Universidade do Minho
Escola de Engenharia

Lúcia Maria Gonçalves Ferreira das Neves

Anaerobic Co-Digestion Of Organic Wastes

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Anaerobic Co-Digestion Of Organic Wastes

Doutoramento em Engenharia Química e Biológica

Trabalho efectuado sob a orientação da
Doutora Maria Madalena dos Santos Alves
e da
Professora Doutora Domingas do Rosário Veríssimo Jacinto Tavares de Oliveira

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|ANAEROBIC CO-DIGESTION OF ORGANIC WASTES

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|SUMMARY

Anaerobic digestion is an already established process but the increasing need of bio-waste recovery has determined the emergence of new substrates, revamping the research in this field. Contrary to some other European countries, in Portugal this technology is still scarcely in use. Nonetheless, the current legislation endorses this application as a waste management and as an energy recovery process. The rapid growth of the world population, in the past decades, and the economical development of several countries resulted in the production of large amounts of related to food waste and animal manure. These wastes are good substrates for biogas production.

This dissertation reports several studies that were performed in order to evaluate and optimize the methane production of organic wastes anaerobic co-digestion. Emphasis was placed on the role of fatty wastes in the co-digestion process. Within this scope, a method is described in order to extract, indentify and quantify Long Chain Fatty Acids (LCFA), in both the solid and liquid phase. Moreover, the feasibility of anaerobic digestion of several bio-wastes that currently are landfilled is also reported.

The substrates used during these research studies were as follows: (1) simulated food waste which was made by blending melted pork lard, white cabbage, chicken breast, and potato flakes to simulate lipids, cellulose, protein, and carbohydrates, respectively; (2) food waste, collected in restaurant of the University of Minho; (3) cow manure; (4) oily waste collected in a canned fish processing industry; (5)five wastes from a coffee substitutes production facility and (6) activated sludge.

It was observed that food waste composition altered the single biomethanation potential, which implies that anaerobic digestion facilities with large variations in lipids input can have significant changes in process performance. However, whilst imposing transient fluctuations in the fat content of food waste co-digestion with cow manure, the results proved that cow manure/food waste co-digestion presents a sufficient buffer capacity to endorse lipids fluctuations, up to concentrations of 7.7 $gCOD_{oil}/L_{Reactor}$ (55% $Oil_{COD}/Total_{COD}$), maintaining an efficient overall reactor performance and stability, when the total chemical oxygen demand (COD) fed was constant. Co-digestion process of cow manure/food waste was improved by addition of oily wastes pulses. The threshold input of oily waste that enhanced the methane production in this co-digestion process was 12 $gCOD_{oil}/L_{reactor}$, considering the mixture of lipids present in the oily waste added. This corresponds to a continuous feeding of 10% ($V_{food\ waste}/V_{manure}$) with intermittent oil pulses of 5% (V_{oil}/V_{manure}). A pulse feeding of 18 $gCOD_{oil}/L_{reactor}$ induced a persistent process inhibition, detected by the decrease in pH to a minimum of 6.5 and by an increase in the effluent soluble COD and volatile fatty acids. Negative linear correlations between the achieved biomethanation % and the solid-associated LCFA or palmitic acid (C16:0), allowed to establish threshold values of 180-220 gCOD-LCFA/kg TS and 120-150 gCOD-C16:0/kg TS, respectively, that should not be surpassed in order to prevent reactor failure.

Four of the five assayed wastes from coffee substitutes, are feasible to be co-digested with activated sludge, instead of landfilled. The coffee substitute solid waste composed of 100% barley is preferable to be co-digested with the organic fraction of municipal solid waste. Although an alkaline pre-treatment before co-digestion with activated sludge is also beneficial to improve methane production, but the cost of this approach should be evaluated.

|SUMÁRIO

A digestão anaeróbia é um processo já bem estabelecido, mas a necessidade crescente de valorizar bio-resíduos em alternativa à sua deposição em aterro, tem determinado um crescente interesse pela investigação neste domínio, nomeadamente ao nível do estudo de biodegradabilidade de substratos potenciais. Ao contrário de alguns países europeus, em Portugal esta tecnologia ainda é muito pouco usada. No entanto, a legislação actual prevê a sua implementação tanto como uma aplicação na gestão integrada de resíduos sólidos, como para a produção de energia. O elevado volume de resíduos biodegradáveis agroindústriais, agropecuários e alimentares produzidos, torna-os interessantes substratos ou co-substratos para a produção de biogás.

Esta dissertação reúne uma série de estudos que foram realizados com o objectivo de avaliar e optimizar a produção de metano na co-digestão de resíduos orgânicos. Foi dada ênfase ao estudo do efeito que resíduos lipídicos têm no processo de co-digestão. Neste contexto, é apresentado um método que foi desenvolvido para extrair, identificar e quantificar ácidos gordos de cadeia longa (AGCL) tanto na fase sólida como liquida. Conjuntamente são apresentados estudos de aplicabilidade da digestão anaeróbia a vários bio-resíduos que são actualmente enviados para aterro.

Os substratos utilizados nos ensaios realizados foram: (1) resíduo alimentar simulado constituído por uma mistura de banha de porco, couve branca, peito de galinha e puré de batata, para simular os lípidos, celulose, proteína e os hidratos de carbono, respectivamente; (2) resíduo alimentar, recolhido no restaurante da Universidade do Minho; (3) chorume de vacas; (4) óleo residual de uma indústria conserveira de peixe; (5) cinco resíduos da indústria de processamento dos substitutos de café e (6) lamas activadas.

Os resultados mostraram que a composição do resíduo alimentar, quando utilizado como mono-substrato, alterou o potencial da produção de metano, sugerindo que digestores anaeróbios que sofram uma grande variação no conteúdo lipídico na corrente de alimentação de um resíduo facilmente biodegradável como o estudado, possam apresentar alterações na sua performance. No entanto, quando se provocaram flutuações ocasionais de gordura na co-digestão de chorume de vacaria com resíduos alimentares, os resultados demonstraram que este sistema apresenta uma boa capacidade tampão para suportar flutuações de lípidos, até concentrações de 7.7 $gCQO_{óleo}/L_{reactor}$ (55% $Óleo_{CQO}/Total_{CQO}$), mantendo a performance e estabilidade dos reactores, quando a carência química de oxigénio (CQO) da alimentação era constante. A produção de metano do processo de co-digestão de chorume de vacaria/resíduos alimentares foi favorecida pela adição de pulsos de óleo até 12 $gCOD_{óleo}/L_{reactor}$. Este valor corresponde a uma alimentação contínua de 10% ($V_{resíduos\ alimentares}/V_{chorume}$) com pulsos intermitentes de 5% ($V_{óleo}/V_{chorume}$). Foi no entanto detectada uma inibição persistente da produção de metano para um pulso de 18 $gCOD_{óleo}/L_{reactor}$. A diminuição de metano foi acompanhada de uma diminuição do pH para valores mínimos de 6.5 e um aumento da CQO solúvel e ácidos gordos voláteis no efluente. Obtiveram-se correlações lineares negativas entre a percentagem de bio-metanização e os AGCL e ácido palmítico (C16:0) associados aos sólidos, que permitiram estabelecer valores limite de 180-220 gCQO-AGCL/kg ST e 120-150 gCQO-C16:0/kg ST, respectivamente, que não devem ser ultrapassados de forma a prevenir a falha do processo de co-digestão.

Quatro dos cinco resíduos de sucedâneos de café que foram testados mostraram ser passíveis de ser co-digeridos com lamas activadas. O resíduo composto só por cevada mostrou melhor eficiência quando co-digerido com resíduos de alimentos. Outra opção para este resíduo, é submetê-lo a uma pré-hidrólise alcalina antes do processo de co-digestão, mas esta opção acarreta custos que devem ser avaliados.

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| LIST OF SYMBOLS AND ABBREVIATIONS

AD	Anaerobic Digestion
AGCL	Ácidos gordos de cadeia longa
BMP	Biochemical methane potential
BW	Barley waste
C/N	carbon-to-nitrogen
C12:0	Lauric acid
C14:0	Myristic acid
C15:0	Pentadecanoic acid
C16:0	Palmitic acid
C16:1	Palmiticoleic acid
C18:0	Stearic acid
C18:1	Oleic acid
C18:2	Linoleic acid
CH_4	Methane
CM	Cow manure
CO_2	Carbon dioxide
COD	Chemical oxygen demand
CSTR	Continuous stirred tank reactor
DCM	Dichloromethane
EEA	European environmental action
FID	Flame ionization detector
FW	Food waste
GC	Gas chromatography
GHG	Greenhouse gas
H_2	Hydrogen
HCl	Hydrochloric acid
He	Helium
HPLC	High performance liquid chromatography
HRT	Hydraulic retention time
IS	Internal standard
Kh	kinetic coefficients of the first order rate of hydrolysis
LCFA	Long chain fatty acids
LOD	Limit of detection
LOQ	Limit of quantification
MMPR	Maximum methane production rate
MTBE	Methyl Tertbutyl Eter
$NaHCO_3$	Sodium bicarbonate
ND	Not determined
OFMSW	organic fraction of municipal solid waste
OIL	Oil waste
R1	Reactor 1
R2	Reactor 2
R3	Reactor 3
R4	Reactor 4
rpm	Revolutions per minute

RSD	Relative standard deviation
SBR	Anaerobic sequencing batch reactor
SMA	Specific methanogenic activity
STP	Standard temperature and pressure conditions
TKN	Total kjeldahl Nitrogen
TS	Total solids
UASB	Upflow anaerobic sludge blanket reactor
v/v	volume/volume
VFA	Volatile fatty acids
VS	Volatile solids
w/v	weigh/volume
WWTP	Wastewater treatment plant

Introduction, Research Aim and Thesis Synopsis

This chapter introduces the relevance of anaerobic digestion and the current European legislation framework supporting the increasing opportunities to anaerobic digestion application. The importance of co-digestion to increase methane production is also highlighted. The critical Portuguese scenario of under use of anaerobic digestion is discussed under the perspective of an expected great evolution in this field, to comply with the legislation on waste treatment and on the use of renewable energy sources. Consequently, the motivation to study anaerobic co-digestion of organic wastes is expressed. Finally, the research aim and thesis synopsis are explained.

1.|INTRODUCTION, RESEARCH AIM AND THESIS SYNOPSIS

1.1.|EUROPEAN LEGISLATION FRAMEWORK AND THE EMERGING OPPORTUNITIES FOR ANAEROBIC DIGESTION APPLICATION

1.1.1.|EUROPEAN ENVIRONMENT ACTION PROGRAMMES

Environmental problems have far-reaching social and economical implications and their solutions demand changes in public awareness and attitudes, as well as new legislative frameworks. Since the last century, the European Union environmental legislation has been guided by frameworks set by different Environmental Action Programmes (EEA). The 1st EEA Programme was launched in 1973, which defined the future direction of European Union policy in the environmental field and set specific proposals that the Commission intended to put forward over the next years. This programme defined principles such as the "Polluter Pays Principle" and recognised that prevention was better than to remediate the problem after it occurred. However, the world in the 1970´s was very different from today as the cold war was still dividing many of the world's most industrialized nations, the Berlin Wall stood firm in Europe, and the e-mail had just been invented, nevertheless, it took more than two decades before its use became widespread. The notion of a personal computer did not exist and the concept of global warming had only just been mentioned for the first time (STUDY OF CRITICAL ENVIRONMENTAL PROBLEMS, 1970). Within these circumstances, it was noteworthy that an international conference on the environment actually took place in Stockholm (1972). The Stockholm declaration on the Human Environment and Principles constituted the first body of 'soft law' in international environmental affairs (LONG, 2000). The essential notion of "sustainable development", which is defined as finding ways of improving our quality of life without causing harm to the environment, future generations or the people of both the rich and developing world, was introduced with the 5th EEA programme (1993-2000). Presently, the European environmental goals continue under the 6th EEA Programme (2002-2012) entitled the Environment 2010: Our Future Our Choice (DECISION 1600/2002/EC). Waste management is undoubtedly an environmental concern and the later Programme identifies waste prevention and management as one of four top priorities.

1.1.2.|EUROPEAN UNION LANDFILL DIRECTIVE

Strong targets were set by the European Union Landfill Directive (COUNCIL DIRECTIVE 1999/31/EC)

concerning organic wastes. This Directive introduced requirements on member states to reduce the amount of biodegradable wastes to be disposed in landfills if they were not subject to some previous recovery action. The first target was in 2006, which required the amount of biodegradable municipal waste sent to the land-fill to be 75% of the amount produced in 1995. This amount would be further reduced to 50 and 35% of 1995 levels in the years 2009 and 2016, respectively. This directive was implemented in Portugal (DECRETO-LEI Nº 152/2002), and is nationally known as ENRRUDBA. The limits of biodegradable municipal waste admissible to landfill in Portugal were 1 689 540 tons in January 2006. Moreover, these limits will be further reduced to 1 126 360 and 788 452 tons by January 2009 and 2016, respectively, in order to achieve the targets set and regarding the biodegradable waste produced in 1995, which was 2 252 720 tons (PROGRAMA NACIONAL ALTERAÇÕES CLIMATICAS, 2006).

According to the 2007 Environmental Policy Review (COM(2008)409), in 2006, 435 kg/per capita of municipal waste were generated in Portugal compared to an average of 517 kg/per capita in the European Union. Of this, 63% was landfilled while 22% was incinerated. The Commission opinion was that additional efforts were needed in order to reach the 2010 targets. Therefore, a new strategic plan for urban solid waste was approved for the period 2007-2016 in Portugal (PERSU II, 2007). In this plan, a moderate scenario for biodegradable waste recovery, meaning, composting plus anaerobic digestion, was developed as presented in Figure 1.1. In this figure, the number of expected plants due to the new waste legislation in Portugal is depicted alongside with the scenario prior to implementation of the ENRRUBDA legislation.

1.1.3.|WASTE DIRECTIVE

The recently revised waste framework directive (COUNCIL DIRECTIVE 2008/98/EC) establishes a real hierarchy management for integrated waste management, with a strong impulse towards a recycling society and also introduces the concept of 'bio-waste'. Bio-waste is defined as biodegradable garden and park waste, food and kitchen waste from households, restaurants, caterers and retail premises, and comparable waste from food processing plants. It does not include forestry or agricultural residues, manure, sewage sludge, or other biodegradable waste such as natural textiles, paper or processed wood. It also excludes those by-products of food production that never become waste (COM(2008)811). Furthermore, as stated in the COUNCIL DIRECTIVE 2008/98/EC, article 22, Member States shall take measures, as appropriate, and in accordance with Articles 4 and 13, to encourage the separate collection of bio-waste with a view towards anaerobic digestion application followed by a composting process. Furthermore, It is stated in the green paper (COM(2008)811), that anaerobic digestion is especially suitable for

treating wet bio-waste, including fat, as for example kitchen waste.

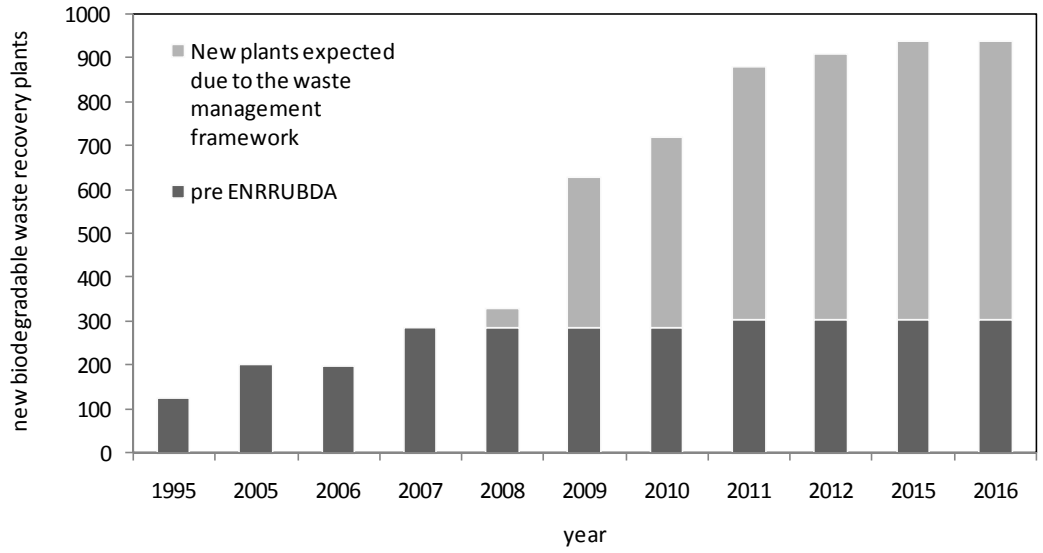

Figure 1.1| Number of new plants (composting+anaerobic digestion) for biodegradable waste recovery expected due to the new waste legislation alongside with the scenario estimated before ENRRUBDA legislation in Portugal (adapted from the PERSU II results).

1.1.4.|GREENHOUSE GASES AND THE KYOTO PROTOCOL

Ideally, anaerobic digestion leads to the overall biodegradation of organic wastes, producing methane (CH_4) and carbon dioxide (CO_2) and traces of other gases, such as H_2S. This gas mixture is commonly defined as biogas. The biogas can be used as a biofuel for multiple uses including transport, after upgrading can be injected into the gas distribution grid, can be used for electrical production either for self-utilization or for injection in the electricity grid. All the uses of biogas allow energy recovery from wastes. However, the effluents from anaerobic digestion are not generally suitable to apply directly to the land. Thus, it is generally accepted that post-treatment after anaerobic digestion is needed to obtain a high-quality, finished product (POGGI-VARALDO ET AL., 1999). The traditional question rises, why not use direct aerobic composting instead of a combined anaerobic-aerobic treatment process? It is true that anaerobic technology requires a larger investment and that the overall process is more complex. However, energy is recovered in anaerobic treatment whereas composting is a net energy consumer (MATA-ALVAREZ ET AL., 2000). Many comparisons have been carried out in the past, with variable results highly dependent on energy costs (MATA-ALVAREZ ET AL., 2000). However, it should be stressed that composting may be associated with anthropogenic "Greenhouse Gas" (GHG) emissions, since nutrients are lost

during the operation and may induce environmental problems. Losses are generated in many ways: ammonia volatilisation (KIRCHMANN & WITTER, 1989), nitrous oxide (CZEPIEL ET AL., 1996) and CH_4 emissions (LOPEZ-REAL & BAPTISTA, 1996) or as leaching of nutrients in drainage water. Composting is also responsible for significant CO_2. However, the latter is not considered as a net source of GHG (PEIGNÉ & GIRARDIN, 2004). Unlike CO_2, CH_4 produced by inadequate composting is a net production from anthropogenic source, and is 23 times more serious in terms of GHG than the first, and, has to be fully computed as a net emission in GHG inventories (CLIMATE CHANGE, 2007). Following water vapour and CO_2, CH_4 is the most abundant GHG in the troposphere, and 17% of global CH_4 emissions originate from improper waste handling (WUEBBLES & HAYHOE, 2002). Variation of emitted quantities of CH_4 illustrates, that composting can be either a negligible source of CH_4 emission or an important source of pollution when anaerobic conditions prevail, e.g. up to 22% of total GHG emission at a dairy farm-scale (AMON ET AL., 2001).

Biogas recovery (60-70% CH_4) produced in anaerobic digestion improves the achievement of the goals set by the Kyoto Protocol signed in 1997. The later requires that developed countries, which are responsible for 80% of global GHG emissions, reduce their emissions by an average of 5.2% until 2008-2012 compared with emissions in 1990 (http://www.kyotoprotocol.com). The 2007 environmental policy review (COM(2008)409) stated that Portugal may not exceed GHG emissions by more than 27% for the period 2008–2012 compared to the base year. In 2005 emissions were 85.5 million tons CO_2 equivalents, corresponding to an increase of 40.4%. Projections for 2010 indicate that Portugal will fail the Kyoto target, unless additional policies are implemented.

Controlled anaerobic digestion of organic material is therefore environmentally beneficial in two ways. First, by containing the decomposition processes in a sealed environment, preventing the potentially damaging CH_4 from entering the atmosphere. Subsequent burning of the gas will release carbon-neutral CO_2 back to the carbon cycle (WARD ET AL., 2008). In addition, the energy gained from combustion of methane will displace fossil fuels, reducing the production of CO_2 that is not part of the recent carbon cycle (WARD ET AL., 2008). TILCHE & GALATOLA (2008) showed that biogas if used as a transport biofuel, may allow for true negative GHG emissions, showing a net advantage with respect to other biofuels. When analysing the potential reduction of GHG emission by anaerobic digestion, it should be noted that biogas leaks or venting represent net increases in GHG balance, while biogas flaring results in neutral or positive (increase) emissions. The only case, in which, there could be a reduction in emission values is when biogas is used for producing useful energy. In this instance, the overall balance will depend on the environmental life cycle profile of the energy source substituted (TILCHE & GALATOLA, 2008).

The unwillingness to implement anaerobic digesters is likely to change rapidly with green energy requirements, oil and gas prices, and carbon credits. Carbon credits may have significant economic impact on anaerobic digestion profitability for the coming decades as global economies use emerging GHG offset markets (JOHNSON & HEINEN, 2004; SCHNEIDER & MCCARL, 2006).

1.1.5.|DIRECTIVE ON ELECTRICITY PRODUCTION FROM RENEWABLE ENERGY SOURCES

The use of renewable energy sources, such as biogas, is becoming progressively more essential, especially with the new economical situation, determining the need to substitute fossil fuels. In the 1970´s, the United States and other developed countries experienced an energy crisis. Allegedly, fossil fuels would soon be depleted and energy consumption might be reduced and the use of alternative energy supplies should very rapidly start, with emphasis on renewable sources. However, as fast as that crisis appeared, it disappeared in the late 1980´s and the problem of the fuel fossil depletion was delayed for a distant future. A revived interest in renewable energy and the principles of green energy are emerging again, in response to the recent petroleum crisis. Biogas or CH_4 is about to become much more important as an energy source than it has been in the past, due to the ever rising cost of natural gas.

In accordance to the Directive on Electricity Production from Renewable Energy Sources (DIRECTIVE 2001/77/EC), all Member States have adopted national targets for the promotion of electricity consumption from renewable energy sources. If all Member States achieve their national targets, 21% of overall electricity consumption in the EU will be produced from renewable energy sources by 2010. With current policies and effort, the European Union will probably achieve a figure of 19% by 2010 (COM(2006)848). Hence, the European Commission put forward a proposal for a new directive, known as RES directive or Renewables Directive to replace the existing measures adopted in 2001. According to the RES Directive proposal (COM(2008)19), each member state should increase its share of renewable energies in an effort to boost the EU's share from 8.5% today to 20% by 2020. A 10% increase in biofuels used in transportation is included within the overall EU objective. In this proposal, definition of biomass included “the biodegradable fraction of products, waste and residues from agriculture (including vegetal and animal substances), forestry and related industries, as well as the biodegradable fraction of industrial and municipal waste”. It is anticipated that biomass will have a major role in the substitution of fossil fuels with renewable sources, and will presumably contribute 83% to the increased use of renewable sources by the year 2010 (KARPENSTEIN-MACHAN 2001). The Portuguese target for energy from renewable sources in 2020 is now 39% compared with the

20.5% in 2005 (COM(2008)19).

Presently, the Portuguese legislation (DECRETO-LEI Nº 225/2007) defined as an average feed in tarif for electricity production from anaerobic digestion of municipal solid wastes, sludge digesters, animal and food wastes digestersa value of 116 Euros/MWh of energy produced (www.dgge.pt).

Within this framework, anaerobic digestion plant operators are urged to increase CH_4 production by improving the existing plants process. In fact, problems such as low CH_4 yield and process instability are often encountered in anaerobic digestion, preventing this process from being more widely applied. Municipal wastewater anaerobic digesters operate at low loading conditions that yield low amounts of CH_4 production, causing low recovery of heat and electrical energy (BOLZONELLA ET AL., 2006). Co-digestion is a very strong option to enhance CH_4 generation in biogas plants already built or in use. That is, the use of a co-substrate can significantly improve the waste treatment efficiency resulting in a better economical balance. Co-substrates may also improve biogas yields due to positive interactions in the digestion medium and also by the addition of missing nutrients. Moreover, the economical advantages derived from equipment sharing are quite significant (MATA-ALVAREZ ET AL., 2000).

There is no recent data available about the anaerobic digestion market in Portugal. Currently, this technology is mostly used in municipal wastewater treatment plants, and there is only one anaerobic digester for municipal solid waste running in Lisbon. Moreover, the co-digestion of wastes is still not used in Portugal. This means that in the next years it is expected to have a great evolution in this field to comply with the legislation on waste treatment and on the use of renewable energy sources. Furthermore, the Portuguese market has the unexplored potential to produce 120 GWh/year of biogas obtained from the food industry waste, 226 GWh/year of biogas from animal production (cows, chicken and pigs), and 157GWh/year of biogas from wastewater treatment plants using co-generation (ADENE/INETI, 2001).

1.2.|RESEARCH AIM

Essentially, this dissertation reports several studies that were undertaken in order to improve CH_4 production in anaerobic co-digestion of organic wastes. The substrates used in the studies performed were as follows:

- simulated food waste which was obtained by blending melted pork lard, white cabbage, chicken breast, and potato flakes to simulate lipids, cellulose, proteins, and carbohydrates, respectively (CHAPTER 3);

- food waste, which was a composite sample (one week based) of the waste produced in the restaurant of the University of Minho, located in "Campus de Gualtar", Braga, Portugal (CHAPTER 3, CHAPTER 5, CHAPTER 6 AND CHAPTER 8);
- cow manure, collected from a dairy farm located in the suburbs of Braga, Portugal (CHAPTER 4, CHAPTER 5 AND CHAPTER 6);
- oily waste effluent collected from a canned fish processing industry in Portugal (CHAPTER 4, CHAPTER 5 AND CHAPTER 6);
- coffee wastes from the Nestlé instant coffee substitute production facility in Avanca, Portugal (CHAPTER 7 AND CHAPTER 8);
- activated sludge from a wastewater treatment plant in Portugal (CHAPTER 7 AND CHAPTER 8).

1.3.|THESIS SYNOPSIS

The present section (CHAPTER 1) provides information on the European legislation, regarding solid waste treatment and the use of renewable energy sources. In many ways, the new legislative framework of these issues favors the application of anaerobic digestion. Consequently, there is a motivation to study anaerobic co-digestion of organic wastes. In other words, the need to improve co-digestion processes to increase the CH_4 production yield was the driving force of this thesis.

A brief description of the anaerobic digestion process, the benefits of co-digestion and an overview of the recent research using the substrates within the scope of this thesis are presented in CHAPTER 2.

In CHAPTER 3, the influence of the composition of kitchen/restaurant waste at mesophilic temperatures on the biomethanation potential is described. The synthetic waste was used to study the effect of waste composition on anaerobic digestion of restaurant waste in batch assays. Four blends of the four components of food waste (proteins, carbohydrates, lipids, and cellulose) with an excess of each component were assayed and compared with a fifth blend containing an equal amount of chemical oxygen demand (COD) of each of the four components. The latter mixture was compared with the real restaurant waste in terms of methane yield.

In several experimental works reported along this thesis, emphasis was placed on the addition of fat and the role of fatty wastes in the co-digestion process. Under this scope, a method was developed in order to extract, indentify and quantify long chain fatty acids (LCFA) in both the solid and liquid phase. In CHAPTER 4, the method is described and applied to samples collected from anaerobic digesters fed with cow manure with occasional inputs of oily waste.

The behavior of cow manure and food waste co-digestion, with increasing concentrations of intermittent pulses of oil is explained in CHAPTER 5. The oil concentration was increased to 9, 12, 15 and 18g $COD_{oil}/L_{reactor}$ after pulse feeding the reactor. The purpose was to study the optimal lipids concentration that can be added to enhance methane production without inhibiting the process.

In CHAPTER 6 the study of LCFA profiles associated to the solid phase of four reactors fed with cow manure and food waste, in two different approaches is reported: First, the lipids composition was forced to change suddenly in three moments without changing the total COD fed to the reactors. Secondly, pulses of lipids were added, raising the concentration in the reactors up to 9, 12, 15 and 18 $gCOD_{oil}/L_{reactor}$ (CHAPTER 5). The ultimate goal was to find a practical value of solids-associated LCFA that a co-digestion anaerobic plant based on cow manure and food waste can endure.

In CHAPTER 7, the feasibility of anaerobic co-digestion of solid waste of five different coffee substitutes and sewage sludge was assessed in mesophilic batch assays. However, one of the wastes, composed of 100% barley, produced a very poor methane yield when compared to the other coffee substitute wastes used in this study. Therefore, two different approaches were used to enhance methane production. First, the barley waste was subjected to alkaline hydrolysis pre-treatment before co-digestion with activated sludge. The second approach consisted of co-digestion with kitchen waste (40% Barley waste, 60% kitchen waste). The results from these two approaches are described in CHAPTER 8.

CHAPTER 9 contains the most significant conclusions withdrawn from the described experiments as well as some perspectives for further investigation in this field of research.

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Anaerobic Digestion Overview

The objective of this chapter is to present an overview of the recent scientific literature on anaerobic co-digestion of organic wastes. Particular attention is given to food/kitchen waste, animal manure and fat/greases role as co-substrates. Co-substrates with high contents in lipids, proteins and carbohydrates are essential for the economy of anaerobic plants but if not handled properly might lead to disturbances. In fact, the performance of an anaerobic digestion process has been shown to be much dependent on the type and the composition of the material to be digested. Hence, experimental studies focused on the improvement of CH_4 production using different feedstocks are of significant importance. A brief description of the anaerobic digestion process is included and finally, the conclusion and perspectives are presented.

2.|ANAEROBIC DIGESTION OVERVIEW

2.1.|ANAEROBIC DIGESTION, A BRIEF HISTORY

Anaerobic digestion is a microbial mediated biochemical degradation of complex organic material into simple organics and dissolved nutrients. This process occurs naturally in the environment and consists in the breakdown of complex organic substrates, through a series of parallel and sequential steps, by several groups of microorganisms, in an oxygen free environment. Ultimate, the organic matter is degraded to carbon dioxide (CO_2), ammonium, sulphide and methane (CH_4), usually, known as biogas.

The science of this process is as old as scientific research can be, and, includes the names of world's most famous researchers. Benjamin Franklin described as early as 1764 that was able to light a large surface of a shallow muddy lake, in New Jersey. This experiment was reported in a letter to Joseph Priestly in England, who published in 1790 his own experiences with the inflammable air (TITJEN, 1975). Alexander Volta was the first researcher who described, scientifically, the formation of inflammable gases in (low-temperature) marshes and lake sediments (BARKER, 1956). In 1804, Dalton gave the correct chemical formula for CH_4. Gayon, in 1883-84, a pupil of Pasteur recorded his experiments success, with fermented manure at 35°C attaining 100 LCH_4/m^3 of manure (TITJEN, 1975). The volume of gas collected was so great, that Louis Pasteur concluded that anaerobic manure fermentation might supply gas for heating and illumination under special circumstances. However, the proposal made in jest by the newspaper "Le Figaro" to improve the street illumination of Paris by manure fermentation from the numerous horses of the taxis and public works was not executed (TITJEN, 1975). In 1856, Reiset found CH_4 being liberated from decomposing manure piles and proposed this process to be studied to help explain the decomposition of organic material in general (BUSWELL AND HATFIELD, 1938).

Even so, it was only at the end of the 19th century that anaerobic digestion was applied for the treatment of wastewater and solid waste (GIJZEN, 2002). The first application of anaerobic digestion for sewage treatment dates back to 1860, with the development of a simple air-tight chamber by Mouras in France (MCCARTY, 2001). In 1897, the local government board of the city of Exeter, England, approved the treatment of the entire wastewater of the city in a similar anaerobic system, referred to as “septic tank”. At the beginning of the 20th century, Imhoff developed a slightly more advanced digester which combined sedimentation and digestion in a single compartment. Primitive anaerobic filters and hybrid systems were also introduced around

the turn of the century (MCCARTY, 2001). Although anaerobic wastewater treatment has been used since the late 19th century, it was for a long time considered to be unstable, inefficient and a slow process (GIJZEN, 2002). The major limitation in the development of high-rate anaerobic digesters was the low yield and long doubling times of the microorganisms, especially for those involved in the acetogenic and methanogenic reactions (GIJZEN, 2002).

The anaerobic digestion arises as a wastewater treatment system in the 1970´s, showing promises as an alternative energy source, in particular from animal waste. However, in the 1980´s and 1990´s, the extensive diffusion of anaerobic digestion technologies was mainly linked to the development of the Upflow Anaerobic Sludge Blanket (UASB) concept and the treatment of a wide range of industrial organic wastewaters, with the main objective of wastewater treatment. It is only at the beginning of the new century that anaerobic digestion returns, once more, to the market for its energy recovery potential, with applications to various bio-wastes and biomass. Actually, in China, about 25 million people use biogas for cooking and lighting for 8-10 months a year (BABEL ET AL., 2009). Biogas production attained by anaerobic digestion of organic wastes has been carried out for decades, and, it is in many cases a mature concept (RIGGLE, 1998). However, it is still capable of showing new features.

2.2. | ANAEROBIC DIGESTION, A CONCISE INTRODUCTION

The microbiology of anaerobic digestion is complex, since it involves a great variety of different species from two entirely different biological kingdoms. The Bacteria and the Archaea (MCCARTHY, 2001), each performing a separate task of the overall degradation process, in order to convert organic wastes through a variety of intermediates into CH_4 gas.

Briefly, the anaerobic digestion process can be divided in four main steps. It begins with hydrolysis of the input materials, in order to break down insoluble and soluble organic polymers in their monomers, making them available for the subsequent steps. In acidogenesis, also known as fermentation, sugars, amino acids and long chain fatty acids are converted into Carbon Dioxide, Hydrogen, Ammonia, Alcohols and Organic acids. Then, in acetogenesis, these intermediates such as alcohols and organic acids are converted into acetic acid, H_2, and CO_2. Finnally, in methanogenesis, these products are converted to CH_4 and CO_2. A simplified schematic representation of anaerobic degradation of organic matter is given in Figure 2.1.

Given the diversity of complex biochemistry involved, only a succinct description of the main characteristics will be given in each degradation step.

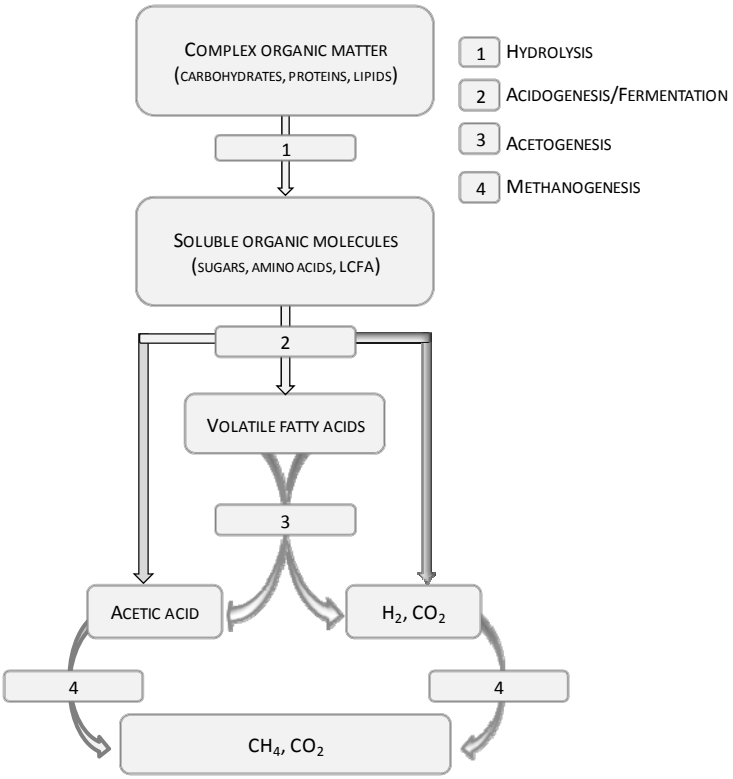

Figure 2.1|Schematic of the four main pathways of degradation of complex substrates under anaerobic conditions.

2.2.1.|HYDROLYSIS

Particulate and soluble complex substrates, such as carbohydrates, proteins and lipids, cannot be directly used by the anaerobic microorganisms. Thus, there is a need of being transformed into smaller molecules. Such process takes place during the hydrolysis step, in which these complex compounds are hydrolyzed into their basic building units. Complex substrates are also products from the disintegration of composite particulates. Other products of the disintegration are inert particulate and soluble compounds. The products of enzymatic degradation of carbohydrates, proteins and lipids are monosaccharides, amino acids and long chain fatty acids (LCFA), respectively (BATSTONE ET AL., 2002).

Hydrolysis of organic polymers is carried out by extra cellular enzymes, hydrolases, which are excreted by fermentative bacteria. The parallel enzymatic steps with cellulases, proteases and lipases account for the difference in hydrolysis rate of the particulate carbohydrates, proteins and lipids, respectively (STRYER, 1995). The cumulative effects of the different processes, taking place during hydrolysis, have traditionally been simplified to single first-order kinetics for the

substrate biodegradation (EASTMAN & FERGUSON, 1981). Table 2.1, presents the kinetic coefficients (*kh*) of the first order rate of hydrolysis of some substrates used under the scope of this thesis. It should be stressed that even using similar substrates, the *k*h is strongly dependent on the experimental conditions, namely temperature, pH, particle size, stirring conditions, inoculum/substrate ratio which makes very difficult any comparison with existing literature values.

Table 2.1|kinetic coefficients of the first order rate of hydrolysis

Substrate	*Kh* (day^{-1})	T (ºC)	Reference
Carbohydrates	0.025-0.2	55	CHRIST ET AL. (2000)
Carbohydrates	0.5-2		GARCIA-HERAS (2003)
Proteins	0.015-0.075	55	CHRIST ET AL. (2000)
Proteins	0.25-0.28		GARCIA-HERAS (2003)
Proteins (gelatine)	0.65	55	FLOTATS ET AL. (2006)
Lipids	0.005-0.010	55	CHRIST ET AL. (2000)
Lipids	0.1-0.7		GARCIA-HERAS (2003)
Lipids	0.76		SHIMIZU ET AL. (1993)
Lipids	0.63	25	MASSE ET AL. (2003)
Proteins	0.25-0.8		GARCIA-HERAS (2003)
Kitchen waste	0.34	35	LIEBETRAU ET AL. (2004)
Food waste	0.55	37	VAVILIN ET AL. (2004)
Household solid waste	0.1	37	VAVILIN & ANGELIDAKI (2005)
Bio-waste	0.12	35	LIEBETRAU ET AL. (2004)
Bio-waste	0.03-0.15	20	VEEKEN & HAMELERS (1999)
Bio-waste	0.24-0.40	40	VEEKEN & HAMELERS (1999)
Cattle manure	0.13	55	VAVILIN ET AL. (2008)

Relatively high hydrolysis rates were reached in anaerobic biodegradability tests with a high inoculum-to-substrate ratio (FERNÁNDEZ ET AL., 2001), showing some degree of dependence of hydrolysis on biomass concentration or activity. Thus, the first order kinetics appears to be not applicable in all circumstances, indicating that an in-depth understanding of the different processes involved is needed to accurately describe hydrolysis.

The hydrolysis of particulate organic material has been considered the rate-limiting step in anaerobic digestion (EASTMAN & FERGUSON, 1981; PARKIN & OWEN, 1986, PAVLOSTATHIS & GIRALDO-GOMEZ, 1991), nonetheless some authors have emphasized that the hydrolytic process still remains as the least well defined step (MIRON ET AL., 2000; GAVALA ET AL., 2003).

2.2.2.|ACIDOGENESIS

The acidogenesis step, or fermentation, is the degradation of soluble substrates largely without

an external electron acceptor. During stable operation the main products of acidogenesis are: acetate, propionate, butyrate, CO_2, H_2 and other organic products, such as lactate and alcohols (HARPER & POHLAND, 1986). Acetate, CO_2, H_2 and single-carbon compounds can be directly utilized by methanogens.

The levels of volatile fatty acids (VFA) and H_2 provide important information on an unbalanced process. The accumulation of organic acids, such as VFA, consequently lowering the pH, can lead to the suppression of the methanogenic activity, and, consequently to process failure (ZOETEMEYER ET AL., 1982). Acetic acid is usually present in higher concentrations than other VFA during anaerobic digestion (WANG ET AL., 1999). However, propionic and butyric acids are inhibitors of the methanogens (WARD ET AL., 2008). In a recent study, PULLAMMANAPPALLIL ET AL. (2001) found that propionic acid was an effect rather than a cause of inhibition of anaerobic processes.

The conversion of the hydrolysis products takes place in the bacterial cells. The fermentation pathways depend on the substrate and microorganisms involved. Acidogens grow relatively faster and are less sensitive to pH variation than acetogens/methanogens (COHEN ET AL., 1980). In general, the acidogenic (fermentative) population represents about 90% of the total microbial population present in anaerobic digesters (ZEIKUS, 1980). These bacteria have a short doubling time (MOSEY, 1983) and therefore acidogenesis is not regarded as a limiting step in the process of anaerobic digestion (GUJER & ZEHNDER, 1983; MOSEY, 1983).

2.2.3.|ACETOGENESIS

In the acetogenic step, the reduced fermentation intermediates, are converted to acetate, CO_2 and H_2 by obligate, H_2-producing acetogenic bacteria. Is not always clear the distinction between acetogenesis and acidification reactions (FOX & POHLAND, 1994). The conversion of fermentation intermediates, such as alcohols and fatty acids is not energetically feasible under standard conditions, and a syntrophic microbial relationship is required for the reactions to proceed. The reactions become feasible when the H_2 partial pressure is in the range of 10^{-4}-10^{-5} atm.

2.2.4.|METHANOGENESIS

Methanogenesis is the final step, where the CH_4 production occurs. This process is carried out by methanogenic archaea, which metabolize the end products of the previous reactions, mainly H_2, CO_2, and acetate, to form CH_4. Other C1 compounds such as formate, methanol and methylamines can be also used by methanogenic archaea. Most of the CH_4 produced in a

digester, about 70%, is generated via the acetotrophic pathway (LALMAN & BAGLEY, 2001) by the acetoclastic methanogens (JETTEN ET AL., 1992) putting forward their importance for the efficient energetic valorisation of waste (waters). On the other hand, methanogenesis from CO_2 and H_2 has a significant role, by keeping a low H_2 pressure that enables the growth of the obligate, H_2-producing acetogenic bacteria. Methanogenic archea are extremely sensitive to temperature, loading rate as well as pH fluctuations, and, are inhibited by a number of compounds. Several authors have reported methanogenesis as being the rate-limiting conversion in anaerobic digestion (FANG ET AL., 1995; HUANG ET AL., 2003). If the substrate is easily hydrolysed, the last degradation step is often rate limiting, since methanogens grow slower than the acidogens upstream in the degradation chain. This can give rise to negative effects. In the case of organic overload or exposure to toxic compounds that may induce a build-up of the metabolic intermediates, mainly VFAs (ROZZI, 1991). The methanogenic species are more inhibited by a decrease in pH than are the acid-producing species (ANDERSON & YANG, 1992), which causes further acid accumulation and eventually leads to process failure.

Methane production is directly related to a decrease of the chemical oxygen demand (COD) in waste(water) during anaerobic degradation. Thus, CH_4 yield can be evaluated from the COD balance in the system, based on the COD removed. In standard conditions, theoretical methane production is about 0.35 m^3/kg of the COD degraded. Distinguishing between available degradable (substrate) and total input COD is very important, as a considerable fraction of the input COD may be anaerobically not biodegradable (GOSSETT & BELSER, 1982).

In sum, for efficient CH_4 production it is important to have a balance between the reactions rates of the different steps involved in the anaerobic digestion of complex organic material. The overall rate of conversion of organic matter is governed by the kinetic characteristics of the slowest step (GIJZEN, 2002).

2.2.5.|OPERATIONAL CONDITIONS

The operational and environmental parameters of the process obviously affect the behaviour, performance and eventually the fate of the microbial community in anaerobic digesters. In fact, the process is strongly influenced by temperature, pH, alkalinity and toxicity are primary control factors. Furthermore, the nature and influence of the seed sludge used for inoculation should also be considered (GUYOT ET AL., 1993).

|BUFFERING CAPACITY AND PH

Buffer capacity is often referred to as alkalinity, which is the equilibrium of CO_2 and bicarbonate

ions that provides resistance to significant and rapid changes in acid/alcali concentrations. The buffering capacity is, therefore, proportional to the concentration of bicarbonate (WARD ET AL., 2008). Buffer capacity is reported to be a more reliable method of measuring digester imbalance than direct measurements of pH, since an accumulation of short chain fatty acids will reduce the buffering capacity significantly before the pH decreases. Increasing a low buffer capacity is best accomplished by reducing the organic loading rate. A more rapid approach is the addition of strong bases or carbonate salts to remove CO_2 from the gas space and convert it to bicarbonate, or alternatively bicarbonate can be added directly (GUWY ET AL., 1997). Direct bicarbonate addition is more accurate, as converting CO_2 to bicarbonate will require a time lag for gas equilibrium to occur which could result in over-dosing (WARD ET AL., 2008).

VFA monitoring, particularly butyrate and isobutyrate, has been demonstrated to indicate process stability (AHRING ET AL., 1995), as an increase in fatty acids can be indicative of an overload of the organic loading rate. BJÖRNSSON ET AL. (2000) conducted a study using three bench-scale digesters fed with municipal wastewater sludge. After stable operation, the organic loading rate of the digesters was increased by addition of carbohydrate-rich food waste. One digester received a pulse load by increasing the organic loading rate from 1.6 to 3.6 kgVS/m^3.day. Partial alkalinity and pH decreased and VFA levels increased while the total alkalinity remained constant. In the other two digesters, the organic loading rate was stepwise increased. Signs of overloading were observed at 5.9 kgVS/m^3.day in one digester and at 5.3 kgVS/m^3.day in the other digester. In both cases, changes in process monitoring parameters were similar to those observed during the pulse load. The authors concluded that partial alkalinity and VFA levels were reliable parameters for monitoring. The pH was a less reliable parameter because of possible variations of the buffering capacity.

The ideal pH range for anaerobic digestion is very narrow between 6.8 and 7.2. The growth rate of methanogens is greatly reduced below pH 6.6 (MOSEY & FERNANDES, 1989). Although, the optimal pH of methanogenesis is around pH 7.0, the optimum pH of hydrolysis and acidogenesis has been reported to be between pH 5.5 and 6.5 (KIM ET AL., 2003; YU & FANG, 2002).

A low pH level and acetate stored in the system cause inhibition of acetogens and hydrogenotrophs. In this case the system is fully inhibited, and the pH is normally below 5, meaning that the overall COD is converted to acids, whereas methanogenesis does not occur. However, there are some waste with a high ammonia load, like the animal slurry, for which the decrease of pH below 7 is usually not observed due to the high buffering capacity of ammonia (MONTUSIEWICZ ET AL., 2008). Such systems with interactions between free ammonia, VFA and pH, operate in an “inhibited steady state”. This means that the digestion process is running stable,

thus a complete failure of the system is not observed, but CH_4 production yield is low (ANGELIDAKI & AHRING, 1993; ANGELIDAKI ET AL., 2006). Essentially, the reason is that methanogens will not be able to metabolise the acetate produced by the acetogenic organisms until the number of methanogenic organisms has increased sufficiently. This is especially true for feedstock that are rapidly hydrolysed. With poorly-degradable feedstock, the hydrolysis stage is more likely to be the limiting step. It has also been demonstrated that the inoculum-to-feed ratio can be modified to maintain a constant pH (GUNASEELAN, 1995).

| TEMPERATURE

Temperature affects the metabolic activities of the microorganisms that, in turn, affect the rate of digestion and CH_4 production (WARD ET AL., 2008). Even small changes in temperature, from 35ºC to 30ºC and from 30ºC to 32ºC reduce biogas production rate (CHAE ET AL., 2008). There are three common temperature ranges for anaerobic digestion: (1) the lower temperature range, which is referred to as psychrophilic, meant for temperatures lower than 20ºC; (2) temperatures within 20–45ºC, named mesophilic temperatures; (3) temperatures in the range of 45–60ºC, are termed thermophilic temperatures. Nonetheless, most of the reactors operate at either mesophilic or thermophilic temperatures, with optima at 35ºC and 55ºC, respectively (WARD ET AL., 2008).

There is not a standard or optimal temperature range for the anaerobic digestion, since there are studies for mesophilic and thermophilic conditions with conflicting results.

For instance, HEGDE & PULLAMMANAPPALLIL (2007) tested a batch digestion of vegetable waste and wood chips mixture. Methanogenesis was more rapidly initiated in the thermophilic digester, 95% of the CH_4 potential was produced in 11 days under thermophilic conditions as opposed to 27 days under mesophilic conditions. It was also reported that a more rapid degradation of VFA was detected at 55ºC than at 38ºC. As well, LI ET AL. (2002) studied the anaerobic degradation of lipids-rich food conducted under mesophilic (35°C) and thermophilic (55°C) conditions. The lipids (fats or oil and grease) content in the influent was changed from 8% to 40% by adding salad oil and lard to the food wastes. The results showed that the food wastes containing high lipids content, was effectively degraded by the high solids co-digestion process and over 85% of lipid was degraded to biogas with 60–65% of CH_4,. Nevertheless, thermophilic fermentation was more effective for reducing lipids and had higher loading capacity compared with mesophilic condition.

On the other hand, PARAWIRA ET AL. (2007) compared two-stage digesters of mesophilic + mesophilic, mesophilic + thermophilic, and thermophilic + thermophilic configurations treating

solid potato waste. The CH_4 yield was higher under mesophilic conditions than in thermophilic conditions. These authors reported that, if the aim was to treat solid potato waste completely within a short period of time, thermophilic conditions would be preferred, but to obtain higher CH_4 yield, mesophilic conditions are preferable. There was also evidence that mesophilic temperature digesters have improved degradation rates, when compared with thermophilic digesters. Moreover, FANG & CHUNG (1999) experiments with proteinaceous wastewater showed that a mesophilic reactor attained higher COD reductions when compared to a thermophilic one.

Microorganisms operating in mesophilic range are more robust and can tolerate greater changes in the environmental parameters than thermophilic ones (NGUYEN ET AL., 2007). The stability of the mesophilic process makes it more popular in current anaerobic digestion facilities due to the fact that thermophilic bacteria are more sensitive to toxicants and temperature fluctuation outside the optimum range (BIEY ET AL., 2003). The operation in mesophilic range (33–37ºC) is reported to be more stable and requires a smaller energy expense (FERNANDÉZ ET AL., 2008). Also, the inhibition by ammonium (ANGELIDAKI & AHRING, 1994; HANSEN ET AL., 1998) and by VFA (FIELDS, 2001) is more unusual than in thermophilic conditions.

KIM ET AL. (2003) studied the feasibility of food waste as a co-substrate in anaerobic digestion of sewage sludge, using batch tests. These authors observed that mixed food waste led to an increase in CH_4 production, both at mesophilic and thermophilic conditions. Based on CH_4 production rate, the optimal mixing ratios of food waste were 39.3% and 50.1% in mesophilic and thermophilic conditions, respectively. However, more recently, BANKS ET AL. (2008) accessed two digesters fed with segregated food waste collected from domestic properties: one at mesophilic (36.5ºC) and other at thermophilic temperature (56º C). The results from both digesters showed high VFA and ammonia concentrations, but, in the mesophilic digester, the pH remained stable at around 7.4, buffered by a high alkalinity of 13 g/L, whereas in the thermophilic digester VFA levels reached 45 g/L causing a drop in pH and digester instability. In the mesophilic digester, VS destruction and specific gas yield were favourable, with 67% of the organic solids being converted to biogas giving a biogas yield of 0.63 $m^3/kgVS_{added}$. The digestion under thermophilic conditions showed potentially better VS destruction at 70% VS and a biogas yield of 0.67 $m^3/kgVS_{added}$, but the shifts in alkalinity and the high VFA concentrations required a reduced loading to be applied

The different experimental works performed under the scope of this thesis were all performed at mesophilic temperature.

2.3. | BENEFITS OF ANAEROBIC DIGESTION

This process of bio-waste-management and of energy production has many environmental benefits and offers significant advantages over other forms of waste treatment, including:

- less biomass sludge is produced in comparison to aerobic treatment technologies (WARD ET AL., 2008);
- well-known advantages for the treatment of high organic concentration wastewaters (SAYED ET AL., 1988; MÉNDEZ ET AL., 1989; RICO ET AL., 1991; HAWKES ET AL., 1995);
- successful in treating wet wastes of less than 40% dry matter (MATA-ALVAREZ, 2002);
- the possibility of nutrient recycling and reduction of waste volumes (GHOSH ET AL., 1975; VAN LIER ET AL., 2001). The slurry produced (digestate) is an improved fertiliser in terms of both its availability to plants (TAFDRUP, 1995) and its rheology (PAIN & HEPHERD, 1985);
- effective pathogen removal (BENDIXEN, 1994; LUND ET AL., 1996; SAHLSTROM, 2003). This is especially true for multi-stage digesters (KUNTE ET AL., 2004; SAHLSTROM, 2003);
- minimal odour emissions (SMET ET AL., 1999);
- high degree of compliance with many national waste strategies implemented to reduce the amount of biodegradable waste entering landfill (WARD ET AL., 2008).

However, problems such as low CH_4 yield and process instability are often encountered in anaerobic digestion, preventing this technique from being widely applied (BOLZONELLA ET AL., 2006).

There is a long tradition of treating sewage sludge anaerobically at wastewater treatment plants to reduce the volume of sludge, but the process has not been focused, until recently, on optimal biogas production. Considering the general problems related to one-source waste fermentation, co-digestion seems to be a promising solution (CECCHI ET AL., 1996). This approach can be a very strong option to improve the CH_4 generation of the biogas plants already constructed. Hence, studies are needed to investigate the effects of variations in the input to a digester, and how the waste composition influences the overall stability of the process (MURTO ET AL., 2004).

2.4. | CO-DIGESTION OF ORGANIC WASTES

The basic principle of co-digestion consists in balancing several parameters in a selected substrate mixture. Such a balance involves qualitative and quantitative characteristics of waste originating from different sources. The quantitative character of individual component indirectly influences the quality of the mixture (MONTUSIEWICZ ET AL., 2008).

Several researchers have studied the anaerobic co-digestion of sewage sludge with the organic fraction of municipal solid waste (OFMSW) or with agricultural wastes and stated that an enhancement in CH_4 yield was achieved (ANGELIDAKI & ELLEGAARD, 2003; BOLZONELLA ET AL., 2006; GÓMEZ ET AL., 2006; PAVAN ET AL., 2007; MACIAS-CORRAL ET AL., 2008; ROMANO & ZHANG, 2008).

Therefore, anaerobic co-digestion of bio-waste and sludge can be considered a sustainable solution for small wastewater treatment plants in rural areas, where several different kinds of bio-waste are available to enhance biogas production (PAVAN ET AL., 2007). Apart from higher biogas yields due to positive synergetic effects on microorganisms (CECCHI ET AL., 1996; MATA-ALVAREZ ET AL., 2000), there are other benefits of co-digestion approach, which are:

- dilution of toxic substances coming from any of the substrates involved (CECCHI ET AL., 1996; MURTO ET AL., 2004), including, possible removal of some xenobiotics (detoxification based on co-metabolism process) (CECCHI ET AL., 1996);
- improved nutrient balance (CECCHI ET AL., 1996, MURTO ET AL., 2004);
- reducing micro and macronutrient deficiency (MONTUSIEWICZ ET AL., 2008);
- improving process stability (MONTUSIEWICZ ET AL., 2008);
- the use of a co-substrate can also help to establish the required moisture contents of the digester feed (SOSNOWSKI ET AL., 2003). Better handling and digestibility can be achieved by mixing solid waste with diluted waste (MURTO ET AL., 2004);
- in addition, economic advantages can be significant, derived from the fact of sharing equipment (MATA-ALVAREZ ET AL., 2000).

There are many examples of success from mixing organic wastes in anaerobic digestion. Co-digestion of cattle manure slurry with fruit, vegetable wastes and chicken manures is a good example of success. CALLAGHAN ET AL. (2002) blended high carbon-to-nitrogen (C/N) ratio and low C/N feedstock and improved digester performance. Also, co-digestion of sisal pulp and fish wastes had shown a 59–94% increased in the CH_4 production yield as compared to sisal pulp and fish wastes digestion alone (MSHANDETE ET AL., 2004). Additionally, BOLZONELLA ET AL. (2006) presented the results of two full-scale applications of the anaerobic co-digestion process of waste activated sludge together with the OFMSW. The experiences were carried out at Viareggio and Treviso wastewater treatment plants, in Italy. In the first plant, 3 tons/day of source sorted OFMSW were co-digested with waste activated sludge, increasing 50% the biogas production. At the Treviso plant, 10 tons/day of separately collected OFMSW were treated using a low-energy consumption sorting line, in which 99% and 90% of metals and plastics respectively were removed. In these conditions, the biogas yield increased from 3 500 up to 17 500 m^3/month. Industrial costs were evaluated less than 50 €/ton of organic waste, while the payback time was

calculated as two years

However, some drawbacks also exist, mainly due transport costs and the problems arising from the harmonisation of different policies of the waste generators (MATA-ALVAREZ ET AL., 2000). Optimization of CH_4 generation from anaerobic systems has been focusing on digester design and operation, although it has been stated that the feedstock is as important as the digester technology, if not more (LISSENS ET AL., 2001).

There are some wastes recognized as suitable substrates for co-digestion and suggested for biogas production improvement. A substrate categorization, according to BRAUN ET AL. (2003) and regarding the application in anaerobic digestion, is depicted in Table 2.2. The biogas yields obtained by theses authors, when accessing some of the substrates at mesophilic temperatures in batch assays, all performed in similar conditions, are displayed as well.

A brief survey of the most recent literature on the co-substrates used in the experimental work reported herein, with special emphasis for food waste, fat substrates and cow manure is presented shortly.

Table 2.2|Categorization of substrates for anaerobic digestion and the biogas yield attained in batch assays at mesophilic temperatures (adapted from BRAUN ET AL., 2003)

Substrate	Excellent	Good	Poor	Biogas yield $m^3/(kg.VS_{added})$ [days]
Biogenic material from agriculture:				
Straw and other plant residues			x	
Green plant material, crops grain and silages		x		
Harvest residues		x		
Animal Manure:				
Chicken manure		x		
Liquid Pig manure	x			
Cow manure		x		
Food industry waste:				
Expired food		x		
Fast food				0.7 [35]
Fast food leftovers				0.5-1.1 [33]
Confectionary		x		
Whey	x			
Waste from canning and frozen foods		x		
Waste from fruit juice production		x		
Animal fat from slaughterhouse	x			1.0 [33]
Wastes from plant and animal fat production:				
Spoil plant oils	x			
Oil seed residues		x		
Fat trap contents		x		
Fats		x		
Edible oil sludge	x			
Edible fat sludge	x			1.1 [30]
Wastes from source separated collection:				
Biogenic wastes		x		0.40 [27]
Garden-yard wastes			x	
Market wastes		x		0.90 [30]
Wastes from wastewater treatment				
Sludges	x			0.30 [30]
Oil and fat trap wastes		x		
Sludge from gelatine production	x			
Sludge from starch production	x			
Waste from rice starch production	x			

2.4.1.| FOOD WASTE

Food waste represents a desirable waste stream that holds a significant potential as a resource for energy production through anaerobic digestion, since it is biodegradable with high moisture content. The amount of waste from household food leftovers in urban communities is increasing with the update of the source-sorted OFMSW management. Also, the growing demand for food products in developed countries has led to an increase in productivity from food processing industries. According to DE BAERE (2000),in Europe, in the early 1990´s anaerobic digestion of bio-waste mixed with grey wastes were similar, about 100 000 tons/year for each, while bio-waste treatment has been prevailing in recent years, reaching levels of 900 000 tons/year in 2001. This fact is due to the introduction of source and/or separate collection of the OFMSW in most of the

urbanised areas of the European Union. However, there are very few reports of anaerobic digestion plants operating entirely on the source segregated food waste fraction (CLIMENHAGA ET AL., 2008). Unfortunately, digestion plants for this purpose are in operation only in few countries, and the capacity of the plants is still limited compared to the organic waste potential (DAVIDSSON ET AL., 2007). In Portugal the anaerobic technology applied to organic wastes is under-utilized. In fact, there is only one facility treating the OFMSW by anaerobic digestion. Valorsul, S.A. is the company responsible for the treatment of the municipal solid waste produced in Lisbon, approximately 800 thousand tons per year. It covers a population of 1 176 180 inhabitants, which represents 12% of the total inhabitants of Portugal (VAZ ET AL., 2008). The anaerobic digestion plant is dimensioned to treat 40 000 ton of biodegradable waste per year in a 1st stage, and, 60 000 ton per year in a 2nd stage. For that purpose the biodegradable waste is selectively collected in restaurants, hotels, supply and retail markets, among other big producers of this waste, in the municipalities of Lisbon Area. VAZ ET AL. (2008), reported that the solids content in the digester is as low as 2.8% in total solids (TS), nevertheless, the biogas production is higher than the one predicted in the design operational parameters.

Even with this excellent result, there are a number of technical constraints associated with anaerobic digestion of food waste. In fact, this high caloric substrate is easily degraded by fermentative bacteria, which produce large amounts of organic acids, that lower the pH in the reactor inhibiting the methanogenic system and limiting the generation of significant amounts of CH_4 (VAVILIN ET AL., 2006; BOUALLAGUI ET AL., 2004; WANG ET AL., 1997). KIM ET AL. (2004A) reported that anaerobic treatment of food waste was not effective due to VFA accumulation, which resulted from the extremely high biodegradability of this waste. However, if used in co-digestion with sewage sludge, food waste improved nutrient balance and biodegradability. Thus, research at a laboratory scale is necessary for determining the operational parameters at full scale, also taking into account that anaerobic digestion has to face the high heterogeneity and seasonality of food production that eventually determine waste composition.

The physical and chemical characteristics of the organic waste should be known for designing and operating anaerobic digesters, since they affect biogas production and process stability. They include, but are not limited to, moisture content, volatile solids (VS) content, nutrient contents, particle size, and biodegradability (ZHANG ET AL., 2007). In Table 2.3 some of the characteristics relating different food waste are depicted.

Table 2.3|Food waste characteristics reported in several works

Food waste substrate from:	pH	TS (%)	COD ($g/g_{dryweight}$)	Organic N kjedhal (%)	Lipids (%)	C/N ratio	reference
Pre-cooked	5.7	42	1.2	2.2	12.9		CARUCCI ET AL. (2005)
Restaurant	4.5	21				13	MURTO ET AL. (2004)
Market waste		22	1.0[(1)]	2.9			PAVAN ET AL. (2007)
Markets; hotels; households; juice centers		15		1.1	8.5[(2)]	36	RAO ET AL. (2000); RAO & SINGH (2004)
Restaurants		31				15	ZHANG ET AL. (2007)
Restaurant Portugal		32	0.9[(1)]				CAPELA ET AL. (2008)
University canteen		28	0.5	3.8	22[(2)]		CLIMENHAGA & BANKS (2008)
University cafeteria	5.9	85		1.4		37	FORSTER-CARNEIRO ET AL. (2008A)
Dining hall	4.6	16	1[(1)]	0.4*			KIM ET AL. (2004A)
University cafeteria		22	1.1[(1)]			3.1	ORTEGA ET AL. (2008)
Households		23				14	BANKS ET AL. (2008)

[(1)]Calculated from the data presented; [(2)] dry basis, % in total solids.

It should be noted, that substrate characteristics change regarding the population habits, the different locations, the lifestyle, the cultural habits, the recycling practices and the type of food waste produced. For instance, CLIMENHAGA & BANKS (2008) reported that the anaerobic digestion of source-separated food wastes collected from a campus catering facility containing a varied mix of fruits, vegetables, meats and fried foods with no micronutrients supplementation exhibited methanogenic failure. Meanwhile, at the same time, duplicates of the reactors with addition of trace elements allowed stable digestion at high total VFA levels. According to these authors pure food waste may not contain all nutrients required to meet microbial metabolic requirements. Nevertheless, earlier, ZHANG ET AL. (2007) stated that food waste contained the required nutrients for anaerobic microorganisms. These authors attained, in batch, a CH_4 yield of 0.435 m^3/kgVS, using as single substrate source-separated food waste from restaurants, food markets (grocery stores), and commercial sources (hotels and businesses) at thermophilc temperatures.

Other authors (RAO ET AL, 2000) have reported that substantial differences were observed in the CH_4 yields and kinetics, depending on the food waste or OFMSW type. FORSTER-CARNEIRO ET AL. (2008A) studied the thermophilic dry anaerobic digestion process, 20% in TS content, for three organic substrates: food waste, shredded OFMSW and OFMSW. The food waste was collected in an university campus restaurant and the OFMSW was collected from Calandrias MSW Treatment plant, both in Spain. Experimental results showed important different behaviour patterns in these wastes, related with the organic matter biodegradation and CH_4 production. The food waste attained the highest production (0.18 m^3CH_4/kgVS). In contrast, the shredded OFMSW

showed the lowest production (0.05 m^3CH_4/kgVS). The CH_4 yield reached by the OFMSW was 0.08 m^3CH_4/kgVS. These authors reported that the nature of organic substrate has an important influence on the biodegradation process and CH_4 yield. Moreover, pre-treatment of OFMSW was not necessary. Some examples of CH_4 yields reported for different types of food waste are presented in Table 2.4.

Table 2.4| Process parameters and CH_4 yields of anaerobic digestion of food waste

Substrate:	TS (%)	System	T (ºC)	Time (days)	Methane Stoichiometry attained (%)	Methane yield m^3/kg $_{vsadded}$	reference
Korean food waste	15-30	Batch	37	28	86	0.47	CHO & PARK (1995)
Korean food waste		Batch	35	40		0.49	HEO ET AL. (2004)
Pre-cooked food waste	10	Batch	M[1]	114	0	0	CARUCCI ET AL. (2005)
markets; hotels; households; juice centers		Batch	26		95	0.66[2]	RAO ET AL. (2000)
markets; hotels; households; juice centers	6	Batch	26	100		0.35-0.4 [2]	RAO & SINGH (2004)
markets; hotels; households; juice centers	6	Batch	32	100		0.45-0.5 [2]	RAO & SINGH (2004)
Restaurants		Batch	50	28	80	0.45	ZHANG ET AL. (2007)
University cafeteria	20	Batch	55		40[4]	0.18 [3]	FORSTER-CARNEIRO ET AL. (2008A)
University cafeteria	20	Batch	55		51[4]	0.22[3]	FORSTER-CARNEIRO ET AL. (2008B)
University cafeteria	30 [5]	Batch	55	60		0.11[6] 0.22 [7] 0.03[8] 0.18[9] 0.29[10] 0.17[11]	FORSTER-CARNEIRO ET AL. (2007)
Households		Batch-continues	56			0.67[12]	BANKS ET AL. (2008)
Households		Batch-continues	37			0.63	BANKS ET AL. (2008)

[1]M- mesophilic ; [2]inoculum:15%(v) cattle dung;[3] 20% TS of food waste:30% of inoculum mesophilic sludge (v/v); [4] Calculated from the data presented; [5] 25% TS of food waste:30% of inoculum (v/v); [6] inoculum: corn silage; [7] inoculum: restaurant waste digested mixed with rice hulls; [8] inoculum: cattle excrement; [9] inoculum: swine excrement; [10] inoculum: digested sludge; [11] inoculum: swine excrement mixed with sludge (1:1); [12] process imbalance reported.

The anaerobic digestion process performance is much dependent on the type and the composition of the material to be digested (MURTO ET AL., 2004) but also the selection of the inoculum used can be as important as the biodegradability of the waste, especially during the start-up phase.

This is clear in the biomethanization studies of food waste performed by FORSTER-CARNEIRO ET AL. (2007) (Table 2.4). More recently, FORSTER-CARNEIRO ET AL. (2008B) analyzed the biomethanation process of food waste from a university campus restaurant, accessing three different TS percentages (20%, 25% and 30%) and two different inoculum percentages (20, 30% of mesophilic sludge). The best performance for food waste biodegradation and CH_4 generation was attained in the reactor with 20% TS: 30% of inoculum (Table 2.4). It was reported that food waste exhibit the classical waste decomposition pattern with a fast start up phase beginning within 0–5 days, an acclimation stage (acidgenic/acetogenic phases) between days 5 and 20–30 days and a subsequent stabilization phase.

CHO AND PARK (1995) determined the CH_4 yield of different food wastes digested for 28 days at 37°C. The CH_4 production was 0.482, 0.294, 0.277, and 0.472 m^3/kgVS for cooked meat, boiled rice, fresh cabbage and mixed Korean food wastes, respectively, corresponding in the same order to 82%, 72%, 73% and 86% of the stoichiometry CH_4 yield. However, to perform successfully the solid-state anaerobic digestion of Korean food wastes, these authors suggest, a two-phase digestion method in order to control the rapid VFAs production at the initial stage of fermentation.

Waste related to the food stream is regularly described as a good co-substrate. The biochemical CH_4 potential (BMP) test was used by HEO ET AL. (2004) to evaluate the biodegradability of a traditional Korean food and activated sludge. The results showed that the biodegradability of the mixture increased from 36.6 to 82.6% as the food waste proportion of the mixture increased from 10 to 90%, respectively. Also, during the operation of a single-stage anaerobic digester for the co-digestion of these substrates, there was no indication of failure such as low pH, insufficient alkalinity, ammonia inhibition, and the accumulation of VFAs. Recently, within the same context, SOSNOWSKI ET AL. (2008) compared the mesophilic co-digestion of a simulated OFMSW (25%) and activated sludge (75%) with the mono-digestion of the wastes. The composition of the simulated OFMSW was as follows: 55% of potato, 28% of fruit and vegetables, 5% of bread, 2% of paper plus 10% of rice and 10% pasta in weight. The cumulative biogas production for sewage sludge (0.181 m^3) was lower than that for co-digestion (0.232 m^3) or than the simulated OFMSW (0.228 m^3). During the fermentation of the latter, accumulation of VFA caused pH decrease and strongly inhibited gas production. The addition of activated sludge

improved the buffering capacity of the system. The results showed that if used in the proper proportion, activated sludge and OFMSW could be co-digested efficiently in existing municipal sewage sludge digesters that are operated under low load conditions. Similar work plan was used by ZHANG ET AL. (2008), reaching similar conclusions. The composition of the simulated OFMSW used by these authors was as follows: kitchen, vegetable market and yard waste.

CARUCCI ET AL. (2005) reported methanogenesis inhibition from no acclimated inoculum, when accessing in mono-digestion fresh vegetables and precooked wastes from a food processing industry. According to the authors this was due to high contents of potassium and lipids in the substrates assayed. However, co-digestion of the fresh vegetable wastes with aerobic sludge, from an agro-industrial wastewater treatment plant (WWTP) was more effective in CH_4 rate and yield, when compared to the single waste digestion. On the other hand, the co-digestion of the pre-cooked wastes with the aerobic sludge, remained strongly inhibited. Nonetheless, a long acclimation period was suggested to overcome this reported inhibition.

It is important to predict process imbalance, especially when the feed is a particularly easy to degrade substrate, as the food waste. Newly, the increased accumulation of non-degraded intermediates as an indication of process imbalances was examined by HECHT & GRIEHL (2009). These authors assessed in laboratory scale the anaerobic degradation of kitchen waste, with a high protein and fat content, using a quasi-continuous co-digestion process with swine manure, where the substrate load was gradually increased. The important finding was the early detection of aromatics, especially phenylacetic acid, even before the monitoring of VFA content, giving an indication of a process imbalance. These metabolites could be identified as intermediates from the anaerobic degradation of the aromatic amino acids phenylalanine, tyrosine and tryptophan.

Food waste is not very suitable to be mono-digested due to the hydrolysis rate and subsequent level of VFA accumulation. In order to avoid probable digestion failure, the best approach is to be co-digested with other waste with sufficient buffer capacity. Also, the addition of food waste to a process with low CH_4 yield will benefit the process. The answer to improve CH_4 production might be how to modulate the feed of anaerobic digestion.

2.4.2.|FATS AND GREASES

A wide variety of industries produce effluents rich in fats, oils and greases, like the restaurant trade (STOLL & GUPTA 1997), the slaughterhouse wastewater (SALMINEN & RINTALA, 2002), dairy industry (VIDAL ET AL. 2000) and the food industry (CAMMAROTA ET AL. 2001). The fatty matter constitutes a potential problem in terms of wastewater management. Their amount in municipal wastewater is approximately 30–40% of the total COD (CHIPASA & MEDRZYCKA, 2006). Fats may solidify at lower temperatures, thus causing operational damage associated with clogging, unpleasant odours and even causing pipes and sewer lines to become blocked (BAIG & GRENNING 1976). A large number of pre-treatment systems (grease-trap, tilted plate separators, dissolved air flotation systems and physical–chemical treatment) are employed to remove oil and grease from the wastewaters prior to the main treatment process itself, which is generally of a biological nature (CAMMAROTA & FREIRE, 2006). Nonetheless, these pre-treatments may fail to retain dissolved and emulsified fats efficiently allowing them to enter the water treatment system. The fats may then interfere with aerobic biological wastewater treatment processes by reducing oxygen transfer rates (CHAO & YANG 1981) and can also reduce the efficacy of anaerobic treatment processes by reducing the transport of soluble substrates to the bacterial biomass (RINZEMA ET AL. 1994). Fats not properly treated by WWTP may enter rivers and oceans with potentially detrimental environmental impacts (STAMS & OUDE 1997).

The lipids, usually in the form of fats and oils, are glycerol bonded to LCFA, alcohols, and other groups by an ester or ether linkage. Triacylglycerides, also called neutral fats, are the most abundant family of lipids and are hydrolyzed by extracellular lipases to yield glycerol and LCFA (CAVALEIRO ET AL., 2008). In anaerobic digestion glycerol is further degraded via acidogenesis, while LCFA are degraded to acetate, H_2 and CO_2 through the β-oxidation process (syntrophic acetogenesis) (STRYER, 1995). Table 2.5 presents some examples of LCFA compositions in fat-containing waste(water), showing that palmitic acid and oleic acid, in this order, are the most abundant LCFA.

A large amount of CH_4 can be produced from LCFA because they are highly reduced organic materials. For example, theoretically 1.01 L of CH_4 at standard temperature and pressure (STP) can be produced from 1 g of oleate, one of the typical LCFA, while only 0.37 L can be produced from 1 g of glucose (KIM ET AL., 2004B). Therefore, waste with a high lipid-content constitutes an attractive substrate for CH_4 production (KIM ET AL., 2004B).

Table 2.5 | LCFA commonly found in fat based waste(water) (% of weight).

	LCFA (represented by their chemical notation)							
waste(water)	Lauric (C12:0)	Myristic (C14:0)	Palmitic (C16:0)	Palmiticoleic (C16:1)	Stearic (C18:0)	Oleic (C18:1)	Linoleic (C18:2)	Reference
Lard		1-2	28-30		12-18	40-50	7-13	LINSTROMBERG (1970)
Tallow		3-6	24-32		20-25	37-43	2-3	LINSTROMBERG (1970)
Sheep Tallow		7.1	19.6		27.4	39.5		BROUGHTON ET AL. (1998)
Sunflower oil			6-8		2-3	11-16	73-77	GOERING ET AL. (1982)
Soybean oil			10.58		4.76	22.52	52.34	CANAKCI (2007)
Soybean oil			14		4	24	52	HUA ET AL. (2008)
Yellow grease[(1)]		2.43	23.24	3.79	12.96	44.32	6.97	CANAKCI (2007)
Trap grease		1.66	22.83	3.13	12.54	42.36	12.09	CANAKCI (2007)
Pet food [(2)]	0.2	2.2	32.9		14.9	33.8	5.6	FERNÁNDEZ ET AL. (2005)
Animal fat [(2)]	nd	3.0	30.0		17.0	38.0	6.0	FERNÁNDEZ ET AL. (2005)
Vegetable fat[(2)]	45.5	18.5	10.4		3.3	8.7	2.2	FERNÁNDEZ ET AL. (2005)
Chicken fat			20	6.2	5.3	39.6	24.7	HUA ET AL. (2008)
Palm oil			35		6	44	15	HUA ET AL. (2008)
Dairy wastewater			27		7	37	13	KIM ET AL. (2004c)

[(1)] yellow grease collected as animal and vegetal fats waste from food restaurant; [(2)] % of other LCFA detected, 10.4, 6.0 and 11.4 for pet food, animal fat and vegetable fat respectively; nd- not detected

Lipid-rich waste(water) do not always accomplish a desirable performance in terms of COD removal and conversion to CH_4. Briefly, several operational problems are described as the main causes for the difficult conversion of lipids to biogas, namely bacterial inhibition and sludge flotation and washout (JEGANATHAN ET AL., 2006; HWU, 1997; TAGAWA ET AL., 2002). These problems result mostly from the accumulation of LCFA onto the microbial aggregates, by mechanisms of adsorption, precipitation or entrapment (HWU, 1997; PEREIRA ET AL., 2005). Besides the potential metabolic inhibition, LCFA accumulation onto the sludge can create a physical barrier, with consequent limitations in the transport of substrates and products (PEREIRA ET AL., 2005). This inhibition was long assumed irreversible (BROUGHTON ET AL., 1998), but lately it has been proved reversible (PEREIRA ET AL., 2004). In fact, it was found that the observed temporary decrease in the methanogenic activity after the contact with LCFA is a reversible phenomenon, being eliminated

after the conversion to CH_4 of the biomass-associated LCFA (PEREIRA ET AL., 2004, 2005).

A discontinuous operation, specifically designed to promote LCFA accumulation during continuous feeding, and subsequent batch degradation of the biomass-associated substrate, was proposed as a strategy to achieve an efficient rate of CH_4 production during the treatment of fatty waste(water)(PEREIRA ET AL., 2004). Recently, CAVALEIRO ET AL. (2008) reported that when three recurrent pulses of a real dairy wastewater, containing 53% of fat, were added to an oleate adapted inoculums in a continuous reactor, an increased capacity of the sludge to convert substrate accumulated in previous additions was evidenced. Each new pulse was added when biogas production from the previously fed stopped. The CH_4 yields achieved were 0.45, 0.88 and 1.29 gCOD-CH_4/gCOD fed, in the first, second and third pulses, respectively. Within this scope, CH_4 enrichment through intermittent pulses of fat is further discussed in Chapter 5.

Grease trap sludge comprises greasy materials separated from wastewaters of e.g. restaurants and institutional kitchens. Earlier, this type of waste was mainly land-filled, but due to stricter environmental legislation, this is no longer allowed. LUOSTARINEN ET AL. (2009) studied the co-digestion of sewage sludge with grease trap sludge from a meat-processing plant and found feasible an addition of grease trap sludge up to 46% of feed VS (hydraulic retention time16 days; maximum organic loading rate 3.46 kgVS/m^3d).The high CH_4 production potential of grease trap sludge (918 m^3/tonVS_{added}), resulted in a significant increase of the specific CH_4 production in the reactor experiments (maximum 463 m^3/tonVS_{added}) compared to digestion of sewage sludge alone (278 m^3/tonVS_{added}). According to these authors, grease trap sludge addition can be beneficial to the WWTP due to increased CH_4 production and possible gate fees. However, the feed should be planned carefully with stepwise increase to the desired feed ratio in order to acclimatise the bacteria and to prevent overloading, since, higher grease trap sludge addition leads to potential LCFA inhibition and incomplete degradation. . DAVIDSSON ET AL. (2008) also studied the co-digestion of sewage sludge but with grease trap sludge from restaurants and institutional kitchens. These authors also reported a successful co-digestion performed both in laboratory batch and continuous pilot-scale digestion tests. The addition of grease trap sludge to sewage sludge digesters was seen to increase the CH_4 yield by 9 to 27%, when 10 to 30% of sludge from grease traps (on VS-basis) was added. Furthermore, single-substrate digestion of grease trap sludge gave high CH_4 potentials in batch tests, but could not reach stable CH_4 production in continuous digestion.

From the literature review, co-digestion of fats can be more profitable than used as a single-substrate.

2.4.5.|ANIMAL WASTE

Animal manure is a major source of anthropogenic GHG, mostly as CH_4 and nitrous oxide (N_2O). Concerning CH_4, livestock manure contributes to 5–10% of total GHG emissions (ROTMANS ET AL., 1992). In fact, the natural degradation of livestock wastes during their storage, leads to the release of CH_4 to the atmosphere, due to the anaerobic decomposition of the organic matter. Anaerobic digestion of liquid manure may result in indirect environmental benefits, since reduces the spontaneous emissions of CH_4, when compared to the conventional handling systems of manure. This reduction is estimated to be 50% or more, depending on the biogas technology utilised (BÖRJESSON, 2008).

Special emphasis should be put on effective management of manure waste, because its share in the total organic waste collected in Europe reaches 91%, exceeding significantly other shares, which amount for 4%, 2% and 3%, respectively, for OFMSW, sewage sludge and industrial organic waste (BRAUN ET AL., 2003). Animal manures are a plentiful source of organic material for use as feedstock in anaerobic digesters (WARD ET AL., 2008). The CH_4 potential of manure comes from the digestion of the organic components in the faeces and in the straw used as bedding material, which are mainly composed of carbohydrates, proteins and lipids (MOLLER ET AL., 2004A). However, agricultural biogas production from manure alone has a relatively low gas yield, thus is economically not viable at current oil prices. Factors which contribute to the CH_4 potential of manures are the species, breed, and growth stage of the animals, feed, amount, and type of bedding and also any degradation processes which may take place during storage (MOLLER ET AL., 2004 A,B).

Manure often contain recalcitrant organic fibre which is difficult to degrade anaerobically (ANGELIDAKI & AHRING, 2000), including variable quantities of straw bedding material, although the volumetric CH_4 yield of bedding straw has been found to be higher than the manure solid fraction (MOLLER ET AL., 2004A,B). In a single- substrate digestion, the CH_4 yield is relatively low and depends both on the waste source and reactor loading rate, ranging from 35.6 to 290 L/kgVS at mesophilic conditions (HANSEN ET AL., 1998, SALMINEN & RINTALA, 2002, ALVAREZ ET AL., 2006). Biogas production in farm biogas plants could be increased by 80–400% by using organic wastes and by-products as co-substrates, depending on their amount and composition (WEILAND, 2000; BRAUN ET AL., 2003).

There are several examples in the literature of CH_4 enhancement of manure using co-substrates. The addition of coconut pith and cattle manure in the ratio 3:2 (dry basis) showed enhanced biogas production with 80 to 85% CH_4 (RADHIKA ET AL. 1983). The addition of waste milk to a batch

digester containing cattle slurry, promoted high CH_4 production levels in all digesters receiving additions of waste milk, with the highest CH_4 production being observed in those digesters receiving the highest load of milk (CALLAGHAN ET AL., 1997). Immediately after addition, the gas production rate transiently increased from 0.3 L/day to a maximum of 1.46 L/day in the digesters receiving the highest load of 29.3 kgCOD/m^3. The results suggested that the up-scaling of co-digestion of waste milk with cattle manure would be feasible, although inhibition by ammonia was a factor to be considered (CALLAGHAN ET AL., 1997).

Combined anaerobic digestion of oil mill effluent with manure, household waste or sewage sludge was also investigated (ANGELIDAKI & AHRING 1997). It was shown that oil mill effluent could be degraded into biogas when co-digested with manure. In co-digestion with household waste or sewage sludge a higher dilution of oil mill effluent was required for its degradation. The results showed that the high buffering capacity of manure, along with the content of several essential nutrients, make it possible to degrade oil mill effluent without previous dilution and no addition of external alkalinity or external nitrogen source.

According to CALLAGHAN ET AL. (2002), the digestion of cattle slurries and a range of agricultural wastes were successfully accomplished. Previous batch studies have shown that co-digestion of cattle slurry with fruit and vegetable wastes and with chicken manure were among the more promising combinations (CALLAGHAN ET AL. 1999). However, in continuous feeding, when the proportion of chicken manure in the feed was increased, the digester performance was compromised.

MURTO ET AL. (2004) co-digested different waste in two laboratory-scale studies. In the first study, sewage sludge was co-digested with an industrial waste from the potato processing industry. When the fraction of starch-rich waste increased, even at a low organic loading rate, a process failure occurred, due to a low buffer capacity. The co-digestion of pig manure, slaughterhouse waste, vegetable waste, and various kinds of industrial waste was the subject of the second study. This resulted in a highly buffered system as the pig manure contributed to high amounts of ammonia. Although the conversion of VFA was incomplete, high gas yields were obtained, 0.8–1.0 m^3/kgVS.

MACIAS-CORRAL ET AL. (2008) reported a methane production of 172 m^3 CH_4/ton dry waste for the co-digestion of OFMSW with cow manure. Single substrates attained lower values, 62 and 37 m^3 CH_4/ton dry waste, cow manure and OFMSW, respectively. The OFMSW was composed of approximately 62% paper, 23% food waste, and 15% yard clippings. In the same work the co-digestion of cotton gin waste with cow manure produced 87 m^3 CH_4/ton dry waste.

ALVAREZ & LIDÉN (2008) investigated a semi-continuous mesophilic wet digestion system processing a mixture of manure, solid slaughterhouse and fruit-vegetable wastes. The substrates were evaluated separately and in mixtures in proportions of 0–100% (VS/VS) of each substrate. The digestion of mixed substrates was in all cases better than of pure substrates. A semi-continuous co-digestion process using these substrates can be expected to result in a reduction of the volatile solid content (between 50% and 65%) with a CH_4 yield of about $0.3 m^3/kgVS_{added}$ at organic loading rates up to 1.3 $kgVS/m^3d$. However, a further increase in the loading rate promoted a decrease in biogas production, indicating organic overload or insufficient buffering capacity in the digester.

Accordingly, manure is an excellent co-substrate, which buffers the system, supplies the nutrients required for bacterial growth and dilutes feedstock as a result of its low dry matter.

2.5. | PRE-TREAMENTS

Not all substrates are easily hydrolysed. To overcome this limitation, several authors denote the need of a pre-treatment, in order to increase substrate bio-availability by the anaerobic consortium. A review of pre-treatments to enhance the digestibility of lignocellulosic biomass was recently published by HENDRIKS & ZEEMAN (2009). Pre-treatment of wastes can increase biogas production, VS reduction and increased solubilisation.

The use of pre-treatments is particularly useful in the digestion of wastes having high cellulose or lignin content, since these recalcitrant polymers can be physically, thermally, or chemically broken down. Nevertheless, additives can enhance the production rate of a reactor or increase the speed of a start up, their additional cost must always be balanced against resultant improvements in efficiency.

2.6. | CONCLUSIONS AND PERSPECTIVES

The co-substrates rich in lipids, proteins and carbohydrates are essential for the anaerobic plants economy, but might lead to disturbances, if not handled properly. Over the years, a range of innovative ideas for the utilisation of these wastes has been put forward. Many researchers throughout the world are putting a great effort in improving methane production, evaluating all kind of wastes, in order to enhance synergies between different substrates. In fact, the performance of an anaerobic digestion process has been shown to be much dependent on the type and the composition of the material to be digested.

There is optimism in Portugal about the future of biogas, even though there is very little public

awareness about its importance as an alternative energy source. In the next years an increment in the number of anaerobic digestion plants is expected, due to the new ENRRUBDA legislation. Furthermore, the present poor scenario related to anaerobic digestion plants is likely to change rapidly with green energy requirements, oil and gas prices, and carbon credits.

In Portugal there are few animal waste biogas plants, although the use of animal manure and other organic-based waste for waste-to-bio-energy conversion processes would allow farmers to take advantage of new markets for traditional waste products. Further, farm digesters may also assist to reduce the greenhouse gas emissions from manure management. However, to make farm-scale digestion more cost effective, co-digestion of cow manure with supplementary materials is of interest, in order to increase CH_4 yields. Addition of co-substrates with a high CH_4 potential, not only increases gas yields but above all increases the income through tipping fees. Anaerobic digestion of organic wastes to produce energy in the form of biogas is, certainly, the most likely to be of commercial interest.

Hence, experimental studies how to improve CH_4 production ore of significant importance.

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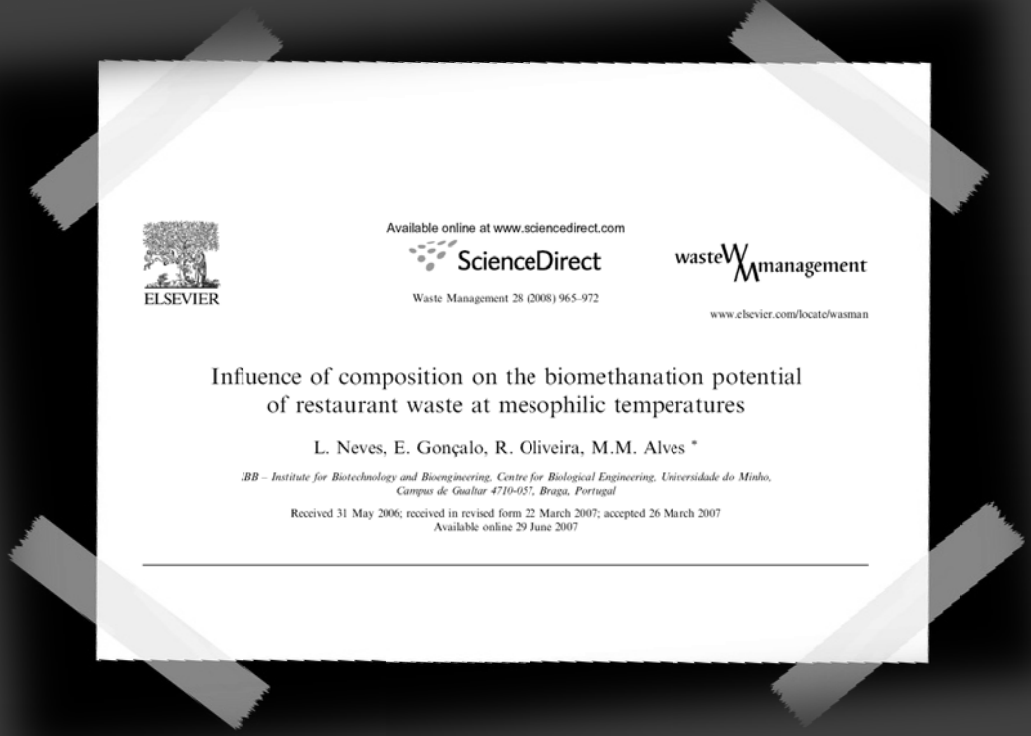

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Influence of composition on the biomethanation potential of restaurant waste at mesophilic temperatures

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A synthetic waste was used to study the effect of waste composition on anaerobic degradation of food waste. It was made by blending melted pork lard, white cabbage, chicken breast, and potato flakes, to simulate lipids, cellulose, protein, and carbohydrates, respectively. Four blends of the four constituents with an excess of each component were assayed and compared with a fifth blend containing an equal amount of COD of each of the four components. The methane production and the time course of soluble COD and VFA were assessed in batch assays. High reduction of VS (between 94 and 99.6%) were obtained in all the assays. The CH_4 yield was between 0.40 m^3CH_4/kg $VS_{initial}$ (excess of carbohydrates) and 0.49 m^3CH_4/kg $VS_{initial}$ (excess of lipids). The degradation of the lipid-rich assays differed from the others. Fifty percent of the biochemical methane potential was obtained after 3-6 days for all the assays, except for the one with excess of lipids which achieved 50% methanation only after 14.7 days of incubation. In the assay with excess of lipids, a considerable fraction of COD remained in the liquid phase, suggesting an inhibition of the methanogenic process that was likely due to the accumulation of LCFA. The hydrolysis rate constants, assuming first order kinetics, over the first 6 days were between 0.12 d^{-1} (excess of lipids) and 0.32 d^{-1} (excess of carbohydrates). The results indicate that anaerobic digestion facilities with large variations in lipid input could have significant changes in process performance that are worth further examination.

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3.|INFLUENCE OF COMPOSITION ON THE BIOMETHANATION POTENTIAL OF RESTAURANT WASTE AT MESOPHILIC TEMPERATURES

3.1.|INTRODUCTION

Although anaerobic digestion of organic solid wastes is an established technology in Europe with 120 full scale plants treating about 4 million ton per year, it represents, on average, only 27.5% of all the biological waste treatment processes (DE BAERE, 2006).

Kitchen waste is a large fraction of municipal solid waste (between 20-65%) (TCHOBANOGLOUS ET AL., 1993). The biomethanation potential of the waste depends on the relative amounts of the four main components – proteins, lipids, carbohydrates, and cellulose. Kitchen and restaurant waste are not homogeneous in its day-to-day composition. It is important to have data to predict how these fluctuations may influence the anaerobic digestion process.

This work aims to study how variations of the major components of restaurant waste influence the methane yield and the process kinetics.

3.2.|MATERIAL AND METHODS

3.2.1.|WASTE CHARACTERIZATION

A synthetic restaurant waste, representing the major components of waste from a real restaurant, was prepared by mixing melted lard of pork, white cabbage, chicken breast, and potato flakes, to simulate lipids, cellulose, protein and carbohydrates, respectively. A preliminary assay was done in order to assess the adequacy of the synthetic waste to simulate a real restaurant waste. The real restaurant waste was a composite sample (1 week based) from the waste produced in the restaurant of the University of Minho, located in “Campus de Gualtar”, Braga, Portugal. The characteristics of each component of the synthetic waste and of the real waste are presented in Table 3.1. The particle size was in the range of 1-3 mm. The different mixtures were prepared immediately before launching the tests. The real restaurant waste was collected, ground to 1-3 mm particle size and stored at 4 ºC during 5 days, until the end of the collection process. Then it was mixed and stored at -18 ºC.

Table 3.1|Characterization of the synthetic and the real restaurant waste.

Waste	Individual components of the synthetic restaurant waste				Real restaurant waste
	Fat (lard)	Cabbage	Chicken breast	Potato flakes	
COD (mg/g_{ww}*)	1632±38	53±7	306±70	1018±106	327±73
TS (mg/g_{ww}*)	970±31	58±1	330±13	930±14	238±1
VS (mg/g_{ww}*)	974±30	56±1	320±28	893±3 1	214±7.0
TKN (mgN-NH_4/g_{ww}*)	0.57±0.10	Not	52.3±3.6	9.3±1.0	13±1
Fat content (mg/g_{ww}*)	977±18	Not	8.5±1.9	16.3±1.2	20±8
Moisture content (%)	3±3	94.2±0.1	67.0±1.3	7.0±1.4	76.2±0.1

TKN – Total Kjeldahl Nitrogen; * g_{ww} – mass of raw waste express in grams of wet weight; Values given are averages and standard deviations based on 25 measurements.

3.2.2.|INOCULUM

The granular sludge used as inoculum was collected from an upflow anaerobic sludge blanket reactor treating a brewery effluent located in Oporto, Portugal. The solid content of the inoculum was 100 mgVS/g of sludge. The VS/TS was 65%. The use of a granular sludge as an inoculum had been tested previously and shown to reduce the risk of over-acidification during batch, high-solids, anaerobic digestion (NEVES ET AL., 2004). Based on these previous studies, the use of the granular sludge as an inoculum appears to lead to rapid digestion of food waste without the need for a lengthy acclimatisation period.

The production of methane due to the residual substrate present in the inoculum was recorded in a parallel blank assay where only the inoculum was incubated without any substrate. The values obtained in this experiment were used to correct the cumulative methane production values in all the assays with the exception of the preliminary assay comparing the real and the synthetic waste. The inoculum was also characterised in terms of the Specific Methanogenic Activity in the presence of acetate and H_2/CO_2, the two trophic groups directly involved in methane production. The obtained values were 0.92±0.20 and 2.96±0.03 gCOD-CH_4/(gVS.d), respectively.

The quantification of the residual methane production was performed by measuring the methane production in sealed vials without substrate. A pressure transducer was used to record the increase in pressure and headspace biogas was sampled periodically to assess the methane content. Strict anaerobic conditions were maintained by using an anaerobic basal medium composed of cysteine- HCL (0.5 g/L), $NaHCO_3$ (3 g/L), with the pH adjusted to 7.0-7.2. Resazurin was added as an indicator of redox potential. This basal medium was prepared by boiling the medium before adding the bicarbonate. The hand held pressure transducer was capable of

measuring a pressure increase or decrease of two atmospheres (0 to ± 202.6 kPa) over a range of -200 to +200 mV. The sensing element is connected to a digital panel module and the device is powered by a 9.0 V DC transformer. The same technique was used to assess the specific methanogenic activity, but individual substrates (acetate-30 mM and H_2/CO_2 80:20 V/V at 1 Bar overpressure) were added to the vials. All the batch assays described were performed in triplicate assays. The volume of methane produced was corrected to Standard Temperature and Pressure conditions (STP - 1 atm and 273 K).

3.2.3.|BATCH EXPERIMENTS

Two preliminary batch experiments were done in order to verify the suitability of the synthetic waste to represent a real restaurant waste. Methane production was followed from the real restaurant waste and from the synthetic restaurant waste after incubation in batch vials in the following conditions: 5% Total Solids (TS), 1.35 g volatile solids $(VS)_{waste}/gVS_{inoculum}$. The synthetic waste was made by blending the different components in equal amount of TS (125 mg). After introducing the correct amounts of waste and seed sludge, a defined amount of anaerobic basal medium (described above) was added under strict anaerobic conditions, in order to give the desired solid content. The vials were then incubated at 37 ºC under stirring conditions (150 rpm) and the pressure increase was recorded using the above mentioned pressure transducer device. The biogas accumulated in the headspace was sampled regularly and the methane content was determined. Pressure and headspace methane content data were used to calculate the volume of methane produced and then corrected to STP conditions. The results were expressed in terms of methane yield ($m^3CH_4/kgVS_{initial}$) and % of methanation that corresponds to the % of methane produced relative to the biochemical methane potential (0.350 $m^3CH_{4\ (STP)}$/kg COD from stoichiometry).

After this preliminary experiment, two sets of assays were performed in order to study the influence of the excess of each component present in the synthetic waste in terms of biogas production and process kinetics. In the first set of batch assays, the biogas production and composition was assessed as previously described. In the second set of batch tests, the liquid composition was assessed in terms of VFA and soluble COD. Table 3.2 presents the experimental conditions prevailing in both assays.

The liquid composition assays were performed in 600 mL flasks, keeping all the ratios and conditions applied in the second set of the methanation assays. Liquid samples were regularly withdrawn, centrifuged, and filtered (0.2 µm pore size membranes) for soluble COD and VFA (acetate, propionate, iso-butyrate and *n*-butyrate) analysis. These batch tests were performed in

duplicate assays.

Table 3.2|Experimental conditions prevailing in the batch assays.

	Waste	Gas production assays	Liquid composition assays
% Total solids		1.8	1.8
Waste/Inoculum (gVS/gVS)		1.35	1.35
Total volume (mL)		160	600
Anaerobic medium added (mL)		10	200
Initial COD (g/L)		16	16
Excess Lipids (mg COD added)	Fat (lard)	70	1400
	Cabbage	30	600
	Chicken breast	30	600
	Potato flakes	30	600
Excess Cellulose (mg COD added)	Fat (lard)	30	600
	Cabbage	70	1400
	Chicken breast	30	600
	Potato flakes	30	600
Excess Protein (mg COD added)	Fat (lard)	30	600
	Cabbage	30	600
	Chicken breast	70	1400
	Potato flakes	30	600
Excess Carbohydrates (mg COD added)	Fat (lard)	30	600
	Cabbage	30	600
	Chicken breast	30	600
	Potato flakes	70	1400
Control (mg COD added)	Fat (lard)	40	800
	Cabbage	40	800
	Chicken breast	40	800
	Potato flakes	40	800

3.2.4.|ANALYTICAL METHODS

COD, TS, VS and Total Kjeldhal Nitrogen (TKN) were determined according to Standard Methods (APHA ET AL., 1989). The closed reflux titration method was used for COD analysis. A defined amount of waste was previously suspended in hot water and homogenised with a Euroturrax T20 Standard (Ika Labortechnik) homogeneizer. For TKN analysis a defined amount of the solid waste was directly digested using selenium as catalyst. The fat content was extracted with a mixture of chloroform:methanol 1:2 (v:v) in a soxtec system, dried and weighed. Methane content of the biogas was measured by gas chromatography using a Porapack Q (180 to 100 Mesh) column, with Helium as the carrier gas at 30 mL/min and a thermal conductivity detector. Temperatures of the detector, injector and oven were 110, 110 and 35ºC, respectively. VFA were determined by high-performance liquid chromatography using a chrompack column (300x6.5

mm) and a mobile phase of sulphuric acid 5 mM at 0.7 mL/min. The column was set at 60ºC and the detection was by spectrophotometry at 220 nm.

3.3.|RESULTS AND DISCUSSION

In the first experiment, the cumulative methane production obtained from the real restaurant waste was compared with the methane production obtained from the synthetic waste (Figure 3.1).

This experiment was planned to indicate the adequacy of the synthetic waste to represent the real waste. Only the initial cumulative methane production was considered, which in eight days reached 56% of the theoretical methane yield for both wastes (196 $mLCH_4/gCOD_{added}$).

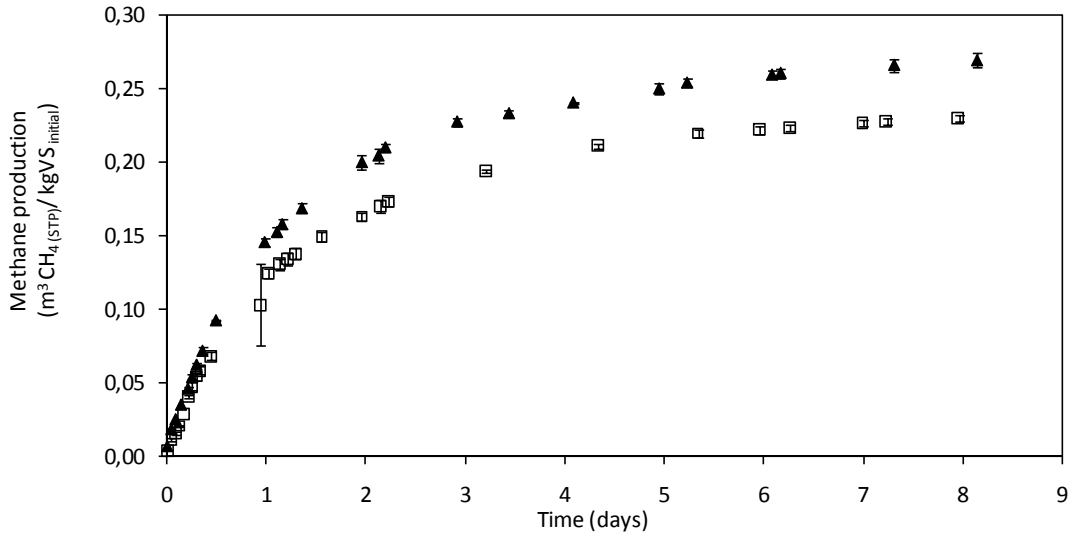

Figure 3.1|Time course of the initial methane production obtained from the synthetic waste (□) and real restaurant waste (▲) when incubated in the following conditions: 5% Total Solids (TS), 1.35 g $VS_{waste}/gVS_{inoculum}$. The synthetic waste was made by blending the different components in equal amount of TS (125 mg). Values represent the average of duplicate experiments and *y*-bars represent the standard deviation.

The similar initial methane production pattern obtained for the two wastes indicates that the synthetic waste was suitable to represent the real one. The advantage of using the synthetic waste in this study is that it allows the concentrations of the major components of a restaurant waste to be changed.

Figure 3.2 presents the time course of the methane production in all the assays.

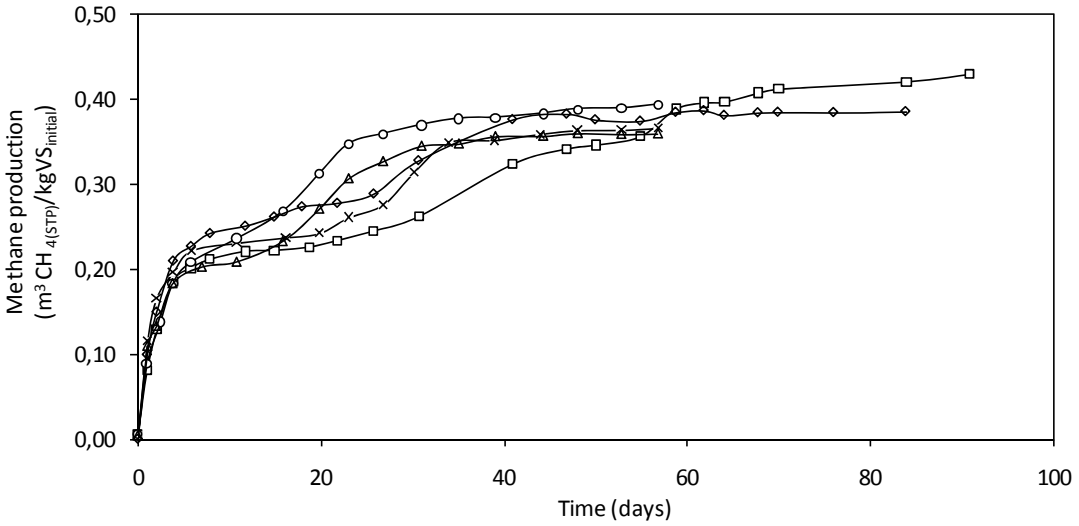

Figure 3.2|Time course of cumulative methane production versus time in the assays with excess of lipids (□), cellulose (Δ), protein (○), carbohydrates (×) and the assay of equal COD amounts (◊).

All the curves of methane production had, in general, similar behaviour and displayed two plateaux. The first plateau was attained by day 3.8 for the assay with an excess of cellulose and around day 5.8-5.9 for the other assays. On average 50±5% of the total methane production was achieved at this time in all the assays, with the single exception of the assay with an excess of lipids (50% methanation only after 14.8 days). Although different solids content was used in the preliminary experiment, a similar result was obtained where 50% of the theoretical methane potential was achieved after 4 days (Figure 3.1). Therefore, it is expected that if the time period was extended in that experiment a similar behaviour would be observed.

Table 3.3 represents the time at which 50% and 85% of the theoretical methane potential were achieved. This % represents the obtained cumulative volume of methane divided per mass of COD added in relation to the biochemical methane potential of 0.35 m^3CH_4/kgCOD. The methane yield and the VS reduction are also presented. All the values presented Table 3.3 in were corrected by subtracting the cumulative methane production obtained in the blank assays without waste.

The first assay to achieve 85% methanation was the one containing an excess of protein (after 23 days), followed by the assay containing an excess of cellulose (after 24 days), an excess of carbohydrates (in day 30), and an equal amount of COD (in day 32). The assay with an excess of lipids achieved 85% methanation only after 57 days. The values of methane yield attained with

the synthetic waste were in the range of values reported in the literature for the anaerobic digestion of the organic fraction of municipal solid waste. For instance, a methane yield of 0.301 m^3/kgSV was reported for the Valorga process (VALORGA, 1985) while MATA-ALVAREZ (2003) reported a methane yield of 0.489 m^3/kgVS for the organic fraction of municipal solid waste for the city of Barcelona.

Table 3.3|Time necessary to achieve 50 and 85% of the theoretical methane potential, final methane yield and VS reduction.

Assay	Time for 50% methanation (days)	Time for 85% methanation (days)	Final Methane yield ($m^3CH_{4(STP)}$/kg$VS_{initial}$)	Volatile solids reduction (%)
Excess Lipids	14.8	57	0.43	96.5
Excess Cellulose	3.9	24	0.36	95.1
Excess Protein	5.9	23	0.39	94.0
Excess Carbohydrates	3.0	30	0.37	99.6
Control	3.4	32	0.39	95.9

The overall volatile solids reduction, including both inoculum and waste, was in the range of 94-99.6% (Table 3.3). The volatile solids change associated with the inoculum is not easy to assess because the inoculum contains substrates that are consumed and also is the source for biomass growth. In any case, the volatile solids change due to the inoculum is not as significant as for the waste.

The assays with an excess of cellulose, protein, carbohydrates, and equal COD presented maximum VFA concentrations between days 5 and 30, followed by a decrease and stabilization near a null value by day 50 (Figure 3.3).

In general, the maximum soluble COD (not shown) and VFA concentrations occurred simultaneously with the first plateau observed in the cumulative methane production. The most significant VFA detected in the assays were acetic and *n*-butyric acids. The concentrations of VFA in assays containing an excess of protein were the highest and peaked at a value twice the amount detected in the other assays. It can be hypothesised that higher levels of proteins induced inhibition of methanogens by ammonia. In general, the concentration of VFA was approximately zero after 45 days of operation and corresponded to the stabilization in cumulative methane production. In the assay with lipids, a considerable amount of COD remained in the liquid phase as VFA after 80 days. This indicates that β-oxidation proceeded until the formation of butyrate, but further acetogenesis and methanogenesis was impaired.

The inhibition of methanogens in the assay with an excess of lipids can also be observed in Figure 3.4 , where the percentage of the acidified COD that was not converted into methane

versus time for all the assays is presented.

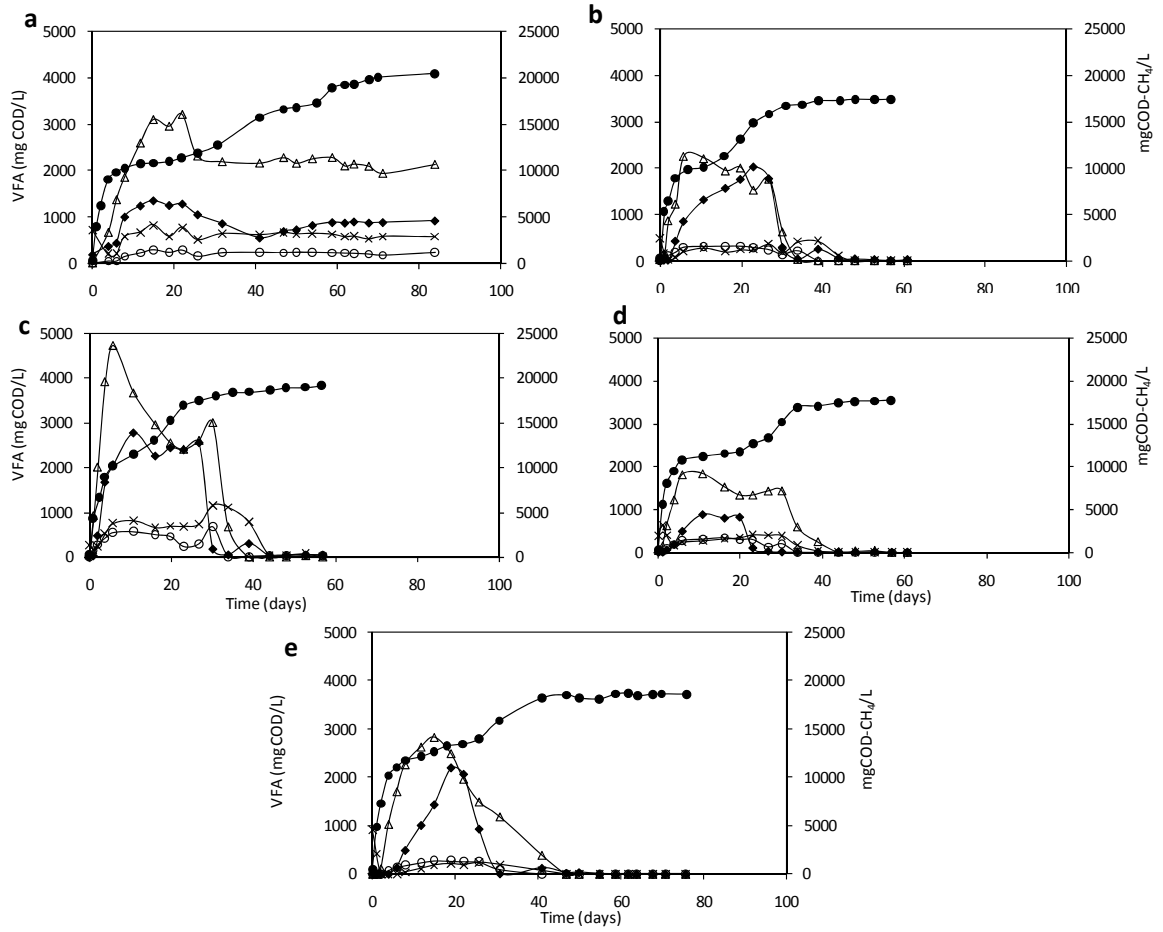

Figure 3.3|Individual VFA concentrations (◆acetic acid, × propionic acid, ○ i-butyric acid, Δ n-butyric acid) and cumulative methane production (●), expressed as mgCOD/L versus time in the assays with excess of lipids (a), cellulose (b), protein (c), carbohydrates (d) and the assay of equal COD amounts (e).

Acidified COD was calculated as the sum of VFA-COD present in each liquid sample and the cumulative Methane-COD production until the time of sampling. At the end of this assay, approximately 15% of the acidified COD that remained was not converted to methane. After hydrolysis of lipids, long chain fatty acids (LCFA) are produced, which are described in the literature as inhibitory to acidogenic and methanogenic populations (RINZEMA, 1988; ANGELIDAKI & AHRING, 1992). This was reported to be a reversible effect and can occur at a metabolic level or at a physical level. The reversibility of physical inhibition requires special conditions and was demonstrated in a study with oleic and palmitic acids (PEREIRA ET AL., 2005). It has been suggested that if the conversion of LCFA is slow, they can form a physical barrier that shields lipid surfaces from lipases, thus affecting the rate of hydrolysis (RIETSCH ET AL., 1977; VERGER, 1980). The

production of biogas was also reported to affect the hydrolysis of lipids due to an emulsion effect. This effect decreases the size of the micelles and increases the available surface, thus increasing the hydrolysis rate constant (SANDERS, 2001).

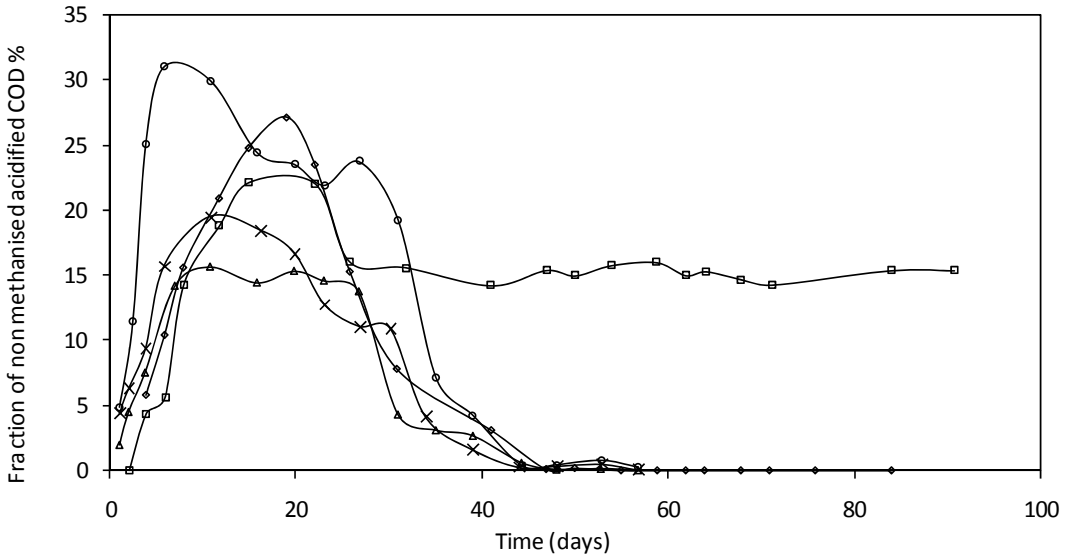

Figure 3.4|Percentage of COD acidified but not methanised in the assays with an excess of lipids (□), cellulose (Δ), protein (○), carbohydrates (×) and the assay of equal amounts of COD (◊).

All the assays except the lipid-rich ones exhibited relatively similar behaviour. In general, between days 5 and 30, there was a percentage of acidified COD that had not been converted into methane. This was more evident in the assay with an excess of protein, where approximately 30% of the acidified COD remained not methanised, most likely due to the presence of ammonium nitrogen that, depending on the pH, can inhibit the methanogenic population. However, after day 38, all the acidified COD was converted into methane, indicating that the inhibitory problems were reversible.

It was possible to calculate the hydrolysis rate constants for each assay assuming a first order kinetics and following the procedure described by SANDERS ET AL., 2003 (Eq. (1)) taking into account the values of initial particulate COD, soluble COD at different time intervals and the cumulative methane production,. In the "classic" batch reactor approach followed, the degree of hydrolysis is calculated from the methane production and the production of soluble COD during digestion according to the following procedure:

$$-\frac{dX}{dt} = Kh \cdot X \qquad (1)$$

Where X is the COD concentration of the particulate substrate present in the assay at each time and Kh is the hydrolysis rate constant. The integration of this equation gives Eq. (2).

$$\ln\left(\frac{X}{X0}\right) = -Kh \cdot t \quad (2)$$

where X_0 is the COD concentration of the particulate substrate initially present in the vial. At each time, the COD concentration of the particulate substrate (X) was calculated according to the Eq. (3):

$$X = X_0 - \sum_{i=1}^{n} \text{Hydrolised COD} \quad (3)$$

Where *i* represents a sample taken from the medium in the liquid composition assays, *n* is the number of samples taken until time *t*, and the cumulative hydrolysed COD $(\sum_{i=1}^{n} \text{Hydrolised COD})$ is the sum of the soluble COD present until time t and the COD that has already been converted into methane, according to the Eq. (4):

$$\sum_{i=1}^{n} \text{hydrolised COD} = (\text{soluble COD})_t + \int_{t=0}^{t} (\text{COD} - \text{CH}_4)dt \quad (4)$$

where $(\text{Soluble COD})_t$ is the soluble COD measured on time t and $\int_{t=0}^{t} (\text{COD} - \text{CH}_4)dt$ is the cumulative methane production until time *t*, expressed as COD. The values of X were then calculated based on the initial particulate COD introduced in the vials (X_0), on the soluble COD measured in each of the *n* samples taken along the liquid composition assays and from the cumulative methane production curves.

Figure 3.5 represents the linear plots of lnX versus initial time. The hydrolysis rate constants were obtained from the slope of each line.

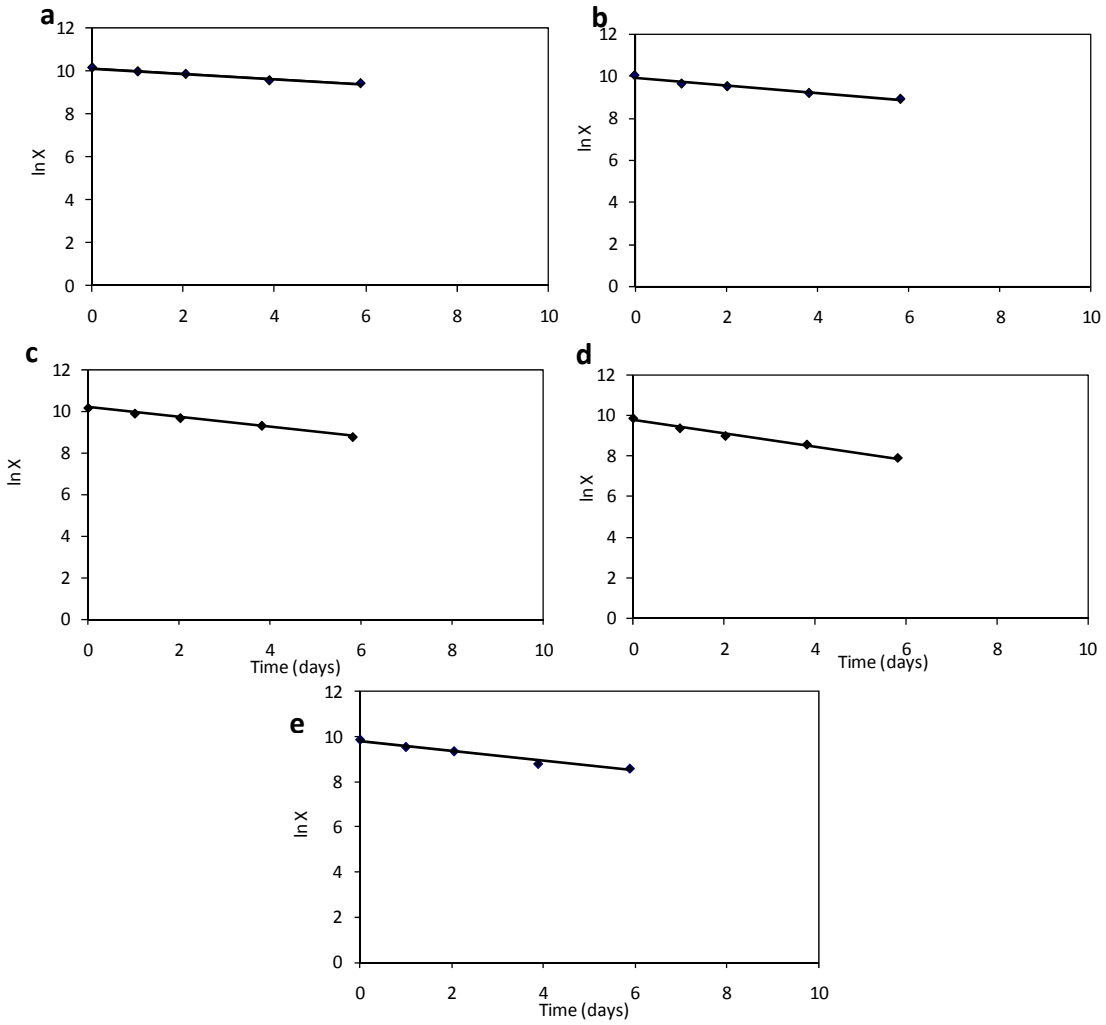

Figure 3.5|Linear plot of ln(X) versus initial time in the assays with an excess of lipids (a), cellulose (b), protein (c), carbohydrates (d) and the assay of equal amounts of COD (e). The hydrolysis rate constants were obtained from the slope of each line.

Table 3.4 compares the obtained values with literature values obtained for similar substrates. The hydrolysis rate constants were in the range of 0.12 d^{-1} to 0.32 d^{-1}. The assays with an excess of carbohydrates and protein presented higher hydrolysis rate constants when compared to the assay containing an equal amount of COD for each component. The lowest values obtained for the hydrolysis rate constant were obtained in the assays with an excess of lipids and cellulose, indicating that when these components are in excess, a slower hydrolysis is induced.

Lipids/LCFA can have a synergic effect on the degradation of all components present, since they adsorb onto solid surfaces and may delay the hydrolysis of other particulate compounds by reducing the accessibility of enzyme attack (PALLENZUELLA, 1999). On the other hand, the possible adsorption of lipids/LCFA on the cells surface can hinder the access of simple substrates such as acetate, and therefore, methanogenesis can also be delayed.

Table 3.4 | Values of the hydrolysis rate constants found in the tested batch assays over the first six days and values reported in the literature to similar substrates (assuming first order kinetics).

Reported literature	Substrate	Kh (d^{-1})	Temperature(ºC)
BOON (1994)	domestic sewer protein	0.2	35
GARCÍA-HERAS (2002)	proteins	0.25-0.8	35
Present work	"excess of protein"	0.24	37
GARCÍA-HERAS (2002)	lipids	0.1-0.7	35
Present work	"excess of lipids"	0.12	37
GRECO ET AL. (1983)	cellulose	0.12	35
Present work	"excess of cellulose"	0.18	37
BOON (1994)	starch	0.20-1.08	35
GARCÍA-HERAS (2002)	carbohydrates	0.5-2	35
Present work	"excess of carbohydrates"	0.32	37
Present work	" equal COD amounts"	0.22	37

3.4. | CONCLUSIONS

Batch degradation of restaurant waste under methanogenic conditions depends on waste composition. If lipids are in excess a slower methane production, a higher concentration of COD in the liquid, and a lower hydrolysis rate constant is observed in comparison with a waste with equivalent amounts of COD of proteins, carbohydrates, lipids, and cellulose. One waste with an excess of carbohydrates and proteins presented hydrolysis rate constants higher (0.32 and $0.22d^{-1}$, respectively) than the wastes with an excess of lipids and cellulose (0.12 and $0.18d^{-1}$, respectively). The most efficient methane production rate and the lowest accumulation of volatile fatty acids were observed for the waste with an excess of carbohydrates. In this assay, after 3 days, 50% of the theoretical methane potential was achieved, although 30 days were needed to attain 85% biodegradability. The results found could be due to the choice of inoculum, and the use of anaerobic consortia that had been adapted to consuming varying loading rates of these organics might not show the same effects. The results point to the need for further work on digestion with other inocula. In addition, these results apply to particular wastes tested at one temperature, solids content, nutrient and toxin condition, and one should be careful before generalizing the findings to other test conditions. The present results indicate that anaerobic digestion facilities with large variations in lipid input could have significant changes in process performance that merit further examination.

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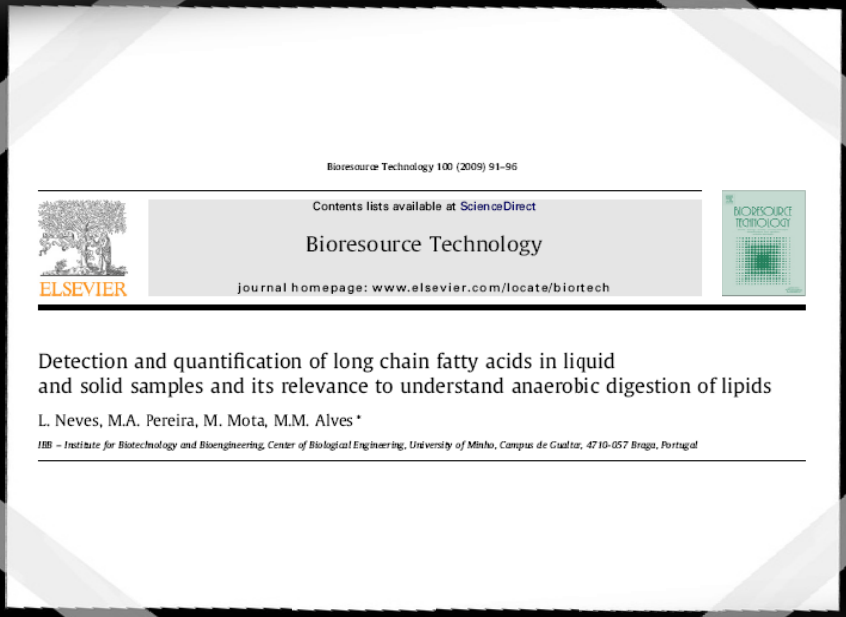

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Detection and quantification of long chain fatty acids in liquid and solid samples and its relevance to understand anaerobic digestion of lipids

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This chapter reports the method developed for the extraction, identification and further quantification by gas chromatography of long chain fatty acids (LCFA) and the demonstration of its application to liquid and solid samples collected from anaerobic digesters. After validation, the usefulness of this method was demonstrated in a cow manure digester receiving pulses of an industrial effluent containing high lipids content. Analysis of LCFA data showed that the conversion of oleic acid, the main LCFA fed to the reactor, by an adapted inoculum became faster and more effective along the successive pulses. Conversely, the accumulation of palmitic acid in the solid phase suggests that degradation of this LCFA, under these conditions, is less effective.

- The results presented in this Chapter have been published in: Neves L., Pereira M.A., Mota M., Alves M.M. (2009) "Detection and quantification of long chain fatty acids in liquid and solid samples and its relevance to understand anaerobic digestion of lipids". Bioresource Technology 100:91-96.

4. | DETECTION AND QUANTIFICATION OF LONG CHAIN FATTY ACIDS IN LIQUID AND SOLID SAMPLES AND ITS RELEVANCE TO UNDERSTAND ANAEROBIC DIGESTION OF LIPIDS

4.1. | INTRODUCTION

In early literature it is suggested that LCFA, produced during hydrolysis of neutral lipids, exert a permanent toxic effect (ANGELIDAKI & AHRING, 1992) and even a bactericidal effect on methanogens (RINZEMA ET AL., 1994). Furthermore, LCFA inhibitory effect on the anaerobic microbial activity, at even low concentrations, has been often reported (KOSTER & CRAMER, 1987; HANANKI ET AL., 1981; HWU ET AL, 1996; LALMAN & BAGLEY, 2000, 2001, 2002; SHIN ET AL., 2003). However, in later studies, ALVES ET AL. (2001), observed that after being continuously fed with oleic acid (C18:1), anaerobic sludge that was encapsulated by a whitish matter, was able to efficiently convert to methane the accumulated substrate when incubated in batch assays at 37ºC, without any added carbon source, evidencing that the anaerobic consortium remained active in such conditions. It was further demonstrated that LCFA, provided they are associated with the sludge and not in the bulk medium, can be efficiently converted to methane, besides the observed temporary decrease in the methanogenic activity after the contact with LCFA is a reversible phenomenon, which is eliminated after the conversion to methane of the biomass-associated LCFA (PEREIRA ET AL., 2004, 2005). This finding was observed for sludge with specific LCFA contents in the range from 1 to 5 gCOD/gVSS. Therefore, the ability of the encapsulated sludge to efficiently mineralise high amounts of adsorbed/accumulated LCFA may represent a potential challenge to the optimisation of methane production when treating effluents with high lipid/LCFA content. These new reported developments were the driving force to develop a method to identify and quantify the LCFA in the liquid phase as well as accumulated onto the biomass, during the anaerobic digestion process.

Several methods have been developed for extraction and quantification of lipids/LCFA in samples from various sources. Some procedures, adapted to environmental samples, report chloroform, methanol, or a mixture of both as the organic solvent used to extract lipids/LCFA (BLIGH & DYER, 1959). Others procedures include the use of a hexane/methyl tertbutyl ether (MTBE) mixture, as alternative to chloroform-based extractants (carcinogen properties) (LALMAN & BAGLEY, 2004), or other extraction agents such as petroleum ether (HWU ET AL., 1998) or heptane (FERNÁNDEZ ET AL., 2005). In most of the reported methods, the formation of fatty acid methyl esters, by methods similar to those described by KALUZNY ET AL. (1985), is a routine

procedure for subsequent gas chromatography (GC) determination.

Some papers reported results of identification and quantification of LCFA in samples from anaerobic reactors fed with lipids/LCFA. Nevertheless, these analysis were performed only in the liquid phase or in the centrifuged and filtrate supernatant (LALMAN & BAGLEY, 2000; HWU ET AL.; 1998, FERNÁNDEZ ET AL., 2005; PROCIDA & CECCON, 2006), and not in the solid matrix. JARVIS & THIELE (1997) described a method for extraction of free LCFA from supernatant, biomass and lipid phase for further quantification by high performance liquid chromatography (HPLC). The sample preparation protocol described in this method is complex, including a derivatization step with 2-nitrophenylhydrazine hydrochloride, before HPLC analysis. Hence, a fast and effective method that is able to extract and quantify the LCFA adsorbed onto the solid matrix, i.e. biomass, and present in the liquid phase, i.e. supernatant, collected from anaerobic reactors, is required. The extraction of LCFA should be as complete as possible and hydrolysis of neutral lipids should be avoided.

It is important to identify the key intermediates in the lipids anaerobic degradation to full understand the pathways involved in this process, most of the papers about anaerobic digestion of lipids do not identify the LCFA involved (SHIN ET AL, 2003), some only analyse the fat content and report LCFA inhibition (GEBAUER & EIKEBROKK, 2006).

In this paper, a method for LCFA extraction from liquid and solid samples and further quantification by capillary gas chromatography is described and validated. Furthermore, the application of the developed method to analyse samples of the solid and liquid phases from an anaerobic reactor fed with dairy cow manure during weakly pulses of an oily effluent from a can fish processing industry, is also presented as an example.

4.2.|EXPERIMENTAL PROCEDURES

4.2.1.|STANDARDS AND REAGENTS

Lauric (C12:0), myristic (C14:0), pentadecanoic (C15:0) palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1) and linoleic (C18:2) acids (puriss p.a. standard for GC analysis), oleic acid sodium salt (puriss p.a. for GC analysis ≥99%) and dichloromethane (DCM) (puriss p.a. stabilized with amylene) were purchased from Fluka (Buchs, Switzerland). Sodium oleate powder purum (assay of fatty acids min 82 %) was purchased from Riedel-deHäen (Seelze, Germany) and highly refined olive oil (low acidity) from Sigma (St. Louis, MO, USA). The hydrochloric acid solution (37%) and 1-propanol (p.a-ACS) were purchased from Panreac (Barcelona, Spain).

4.2.2.|CALIBRATION

Calibration curves were produced from a series of standard solutions (25, 50, 100, 250, 500 and 1000 mg/L) prepared with the following acids (represented by their chemical notation): C12:0, C14:0, C16:0, C16:1, C18:0, C18:1 and C18:2, in a DCM solution. Pentadecanoic acid (C15:0) was used as the LCFA internal standard (IS).

4.2.3.|SAMPLE PROCESSING

The standards (DCM solution), liquid (aqueous solution) and solid (anaerobic biomass) samples were submitted to a similar procedure, ensuring that the organic phase and the aqueous phase always comprised equal amount (3.5 mL), in a total volume of 7 mL. For the standards and liquid samples, once homogenised, 2 mL were transferred into glass vials. Afterwards, 1.5 mL of the IS solution (1000 mg/L) and 1.5 mL of HCl:1-Propanol (25 % v/v) were added. For the liquid samples, 2 mL of DCM was subsequently added, whereas, for the standard solutions, 2 mL of ultra-pure water was added instead. For the solid samples, a defined amount was transferred to the glass vials and dried for 12 h at 85 ºC. The content of the vial was weighed and the solutions of IS (1.5 mL), HCl:1-Propanol (1.5 mL), DCM (2 mL) and ultra-pure water (2 mL) were further added.

The mixture was vortex-mixed, to promote good contact between the two phases, and was digested at 100 ºC for 3.5h. After digestion, the content of the vial was transferred with 2 mL of ultra-pure water to a different vial, rubber covered, and the contact between the two phases was further promoted. These new vials were kept in inverted position for 30 min, after which 1 mL of the organic phase was collected. 1µL of this sub sample was analysed by GC.

4.2.4.|GAS CHROMATOGRAPHY

This analysis was carried out in a GC system (CP-9001 Chrompack) equipped with a flame ionization detector (FID). LCFA were separated using an eq.CP-Sil 52 CB 30 m x 0.32 mm x 0.25 µm column (Teknokroma, Tr-wax), with He as the carrier gas at 1.0 mL/min. Temperatures of the injection port and detector were 220 and 250 ºC, respectively. Initial oven temperature was 50 ºC for 2 min, with a 10 ºC/min ramp to 225 ºC, and a final isothermal for 10 min.

4.2.5.|VALIDATION PROCEDURE

The presented method was validated in terms of linearity, limit of detection and quantification,

precision, accuracy and selectivity. Linearity was evaluated by the correlation coefficient of the calibration curves obtained. Detection limit and quantification limit were estimated as the LCFA concentration for which the area of the chromatographic peak was equal to 3 and 10 times, respectively, the standard deviation of the most diluted standard.

Precision of the method was evaluated using two criteria: reproducibility and repeatability (MILLER & MILLER, 1991; CAULCUTT & BODDY, 1983). According to CASTILLO & CASTELLS (2001), precision results are reported as relative standard deviation (RSD, %), which should not exceed the 20% (SHAH ET AL, 1992). Reproducibility of the method was evaluated by the RSD of the slope of 5 calibration curves constructed over a year period by 3 different analysts (3+1+1). Repeatability (measured as % RSD) and accuracy (measured concentration/real concentration x 100) were assessed by means of LCFA recovery experiments performed in liquid and solid samples. Blank samples (supernatant and biomass from anaerobic reactors) with and without the addition of LCFA were also processed to test for interferences of the liquid and solid biological matrices, evaluating the selectivity of the method. Unwanted hydrolyses of neutral lipids during the LCFA extraction procedure was also evaluated by processing solutions containing olive oil.

An additional series of experiments was carried out to optimise the extraction of the LCFA from the solid phase to the organic phase. Different digestion times, different volumes of the organic phase and different amounts of dry sample were tested.

Table 4.1 and Table 4.2 summarises the assays performed with liquid and solid samples, respectively. The solid samples consisted of anaerobic suspended (S) and granular (G) biomass collected from three different reactors: (i) a lab scale reactor fed with sodium oleate, designated as biomass SL, which was expected to have a high amount of adsorbed/accumulated LCFA, because it was visibly encapsulated by a whitish matter; (ii) two wastewater treatment plants, designated as biomass S1 and S2; and (iii) an UASB (upflow anaerobic sludge blanket) reactor treating effluent from a brewery company (Oporto, Portugal), designated as biomass G.

4.2.6.|STATISTICAL ANALYSIS

Single factor analysis of variances (ANOVA) was used to determine if significant differences existed between results obtained under different experimental procedures. Statistical significance was established at the $P< 0.05$ level.

Table 4.1|Assays performed to validate the method for liquid (l) samples.

Assay #	Oleic acid sodium salt (mg/L)	Sodium oleate powder (mg/L)	LCFA mixture [(a)] (mg/L)	Olive oil (mg/L)	C16:0 (mg/L)	C18:1 (mg/L)	Solvent
1(l)	-	-	≅500	-	-	-	Water
2(l)	-	-	≅1000	-	-	-	Water
3(l)	258	-	-	-			Water
4(l)	-	590	-	-	-	-	Water
5A(l)	-	-	-	-	-	-	Supernatant
5B(l)	-	-	≅500	-	-	-	Supernatant
6(l)	-	-	-	1173	-	-	DCM
7(l)	-	-	-	1516	938	1184	DCM

[(a)] C12:0, C14:0, C16:0, C16:1, C18:0, C18:1 and C18:2.

Table 4.2|Assays performed to optimize and validate the method for solid (s) samples.

Assay #	Biomass	Total Solids (g)	Digestion time (h)	Volume organic phase (DCM) (mL)	Added LCFA [(b)] (mg)
1(s)	SL [(a)]	≅ 0.05	3.5	3.5	-
2(s)	SL	≅ 0.05	5	3.5	-
3(s)	SL	≅ 0.05	7	3.5	-
4(s)	SL	≅ 0.05	3.5	4.0	-
5(s)	SL	≅ 0.05	3.5	4.5	-
6(s)	SL	≅ 0.1	3.5	3.5	-
7(s)	SL	≅ 0.5	3.5	3.5	-
8(s)	S1 [(a)]	≅ 0.05	3.5	3.5	-
9(s)	S1	≅ 0.1	3.5	3.5	-
10(s)	S1	≅ 0.2	3.5	3.5	-
11A(s)	G [(a)]	≅ 0.05	3.5	3.5	-
11B(s)	G	≅ 0.05	3.5	3.5	≅1
12A(s)	S2 [(a)]	≅ 0.05	3.5	3.5	-
12B(s)	S2	≅ 0.05	3.5	3.5	≅1

[(a)] SL- highly loaded with biomass-associated LCFA; S1 and S2- suspend sludge from two different wastewater treatment plants; G-granular sludge from a UASB reactor treating brewery wastewater; [(b)] C12:0, C14:0, C16:0, C16:1, C18:0, C18:1 and C18:2.

4.2.7.|APPLICATION OF THE METHOD TO MONITOR ANAEROBIC DIGESTION OF LIPIDS

A 26 L mesophilic continuously stirred tank reactor (CSTR) was fed with dairy cow manure (1.4 gCOD/gTS) for 122 days, at an organic loading rate of 1.2 gCOD/(Lday). The hydraulic retention time (HRT) was set at 26 days. From day 123 on, pulses of an oily effluent from a can fish processing industry (2.7 $gCOD/g_{waste}$ and 99.8% of fat content) were added once a week, every 7 days. In those days, the organic loading rate applied to the reactor was of 5.0 gCOD/(Lday). Chemical oxygen demand (COD) and total solids (TS) were determined according to Standard

Methods (APHA, 1989). The fat content from the oily effluent was extracted with a mixture chloroform:methanol 1:2 (v/v) in a soxtec system, dried and weighed.

Samples from the biomass and liquid phase (supernatant) were collected from the bottom of the reactor and analysed for LCFA content. The cow manure and oily effluent fed to the reactor were also analysed for LCFA.

4.3.|RESULTS AND DISCUSSION

4.3.1.|CALIBRATION AND VALIDATION

The tested LCFA were detected by gas chromatography in a 25 min single run analysis, with a good separation between peaks.

The generated calibration curves were linear over the concentration range studied with coefficients of correlation ≥ 0.997 for all the analyzed LCFA. Examples of the typical regression equations obtained are shown in Table 4.3, as well as the correspondent LCFA linear ranges, detection (LOD) and quantification limits (LOQ). Reproducibility was further evaluated by constructing 5 calibration curves over one year period by 3 different analysts. The relative standard deviation of the slopes of the calibration curves generated for each LCFA, ranged between 4.1 to 13.3% (Table 4.3), verifying the day-to-day precision of the method. The described LCFA extraction and quantification procedure was further validated for application to liquid and solid samples.

Table 4.3|Parameters from the calibration curves obtained for the analyzed LCFA.

LCFA	Regression equation(*)	r^2	Linear ranges (mg/L)	LOD (mg/L)	LOQ (mg/L)	RSD of slope (*n*=5) (%)
C12:0	y = 1.095x - 0.008	0.999	25.0-1001.0	5.0	16.6	13.3
C14:0	y = 1.039x + 0.003	0.999	25.6-1024.0	4.0	13.3	5.4
C16:0	y = 0.949x + 0.014	1.000	27.7-1108.0	10.4	34.6	5.2
C16:1	y = 0.863x + 0.004	1.000	31.7-1270.0	7.2	24.0	5.4
C18:0	y = 0.879x + 0.016	0.999	23.1-925.0	14.4	48.1	4.1
C18:1	y = 0.885x + 0.019	0.999	27.9-1118.0	14.7	49.1	7.2
C18:2	y = 0.794x – 0.014	0.997	48.1-961.0	24.2	80.7	9.6

(*) *y*=LCFA peak area/IS peak area, *x*=[LCFA]/[IS], IS-internal standard (C15:0); LOD - limit of detection; LOQ - limit of quantification; RSD – relative standard deviation.

Table 4.4 presents the results obtained from the recovery assays carried out with liquid samples (Table 4.1). Satisfactory yields were achieved in the tests performed to evaluate the extraction of LCFA from the aqueous phase. Mean recoveries in the range of 91-101% and 99-110% were obtained at LCFA concentration levels of about 500 (assay 1(l)) and 1000 mg/L (assay 2(l)),

respectively, with good precision ($RSD_{(500)}$<9.5% and $RSD_{(1000)}$<3.0%). A high mean recovery of oleic acid (C18:1), i.e. 99%, was also attained from aqueous solutions prepared with oleic acid sodium salt (puriss p.a. ≥99%), with a RSD value of 1.9% (assay 3(l)). Furthermore, analysis of a solution prepared with sodium oleate powder (assay 4(l)) revealed that oleic acid represented 80±1 % of the total LCFA detected, which corresponds to a mean recovery of 98% in relation to the minimum 82% of C18:1 expected, as specified by the manufacturer.

Table 4.4|Mean detected LCFA concentrations, relative standard deviation (RSD) and mean recoveries obtained in the validation assays performed with liquid (l) samples.

Assay # (*n*=4)	Detected concentration (mg/L), [RSD (%)], (Recovery (%))						
	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2
1(l)	437, [9.5], (94)	518, [1.1], (94)	341, [1.5], (94)	414, [4.3], (92)	346, [3.2], (99)	547, [2.7], (91)	375,[1.9], (101)
2(l)	935, [1.5], (100)	1187, [1.0], (107)	745, [0.8], (102)	902, [0.7], (100)	765, [3.0], (109)	1195, [2.2], (99)	820, [2.3], (110)
3(l)	ND	ND	ND	ND	ND	237, [1.9], (99)	ND
4(l)	ND	21 [19.2]	33 [14.3]	16 [18.4]	13 [2.3]	331 [6.8], (98)(*)	ND
5A(l)	ND	ND	ND	ND	ND	ND	ND
5B(l)	480, [5.9], (106)	537, [4.9], (100)	486, [5.0], (105)	646, [5.2], (100)	431, [3.3], (96)	564, [9.0], (96)	550, [7.7], (111)
6(l)	ND	ND	ND	ND	ND	ND	ND
7(l)	ND	ND	981, [1.6], (105)	ND	ND	1332, [3.8], (112)	ND

ND- non-detectable; (*) percentage of C18:1 recovered in relation to a minimum of 82% of C18:1 in the total LCFA present, expected in the sodium oleate powder, as specified by the manufacturer.

Supernatant samples, collected from an anaerobic reactor, with and without the addition of LCFA were also analysed to test for interferences of the liquid biological matrix. No LCFA was detected in the pure supernatant samples (assay 5A(l)). After processing these samples supplemented with the standard LCFA at a concentration level of about 500 mg/L (assay 5B(l)), mean recoveries above 96% and RSD lower than 9.0% were obtained. Additionally, comparison of the chromatograms obtained after processing the matrix solution, i.e. supernatant (assay 5A(l)), and the matrix solution to which the LCFA had been added (assay 5B(l)) revealed no interferences of the biological liquid matrix in the LCFA analysis (not shown).

Olive oil solutions were also analysed to ensure that the method procedure did not promote hydrolysis of neutral lipids. The obtained results confirmed the desired condition, as no free LCFA were detected after processing the pure olive oil solutions (assay 6(l)). Moreover, high oleic (unsaturated LCFA, C18:1) and palmitic acid (saturated LCFA, C16:0) recoveries were achieved after processing olive oil solutions supplemented with the two acids (assay 7(l)).

Table 4.5 presents the results obtained in the assays carried out with solid samples (Table 4.2), i.e. anaerobic biomass. First, to optimise LCFA extraction from the solid matrix, different digestion times, volumes of organic phase and amounts of dry sample were studied, using biomass expected to be highly loaded with biomass-associated LCFA, designated as SL (assays 1(s)-7(s)).

Table 4.5|Mean detected LCFA contents, relative standard deviation (RSD) and mean recoveries obtained in the validation assays performed with solid (s) samples.

Assay #	Detected content (mg/gTS), [RSD (%)]							
	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	Total LCFA
1(s)	ND	11, [2.7]	407, [1.5]	ND	45, [1.8]	8, [15.4]	ND	471, [1.3]
2(s)	ND	10, [3.1]	356, [1.7]	ND	41, [2.2]	9, [21.8]	ND	416, [0.7]
3(s)	ND	11, [2.9]	381, [6.1]	ND	43, [6.9]	10, [1.0]	ND	445, [5.6]
4(s)	ND	10, [2.9]	392, [2.8]	ND	42, [5.7]	6, [121]	ND	450, [4.6]
5(s)	ND	11, [4.4]	416, [6.0]	ND	43, [4.0]	5, [97.9]	ND	475, [4.5]
6(s)	ND	8, [2.4]	281, [7.0]	ND	34, [2.4]	8, [15.8]	ND	331, [6.6]
7(s)	ND	2, [10.5]	62, [4.3]	ND	5, [106]	ND	ND	72, [14.3]
8(s)	ND	ND	3.2, [43.8]	ND	ND	ND	ND	3.2, [43.8]
9(s)	ND	ND	2.9, [13.8]	ND	ND	ND	ND	2.9, [13.8]
10(s)	ND	ND	2.5, [12.0]	ND	ND	ND	ND	2.5, [12.0]
11A(s)	ND	ND	ND	ND	ND	ND	ND	ND
12A(s)	1.8, [64.7]	1.9, [79.7]	6.5, [26.1]	1.8, [79.9]	1.4, [96.9]	3.9, [41.0]	0.87, [200]	18.2, [56.4]
	Detected amount (mg), [RSD (%)], (Recovery (%))							
	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	
11B(s)	0.9, [2.8], (95)	1.1, [4.4], (98)	0.7, [1.4], (93)	0.9, [1.5], (94)	0.7, [1.9], (95)	1.2, [2.0], (96)	0.8, [1.6], (106)	
12B(s)	0.9, [3.4], (100)	1.1, [2.5], (99)	1.1, [10.3], (104)	1.3, [1.8], (101)	0.9, [4.1], (93)	1.2, [5.2], (101)	1.1, [3.3], (95)	

ND- non-detectable ;(*) Assays 1 to 7, *n*=3; Assays 8 to 12, *n*=4.

No statistically significant differences at a 0.05 level (*P*=0.075) were observed when a dry amount of ≅ 0.05 g of this biomass was submitted to different digestion times of 3.5 (assay 1(s)), 5 (assay 2(s)) and 7 h (assay 3(s)). Likewise, when tested for different organic phase volumes of 3.5 (assay 1(s)), 4.0 (assay 4(s)) and 4.5 mL (assay 5(s)), the detected LCFA content showed statistic similar results (*P*=0.429). Furthermore, in all experiments the amount of palmitic acid

(C16:0) detected represented 86±1 % of the total LCFA extracted. However, when the amount of dry biomass was increased to ≅ 0.1 g (assay 6(s)) and ≅ 0.5 g (assay 7(s)) a decrease on the LCFA content detected to 30% and 85%, respectively, was found. Nevertheless, in both cases, the percentage of C16:0 detected in the total LCFA extracted was identical, i.e. 85±1%. From the obtained results, the solid phase LCFA extraction procedure was set-up with 3.5 h of digestion and 3.5 mL of organic phase, as previously established for the liquid phase extraction procedure. The amount of dry biomass used in this analysis should be such that allows complete LCFA extraction from the solid phase to the organic phase, therefore depending on the amount of LCFA present in the sample. For highly LCFA loaded biomass, i.e. up to a maximum of approximately 500 mgLCFA/gTS, as observed for biomass SL, it was found that a dry amount as low as 0.05 g was needed in order to avoid LCFA extraction saturation. Due to the heterogeneity associated to the random LCFA accumulation into the biomass, high standard deviations were obtained when analysing the biomass samples.

To validate the lower sensitivity of the method, biomass expected to have a low LCFA content, designated as S1, was also tested using different amounts of dry sample (assays 8(s)-10 (s)). The results obtained showed no statistically significant differences (P= 0.609) in the LCFA content detected when analysing dry amounts of 0.05, 0.1 or 0.2 g, further demonstrating that the described method was feasible even when the biomass has a total LCFA content as low as 3 mg/gTS. In this case, it was found that dry sample amounts higher than 0.05 g were needed in order to increase the obtained precision (RSD<20%).

Anaerobic biomass samples with and without the addition of LCFA were also analysed to test for interferences of the solid biological matrix. Two different structure types of biomass, granular (biomass G) and suspended (biomass S2), were tested. No LCFA were detected in the granular biomass (assay 11A(s)), whereas in the suspended biomass (assay 12A(s)) a total LCFA content of 18 mg/gTS was found. The individual LCFA contents present in this biomass were discounted in the experiments performed with the biomass samples fortified at individual LCFA amounts of about 1mg. In these experiments, LCFA mean recoveries above 93%, with RSD <11%, were attained for both biomass types. As previously found for the biological liquid matrix, no interference of both biological solid matrices in the LCFA analysis was observed.

4.3.2.|APPLICATION OF THE METHOD TO MONITOR ANAEROBIC DIGESTION OF LIPIDS

The proposed method was applied to monitor LCFA degradation/accumulation in an anaerobic CSTR reactor treating dairy cow manure, when submitted to pulses of an oily effluent from a can fish processing industry. Analysis of the fed wastes revealed that LCFA represented 3% and 77%

of the COD in the cow manure and in the oily effluent, respectively (Table 4.6). Previous to the pulses, analysis of the digested manure showed a LCFA reduction of 80%, when compared to the fresh manure. This reduction was identical for all LCFA detected in the analysed samples.

Table 4.6|LCFA content in the reactor feed.

LCFA	Cow manure (mgCOD/gTS)	Oily effluent (mgCOD/g_{waste})
C14:0	3	19
C16:0	14	260
C16:1	0	27
C18:0	25	75
C18:1	0	891
C18:2	0	790

The chromatograms obtained from the LCFA analysis (not shown) displayed a good separation between peaks, indicating that the peak integration was not interfered by the solid matrix, in contrast to the HPLC chromatograms presented by JARVIS & THIELE (1997). In addition, the retention times of the LCFA detected were in concurrence to the GC chromatogram presented by PROCIDA & CECCON (2006), for the analysis of free fatty acids in liquid phase of olive mill wastewaters.

Successive pulses of the oily effluent were applied to the reactor on days 123, 130, 137, 144, 151 and 158. In each applied pulse, the main LCFA fed to the reactor were C18:1 (43.2±0.4%), followed by C18:2 (38.3±0.5%) and C16:0 (12.5±0.1%) (Table 4.6). During the pulses trial, no LCFA were detected in the liquid phase collected from the reactor, suggesting a fast accumulation of these compounds into the solid phase. This finding is in accordance WITH HANAKI ET AL. (1981) that reported that these compounds could fast adsorb to the biomass, within 24 h. In this study, LCFA accumulation onto the biomass was confirmed by the results obtained from LCFA analysis in the solid matrix collected from the bottom of the reactor. The LCFA detected in this phase were C14:0, C16:0, C18:0, C18:1 and C18:2. It was further demonstrated that C16:0 and C18:1 were the main LCFA adsorbed/accumulated onto the sludge, jointly accounting for about 60 to 100% of the total LCFA detected in the solid phase (Figure 4.1).

Along the trial period, a decrease of C18:1 in the solid phase was observed, which became more evident in the last two pulses. These data suggests that the conversion of C18:1, the main LCFA fed to the reactor, by the adapted biomass became faster and more effective along the successive pulses. Conversely, the accumulation of C16:0 in the solid phase suggested that degradation of this LCFA, under these conditions, was less effective.

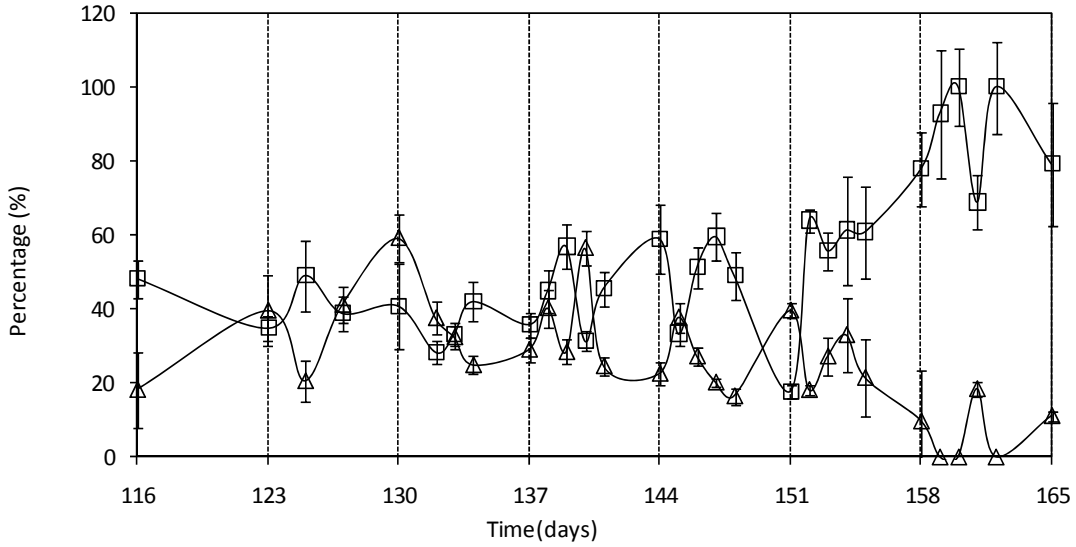

Figure 4.1|Percentage of C16:0 (□) and C18:1(Δ) in the total LCFA detected in the solid phase present in the bottom of the reactor. Error bars indicate standard error of the mean of the replicates.

Nevertheless, the specific content of C16:0 accumulating in the last two pulses was relatively low, ranging from 7 to 18 mgCOD/gST. The presented method for LCFA detection and quantification constitutes a valuable tool to identify key intermediates in the still obscure anaerobic accumulation/degradation of LCFA as was shown in the given example.

4.4.|CONCLUSIONS

The analytical method reported is based on the extraction and gas cromatography analysis of long chain fatty acids present in solid and liquid samples in anaerobic digesters. Relative standard deviation values lower than 15% and mean LCFA recoveries above 90% were obtained. After validation, the usefulness of this method was demonstrated in a cow manure digester receiving pulses of an industrial effluent containing high lipid content. The conversion of oleic acid (C18:1), the main LCFA fed to the reactor, became faster and more effective along the successive pulses. Conversely, the accumulation of C16:0 in the solid phase suggests that degradation of this LCFA, under these conditions, is less effective. The application of this method, as well as the identification of the key intermediates will contribute to a better understanding of LCFA adsorption and degradation processes that occur during the anaerobic digestion of lipids.

4.5.|REFERENCES

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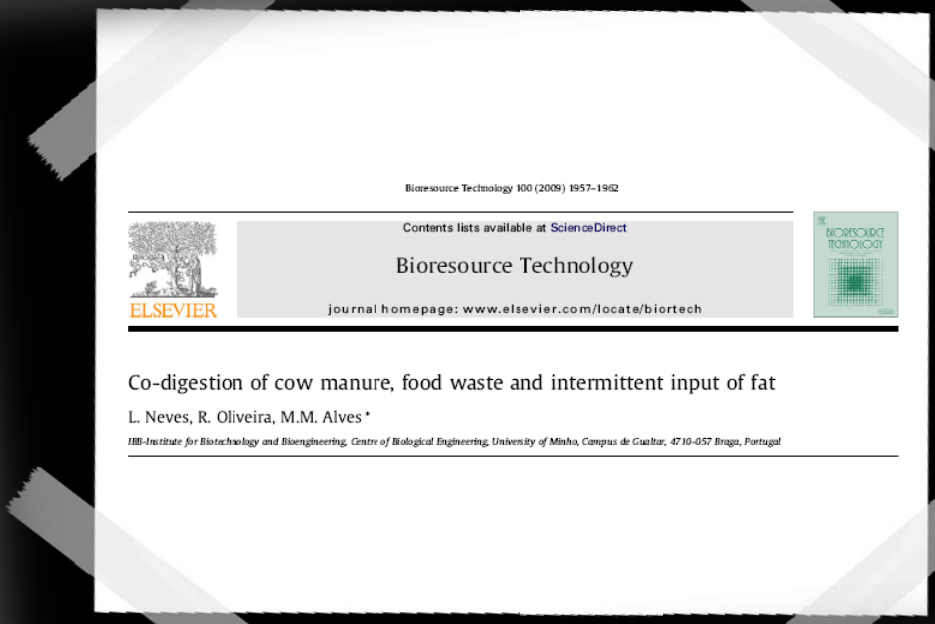

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Co-digestion of cow manure, food waste and intermittent input of fat

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Pulses of oil were added to completely mixed reactors fed with dairy cow manure and food waste, after achieving a stable performance at an organic loading rate of 4.6 ± 0.1 gCOD/($L_{reactor}$.day), an oily waste effluent from a canned fish processing industry was fed in the form of pulses. The oil concentration rose up to 9, 12, 15 and 18 g $COD_{oil}/L_{reactor}$, after the pulse feeding in the reactor. The highest fat concentration of 18 $gCOD_{oil}/L_{reactor}$ promoted a persistent inhibition in the process in the continuous reactor, although in batch assays, the reactor content evidenced a capacity to degrade more oil and to degrade the accumulated organic matter. All the other pulses had a positive effect in the methane production. From a practical point of view, this work demonstrates that controlled intermittent inputs of oil can enhance the methane production in a co-digestion of cow manure and food waste.

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5.|CO-DIGESTION OF COW MANURE, FOOD WASTE AND INTERMITTENT INPUT OF FAT.

5.1.|INTRODUCTION

Anaerobic digestion is attractive for the treatment of organic wastes such as cow manure, since it produces biogas, a renewable energy source and a digestate that can be used as organic fertilizer. Because biogas plants are difficult to run with economically profitable results if the process is based only in livestock manure, co-digestion strategies are widely applied in order to enhance the methane production in agricultural biogas plants. For instance, in Denmark (RAVEN & GREGERSEN, 2007) and Germany (WEILAND, 2006) the digestion of manure and organic waste is a well-established technological practice. This process consists of combining several wastes with complementary characteristics in order to improve the methane production.

In many cases the addition of co-substrates is however based on trial and error practice. Biogas plant operators know well the advantages of adding fat residues or food waste to their biogas plants. Food waste has a high potential for methane production and can be digested rapidly making it a good source of material for anaerobic co-digestion (LAY ET AL., 1997). According to ZHANG ET AL. (2007), food waste collected from restaurants is a highly desirable substrate for anaerobic digesters, accomplishing 80% of the theoretical methane yield in 10 days digestion time.

Among the co-digested wastes, lipids are also one of the most used (FERNÁNDEZ ET AL., 2005). Lipids (fats, oils, greases) are one of the major organic matters found in food waste and some industrial wastewaters, such as slaughterhouses, dairy industries or fat refineries (LI ET AL., 2002). When compared to other organic wastes of different biochemical composition, lipids are theoretically more interesting for biogas production, since they are reduced organic materials and have higher methane potential (PEREIRA ET AL., 2003). Inhibition related to the long chain fatty acids concentration in anaerobic digestion of lipid rich wastes has been reported in literature, especially in reactors with a continuous feeding (HWU ET AL., 1998; RINZEMA ET AL., 1989; PEREIRA ET AL., 2003) or even in batch assays (KOSTER & CRAMER, 1987; LALMAN & BAGLEY, 2000, 2001, 2002; SHIN ET AL., 2003). On the other hand, BROUGHTON ET AL. (1998) stated that anaerobic digestion of sheep tallow with high lipid content was amenable to mesophilic digestion in batch. At the same time FERNÁNDEZ ET AL. (2005) reported that fats from animal and vegetable origin were almost completely degraded in high percentages in co-digestion with simulated organic fraction of municipal solid waste, confirming that anaerobic digestion of lipids is possible.

Moreover, NIELSEN & AHRING (2006) followed the performance of reactors fed with a mixture of pig and cattle manure with increasing pulses of 0.5 and 2.0 g_{oleate}/L and conclude that these pulses had a stimulating effect on the overall process.

The aim of this work was to study the behaviour of co-digestion of cow manure with food waste, by applying increasing concentrations of intermittent pulses of residual oil, from a canned fish processing industry. The establishment of the optimal lipids concentration that can be added ensuring the methane enhancement, without inhibiting the process, was the specific objective of the present work.

5.2.|MATERIAL AND METHODS

5.2.1.|SUBSTRATES

Three different co-substrates were used in the anaerobic co-digestion process.

(i) Cow Manure, collected in a dairy farm in the suburbs of Braga (Portugal) and stored at 4 ºC, until use to minimize the decomposition of substrate; (ii) food Waste, which was a composite sample (one week based) from the waste produced in the restaurant of the University of Minho, located in "Campus de Gualtar", Braga, Portugal. Food waste was crushed to 1-3 mm particle size and stored at 4 ºC during 5 days, until the end of the collecting process. Then it was mixed and stored at -18 ºC; (iii) fat, was an oily waste effluent collected from a canned fish processing industry. The characteristics of each substrate are presented in Table 5.1.

Table 5.1|Characterization of the co-substrates (results are given as means of triplicates with standard deviations).

Waste	Cow Manure (g/L)	Food Waste (g/kg_{waste})	Oily Waste (g/kg_{waste})
Chemical Oxygen Demand (COD)	39±8	327±73	2690 ± 61
Total Solids (TS)	28±5	238±1	971±5
Volatile Solids (VS)	21±4	214±7	972±4
Total Kjeldahl Nitrogen (TKN)	2 ±1	13±1	170±83
Fat content	-	20±8	877±32

5.2.2.|START-UP AND OPERATION

Four 5 L mesophilic (37ºC) continuously stirred tank reactors with hydraulic retention time of 15 days were fed with cow manure and food waste. The digesters were inoculated with the effluent from a stable laboratory mesophilic anaerobic digester fed with cow manure and food waste.

The ratio cow manure/food waste in the feed was 1 expressed as total solids (TS), meant for equal amount of both co-substrates expressed as TS. The organic loading rate in the four reactors was 4.6±0.1gCOD/($L_{reactor}$.day) with a TS/VS content in the feed of 5.2%/4.5% (w/v).

After a stable operation of the four reactors for 148 days, the intermittent feeding of fat was initiated. It should be noted that variations encountered within the test results can be justified by the heterogeneity of the real wastes used as co-substrates in this work.

Reactor 1 (R1) was used as control and so no oily waste was added. In reactors R2, R3 and R4, pulses of oil, were applied, according to Table 5.2. After the 7th pulse (day 204) methane production in R4 decreased drastically and so no more oily waste was added to this reactor.

Table 5.2|Punctual loading concentrations of fat ($gCOD_{oil}/L_{reactor}$)

Day (pulse)	R1 ($gCOD_{oil}/L_{reactor}$)	R2 ($gCOD_{oil}/L_{reactor}$)	R3 ($gCOD_{oil}/L_{reactor}$)	R4 ($gCOD_{oil}/L_{reactor}$)
148 (1st)	0	9	12	15
168 (2nd)	0	9	12	15
176 (3rd)	0	9	12	15
183 (4th)	0	9	12	15
190 (5th)	0	9	12	15
197(6th)	0	9	12	15
204(7th)	0	12	15	18
211 (8th)	0	12	15	0
218(9th)	0	12	15	0
225(10th)	0	12	15	0

5.2.3.|ANALYTICAL METHODS

The routine analysis (COD, pH, TS, VS and TKN) was performed according to Standard Methods (APHA ET AL., 1989).

Methane content of the biogas was measured by gas chromatography using a Porapack Q (180 to 100 Mesh) column, with He as the carrier gas at 30 mL/min and a thermal conductivity detector. Temperatures of the detector, injector and oven were 110ºC, 110ºC and 35ºC, respectively. Biogas flow rate was measured by using a Ritter Milligascounter (Dr. Ing. Ritter Apparatebau GmbH, Bochum, Germany).

Volatile fatty acids (VFA) (acetate, propionate, iso-butyrate and n-butyrate) were determined by high-performance liquid chromatography using a Chrompack column (300x6.5 mm) and a mobile phase of sulphuric acid 5 mM at 0.7 mL/min. The column was set at 60 ºC and the detection was

by spectrophotometry at 220 nm.

The total fat content was extracted with diethyl ether in a Soxtec System HT2 1045 extraction unit produced by Tecator (OFFICIAL METHODS OF ANALYSIS 2003.05, 2007). The Soxtec System is an extraction unit in which a thimble containing the oil matrix was immersed in a boiling solvent. The oily waste was added to thimbles previous weight and placed in the apparatus. A previous weight cup containing boiling stones and 50 mL of diethyl ether was placed in the apparatus. The thimbles were lowered to the "boiling" position, and heat was applied to the plates. The temperature of the circulating heating fluid was 90ºC. The condensers were connected to a recirculation cold bath. After 40min the thimbles were raised to the "rinsing" position for 40min. The solvent collection knob was closed and when no additional solvent could be seen collecting in the condensers, the cups were removed and placed in the hood to eliminate last traces of solvent. The samples were weighed and dried a second time for 15min or until a constant weight was reached.

5.2.4.|METHANE POTENTIAL ASSAYS

Two distinct methane potential assays were performed with biomass collected from R1 and R4 in day 203 (end of 6^{th} pulse) and in day 224 (9^{th} pulse). In the first type of methane potential assays, the samples collected from the reactors were incubated in 125 mL batch vials at 37ºC, with a stirring speed of 150 rpm under strict anaerobic conditions, without any added substrate. The methane production was regularly measured by gas chromatography. The maximum methane yield was calculated per kgVS added in each vial. The maximum methane production rate (MMPR) was determined using the values of the initial slope of the methane production curve.

The second type of methane potential tests included the addition of 4.8 gCOD/L of oily waste to the same biomass samples. These assays were assessed as previously described. All batch experiments were performed in triplicate.

5.2.5|SPECIFIC METHANOGENIC ACTIVITY TEST (SMA)

The SMA of the biomass from the four reactors was assessed in day 203 (end of 6^{th} pulse) and in day 224 (9^{th} pulse), in the presence of 30 mM of acetate and pressurized with H_2/CO_2 (80/20 (v/v)) at 1 bar. Blank controls were used for acetate (no added substrate) and for the gaseous substrate (pressurized with N_2/CO_2-80/20 (v/v) at 1 bar). Strict anaerobic conditions were maintained.

SMA values were determined by dividing the initial linear slope of the methane production curve by the VS content of each vial at the end of the experiment. The volume of methane produced was corrected to the Standard Temperature and Pressure conditions (STP - 1 atm and 273 K).

5.3.|RESULTS AND DISCUSSION

5.3.1.|REACTORS PERFORMANCE

Before the trial all the four reactors were run for 148 days and had achieved a stable performance in terms of methane production (3.2±0.2 LCH_4/day) and VFA concentrations (≤0.5g COD/L), the pH was stable and between 7.7-7.9. The % of solids reduction in the four reactors was 47, 48, 50 and 48 for TS and 60, 59, 60 and 59 for VS in R1, R2, R3 and R4, respectively.

The effect of oil pulses in the methane production is presented in Figure 5.1 and Table 5.3 summarizes the obtained peaks of methane production after the pulse feeding, in terms of % of increase, relative to the control R1 in the same day.

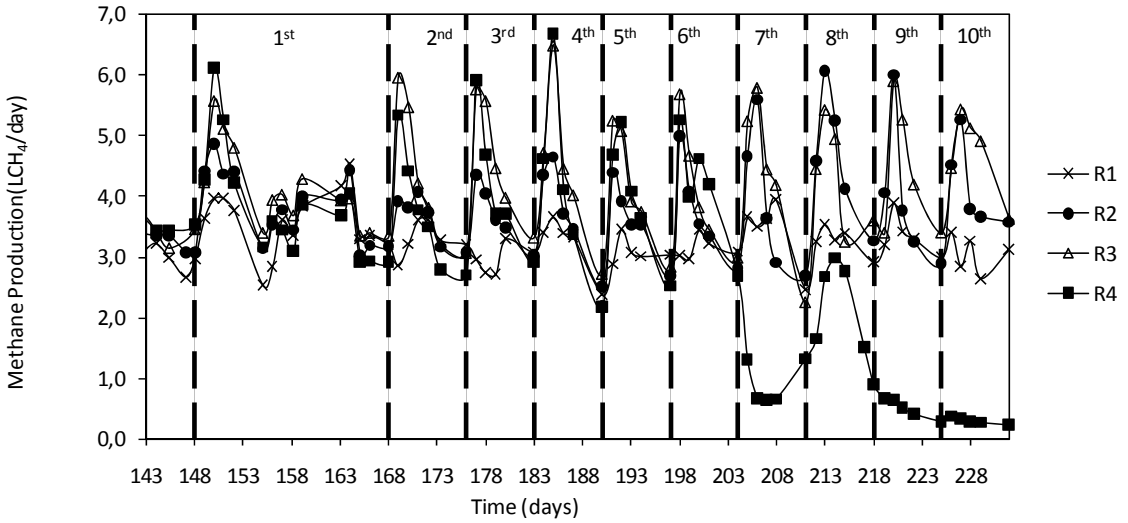

Figure 5.1|Time course of methane production (LCH_4/day). [1st to the 6th pulse were 0, 9, 12 and 15 $gCOD_{oil}/L_{reactor}$ in R1, R2, R3 and R4, respectively; 7th pulse was 0, 12, 15 and 18 $gCOD_{oil}/L_{reactor}$ in R1, R2, R3 and R4, respectively; 8th to 10th pulse were 0, 12, 15 and 0 $gCOD_{oil}/L_{reactor}$ in R1, R2, R3 and R4, respectively].

A comparison with the expected values is also provided. In the first six pulses, the expected methane enhancement in R2 and R3 was attained. On the other hand, when compared to R1, the 15 $gCOD_{oil}/L_{reactor}$ pulse should enhance 135% the methane production in R4. The expected peaks values ought to be 5.25 LCH_4 higher than R1, nonetheless this was not observed.

Table 5.3|Peak of methane production after the pulse feeding, in terms of % of increase relative to the control R1 in the same day. [Expected theoretical increase %]. (Results are given as means with standard deviations)

Pulse #	Day #	R1	R2	R3	R4
1^{st} to 6^{th}	148, 168, 176, 183, 190, 197	-	42±15[41]	82±12[88]	80±9[135]
7^{th} to 10^{th}	204, 211, 218, 225	-	70±15[88]	69±18[135]	[182]

From the 7^{th} to the 10^{th} pulse the obtained methane peaks were somewhat lower than expected, although the same behaviour was observed. In R4, when the oil pulse concentration increased up to 18 $gCOD_{oil}/L_{reactor}$ (7^{th} pulse-day 204), the methane production decreased to values of $0.68LCH_4$/day, about 18% of the value obtained in R1. On account of that, no more pulses of oil were added to this reactor. From day 212 to 215, a slight increase in the methane production was observed, although it did not achieve the values obtained in R1, which was being fed in the same conditions. It can therefore be concluded that in R4 a long term inhibition from lipids was observed. The oily added in the 12 $gCOD_{oil}/L_{reactor}$ pulse was 86% converted into methane, whereas in the 15 $gCOD_{oil}/L_{reactor}$ pulse only 55% of methane recovery was observed. Therefore, it is suggested that the threshold to enhance methane production, using intermittent inputs of the oily waste in a co-digestion process of manure and food waste, was 12 $gCOD_{oil}/L_{reactor,}$ in order to avoid long term accumulation of lipids.

Table 5.4 presents the overall average TS and VS percentage of reduction in the four reactors during the experiment, considering samples collected twice a week during all the trial. In general oily waste inputs did not influence TS or VS removal in the reactors.

Table 5.4|TS and VS reduction (%).

Reduction (%)	R1	R2	R3	R4
TS	46.7±6.7	45.8±5.6	43.7±6.2	45.1±5.4
VS	53.5±6.1	52.1±5.0	49.1±5.7	51.0±4.8

The effluent soluble COD, total VFA, individual VFA (acetate, propionate, i-butyrate and n-butyrate) and pH are depicted in Figure 5.2 for all four reactors.

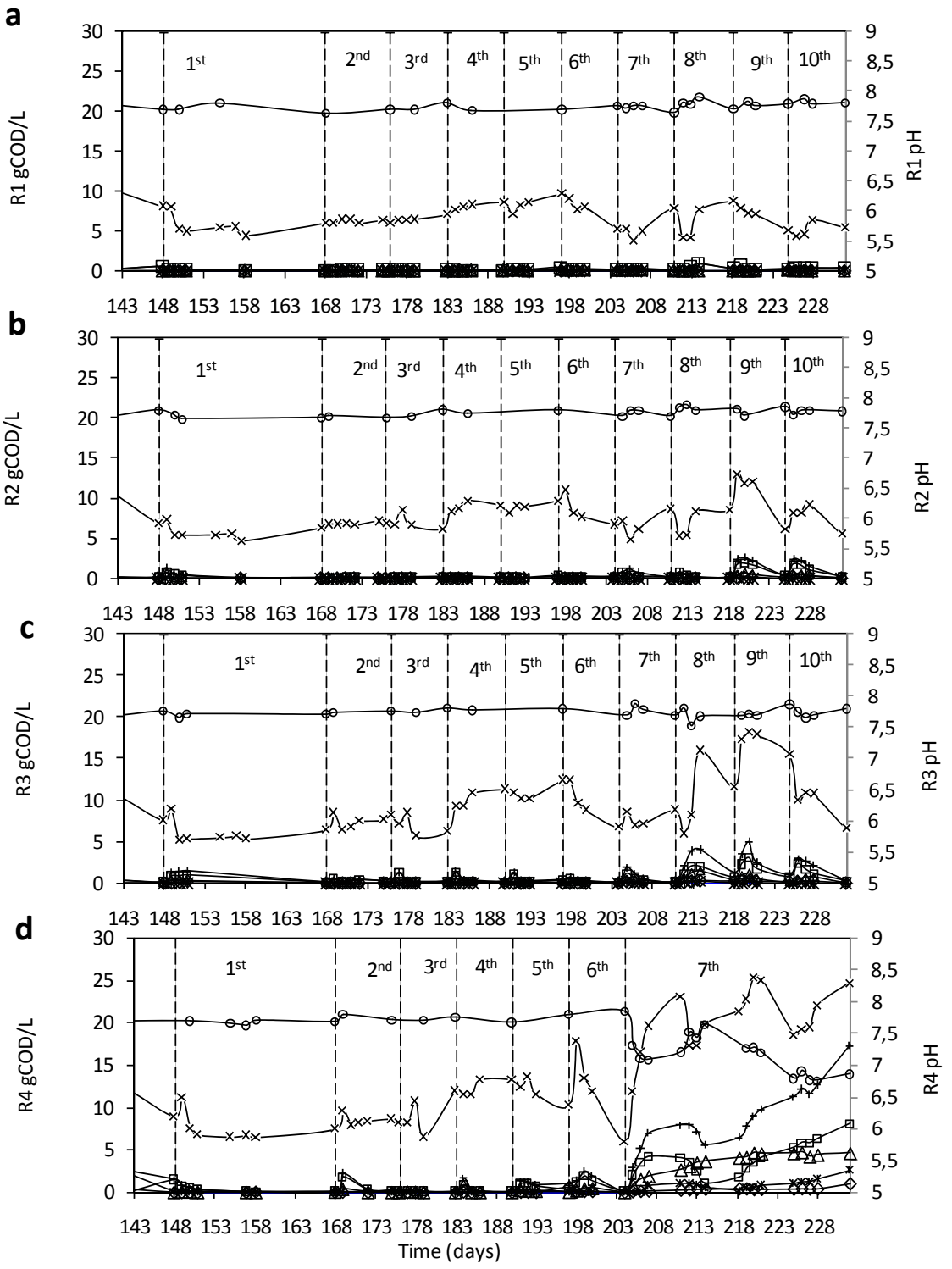

Figure 5.2|Effluent soluble COD (-×-), total VFA (-+-), acetic acid (-□-), propionic acid (-Δ-), iso-butyric acid (-◊-) n-butyric acid (-✳-) and pH (-o-) in R1 (a), R2 (b), R3 (c) and R4 (d). [1st to the 6th pulse were 0, 9, 12 and 15 $gCOD_{oil}/L_{reactor}$ in R1, R2, R3 and R4, respectively; 7th pulse was 0, 12, 15 and 18 $gCOD_{oil}/L_{reactor}$ in R1, R2, R3 and R4, respectively; 8th to 10th pulse were 0, 12 and 15 $gCOD_{oil}/L_{reactor}$ in R1, R2, and R3, respectively]

In the first six pulses, the effluent soluble COD is very similar for all the four reactors, only R4 after the 6th pulse presents a slightly higher value, achieving a peak of 17.9 gCOD/L decreasing, afterwards, to values similar to the other reactors. This peak value was twice the value obtained in R1 at the same day. When the concentration of lipids applied was 9 $gCOD_{oil}/L_{reactor}$, the value attained for the soluble COD was very similar to the control reactor R1. In the pulses of 12 and 15 $gCOD_{oil}/L_{reactor}$ the maximum value of soluble COD attained was 13 and 18 gCOD/L. An increase in the soluble COD was detected in R4, matching the methane production decline. The values of soluble COD in this reactor did not decrease until the end of the experiment even with no more addition of oil, confirming the persistent inhibition of the system.

VFA dynamics followed the general trend presented by the soluble COD. After the 7th pulse, the VFA levels in R4 increased significantly, attaining values of 8, 11, 17 gCOD/L on days 210, 224 and 232 respectively. In this case, the VFA composition in acetate was 51% on day 210 and 47% on days 224 and 232.

After the day 204, the pH values in R4 decreased (Figure 5.2 (d)), although the measured values were always higher than 6.5, this parameter did not recover until the end of the experiment, similarly to the soluble COD and VFA contents.

5.3.2.|METHANE POTENTIAL ASSAYS

The time course of the cumulative methane production in the methane potential tests is depicted in Figure 5.3 for the controls and for the tests with 4.8 gCOD/L of oily waste. Biomass collected from R1 and R4 on days 203 and 224 was used in the assays. The control tests without addition of oily waste, accounted for the methane production due to the residual substrate. All the tests were performed with the reactor effluent, without any pre-treatment of degassing or washing.

The experiment with the biomass collected from R4 in day 224 was done to determine if the inhibition observed in the methane production in the reactor was permanent or reversible (Figure 5.3 (d)).

From Figure 5.3 (d) it is clear that, in batch conditions, after a lag-phase of approximately 10 days, the consortium collected from R4 on day 224 (when methane production was already inhibited) started to mineralize the residual substrate, likely including lipids or long chain fatty acids adsorbed or entrapped onto the biomass and fibres, as was previously suggested by PEREIRA ET AL. (2005).

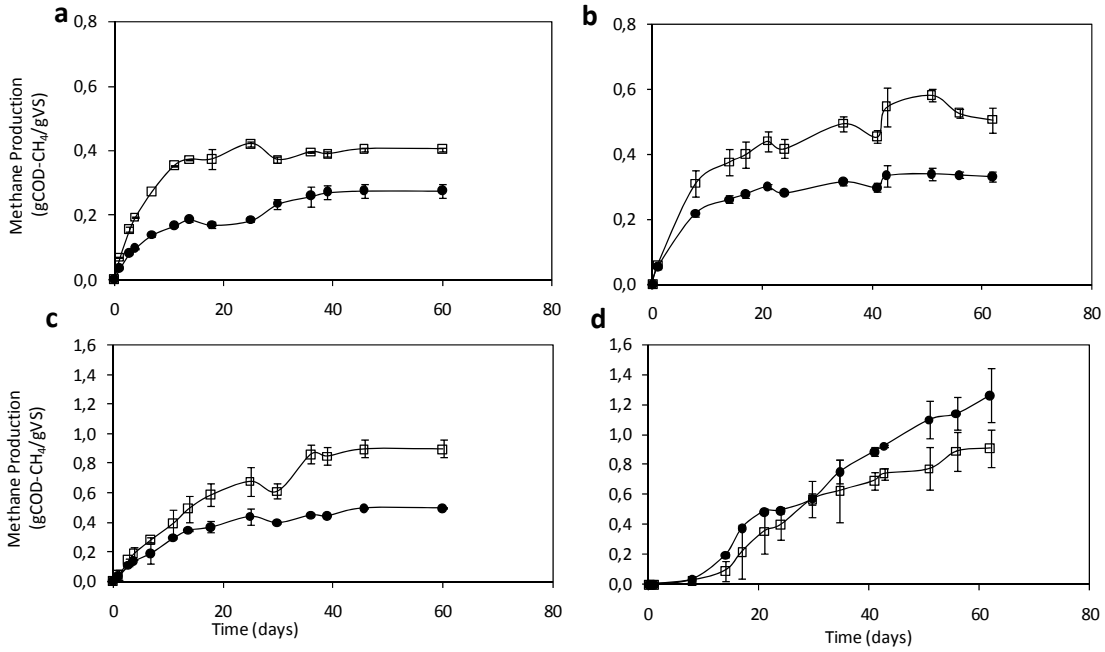

Figure 5.3|Methane production (gCOD-CH_4/gVS) from the methane potential tests in R1 day 203 (a), R1 day 224 (b), R4 day 203 (c) and R4 day 224 (d) (-●- biomass collected from the reactor, -□- biomass with additionally 4.8 gCOD/L of oily waste). The error bars represent the standard deviation.

The information presented in Table 5.5 evidences that the presence of oil enhanced the maximum methane production rate (MMPR) in all assays, with the exception of the sample collected from R4 on day 224. Therefore, the presence of oily waste increased the rate of methane production in the co-digestion of cow manure and food waste, except when the solid matrix was severely loaded with lipids as was the case of R4.

The response of R1 due to the addition of 4.8 $gCOD_{oil}$/L. was similar in the days 203 and 224 with an average increase of 0.13 gCOD-CH_4/gVS. In the biomass collected from R4 on day 203, the addition of 4.8 $gCOD_{oil}$/L, equivalent to 0.38 $gCOD_{oil}$/gVS, promoted an increase of 0.4 gCOD-CH_4/gVS, suggesting a complete mineralisation of the added oily waste, whereas in R1, only 34% of the expected methane production was observed. Therefore, the anaerobic community that was previous submitted to the oily pulses performed a better conversion of the fat added to methane.

Table 5.5|MMPR (gCOD-CH_4/gVS.day) and maximum methane yield (gCOD-CH_4/gVS) obtained in biodegradability assays (results are given as means of triplicates with standard deviation).

Day	Biomass	MMPR (gCOD-CH4/gVS.day)	Maximum methane yield (gCOD-CH4/gVS)
203	R1	0.020±0.001	0.28±0.02
	R1+ 4.8g COD_{oil}/L	0.039±0.001	0.40±0.01
	R4	0.037±0.001	0.50±0.01
	R4+ 4.8g COD_{oil}/L	0.051±0.007	0.90±0.06
224	R1	0.026±0.001	0.37±0.01
	R1+ 4.8g COD_{oil}/L	0.038±0.003	0.50±0.04
	R4	<0.010	nd
	R4+ 4.8g COD_{oil}/L	<0.010	nd

nd – not determined

5.3.3.|SMA TESTS

On days 203 and 224, respectively the end of 6th and 9th pulses SMA tests were performed with acetate and H_2/CO_2 as individual substrates (Figure 5.4).

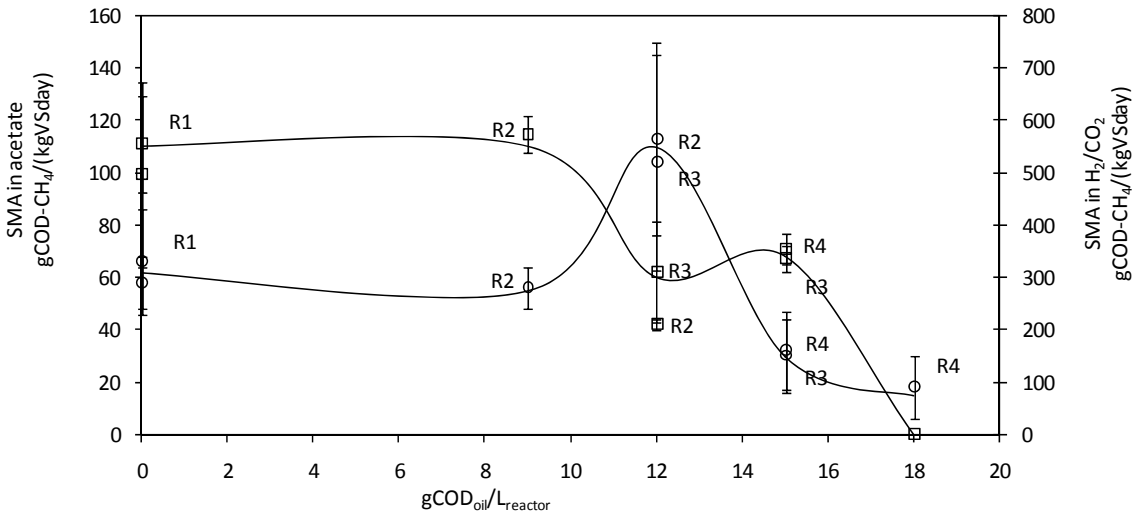

Figure 5.4|SMA measured in the presence of acetate (-o-) and H_2/CO_2 (-□-) after addition of different concentrations of oily waste pulses. The error bars represent the standard deviation. [0gCOD_{oil}/$L_{reactor}$ samples collected on days 203 and 224 in R1, which was devoid from oil pulses; 9gCOD_{oil}/$L_{reactor}$ samples collected on day 203 in R2; 12gCOD_{oil}/$L_{reactor}$ samples collected on days 203 and 224 in R3 and R2, respectively; 15gCOD_{oil}/$L_{reactor}$ samples collected on days 203 and 224 in R4 and R3, respectively; 18gCOD_{oil}/$L_{reactor}$ samples collected on day 224 in R4]

Samples collected from all the oily waste concentration pulses applied were assessed. The

samples are from the solid matrix (not as homogenous as a liquid matrix) explaining the differences between the triplicates and the significant standard deviations obtained.

For the pulses of 12 and 15 $gCOD_{oil}/L_{reactor}$, two different reactors were analysed and the behaviour was similar, irrespectively of the past different history of each reactor.

From Figure 5.4 it is feasible to realize that the result of the SMA in acetate presents an enhancement for the pulse concentration of 12 $gCOD_{oil}/L_{reactor}$. Above this value, the SMA value in the presence of acetate decreases and for the pulse concentration of 18 $gCOD_{oil}/L_{reactor}$ attained the lowest value. Possibly, the drop in methane production in R4 is due to the fact that lipids adsorbed, accumulated or entrapped onto the biomass promote a physical/chemical barrier delaying the transfer of substrates and products, as previously described by PEREIRA ET AL. (2005).

5.4.|CONCLUSIONS

Co-digestion processes of manure and food waste can be improved by addition of oily wastes. The threshold input of oily waste to enhance the methane production in the co-digestion of cow manure and food waste was 12 $gCOD_{oil}/L_{reactor}$, considering the mixture of lipids present in the oily waste added. This corresponds to a continuous feeding of 10% ($V_{food\ waste}/V_{manure}$) with intermittent oil pulses of 5% (V_{oil}/V_{manure}). A pulse feeding of 18 $gCOD_{oil}/L_{reactor}$ induced a persistent inhibition of the process, detected by the decrease in pH to a minimum of 6.5 and an increase in effluent soluble COD and VFA.

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Fate of LCFA in the co-digestion of cow-manure, food waste and discontinuous/recurrent addition of oil

Different concentrations of oily waste were added in a discontinuous mode and recurrently to anaerobic continuous stirred tank reactors fed with cow manure and food waste. Four continuous stirred tank reactors were run in parallel. A control reactor (R1) received no additional oil and R2, R3 and R4 received increasing concentrations of oil in two different experimental approaches. First, the lipids composition was forced to change suddenly, in three moments, without changing the total chemical oxygen demand (COD) fed to the reactors. The only long chain fatty acid (LCFA) detected onto the R1 solid matrix was palmitic acid (C16:0). Nevertheless in the solid matrix of R2, R3 and R4 C16:0 and stearic acid were detected. For occasional increase in the oil concentration up to 7.7 $gCOD_{oil}/L_{reactor}$ (55% $Oil_{COD}/Total_{COD}$) no statistical differences were detected between the reactors, in terms of methane production, effluent soluble COD, effluent volatile fatty acids and total and volatile solids removal. Therefore this experiment allowed to conclude that cow manure-food waste co-digestion presents sufficient buffer capacity to endure solid-associated LCFA concentration up to 20-25 gCOD-LCFA/kgTS.

In a second experiment higher concentrations of oil were added, raising occasionally the concentration in the reactors to 9, 12, 15 and 18 $gCOD_{oil}/L_{reactor}$. All pulses had a positive effect in methane production, with the exception of the highest oil pulse concentration, that persistently impaired the reactor performance. This experiment demonstrates that threshold values for LCFA and C16:0 accumulation onto the solid matrix, of about 180-220 gCOD-LCFA/kg TS and 120-150 gCOD-C16:0/kg TS, should not be surpassed in order to prevent persistent reactor failure, as occurs in some full scale co-digestion plants.

1 2 3 4 5 6 7 8 9

6.|FATE OF LCFA IN THE CO-DIGESTION OF COW-MANURE, FOOD WASTE AND DISCONTINUOUS/RECURRENT ADDITION OF OIL

6.1.|INTRODUCTION

Several organic wastes are treated today in co-digestion processes together with manure (WEILAND, 2004). The biogas yield from raw manure alone is only 20–30 m^3/ton and the operation of the plant is only economically feasible when the biogas yield is higher than 30 m^3/ton (DANISH ENERGY AGENCY, 2005). Co-digestion of manure with biodegradable waste appears as a robust process technology that can increased by 80-400% the biogas production in anaerobic biogas plants. (BRAUN ET AL. 2003; WEILAND, 2004), like food waste. Although co-digestion is an established and applied process, it is important to understand how the changes in the composition will affect the overall process. Food waste, composed by carbohydrates, cellulose, proteins, and lipids, can be highly variable depending on their sources and are not homogeneous in their day-by-day composition. Among the food components, lipids degradation still requires a deeper understanding. It is realistic to consider possible transient, occasional, accidental or even on purpose increase in the lipid content of a food waste stream in a real context.

Lipids as substrate or co-substrate for anaerobic digestion processes constitutes an important issue due to the higher theoretical methane yield (0.99 LCH_4/g) as compared to carbohydrates (0.42 LCH_4/g) and proteins (0.63 LCH_4/g) (ALVES ET AL., 2009). In this context lipid-rich wastes can be regarded as a large potential for renewable energy source (HANSEN ET AL., 1999). Nevertheless, in practice, the anaerobic digestion of lipids is often hampered as the theoretical methane production is not easily achieved. Frequently, the most reported problem is the failure of the system due to the presence/accumulation of the long chain fatty acids (LCFA), ensuing the lipids hydrolysis. LCFA have been reported as inhibitory/toxic to microorganisms even at low concentrations (HANAKI ET AL., 1981, ANGELIDAKI & AHRING, 1992 RINZEMA ET AL., 1994). More recently it was reported that LCFA inhibition was reversible. The observed transient inhibition was partially assigned to transport limitation, due to LCFA adsorption, instead of exclusively to metabolic phenomena (PEREIRA ET AL., 2004, 2005). Adsorption of LCFA is a wide reported phenomena in the anaerobic digestion processes and is frequently the reason appointed to process failure (MIRANDA ET AL., 2006; HWU ET AL., 1998, CIRNE ET AL., 2007). Nonetheless, there are examples of successful anaerobic digestion of lipids in the literature. LI ET AL. (2002) reported that food wastes containing high lipids content, ranging from 8% to 40% by adding salad oil and lard

of pork, were effectively degraded by high solids co-digestion process and over 85% of the lipids content was degraded. AHRING (2003) described a significant increase in the yield of methane, from 25 to 50 $L_{biogas}/L_{cattlewaste}$, when fish oil in a total concentration of 5%, was added to a manure digester. Recently, NIELSEN & AHRING (2006) also showed that the addition of oleate pulses to thermophilic reactors treating mixtures of cattle and pig manure had a stimulating effect on the overall process. In a previous work (NEVES ET AL, 2009) pulses of oily waste from a canned fish processing industry were added to completely mixed reactors fed daily with dairy cow manure and food waste. Concentrations up to 15 $gCOD_{oil}/L_{reactor}$ had a positive effect in methane production, whereas after a sudden addition of oil at 18 $gCOD_{oil}/L_{reactor}$ a decay in methane production was observed, which persisted for a long time suggesting an irreversible inhibition in the time scale of the experiment. This is extremely important as far as the co-digestion of lipids is concerned. Prevention of inhibition by LCFA rather than recovery after inhibition should be the right operational strategy to recover the full potential of methane production from lipids in full scale continuous anaerobic digestion plants.

So far, the exact behaviour of LCFA in co-digestion processes is not well understood. Inhibitory LCFA concentrations, ratio between solid phase-associated LCFA and methane production or/and fate of individual LCFA in co-digestion processes with lipids, are issues that demand additional research efforts.

The present study focus on assessing the individual profiles of LCFA associated to the solid phase of four reactors fed with cow manure and food waste, in two different approaches: First, the lipids composition was forced to change suddenly in three moments without changing the total chemical oxygen demand (COD) fed to the reactors. Secondly, pulses of lipids were added, raising the concentration in the reactors up to 9, 12, 15 and 18 $gCOD_{oil}/L_{reactor}$. Lipid concentrations were manipulated by adding an oily waste from canned fish industry. One of the reactors was used as control and was devoid from the oily waste during both experiments. The ultimate goal was to find a practical value of solids-associated LCFA that a co-digestion anaerobic plant based on cow manure and food waste can endure.

6.2.|MATERIALS AND METHODS

6.2.1.|SUBSTRATES

Three substrates were used in the anaerobic co-digestion process. (i) Cow manure, collected in a dairy farm in the suburbs of Braga (Portugal) and stored in a refrigerator (4ºC) until use to minimize the decomposition of substrate; (ii) Food waste, which was a composite sample (one

week based) from the waste produced in the canteen of the University of Minho, located in "Campus de Gualtar", Braga, Portugal. It was crushed to 1-3 mm particle size and stored at 4 ºC during 5 days, until the end of the collecting process. Then it was mixed and stored at -18 ºC; (iii) Oily waste collected in a canned fish processing industry was used to simulate the variation of lipids content. The characteristics of each substrate are presented in Table 6.1.

Table 6.1|Characterisation of cow manure, food waste and oily waste used in the continuously stirred tank reactors experiments

Substrate	Cow Manure (g/L)	Food Waste (g/kgwaste)	Oily Waste (g/kgwaste)
Chemical Oxygen Demand (COD)	39±8	327±73	2690±61
Total Solids (TS)	28±5	266±1	971±5
Volatile Solids (VS)	21±4	254±7	972±4
Fat content	ne	20±8	877±32
Long Chain Fatty Acids (LCFA)	Cow Manure (gCOD/kgTS)	Food Waste (gCOD/kgTS)	Oily Waste (gCOD/kgTS)
Myristic acid (C14:0)	3±1	0	19± 1
Palmitic acid (C16:0)	14±4	14 ± 4	260± 7
Palmitoleic acid (C16:1)	0	0	27± 1
Stearic acid (C18:0)	26±9	6 ± 2	75± 2
Oleic acid (C18:1)	0	16 ± 5	891± 17
Linoleic acid (C18:2)	0	8 ± 2	790± 33

ne: not evaluated; Data are expressed as mean ± standard deviation of five replicates

6.2.2.| REACTOR START-UP

Four 5L mesophilic continuous stirred tank reactors with hydraulic retention time of 15 days were fed with cow manure and food waste. The digesters were inoculated with the effluent from a stable laboratory mesophilic anaerobic digester feed with cow manure and food waste. The biogas flow rate generated was measured by a Ritter Milligascounter (Dr. Ing. Ritter Apparatebau GmbH, Bochum, Germany). After a stable operation of the four reactors, for 48 days, the experiments with occasional feeding of fat were initiated.

6.2.3.|EXPERIMENTAL PLAN

Two sets of experiments with addition of different lipid concentrations were performed. In both experiments, the feed to the four reactors had equal proportions of cow manure and food waste in a TS basis during the all trial. The organic loading rate of the four reactors was 4.6±0.1 gCOD/($L_{reactor}$.day). The lipid content was changed by adding pulses of oily waste to three of the

four reactors fed with the mixture cow manure/food waste as depicted inTable 6.2.

Table 6.2|Oil waste ($gCOD_{oil}/L_{reactor}$, % fat content in $OIL_{COD}/Total_{COD}$) fed to the reactors in the days represented. LCFA concentration (gCOD/kgTS) immediately after the feeding in each reactor calculated due to the oily waste addition.

	Day		Oily waste* ($gCOD_{oil}/L_{reactor}$)*	$Oil_{COD}/Total_{COD}$ (%)	LCFA (gCOD/kgTS) C14:0	C16:0	C16:1	C18:0	C18:1	C18:2
First experiment	49	R1	0	-	0	0	0	0	0	0
		R2	0.4	6	0	1	0	0	4	3
		R3	0.6	9	0	2	0	0	6	5
		R4	0.8	13	0	3	0	1	9	8
	56	R1	0	-	0	0	0	0	0	0
		R2	1.6	25	0	5	0	2	18	16
		R3	2.4	38	1	9	1	3	31	27
		R4	3.3	51	1	11	1	3	36	32
	84	R1	0	-	0	0	0	0	0	0
		R2	3.6	36	1	13	1	4	45	40
		R3	5.4	46	2	20	2	6	68	61
		R4	7.7	55	2	28	3	8	95	85
Second experiment	148, 168,176, 183,190, 197 (**)	R1	0	-	0	0	0	0	0	0
		R2	9.0	60	2	31	3	9	104	94
		R3	12.0	65	3	39	4	11	137	121
		R4	15.0	70	4	50	5	14	170	151
	204	R1	0	-	0	0	0	0	0	0
		R2	12.0	65	3	39	4	11	137	121
		R3	15.0	70	4	50	5	14	170	151
		R4	18.0	74	4	60	6	17	207	183
	211,218 225 (***)	R1	0	-	0	0	0	0	0	0
		R2	12.0	65	3	39	4	11	137	121
		R3	15.0	70	4	50	5	14	170	151
		R4	0	-	0	0	0	0	0	0

(*) Oil concentration in the reactor immediately after feeding; (**) Six pulses added in each reactor on the specified days; (***) Three pulses added in each reactor on the specified days.

One of the reactors (R1) was used as control and was devoid from the oily waste during the both experiments. The feed composition was changed on specific days as follows. In the first experiment, the oil composition was forced to change suddenly in three moments of the reactors operation. The total COD fed to the system was approximately constant, even when sudden increases of oil concentration were imposed, meant that the amount of cow manure and food waste fed had to decrease in the moments of oil addition. Nevertheless, the ratio cow manure/food waste was still equal to 1 in a TS basis. After the first experiment the reactors operated in batch mode for 39 days. Afterwards, the cow manure/food waste feed re-started. In the second set of experiments, in reactors R2, R3 and R4, pulses of oil were applied, raising the concentration up to 18 $gCOD_{Oil}/L_{reactor}$ after pulse feeding. Specifically, six similar pulses of oil were added to R2, R3 and R4. A 7^{th} pulse, with a higher quantity of oily waste compared to the

first six pulses, was also added to R2, R3 and R4. From day 204 on, R4 did not receive any more inputs of oily waste, on account of the detected methane production decay. However, R2 and R3 received three more pulses of oily waste, each one equal to the 7^{th} pulse concentration.

6.2.4.|ANALYTICAL METHODS

COD, pH, total solids (TS) and volatile solids (VS) were performed according to Standard Methods (APHA ET AL., 1989).

The total fat content was extracted with diethyl ether in a Soxtec System HT2 1045 extraction unit produced by Tecator (OFFICIAL METHODS OF ANALYSIS 2003.05, 2007)

Methane content of the biogas was measured by gas chromatography using a Porapack Q (180 to 100 Mesh) column, with He as carrier gas at 30 mL/min and a thermal conductivity detector. Temperatures of the detector, injector and oven were 110ºC, 110ºC and 35ºC, respectively.

VFA (acetate, propionate, iso-butyrate and n-butyrate) were determined by high-performance liquid chromatography using a Chrompack column (300x6.5 mm) and a mobile phase of sulphuric acid 5 mM at 0.7 mL/min. The column was set at 60ºC and the detection was by spectrophotometry at 220 nm.

LCFA (lauric (C12:0), myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1) and linoleic (C18:2) acids) analyses were done as described in NEVES ET AL. (2009A).

6.2.5.|BIODEGRADABILITY TESTS

Biodegradability assays were assessed separately, with the food waste and cow manure. Methane production produced by food waste was followed after incubation in batch vials in the following conditions: 5% TS, 1.35 $gVS_{waste}/gVS_{inoculum}$. The granular sludge used as inoculum was collected from an upflow anaerobic sludge blanket reactor treating a brewery effluent located in Oporto, Portugal. The use of granular sludge inoculum reduces the risk of over-acidification during batch, high-solids, anaerobic digestion (NEVES ET AL., 2004). After the introduction of the correct amounts of food waste and inoculum, a defined amount of an anaerobic basal medium was added under strict anaerobic conditions, in order to attain the desired solid content. The anaerobic basal medium was composed of cysteine- HCL (0.5 g/L), $NaHCO_3$ (3 g/L), with the pH adjusted to 7.0-7.2. Resazurin was added as an indicator of redox potential. This basal medium was prepared by boiling the medium before adding the bicarbonate. The vials were then incubated at 37ºC with a stirring speed of 150 rpm, and the methane production was regularly measured by gas chromatography.

Methane production from cow manure was followed after incubation in batch vials supplemented with anaerobic basal medium in order to achieve 5% TS, without any extra inoculum source, using the method described above. The volume of methane produced was corrected to Standard Temperature and Pressure conditions (STP - 1atm and 273K). The results were expressed in terms of methane production in $m^3CH_4/kgVS_{initial}$ and % of methanisation, which corresponds to the % of methane produced relatively to the biochemical methane potential (0.350 $m^3CH_{4\ (STP)}$/kgCOD). All batch experiments were performed in triplicate.

6.2.6.|STATISTICAL ANALYSIS

Single factor analysis of variances (ANOVA) was used to determine if significant differences existed between results obtained under different experimental procedures. Statistical significance was established at a $P< 0.05$ level.

6.3.|RESULTS AND DISCUSSION

6.3.1.|METHANE PRODUCTION AND REACTORS PERFORMANCE IN THE FIRST EXPERIMENT.

Before the experiments, all four reactors achieved a stable performance in methane production. The values obtained for R1 were 0.25±0.05 m^3kg/VS_{added} along with 0.21±0.05, 0.22±0.05 and 0.26±0.04 $m^3/kgVS_{added}$ for R2, R3 and R4, respectively, with no statistical differences (P=0.09). The VFA concentrations were lower than 1.2 gCOD/L and the pH was stable between 7.7-7.9. The percentage of solids reduction in the four reactors was 46, 47, 48, 46 for TS and 66, 65, 67 and 64 for VS. The TS and VS concentrations were not statistically different among the reactors (P=0.951 plus P=0.549 for TS and VS, respectively).

In the first set of experiments the % of oil varied between 6 and 55% $Oil_{COD}/Total_{COD}$, but the total COD fed to the reactors was kept constant. The methane production fluctuated between 0.20 and 0.35 $m^3/kgVS_{added}$. R1 attained an average value of 0.26±0.06 $m^3CH_4/kgVS_{added}$ and R2, R3 and R4 displayed similar behaviours with methane productions of 0.26±0.06, 0.28±0.07 and 0.29±0.04 $m^3CH_4/kgVS_{added}$, respectively. The methane production profile was very similar throught all the experiment. Using ANOVA to analyse all the values of methane production obtained in the four reactors, no statistical differences were observed. Comparing the methane production of all four reactors no dissimilarity was detected due to sudden increase in the lipid content. The values attained are in accordance to literature on dairy cattle manure co-digestion with bio-waste (household sorted) in mesophilic continued stirred tank reactors (0.2-0.3

m^3CH_4kg/VS_{added}) fed with 20%/80% (in VS) bio-waste/cow manure (PAAVOLA ET AL., 2006). The food waste used in the present work, without any extra added oily waste had 7.5% (dried weight) fat content. In the moments of increasing the oil concentration, this value was clearly exceeded. For instance on day 84, in R4, oil increased transitorily to 55% of the total COD fed. The operational response did not exhibit any sign of inhibition in terms of methane production. Inhibition by lipids in co-digestion processes was reported previously. For instance CARUCCI ET AL. (2005), reported that anaerobic co-digestion of pre-cooked food wastes along with the aerobic sludge from the food factory wastewater treatment plant (5% TS) inhibited methanogenesis, probably due to the high content in lipids (13% of dry weight in the pre-cooked food wastes) in batch studies. Those authors also reported that methanogenesis inhibition was overcome by long acclimation periods.

The methane content in the biogas produced in the first experiment was between 54-61%, 54-64%, 57-64% and 55-63% in R1, R2, R3 and R4, respectively. These values are somewhat higher than the ones reported (51-57% of methane) by PAAVOLA ET AL. (2006). The flutuactions imposed in the lipids content did not change the biogas production and respective methane content. These results cleary indicate that cow manure buffer capacity was able to overcome lipids transient fluctuations that can occur in food waste.

The biodegradability assays performed for food waste and cow manure gave 0.29±0.01 and 0.11±0.01 $m^3CH_4/kgVS_{initial}$, corresponding to 56% and 18% of the theoretical methane potential respectively. The values obtained for cow manure are small, but comparable with reported literature values for dairy cattle manure. MÖLLER ET AL. (2004) reported an ultimate methane yield for dairy cattle manure of 0.148±0.04 $m^3CH_4/kgVS$, whereas HILL (1984) found a value of 0.131 $m^3CH_4/kgVS$. This may be explained by the fact that there are big differences in the feeding practice and productivity between dairy farms. Hence, cows fed on only roughage give lower yields than cows fed on both roughage and concentrates (MÖLLER ET AL. 2004).

The daily methane production achieved was 3.02 ±0.58, 3.06±0.70, 3.02±0.70 and 3.09 ± 0.51 LCH_4/day in R1, R2, R3 and R4, respectively. Considering the daily feed, the theoretical maximum methane production expected was 5.46 LCH_4/day. This means that 55-57% of the theoretical methane production was achieved.

Solids reduction in the four reactors was 47, 48, 50, 49 for TS and 59, 59, 60 and 60 for VS. No statistical significant differences were detected in TS or VS removal (P=0.131 and P=0.857, respectively) in all reactors, when compared to R1.

The VS reduction obtained was higher than the ones reported by Mladenovska et al. (2003).

These authors reported values of 31±2% of VS reduction, obtained in a lab scale continued stirred tank reactors at 37ºC treating only manure or 51±3% of VS reduction when treating manure with 2% of lipids.

The swift lipid change in the feed did not promote any fluctuation in the COD and VFA profiles, since no significant differences were detected (P=0.705 for soluble COD and P=0.202 for VFA). A soluble COD between 5 and 9 g/L was measured throughout all the experiment in all the four reactors.

Acetic and propionic acids were the only VFA detected, with acetic acid counting for 75-100% of the total. However, the VFA content was always lower than 1.4 gCOD/L corresponding to a maximum of 15% of the soluble COD. Accumulation of VFA is often reported as the main reason for failure of food waste anaerobic digestion processes (KIM ET AL., 2004). This substrate is easily degraded by fermentative bacteria which generate large amounts of VFA lowering the pH thus inhibiting the methanogenic system and limiting the generation of CH_4 (VAVILIN ET AL., 2006; BOUALLAGUI ET AL., 2004). None of these reported effects have been observed. The pH values were stable and always between 7.6-7.8 in the four reactors, during the all trial, evidencing once more the benefits of the co-digestion of food waste with transient/variable lipids concentration with cow manure.

6.3.2.|METHANE PRODUCTION IN THE SECOND EXPERIMENT.

In the second set of experiment the addition of oil as pulses increased largely the values applied in the first experiment (Table 6.2). The COD content of the feed suffered corresponding increases. In this experiment the highest oily waste concentration added as a pulse, 18 $gCOD_{oil}/L_{reactor}$, (74% $OIL_{COD}/Total_{COD}$) promoted a decay in methane production, attaining almost null production. All the other pulses (9, 12 and 15 $gCOD_{oil}/L_{reactor}$) at lower oily waste concentrations had a positive effect in the methane production.

In Table 6.3 is presented the percentage of biomethanation calculated for each set of oil concentration added as a pulse, which corresponded to the percentage of methane produced relative to the theoretical biochemical methane potential.

Table 6.3|Concentration of oily waste inside the reactors after feeding pulse ($gCOD_{oil}/L_{reactor}$) and the percentage of biomethanation achieved due to each set of oil pulse concentration (adapted from previous results presented in NEVES ET AL., 2009).

Day	R1	R2		R3		R4	
	$gCOD_{oil}/L_{reactor}$	$gCOD_{oil}/L_{reactor}$	%*	$gCOD_{oil}/L_{reactor}$	%*	$gCOD_{oil}/L_{reactor}$	%*
148	0	9		12		15	
168	0	9		12		15	
176	0	9	102±15	12	79±15	15	51±18
183	0	9		12		15	
190	0	9		12		15	
197	0	9		12		15	
204	0	12		15		18	
211	0	12	93±12	15	59±7	0	NP
218	0	12		15		0	
225	0	12		15		0	

*%-Percentage of Biomethanation (average ± standard deviation); NP-almost null production, inhibition detected.

6.3.3.|LCFA ANALYSIS IN FIRST AND SECOND EXPERIMENTS

Analyses of the fed wastes revealed that LCFA content represented 3, 4 and 77% of the total COD in cow manure, food waste and in the oily waste, respectively (Table 6.1). The main contribution of oily waste added to the reactors in terms of LCFA was C18:1 (43.2±0.4%), followed by C18:2 (38.3±0.5%) and C16:0 (12.5±0.1%). The oily waste pulses led to an increase of LCFA content in the reactors.

LCFA were analysed in samples collected after each oil addition, once a day, on the subsequent 4/5 days. No LCFA were detected in the liquid phase. In less than 24 hours an exclusive detection of these compounds onto the solid matrix was observed (Figure 6.1).

The results obtained from R1 solid matrix, showed that LCFA content was constant during the experiment, with 9±3 gCOD-C16:0/kgTS and 3±1gCOD-C18:0/kgTS (Figure 6.1). The anaerobic co-digestion of cow manure and food waste accomplished a reduction of 36 and 80% in C16:0 and C18:0, respectively. The acids C18:1 and C18:2 corresponded to 36% and 18% of the total LCFA detected in food waste, but were never detected in R1.

In the oily waste, linoleic acid (C18:2) represented 38% of total LCFA. However, this acid was never detected in the analyzed solid samples in any reactor, suggesting that its conversion in shorter chain acids was faster and easier when compared to others LCFA.

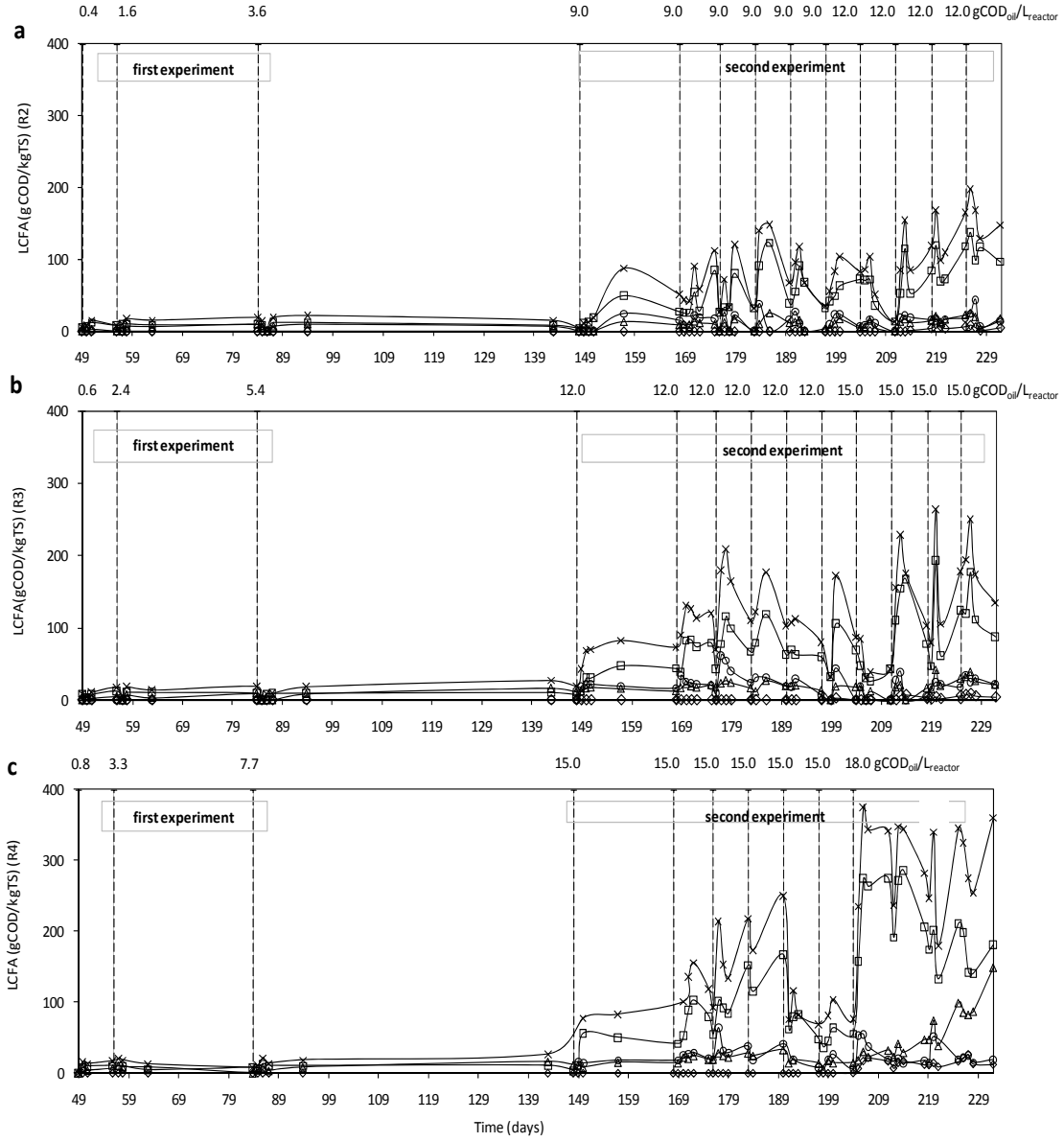

Figure 6.1|Biomass-associated LCFA (gCOD/kgTS) in R2 (a), R3 (b) and R4(c). (-◊- C14:0; -□- C16:0; -Δ- C18:0; -○- C18:1; -×- Total LCFA) [Indication of reactor COD after oil addition is depicted in each graph]

In the first experiment the maximum level of COD-LCFA accumulation detected was 25 gCOD-LCFA/kgTS, composed of C16:0 and C18:0. With no statistical differences between R2, R3 and R4, using ANOVA (*P*=0.51 for C16:0, *P*=0.30 for C18:0, and *P*=0.56 for C16:0+C18:0). Since the three reactors received different LCFA concentrations, and accumulated similar amounts, it is reasonable to presume that biomass from R4 was more efficient in converting LCFA.

Analysing the results of the first experiment it is concluded that the co-digestion system, cow manure-food waste, could endorse higher lipid content

In the second experiment the amount of LCFA added was higher and, therefore, higher LCFA concentrations were detected (Figure 6.1).

For the pulse of $9gCOD_{oil}/L_{reactor}$, with the exception of C16:0, all the other detected acids were about zero after 7 days, indicating that the time to degrade the accumulated LCFA was attained between pulses, and justifying the 102% biomethanation achieved in this set of pulses. It is important to highlight the consistent behaviour in terms of individual LCFA of the R2 and R3 response to several pulses of $12gCOD_{oil}/L_{reactor}$ and $15gCOD_{oil}/L_{reactor}$ The increase from 12 to $15gCOD_{oil}/L_{reactor}$ did not change the LCFA patterns, being the major acid detected palmitic acid (C16:0) representing 72$\pm$ 13% of the total LCFA detected.

The highest amount of LCFA adsorbed/accumulated onto the solid matrix ranged from 61% to 77% of the total LCFA fed as a pulse, with the lowest value attained at a pulse of $9gCOD_{oil}/L_{reactor}$ and the highest when the feeding corresponded to $18gCOD_{oil}/L_{reactor}$. As mentioned before, accumulation of LCFA onto solids is a well known phenomenon. For instance, PETRUY & LETTINGA (1997) also reported that 70% of lipids were adsorbed by granular sludge, within approximately one day.

C16:0 was always the major detected LCFA adsorbed/accumulated onto the solid matrix and achieved higher concentration than the ones corresponding to the influent. This suggests that palmitic acid was also in this case a major intermediate from the conversion of C16:1, C18:0, C18:1 and C18:2, as detected by other authors (LALMAN & BAGLEY, 2000; PEREIRA ET AL., 2002, SALMINEN ET AL., 2001). JEGANATHAN ET AL. (2006) reported that when treating complex oily wastewater in upflow anaerobic sludge blanket reactors, although attaining COD removal efficiencies of 80%, reactor failure was detected after high organic loading rate due to the fat accumulation onto the biomass, which was identify mainly as C16:0 ($>$ 60%), whereas, the fed LCFA contained only 30% C16:0 and 50% of C18:0.

NIELSEN & AHRING (2006) observed that an addition of 2.0 g_{oleate}/L (5.8 gCOD/L) to reactors fed with a mixture of cattle and pig manure led to an inhibition illustrated by an instant drop in the methane production, although the reactors performance recovered after a period of time. In the present work the oleate COD present in the pulse feed of $18gCOD_{oil}/L_{reactor}$ was equivalent to $5.9gCOD/L_{reactor}$, comparable to the amount reported by the former authors, with the exception that C18:1 acid was within a complex mixture of LCFA, and no recover was detected in R4 after 30 days.

The major acid identified onto the solid matrix was C16:0 at 273 gCOD-C16:0/kgTS in R4 (Figure 6.1 (c)). However, an overall decrease in the accumulation of C16:0 was observed, which became

more evident in the last days of operation, to values near the ones detected for the other pulses. Nevertheless, the methane production did not recover and conversely to the other reactors, stearate (C18:0) concentration increased, even without any extra addition of oily waste. At the end of the experiment, this acid was 41% at a concentration of 140gCOD-C18:0/kgTS jointly with the 180gCOD-C16:0/kgTS corresponding to 50% of the detected acids.

The accumulation of C18:0 in the presence of high concentrations of C16:0 has been reported in fat degradation in rumen metabolism (VAN NEVEL & DEMEYER 1995, 1996). Linoleic and oleic acids in the feeding (Table 6.2) seemed to be first converted to the intermediate palmitic acid and, in the last 10 days of operation, an higher proportion of stearic/palmitic acid was detected, suggesting a different dynamics of intermediates accumulation during the operation in R4. Although reactor R4 was not like a rumen systems, some parallelism can be done between the two processes. Unsaturated fatty acids have relatively short half-lives in ruminal contents because they are rapidly hydrogenated by microorganisms to more saturated end products (HARFOOT & HAZLEWOOD 1988). The extent to which biohydrogenation of double bonds occurs is variable and its optimum pH is 6.5. R4 was the only reactor that presented altered patterns after day 204, the soluble COD and volatile fatty acids increased considerably and pH values decreased attaining 6.5 (NEVES ET AL., 2009).

Methane production was hampered when the total adsorbed/accumulated LCFA attained 375 gCOD-LCFA/kgTS, which occurred after the $18gCOD_{oil}/L_{reactor}$ pulse. Furthermore, a clear linear negative correlation was observed between the achieved % of biomethanation and the highest transient solids-associated LCFA detected in all the pulses (Figure 6.2 a). From all the LCFA detected, palmitic acid (C16:0) was the main responsible for the observed decay in the methane production (Figure 6.2 b).

According to these results, the quantification of solids-associated LCFA or C16:0, allows the prediction of the biomethanation %, suggesting to be key indicators of biomethanation failure. Threshold values for LCFA and C16:0 accumulation onto the solid matrix, of about 180-220 gCOD-LCFA/kg TS and 120-150 gCOD-C16:0/kg TS, should not be surpassed in order to prevent failure of the system.

These value are however lower than the optimal reported by PEREIRA ET AL., (2004), for the amount of biomass-associated LCFA - 1000 gCOD-LCFA/kgVS-, that could be degraded in batch assays at a maximal rate around 250 gCOD-LCFA/kgVS.day. However process conditions were different and feeding was only oleate.

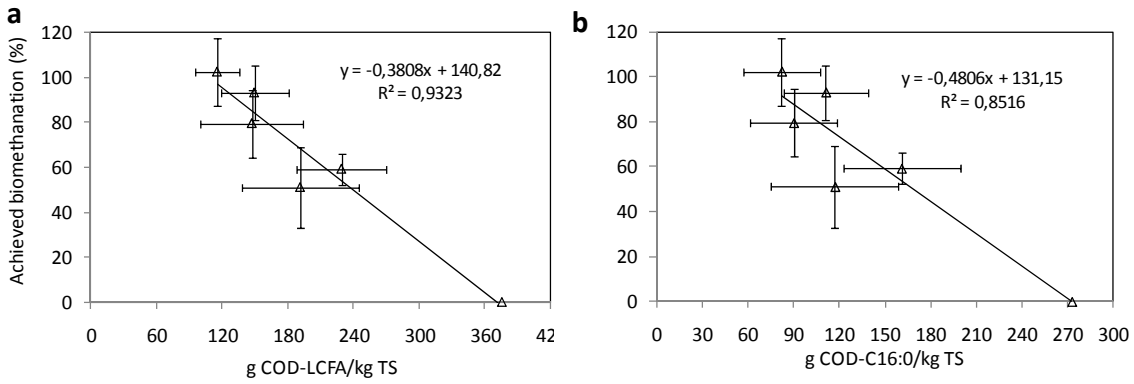

Figure 6.2|Influence of maximum transient accumulation of LCFA(a) and palmitic acid (b) in the achieved biomethanation (%), for all applied pulses. Values represent the average of 6 pulses of 9 $gCOD_{oil}/L_{reactor}$ (R2), 10 pulses of 12 $gCOD_{oil}/L_{reactor}$ (R2+R3), 10 pulses of 15 $gCOD_{oil}/L_{reactor}$ (R3+R4) and 1 pulse of 18 $gCOD_{oil}/L_{reactor}$ (R4). Bars represent the standard deviation

6.4.|CONCLUSIONS

The results of these experiments demonstrate that cow manure/food waste co-digestion presents a sufficient buffer capacity to endorse lipids fluctuations, up to concentrations of 7.7 $gCOD_{oil}/L_{Reactor}$ (55% $Oil_{COD}/Total_{COD}$), maintaining an efficient overall reactor performance and stability, when the total COD fed is constant. The same co-digestion system can endure recurrent pulses of oil (once a week) up to 15 $gCOD_{oil}/L_{reactor}$. At a pulse of 18 $gCOD_{oil}/L_{reactor}$ reactor failure was persistent. Negative linear correlations between the achieved biomethanation % and the solid-associated LCFA or palmitic acid, allowed to establish threshold values of 180-220 gCOD-LCFA/kg TS and 120-150 gCOD-C16:0/kg TS, respectively, that should not be surpassed in order to prevent reactor failure. The approach of occasionally add lipids to the anaerobic co-digestion can be a feasible option to biodegrade fats/greases, recovering the methane potential of these substrates.

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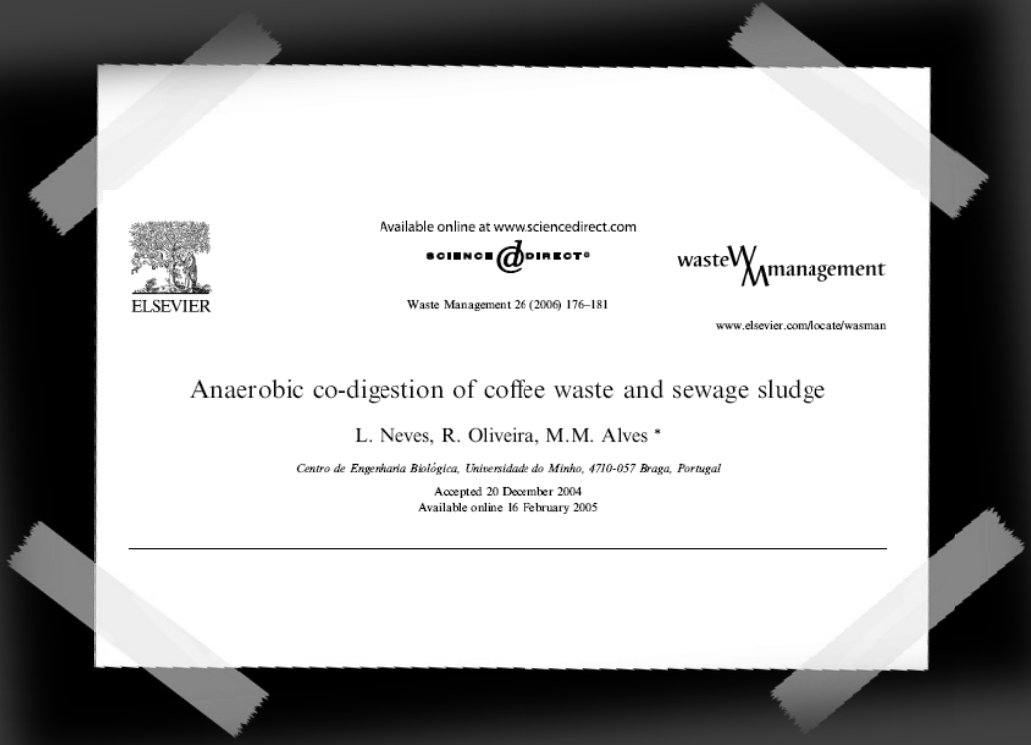

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Anaerobic co-digestion of coffee waste and sewage sludge

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The feasibility of the anaerobic co-digestion of coffee solid waste and sewage sludge was assessed. Five different solid wastes with different chemical properties were studied in mesophilic batch assays, providing basic data on the methane production, reduction of total and volatile solids and hydrolysis rate constant. Most of the wastes had a methane yield of 0.24-0.28 $m^3CH_{4(STP)}/kg_{VSinitial}$ and 76 and 89% of the theoretical methane yield was achieved. Reduction of 50-73% in total solids and 75-80% in volatile solids were obtained and the hydrolysis rate constants were in the range of 0.035-0.063 d^{-1}. One of the solid waste, composed of 100% barley, achieved a methane yield of 0.02 $m^3CH_{4(STP)}/kg_{VSinitial}$, reductions of 31% in total solids, 40% in volatile solids and achieved only 11% of the theoretical methane yield. However, this waste presented the highest hydrolysis rate constant. Considering all the wastes, a inverse linear correlation was obtained between the methane yield and the hydrolysis rate constant, suggesting that hydrolysis was not the limiting step in the anaerobic biodegradability of this kind of waste.

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7.|ANAEROBIC CO-DIGESTION OF COFFEE WASTE AND SEWAGE SLUDGE

7.1.|INTRODUCTION

Due to the strict legislation currently in use for landfilling, anaerobic digestion has a strong potential as an alternative treatment for biodegradable waste. The instant coffee production process involves roasting the beans and extracting the soluble fraction with hot water, giving rise to the generation of large amounts of a dark coloured liquid waste containing about 20% of insoluble solids. When instant coffee substitutes are produced the raw material contains barley, rye, malted barley, chicory and coffee, the relative amount of each depending on the specific substitute to be produced. Whatever the raw material used, the waste is mainly composed of carbohydrate fibers such as cellulose, hemi-cellulose and also lignin (DINSDALE ET AL., 1996). Cellobiose and glucose are the hydrolysis products from cellulose, whereas hemi-cellulose hydrolyses to pentoses, hexoses and uronic acid. Lignin is highly recalcitrant and its degradation is considered the limiting step in the decomposition of lignocellulosic substrates (PAVLOSTHATIS & GIRALD-GOMEZ, 1991).

Coffee waste is produced at high temperatures (70 °C), the pH is near 4 and, due to the roasting process, a number of phenol heterocyclic compounds may appear. The anaerobic digestion of coffee waste has been reported at mesophilic temperatures (LANE, 1983; RAETZ, 1990) and also at thermophilic temperatures (KIDA ET AL., 1992; KOSTENBERG & MARCHAIM, 1993). BOOPATHY (1987) studied different inoculum sources and found that the biomass from a sewage digester appeared to acclimatise quickly to the coffee pulp. When studying the digestion of coffee waste in a continuous reactor at mesophilic temperatures, LANE (1983) found a decline in the gas production after 80 days, due to some inhibitory compounds. Similarly, RAETZ (1990) working at thermophilic temperatures in batch studies, also refers problems in achieving stable gas production, either due to pH problems or inhibition. The anaerobic digestion of the liquid stream of instant coffee substitutes industry was first attempted by KOSTENBERG & MARCHAIM (1993). The aim of their study was to evaluate the potential of the digested material as a growth medium for horticulture after thermophilic anaerobic digestion. These authors reported some problems in the experiments due to the high level of solids and to the high percentage of fiber. In spite of the experimental problems a good biogas production with a composition of 70% CH_4 and relatively stable volatile fatty acids (VFA) concentrations were achieved.

All the studies mentioned above are reported with regards to coffee waste, but most of instant

coffee substitutes are produced from a blend of barley, rye, malted barley, chicory and coffee. Due to the different raw matter used to produce the different substitutes, the waste composition changes sequentially, being important to evaluate their individual performance as far as the anaerobic digestion process is concerned. Therefore, the aim of this work is to study the anaerobic biomethanation process of five wastes from the instant coffee substitute production under mesophilic conditions, co-digested with the excess of activated sludge from a wastewater treatment plant located in the same factory.

7.2.|MATERIALS AND METHODS

7.2.1.|WASTE SOURCE

Five "coffee" wastes from the instant coffee substitute production were obtained from the Nestlé factory in Avanca, Portugal. About 40 ton/day (dry matter between 13 and 22%) of waste are, in average, produced in this factory. A wastewater treatment plant is installed in the same factory, producing an excess of activated sludge of about 3.9 ton/day with a dry matter content of 22%. Table 7.1 shows the composition of the 5 wastes, from W1 to W5. All the different wastes presented pH values between 4.5 and 5.0 and the fiber content may be up to 45% (dry weight).

Table 7.1|Composition of the insoluble matter of the five wastes studied.

Waste #	Coffee (%)	Barley (%)	Rye (%)	Malted barley (%)	Chicory (%)
W1	0	40	5	30	25
W2	45	32	0	0	23
W3	0	100	0	0	0
W4	20	45	0	0	35
W5	20	45	0	0	35

The characterization in TS (total solids), VS (volatile solids) and COD (chemical oxygen demand) of each waste, and of the diluted sludge used in the assays (S) are presented inTable 7.2.

Table 7.2|Characterization of each type of waste in TS, VS and COD.

Waste #	TS (g/kg waste)	VS (g/kg waste)	COD (g/kg waste)
W1	131±4	127±4	111±4
W2	217±5	215±5	208±9
W3	214±2	208±2	123 ±1
W4	144±8	141±8	130 ± 6
W5	139±11	136±11	109±9
S	7±1	6±1	6±1

7.2.2.|INOCULUM

The granular sludge was collected from an UASB (upflow anaerobic sludge blanket) reactor treating a brewery effluent located in Oporto, Portugal. The production of methane due to the residual substrate present in the inoculum was 20±1 ml CH_4/g $VS_{sludge.}$ The quantification of the residual methane production was performed using a pressure transducer technique (COLLERAN ET AL., 1992). The test involves the monitoring of the pressure increase developed in sealed vials without substrate. Strict anaerobic conditions were maintained, using an anaerobic basal medium composed of cysteine-HCL (0.5 g/l), $NaHCO_3$ (3 g/l), with the pH adjusted to 7.0-7.2. Rezasurin was added as an indicator of redox potential. The hand held pressure transducer used was capable of measuring a pressure increase or decrease of two atmospheres (0 to ± 202.6 kPa) over a range of -200 to +200 mV. The sensing element is connected to a digital panel module and the device is powered by a 9.0 V DC transformer. The tests for the quantification of residual methane were performed in 25 ml vials, in triplicate. The volume of methane produced was corrected to the standard temperature and pressure conditions (STP).

7.2.3.|BATCH EXPERIMENTS

|METHANE PRODUCTION ASSAYS

The methane production assays were performed in 160 ml vials, in duplicate. A constant ratio of 7 $gTS_{coffeewaste}/gTS_{sludge}$ was kept in the assays, which reflect the relative daily production of the two waste streams. In each assay, the ratio substrate/inoculum was kept constant at 2.3 $gTS_{substrate}/gTS_{inoculum}$. The pH was corrected to 7 and 0.75 $gNaHCO_3$/gTS was added to give suitable alkalinity. The vials were then incubated at 37 ºC under stirring conditions (150 rpm) and the pressure increase was monitored using the above mentioned pressure transducer device. At regular time intervals, the vials were depressurised and the biogas composition was analyzed for

CH_4 and CO_2 content. The batch assays had a total solid content in the range 6 to 9%. The volume of methane produced was corrected to the standard temperature and pressure conditions. The results from the biomethanation process were expressed in terms of methane yield ($m^3CH_4/kgVS_{initial}$) and in terms of % methanation that corresponds to the % of methane produced relative to the biochemical methane potential (350 LCH_4/kgCOD).

|LIQUID COMPOSITION ASSAYS

Parallel assays, with 500 ml working volume, were set up to assess the liquid composition in terms of soluble COD and VFA (acetate, propionate, iso-butyrate, n-butyrate and valerate).

7.2.4.|ANALYTICAL METHODS

COD, TS and VS, were determined according to Standard Methods (APHA ET AL., 1989). Methane and carbon dioxide content of the biogas was measured by gas chromatography using a Porapack Q (180 to 100 Mesh) column, with He as the carrier gas at 30 mL/min and a thermal conductivity detector. Temperatures of the detector, injector and oven were 110, 110 and 35 ºC, respectively. VFA were determined by high-performance liquid chromatography using a chrompack column (300x6.5 mm) and a mobile phase of sulphuric acid 5 mM at 0.7 mL/min. The column was set at 60 ºC and the detection was by spectrophotometry at 220 nm.

7.3|RESULTS AND DISCUSSION

Figure 7.1 shows the methane production curves obtained for the different assays.

Table 7.3 shows the methane yield, the percentage of methanation, the reduction of TS and VS obtained in each assay, after the correction of the methane production due to the residual substrate present in the inoculum (blank assays).

Among the different wastes, the assay SW2 showed the highest methane yield, 0.28 $m^3/kgVS_{initial,}$ which agrees with the higher VS reduction (80%) and the higher initial COD content of this waste. This assay also achieved 85% of the theoretical methane production, although it took 144 days to attain the “plateau”. In the assays SW1, SW4 and SW5 similar methane yields were obtained (0.24-0.25 $m^3/kgVS_{initial}$), the VS reduction was in the range 75-79% and the percentage of methanation in the range 75-89%. The assay SW1 was faster than the others, since it stabilised after about 50 days, whereas the other assays needed about 100 days.

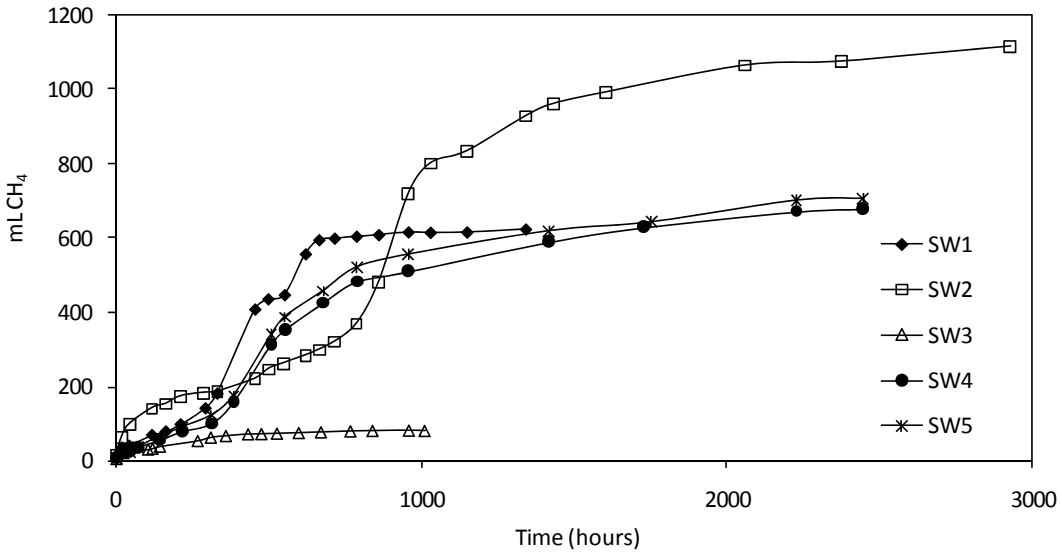

Figure 7.1|Cumulative methane production during the co-digestion assays of coffee waste and sewage sludge.

The methane yield achieved in the assay SW3 was very poor (0.02 m^3CH_4/KgVS), which corresponded to only 11% of the theoretical methane production. This is not surprising because carbohydrates from barley are about 69% composed by fiber (http://www.nutritiondata.com/facts-001-02s04dq.html), being about 6% indigestible fiber (POTTER & HOTCHKISS, 1995). In this assay the lowest values of TS and VS reduction were obtained.

Table 7.3|Methane yield, % of methanation, % reduction of TS and VS of the different coffee wastes in the batch assays.

Assay #	Methane yield ($m^3CH_{4\,(STP)}$/kg $VS_{initial}$)	Methanation (%)	Reduction of TS (%)	Reduction of VS (%)
SW1	0.24	76	73	78
SW2	0.28	85	67	80
SW3	0.02	10	31	40
SW4	0.25	75	50	79
SW5	0.25	89	54	75

Figure 7.2 shows the evolution of methane, VFA and soluble COD, all expressed as COD.

The assay that reached the higher concentration in VFA was SW2 (29 g/L) and this value did not seem to inhibit the subsequent methanogenic process. The poor methane yield of 11% obtained in the assay SW3 was likely due to the presence of products from the hydrolysis of complex heterocyclic compounds rather than to the levels of VFA which peaked at 22 g/L, value lower

than in assay SW2. All the other assays achieved VFA concentrations of around 13-15 g/L. The final pH in all the assays was in the range of 7.3 to 7.8 indicating that irreversible acidification did not occur. At the end of the assays, the VFA concentration was very low (almost near zero in some of the assays), except for SW3 that was still at 20g/L, about 41 days after beginning the test.

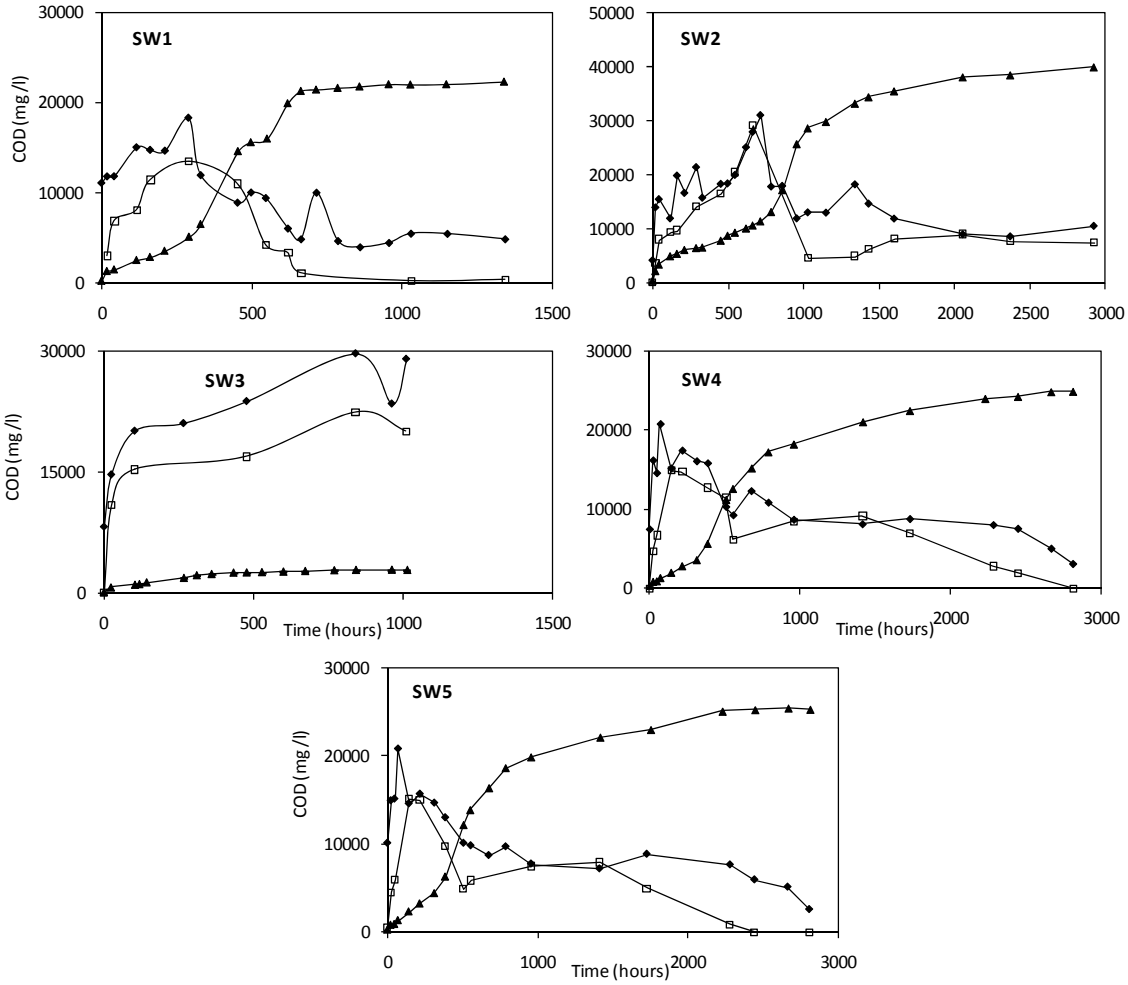

Figure 7.2|Time course of soluble COD (◆), volatile fatty acids COD (□) and methane COD (▲).

Figure 7.3 shows the cumulative methane as COD, hydrolysed COD and acidified COD for all the assays. From this Figure, the relative kinetics of hydrolysis, acidification and methanation can be assessed.

In general, it is accepted that hydrolysis of particulate organic matter is the rate-limiting step in the anaerobic digestion of particulate substrates. However, in the present work this did not occurred, since the curve of cumulative hydrolysed COD increased at a higher rate than the

corresponding cumulative methane production curve.

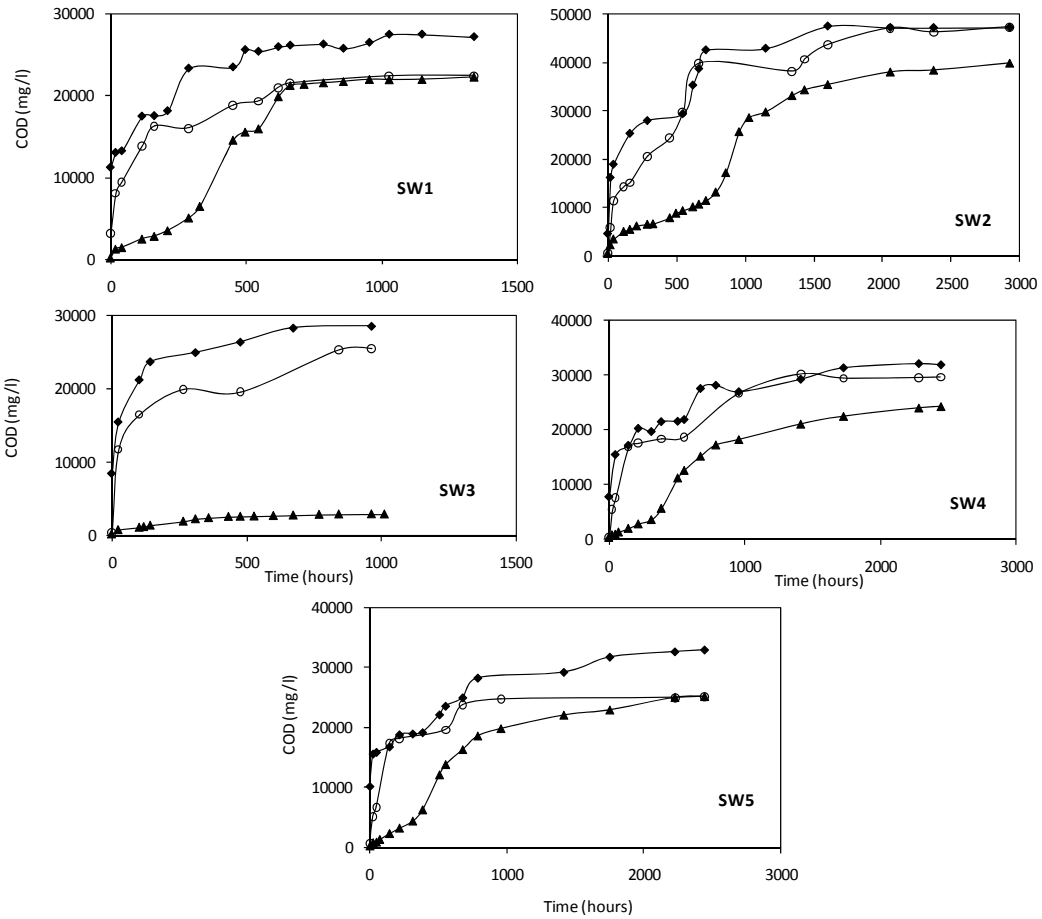

Figure 7.3|Cumulative hydrolyzed COD (◆= methane +soluble COD), acidified COD (○= methane+VFA) and methane COD (▲).

For all the wastes, 84-97 % of the initial COD was hydrolysed, but the percentage of methanation was lower, in the range 75-89%, with the exception of SW3 where only 10% of methanation was observed.

Although the rate of hydrolysis is a function of pH, temperature, concentration of hydrolytic bacteria, and type of particulate organic matter (PAVLOSTATHIS & GIRALD-GOMEZ, 1991), how the physicochemical properties of particulate organic substrates quantitatively affect the rate of hydrolysis (VEEKEN & HAMELERS, 1999), is not well understood. In this study all the mentioned above parameters were the same in all the assays, except the physicochemical properties of the organic waste. The hydrolysis rate constant for each assay was determined, assuming a first order kinetics (Table 7.4).

Table 7.4|Hydrolysis constant rates (assuming first order kinetics).

Assay #	Hydrolysis rate constant (d^{-1})
SW1	0.063
SW2	0.035
SW3	0.084
SW4	0.040
SW5	0.036

Figure 7.4 shows a negative correlation between the hydrolysis rate constant and the methane yield for all the assays.

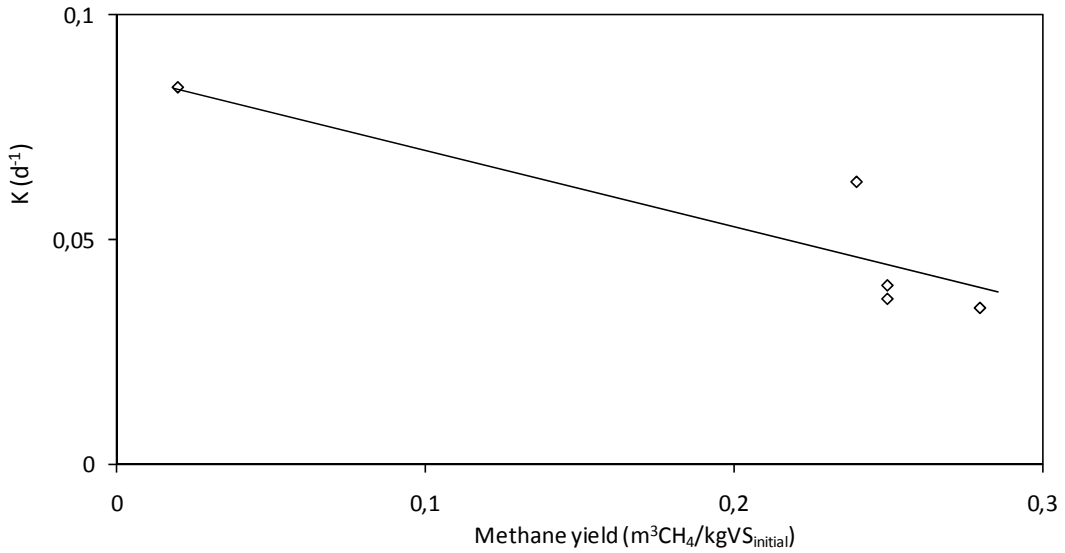

Figure 7.4|Linear correlation between the hydrolysis constant rates and the methane yields.

This indicates that when hydrolysis was faster, the methane yield was lower, likely because the faster hydrolysis induced a more important accumulation of intermediates potentially toxic to the methanogenic population. VEEKEN & HAMELERS (1999), when studying the anaerobic biodegradability of six components of bio-waste containing lignocellulosic material found that grass was less biodegradable (≈ 47%) than leaves (≈ 35%), although having a higher hydrolysis rate constant in mesophilic conditions. According to TONG ET AL. (1990), the biodegradability depends on the structure of the lignocellulosic complex. Cellulose is readily degradable but becomes less degradable or even refractory when incorporated in a lignocellulosic complex. Moreover, AZHAR & STUCKEY (1994) studied the influence of chemical structure of instant coffee

wastes on anaerobic catabolism and found that the individual chemical structure of compounds greatly influences and determines the rate and mechanisms of methanogenic degradation.

7.4.|CONCLUSIONS

When studying five coffee wastes from the production of instant coffee substitutes, methane yields in the range of 0.24-0.28 $m^3/kgVS_{initial}$ were obtained with the exception of a barley rich waste (SW3) that achieved only 0.02 $m^3CH_4/kgVS_{initial}$. Four of the five wastes (SW1, SW2, SW4, SW5) also presented a high reduction of TS (50-73%), VS (75-80%) and 75-89% of the theoretical methane potential (350 L/kgCODremoved). Hydrolysis constant rates in the range of 0.035-0.063d^{-1} were obtained.

In the authors's point of view, these wastes (SW1, SW2, SW4 and SW5) should be treated by anaerobic co-digestion rather than landfilled.

The SW3 waste achieved a methanation of 10% and reduction of TS and VS of 31 and 40%, respectively. However, this waste presented the highest hydrolysis rate constant (0.084d^{-1}), indicating that hydrolysis was not, in this case, the rate limiting step in the anaerobic digestion process. This was evidenced by plotting the hydrolysis rate constants and the methane yields that were inversely correlated, suggesting that intermediates formed during the hydrolysis step, were likely toxic to the methanogenic population.

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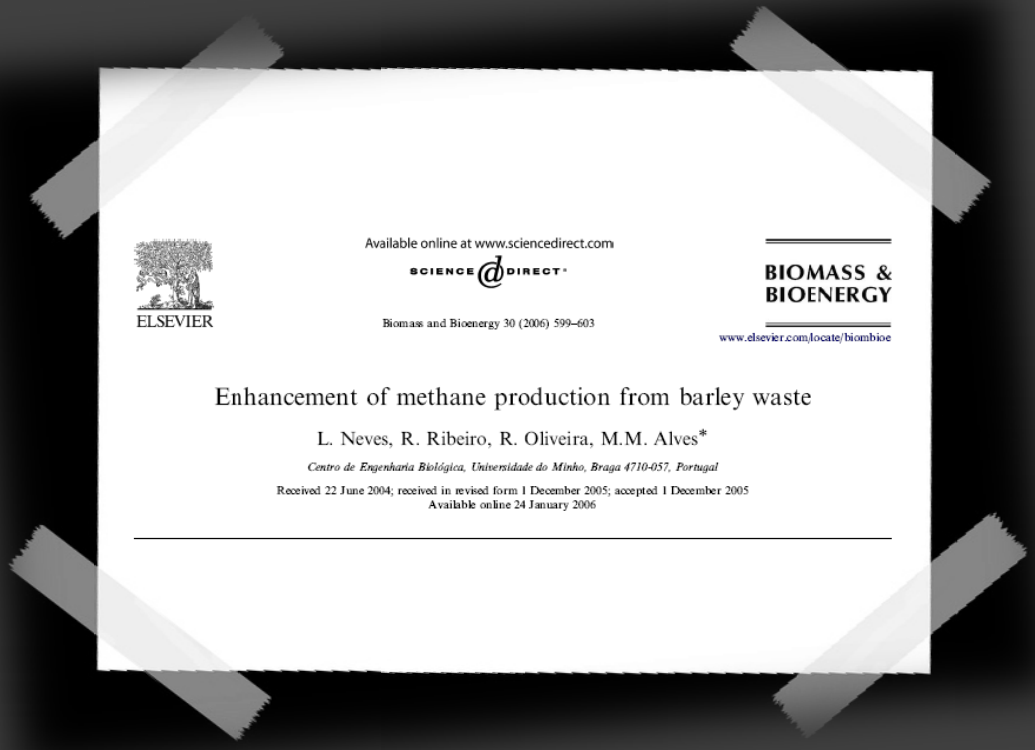

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Enhancement of methane production from barley waste

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Two different approaches were attempted to try and enhance methane production from an industrial waste composed of 100% barley, which results from production of instant coffee substitutes. In previous work this waste was co-digested with an excess of activated sludge produced in the Wastewater Treatment Plant located in same industrial unit, resulting in a very poor methane yield (25 $LCH_{4(STP)}/kgVS_{initial}$), and low reductions in total solids (31%) and in volatile solids (40%).

When the barley waste was subjected to alkaline hydrolysis pre-treatment before co-digestion with activated sludge the methane production increased to 222 $LCH_{4(STP)}/kgVS_{initial}$ and the total and volatile solids reductions increased to 67 and 84%, respectively.

The second approach, followed in the present work, consisted of co-digestion with kitchen waste (40% Barley waste, 60% kitchen waste). The methane production was 363 $LCH_{4(STP)}/kgVS_{initial}$ and the total and volatile solids reductions were 61 and 67%, respectively

1 2 3 4 5 6 7 8 9

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8.|ENHANCEMENT OF METHANE PRODUCTION FROM BARLEY WASTE

8.1|INTRODUCTION

EU legislation, through the COUNCIL DIRECTIVE 1999/31/EC, states that the amount of biodegradable organic waste that is disposed in landfills should be decreased by 65%, relatively to the total amount of organic fraction of municipal solid waste (OFMSW) produced in 1995, by July 2016. In this framework, anaerobic digestion (biomethanation) can be an alternative potential treatment for biodegradable solid waste.

The anaerobic digestion (AD) process was first employed in the treatment of wastewater. However, in the last two decades, this technology has been started to be used in the management of solid waste. This differs from wastewaters due to its high insoluble organic matter content and chemical oxygen demand (COD). This process is considered as organic recycling as it provides renewable energy (biogas) and organic compost after aerobic stabilisation of the digestate. Nevertheless, some organic solid wastes present a low biodegradability in spite of the high COD content and, therefore, studies to enhance the biomethanation process of such wastes are still required.

Coffee waste is a typical example of such a waste. In previous work on the co-digestion of five different wastes from an instant coffee substitutes production industry with sewage sludge, the authors verified that four of the tested wastes gave a methane yield of 240-280 $LCH_{4(STP)}/kgVS_{initial}$, corresponding to 76%-89% of the theoretical methane production. However, a waste composed of 100% barley, attained only 11% of the theoretical methane production, which corresponds to a methane yield of 25 $LCH_{4(STP)}/kgVS_{initial}$ (NEVES ET AL., 2006). This poor methane yield was likely due to the presence of products from the hydrolysis of complex heterocyclic compounds rather than to the levels of volatile fatty acids (VFA), that were lower than in the other tested assays. Moreover, in a study relating the influence of the chemical structure of instant coffee wastes with the anaerobic catabolism, it was found that the individual chemical structure of compounds greatly influences and determines the rate and mechanisms of methanogenic degradation (AZHAR & STUCKEY, 1994)

Acid or alkaline hydrolysis can be applied as a pre-treatment to enhance the anaerobic biodegradability of a recalcitrant waste. Alkaline hydrolysis at ambient temperatures has been proposed as a chemical pre-treatment more compatible with the AD process, since the

bioconversion generally requires an adjustment of pH by increasing alkalinity (PAVLOSTATHIS & GOSSET, 1985). On the other hand, co-digestion with biodegradable wastes has also been successfully and increasingly applied to several agricultural and industrial organic wastes (DE BAERE, 2000).

The aim of this work was to attempt to enhance methane production from a waste composed of 100% barley by using two different approaches: first, an alkaline pre-treatment before co-digestion with sewage sludge, and second co-digestion with kitchen waste, which is the greatest fraction of the OFMSW and is a typical biodegradable waste.

8.2.|MATERIALS AND METHODS

8.2.1.|ANALYTICAL METHODS

The COD, total solids (TS), volatile solids (VS), and total kjeldhal nitrogen (TKN) were determined according to Standard Methods (APHA ET AL., 1989). The methane content of the biogas was measured by gas chromatography using a Porapack Q (180 to 100 Mesh) column, with He as the carrier gas at 30 mL/min and a thermal conductivity detector. Temperatures of the detector, injector and oven were 110, 110 and 35 ºC, respectively. VFA (acetate, propionate, iso-butyrate and n-butyrate) were determined by high-performance liquid chromatography using a chrompack column (300x6.5 mm) and a mobile phase of sulphuric acid 5 mM at 0.7 mL/min. The column was set at 60 ºC and the detection was made by spectrophotometry at 220 nm.

8.2.2.|WASTE CHARACTERIZATION

The barley waste (BW) (100% barley) was generated from the production of instant coffee substitutes. In this industrial plant there is a Wastewater Treatment Plant (WWTP) to treat domestic and other liquid effluents, producing an excess of activated sludge of about 3.9 tonne/day with a dry matter content of 22%.

The kitchen waste was a blended sample (one week based) from the waste produced in the restaurant of the University of Minho, located in “Campus de Gualtar”, Braga, Portugal.

The values of COD, TS, VS, and TKN of the kitchen waste, BW and sewage sludge are given in Table 8.1.

Table 8.1|Characterization of each type of waste used in terms of COD, TS, VS, TKN

Waste #	Kitchen Waste	Barley Waste	Sewage sludge
COD (mg/g)	327±73	123±1	6±1
TS (mg/g)	238±1	214±2	7±1
VS (mg/g)	214±7	208±2	6±1
TKN (mg N-NH_4/g)	13±1	98±2	-ND

ND- Not Determined

8.2.4.|INOCULUM

The granular sludge used as inoculum was collected from an UASB (upflow anaerobic sludge blanket) reactor treating a brewery effluent located in Oporto, Portugal. The production of methane due to the residual substrate present in the inoculum was 20 $LCH_{4(STP)}/kgVS_{sludge}$. The quantification of the residual methane production was performed using a pressure transducer technique (COLLERAN ET AL. 1992). The test involves the monitoring of the pressure increase developed in sealed vials without substrate. Strict anaerobic conditions were maintained by using an anaerobic basal medium composed of cysteine-HCL (0.5 g/L), $NaHCO_3$ (3 g/L), with the pH adjusted to 7.0-7.2. Rezasurin was added as an indicator of redox potential. This basal medium was prepared by boiling the medium before adding the bicarbonate. The handheld pressure transducer used was capable of measuring a pressure increase or decrease of two atmospheres (0 to ± 202.6 kPa) over a range of -200 to +200 mV. The sensing element is connected to a digital panel module powered by a 9.0 V DC transformer. Tests for the quantification of residual methane were performed in 25 ml vials, in triplicate. The volume of methane produced was corrected to the standard temperature and pressure (STP) conditions.

8.2.4|EXPERIMENTAL CONDITIONS

|ALKALINE HYDROLYSIS PRE-TREATMENT

For the alkaline hydrolysis pre-treatment the BW was left overnight in a solution of 0.3 $gNaOH/gTS_{BW}$, at 25 ºC. Batch assays of the treated BW were set up keeping the ratio 7 gTS_{BW}/gTS_{sludge}. This reflects the relative daily production of BW and excess of sewage sludge. The pH was adjusted to 7 and 0.75 $gNaHCO_3/gTS_{initial}$ was added to provide suitable alkalinity. The assays were carried out at 37 ºC under stirring conditions (150 rpm) and the pressure increase was monitored using the pressure transducer device. At regular time intervals, the vials were depressurised and the biogas composition was analyzed for CH_4 content. The volume of methane produced was corrected to STP conditions. The results from the biomethanation

process were expressed in terms of methane yield ($LCH_4/kgVS_{initial}$) and in terms of % methanation, which corresponds to the percentage of methane produced relative to the biochemical methane potential (350 $LCH_4/kgCOD_{initial}$). All the assays were performed in duplicate. These conditions were similar to the ones applied in the first study of co-digestion of the BW with sewage sludge (NEVES ET AL., 2006).

|CO-DIGESTION OF KITCHEN WASTE AND BARLEY WASTE; BATCH REACTOR CONFIGURATION AND OPERATION:

The co-digestion of BW and kitchen waste was studied in a batch anaerobic digester of 120 L with a work volume of 80 L. The waste initially loaded was composed of 60% kitchen waste and 40% BW (digester I). The digester had an internal water jacket to keep the temperature at 37 ºC and was mechanically stirred 3 times a day. For comparative purposes, 100% kitchen waste was fed in a second digester (digester II), which was run under the same conditions as digester I.

In both digesters the solid content (TS) of the waste was 22% and 5 $gNaHCO_3/L$ were added to provide suitable alkalinity.

Once a week, the content of the reactors was sampled for pH, soluble COD, VFA, TS and VS. The cumulative biogas production and the corresponding methane content were determined.

8.3.|RESULTS AND DISCUSSION

8.3.1.|ALKALINE HYDROLYSIS PRE-TREATMENT

Figure 8.1 presents the cumulative methane production obtained in the co-digestion of the pre-treated BW with sewage sludge.

The comparison of the methane production, % methanation as well as the TS and VS reduction obtained in this assay, with the previously reported assay where the barley waste was not pre-treated is given in Table 8.2.

The alkaline hydrolysis pre-treatment increased the methane production up to 222 $LCH_{4(STP)}/kgVS_{initial}$, achieving 100% of the theoretical methanation. Furthermore, this pre-treatment improved the reduction of the TS and VS to 67 and 84%, respectively.

The pre-treatment of lignocellulosic materials with dilute alkali leads to saponification of esters of uronic acid associated with xylan chains resulting in the breaking of cross-linking (DATA, 1981). Consequently, there is a marked increase in the swelling capacity and pore size, improving diffusivity of the hydrolytic enzymes and facilitating enzyme-substrate interactions. Hence, acidogenic bacteria can ferment the pre-treated lignocellulose even though no delignification or

cellulose hydrolysis occurs during the pre-treatment (DATA, 1981).

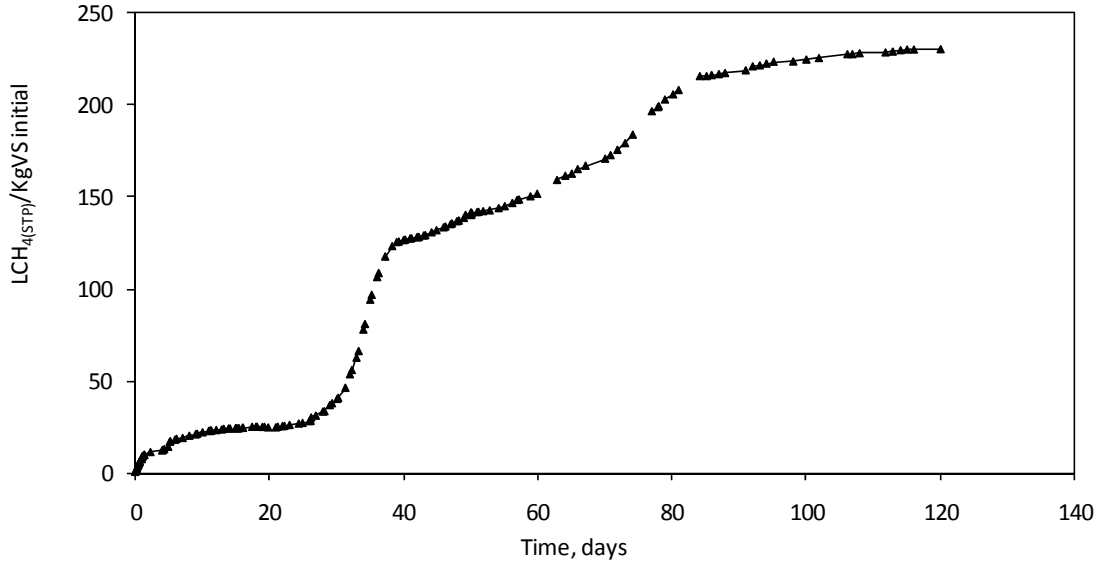

Figure 8.1|Cumulative methane production ($LCH_{4(STP)}/KgVS_{initial}$) obtained in the co-digestion of the pre-treated barley waste with sewage sludge.

The present results show that the alkaline pre-treatment of wastes like barley is beneficial as it significantly improved anaerobic biodegradability. In the assay without pre-treatment reported in the previous work it was observed that hydrolysis was not the rate limiting step in anaerobic biodegradation of barley waste (NEVES ET AL., 2006). The observed inhibition of methane production was likely caused by the sub-products of the natural hydrolysis process (first step of AD process) that were not suitable for the methanogenic population. The outcome from these two assays elicits the conclusion that the products of the alkaline hydrolysis are less toxic and/or inhibitory for the subsequent stages of the AD process.

Table 8.2|Methane production, % methanation, TS and VS reduction in the co-digestion assays with and without pre-treatment. Methane production due to the residual substrate present in the inoculum was discounted

Co-digestion assay #	Methane production ($LCH_{4(STP)}/kgVS_{initial}$)	Methanation (%)	TS Reduction (%)	VS Reduction (%)
BW without pre-treatment*	25	11	31	40
BW with pre-treatment	222	100	67	84

8.3.2.|CO-DIGESTION OF KITCHEN WASTE AND BARLEY WASTE

The results obtained for the weekly monitoring of pH, TS, VS and soluble COD are presented in Figure 8.2. All the studied parameters presented an identical behaviour in both digesters.

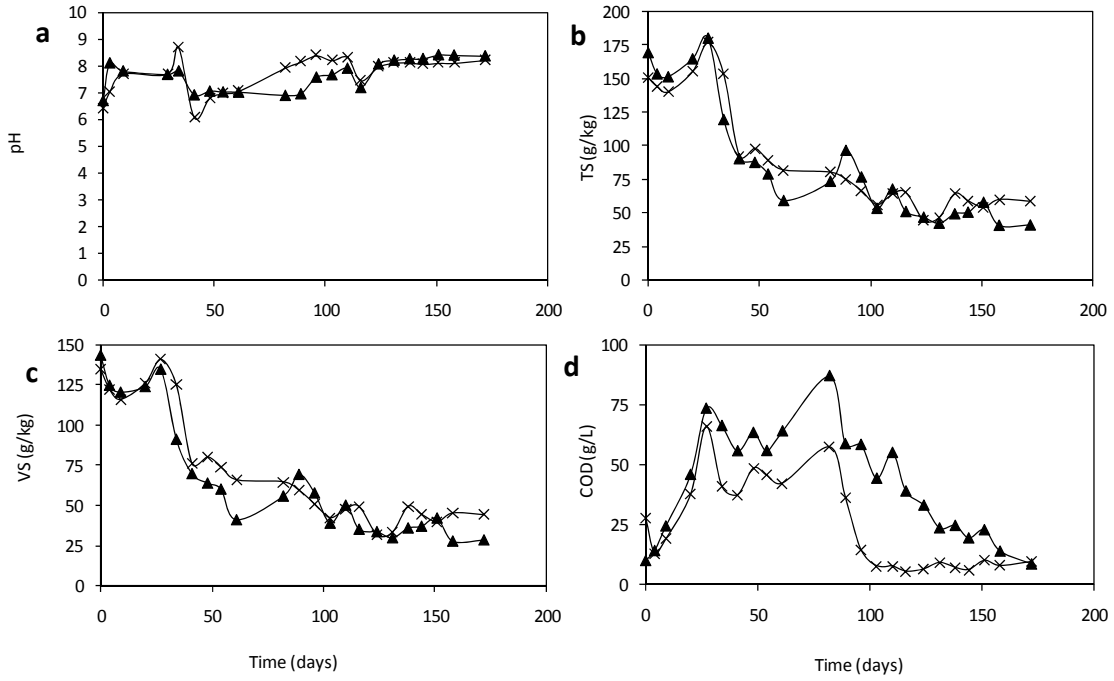

Figure 8.2|Time course of the pH (a), TS (b), VS (c) and soluble COD (d) in the anaerobic digester I (✕) and II (▲)

Although the pH in the co-digestion process (digester I) was slightly higher than in the digester II, at the end of AD process (100 days) the pH was similar in both digesters. No acidification occurred during the AD process, which is an indication that the provided alkalinity was suitable.

The evolution of TS (Figure 8.2(b)) and VS (Figure 8.2(c)) shows that these parameters presented a significant reduction. The profile of soluble COD was different for the two assays. In digester I, soluble COD values were systematically lower than in digester II and attained a residual value of 8 g/L around day 100, whereas in digester II this was only attained by day 172. This indicates that the co-digestion process of BW and kitchen waste was faster than the single digestion of the kitchen waste. This behaviour can also be observed in the cumulative methane production curves (Figure 8.3(a). In this Figure the methane content of the biogas produced is also presented (Figure 8.3(b).

The methane content is somewhat different for the two digesters. In the single digestion of

kitchen waste (digester II), the methane content is about 11% lower than in the co-digestion process (digester I), until day 100. However, thereafter, the biogas from the digester II presented a methane content about 15% higher than the one of the digester I.

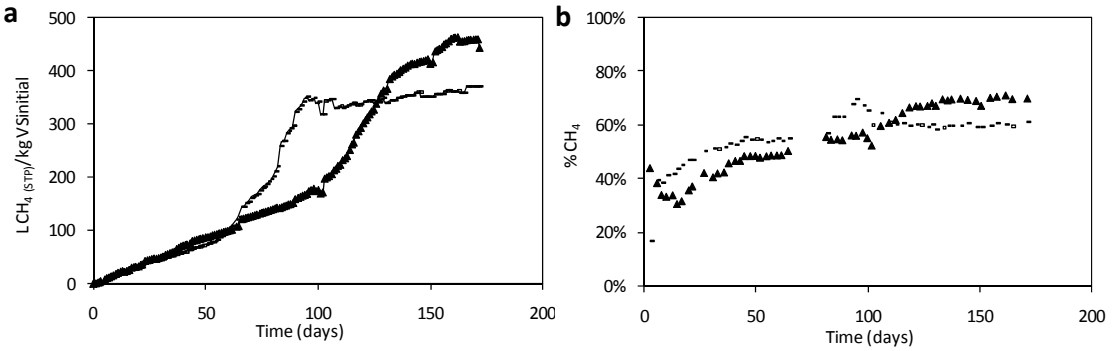

Figure 8.3|Cumulative methane production (a) ($LCH_{4(STP)}/KgVS_{initial}$) and methane content (b) (%) and in the anaerobic digester I (_) and in the anaerobic digester II (▲).

Around day 100, the cumulative methane production of the digester I stabilized at the final value that was, at that time, about 99% higher than that observed in digester II. Nevertheless, at the end, the cumulative methane production was about 20% higher in digester II compared to digester I.

Methane production, % of methanation along with the TS and VS reductions for the two digestion processes are presented in. Table 8.3.

Table 8.3|Methane production, % methanation, TS and VS reduction in the digester I and II. Methane production due to the residual substrate present in the inoculum was discounted

Anaerobic Digester #	Methane production ($LCH_{4(STP)}/kg\ VS_{initial}$)	Methanation(%)	TS Reduction (%)	VS Reduction (%)
I	363	92	61	67
II	432	83	75	80

The co-digestion of the BW with the kitchen waste was beneficial when compared to co-digestion with sewage sludge. Methane production increased from 25 to 363 $LCH_{4(STP)}/kgVS_{initial}$ and the total and volatile solids reductions increased from 31% to 61% and from 40% to 67%, respectively.

Compared with the alkaline pre-treatment, the co-digestion with KW attained more 64% of methane production. However, the TS and VS reductions were 9% and 20% lower, respectively.

From the results of these two approaches, it seems that no inhibition of methanation occurred, as happened when the BW was co-digested with sewage sludge, without pre-treatment. It is feasible to suppose that different intermediates, likely presenting a lower toxicity to the methanogenic populations, were formed in the two approaches studied in the present work.

8.4.|CONCLUSIONS

An alkaline hydrolysis pre-treatment and co-digestion with kitchen waste were beneficial to enhance the methane production of a BW.

Although the best outcome in TS and VS reduction was in the assay with the alkaline hydrolysis, the pH correction in industrial applications can be a costly process when treating large amounts of waste. The co-digestion of the BW with the OFMSW seems to be attractive from an integrated solid waste management point of view because it only decreased the estimated methane production about 20%, reducing the amount of wastes to be landfilled.

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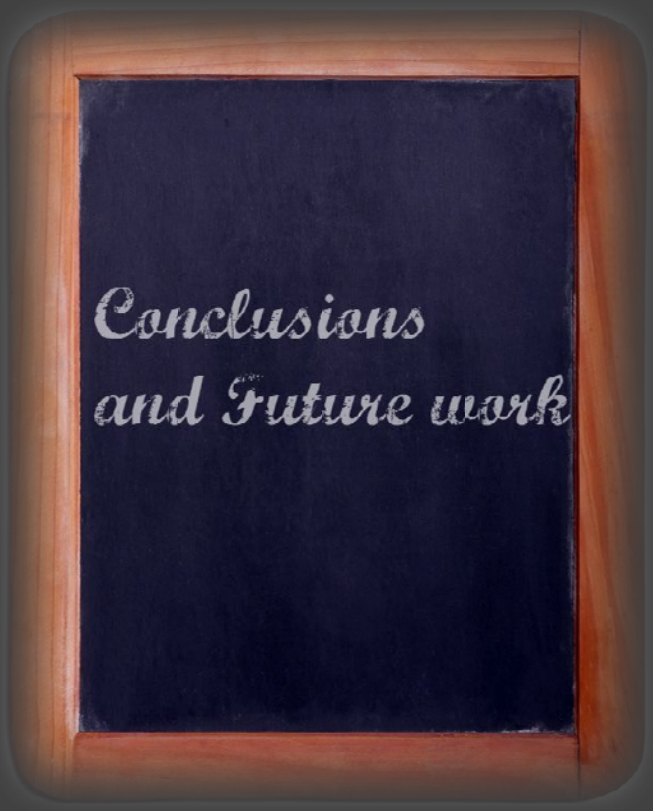

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This chapter reports the main conclusions obtained and also presents some suggestions for future work.

9.|FINAL CONCLUSIONS AND PERSPECTIVES OF FUTURE WORK

9.1.|FINAL CONCLUSIONS

Anaerobic digestion can be an integral part of the solution for two of the most pressing environmental concerns of urban areas: waste management and renewable energy availability. Regardless the fact that national and EU policy has highlighted the need to implement anaerobic digestion technology, not many plants are implemented in Portugal. This situation is in opposition to the scenario in other European countries, where anaerobic digestion is widely spread, such as Germany. Nevertheless, it urges to make the process more profitable. Portugal has significant quantities of bio-wastes and environmental management has become an important issue. These wastes can be employed as feedstock in anaerobic digestion processes with the added value of biogas production.

The studies reported in this dissertation focused the improvement of CH_4 production in co-digestion processes, with a special emphasis on the role of fatty wastes in process performance. Therefore, a method for LCFA extraction, identification and further quantification by gas chromatography was developed (CHAPTER 4). Moreover, the feasibility of anaerobic digestion of some bio-wastes that currently are landfilled was also evaluated.

From the overall results obtained some major conclusions can be withdrawn:

In CHAPTER 3 is showed that the performance of batch anaerobic degradation of food waste under mesophilic conditions depends on waste composition. If lipids are in excess, a slower CH_4 production, and a lower hydrolysis rate constant is observed, in comparison with a waste with equivalent amounts of proteins, carbohydrates, lipids, and cellulose COD. The most efficient methane production rate and the lowest accumulation of VFA were observed for the waste with an excess of carbohydrates. The results of this specific study suggest that anaerobic digestion facilities with large variations in lipids input can have significant changes in process performance.

In CHAPTER 5, pulses of oil were added to completely mixed reactors fed with dairy cow manure and food waste. The oil concentration increased up to 9, 12, 15 and 18 $gCOD_{oil}/L_{reactor}$, after pulse feeding the reactor. From a practical point of view, this work demonstrated that co-digestion process of manure and food waste can be improved by intermittent addition of oily wastes. The threshold input of oily waste to enhance the methane production in the co-digestion of cow manure and food waste was 12 $gCOD_{oil}/L_{reactor}$, considering the mixture of lipids present in the oily waste added. This corresponds to a continuous feeding of 10% ($V_{food\ waste}/V_{manure}$) with

intermittent oil pulses of 5% (V_{oil}/V_{manure}). A pulse feeding of 18 $gCOD_{oil}/L_{reactor}$ induced a persistent inhibition of the process, detected by the decrease in pH to a minimum of 6.5 and an increase in effluent soluble COD and VFA. Negative linear correlations between the achieved biomethanation % and the solid-associated LCFA or palmitic acid, allowed to establish threshold values 180-220 of gCOD-LCFA/kg TS and 120-150 gCOD-C16:0/kg TS, respectively, that should not be surpassed in order to prevent reactor failure (CHAPTER 6). The approach of occasionally add lipids to the anaerobic co-digestion can be a feasible option to biodegrade fats/greases, recovering the methane potential of these substrates.

It was also demonstrated that cow manure/food waste co-digestion presents a sufficient buffer capacity to endorse lipids fluctuations, up to concentrations of 7.7 $gCOD_{oil}/L_{Reactor}$ (55% $Oil_{COD}/Total_{COD}$), maintaining an efficient overall reactor performance and stability, when the total COD fed was constant (CHAPTER 6).

In CHAPTER 7 the feasibility of the anaerobic co-digestion of coffee substitute solid waste and sewage sludge was assessed. Five different solid wastes from coffee substitutes with different chemical properties were studied in mesophilic batch assays. Four of the five wastes, attained 76-89% of the theoretical CH_4 yield. Thus, these wastes could be treated by anaerobic co-digestion, instead of being landfilled. On the other hand, one of the wastes assayed composed of 100% barley, attained only 11% of the theoretical CH_4 yield. In order to improve CH_4 production, two different approaches were attempted (CHAPTER 8). In the first approach, the barley waste was subjected to an alkaline hydrolysis pre-treatment before the co-digestion with activated sludge. The second approach consisted of co-digestion with kitchen waste (40% barley waste, 60% kitchen waste). Both approaches were beneficial to enhance the CH_4 production. However, the best outcome in TS and VS reduction was in the assay with the alkaline hydrolysis. Still, the pH correction in industrial applications can be a costly process when treating large amounts of waste. The co-digestion of the barley waste with the OFMSW seems to be attractive from an integrated solid waste management point of view, because, it only decreased the estimated methane production about by 20%, when compared to the OFMSW single-substrate bio-methanation.

9.2.|FUTURE WORK

Waste to energy approach through anaerobic digestion is and will be forever one of the best options for recovering the value of bio-waste both for energy and fertilizer production. Anaerobic co-digestion is defined as the microbiological production of methane from a mixture of various substrates, generally different types of bio-waste. However, the mixing of wastes can

result in both synergistic and antagonistic interactions that influence methane production. Successful application of co-digestion therefore requires careful management. No single set of operating conditions can be applied to all waste even single feeds can significantly impact the digester performance. Under this rationale, and, because there is a wide range of waste types that can be co-digested, further research assessing different substrates should be performed.

There is a need for simulation tools based on theoretical and practical information. Modelling co-digestion processes taking into account synergies and inhibition, is still lacking as a useful and robust tool for anaerobic digestion plants operators.

Including anaerobic digestion in the management of animal wastes is a practice widely applied in several other European countries. The potential rewards from application of anaerobic digestion are high not only for the farmer but also for the environment, since it revenues in new incomes to the farmers. So, studies on how to make these projects more profitable are needed. Future studies on this subject should be carried out in pilot reactors installed in farms, in order to spread the anaerobic digestion process benefits near potential end users. There is a need to encourage research activities: (1) on improving biogas yield and electricity conversion efficiency, and reducing the cost of anaerobic digestion; (2) on small-scale biogas recovery systems to fit the needs of small scale anaerobic digestion units.

SCIENTIFIC OUTPUT

The overall work developed during this PhD thesis give origin to the following publications:

|PAPERS IN JOURNALS WITH PEER REVIEW:

- Neves L., Oliveira R., Alves M.M. (2009) "Co-digestion of cow manure, food waste and intermittent input of fat." Bioresource Technology 100 (6):1957- 1962
- Neves L., Pereira M.A., Mota M., Alves M.M. (2009) "Detection and quantification of long chain fatty acids in liquid and solid samples and its relevance to understand anaerobic digestion of lipids" Bioresource Technology 100:91-96.
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- Neves L., Ribeiro R., Oliveira R., Alves M.M. (2006) "Enhancement of methane production from barley waste" Biomass and Bioenergy 30:599-603.
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