickson, 2006).

In this work the main progress in competence training for chemical engineers of Valladolid University got up to now is presented. The partial objectives to be carried out in order to get this global aim are the followings:

1. Identify the main transferable skills for chemical engineering curricula
2. Adapt the subject programmes to help students to develop these transferable skills
3. Evaluate the student's competence acquisition level

Taking as reference the analysis extracted from numerous studies related to accreditation of engineering programs contributed by leaders of universities, associations or professional bodies (ICheme, ABET, ANECA, WCEC and UVa) have revealed that the most important generic attributes for chemical engineers are connected with the capacity to communicate effectively, including in english, the ability to work in multidisciplinary teams, the capacity to understand the basic principles underlying chemical engineering and be able to use them to set up and to solve problems and the ability to learn on his/her own, and have a recognition of the need for life-long learning (Cartón *et al.*, 2007).

To help student to develop these transferable skills an integrated and global teaching and learning methodology has been carried out at Valladolid University. The learning objectives were stablished not only for every one of the specific subjects considered but also for the general skills to be acquired for the student. So, the learning methodology includes classical and theoretical and practical classes but also propose individual and team works. Different activities such as oral presentations, written reports, discussion sessions, conferences and technical visits have been planned for all the semester subjects (Lucas *et al.*, 2008). Moreover, new methodologies for improving these transferable skills were applied. In this sense, as an example, Based Problem Learning or appropiate computational tools facilitate the application of basic principles to problem resolution.

An external evaluation to check the efficiency of this integrated learning methodology is required. It is based on specific questionaries carried out by students and teachers at the beginning and at the end of the learning process. The main aspects considered in the inquiries are mainly related to the relevance of transferable skills in their professional future and the level of development acquired, academic information, motivation of students, the working metodology and the organization and planification tasks.

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