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Influence of volumetric oxygen transfer coefficient (k_La) on xylanases batch production by *Aspergillus niger* van Tieghem in stirred tank and internal-loop airlift bioreactors

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ABSTRACT

Oxygenation is an important parameter involved in the design and operation of mixing-sparging bioreactors and it can be analyzed by means of the oxygen mass transfer coefficient (k_La). In this study, batch fermentations in stirred tank bioreactor (STB) and airlift bioreactor (ALB) were operated under a range of k_La values, in attempts to optimize and compare the activities of extracellular xylanase and β-xylosidase synthesized by the fungus *Aspergillus niger* van Tieghem, using corncob as inducer source. The higher xylanase levels were observed on STB (9000 U l^{-1}; $k_La = 30\ h^{-1}$), while the β-xylosidase production was similar in both bioreactors. However, when the enzymatic activity was compared for the same k_La value (12 h^{-1}) in STB and ALB, the xylanase and β-xylosidase productions were higher in ALB (7000 U l^{-1} xylanase; 2100 U l^{-1} β-xylosidase) than STB (4500 U l^{-1} xylanase; 1800 U l^{-1} β-xylosidase).

1. Introduction

Microbial hemicellulases, especially xylanases, have important applications in several industrial sectors due to their enormous potential to modify and transform the lignocellulosic material, which has been used in a wide variety of industrial processes [1]. The major current industrial application of xylanases is the bleaching of cellulose pulp where xylanase pretreatment reduces the use of chlorine-based chemicals, reducing also the generation of pollutant organic chlorine compounds from lignin degradation, which are highly toxic and mutagenic, requiring treatment of the effluents [1–3]. The degradation of xylan involves a hemicellulytic system that includes among others enzymes the endo-1,4-β-xylanase, which cleaves internal bonds in the xylan chain, and β-xylosidases, which cleave xylobiose and xylooligosaccharides to produce xylose [4].

Increasing industrial demand for xylanolytic enzymes requires the development of new processes to ensure the economic feasibility of xylan-containing material hydrolysis at commercial scale. Stirred tank bioreactors are widely used in biotechnological processes providing high values of heat and mass transfer rates (due to efficient mixing). One of the most important challenges in the design and operation of these bioreactors is the non-uniform distribution of the energy dissipated inside the broths by mixing, with direct consequences on the distribution of mixing efficiency and mass/heat transfer rate [5]. Recently, alternative configurations for bioreactors have started to find applications in full-scale plants. One of the examples are airlift bioreactors, these are modified bubble column bioreactors [6], simple to design and build, and provide good mixing that facilitates high mass and heat transfer rates with low energy consumption [7–9]. Mixing in airlift bioreactors is induced by air bubbles supplied through diffusers at the bottom of the reactor [10]. These bioreactors are normally used in operations that involve low-viscosity fluids that do not require mechanical stirring and, therefore, provide low shear forces [11].

The study of parameters such as the agitation and/or aeration on bioreactors are important due their relation with the supply of oxygen to microorganisms during the process, influencing substrate consumption, and enabling or inhibiting product synthesis

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[12]. Oxygen supply in aerobic fermentations constitutes a decisive factor for microorganism growth and plays an important role in the scale-up of bioprocesses. Aeration efficiency depends on oxygen solubilization and diffusion rate into the broth, as well as on the capacity of the bioreactor to satisfy oxygen demands of the microbial population [13].

Oxygen transfer can be analyzed and described by means of the volumetric oxygen transfer coefficient, k_La, being this, the most important parameter for the design and operation of mixing/sparging equipment for aerobic bioreactors. k_La values are affected by many factors, such as mechanical design, geometry of air distributor and impellers, and operating conditions, such as agitation velocity and/or aeration rate. Moreover, medium composition, microorganism concentration and morphology, as well as biocatalyst properties (e.g. particle size and porosity) are also factors that influence k_La values [5,13–15].

Few studies relate the xylanolytic enzyme production in bioreactors to volumetric oxygen transfer coefficient (k_La). In this context, the aim of this work was to evaluate the influence of k_La on batch production of xylanase and β-xylosidase from *Aspergillus niger* van Tieghem in stirred tank and internal-loop airlift bioreactors, using corncob as inducer source, in order to obtain high enzymatic production using a cheap and widely available substrate.

2. Materials and methods

2.1. Material, microorganism and culture conditions

Corncob was kindly supplied by a local farmer (Caide, Lousada, Portugal). This material was dried at 40 °C for 12 h, cut into small chips (1–3 cm), and milled using a knives mill to pass through a 1.0 mm screen. After this, it was stored at room temperature until use.

The microorganism used in this work, collected from decomposition materials in Ribeirão Preto region (São Paulo, Brazil), was classified and deposited as *A. niger* van Tieghem by the Micology Culture Collection URM from Federal University of Pernambuco, Brazil. Stock cultures were propagated on PDA medium (Difco Laboratories, Becton, Dickinson and Co., Sparks, MD, USA), at 30 °C for 1 week, and stored at 4 °C.

The microorganism was grown in SR (Segatto-Rizzatti) medium as described by Rizzatti et al. [16], at pH 6.0, containing 1% (w/v) of corncob as carbon source. The spore concentration in the suspension was determined in a Neubauer counting chamber. Around 9×10^9 spores ml^{-1} were inoculated in Erlenmeyer flasks, containing a medium total volume of 10% (v/v) of bioreactor volume, and incubated on a rotary shaker (100 rpm, 30 °C) for 72 h. Then, these cultures were used as inoculum by pouring directly into the bioreactors (STB and ALB).

2.2. Bioreactors configuration and operating conditions

2.2.1. Stirred tank bioreactor

Stirred tank bioreactor (STB) was a 8-l Biostat R, type A5 from B. Braun Biotech International (Melsungen, Germany) with a working volume of 5 l, equipped with automatic monitoring and control units for temperature, pH and agitation speed. The bioreactor vessel was equipped with two six-bladed Rushton turbines. Aeration was regulated using a 100 SLPM mass flow controller (Alicat, Tucson, AZ, USA) and temperature was fixed at a set point of 30 °C. Mettler-Toledo pH and dissolved-oxygen (DO) probes were used to monitor the pH and the DO, respectively. A diagram of the STB can be conferred in Fig. 1A. Batch fermentations were performed using also SR medium, at pH 6.0, containing 1% (w/v) of corncob as carbon source with the stirring speeds set at 300 or 400 rpm and airflows set at 0.2 vvm ($1\,l\,min^{-1}$) or 0.4 vvm ($2\,l\,min^{-1}$). One milliliter of antifoam 204 (Sigma–Aldrich, St. Louis, MO, USA) was used at the beginning of fermentation. Samples were collected each 24 h for 15 days, filtered and used for enzymatic assays.

2.2.2. Internal-loop airlift bioreactor

A 9.5 l internal loop airlift bioreactor (ALB) with concentric draft tube designed and constructed at the Department of Biological Engineering in the University of Minho (UMINHO, Portugal) was used. It was made of Perspex (polymethylmethacrylate), equipped with automatic monitoring facilities for temperature and pH, and aeration was controlled by 100 SLPM mass flow controller. The working volume was 6 l. A diagram of the ALB is presented in Fig. 1B and other details are available in Michelin et al. [17]. The composition of the culture medium was the same as in the STR. The bioreactor was programmed to work at 30 °C in batch mode, being the air distributor a needle plate at the bottom of the bioreactor. The distributor was composed by three needles with an internal diameter of 0.5 mm. Sterilized airflow rate ranged between 0.2 vvm ($1.2\,l\,min^{-1}$) and 0.5 vvm ($3\,l\,min^{-1}$) promoting efficient air diffusion throughout the bioreactor. The pH and dissolved-oxygen were monitored with Mettler-Toledo probes. Antifoam 204 (Sigma–Aldrich, St. Louis, MO, USA) was also used at the beginning of fermentation. Samples were collected each 24 h for 15 days, filtered and used for enzymatic assays.

2.3. Enzymatic assays

Xylanase activity was determined as described by Miller [18] using 1% (w/v) birchwood xylan (Sigma–Aldrich, St. Louis, MO, USA) as substrate. The reaction mixture was performed at 60 °C during 20 min, by incubating 0.2 ml of substrate in McIlvaine buffer, at pH 5.5, and 0.2 ml of the enzymatic extract. β-Xylosidase activity was determined as described in Kersters-Hilderson et al. [19] using 0.25% (w/v) *p*-nitrophenyl-β-D-xylopyranoside (PNP-xyl; Sigma–Aldrich, St. Louis, MO, USA) as substrate. The reaction mixture contained 50 μl of PNP-xyl suspended in distilled water, 150 μl of McIlvaine buffer, at pH 4.0 and 200 μl of the enzymatic extract. The samples were incubated at 70 °C for 15 min.

One unit of enzyme activity (U) was defined as the amount of enzyme that releases 1 μmol of product per minute under the assay conditions and the activities were expressed in $U\,l^{-1}$. Productivity was defined as the ratio between maximum xylanase activity and corresponding fermentation time. The samples were analyzed in triplicate. The values presented in this work correspond to mean values of three replicates experiments and had a standard deviation below 5%.

2.4. k_La measurement

The volumetric oxygen transfer coefficient (k_La) was measured at 30 °C in both bioreactors (STB and ALB) in cell-free medium by the dynamic gassing-out method [20]. This method was performed by sparging nitrogen until the dissolved oxygen concentration falls close to zero and then monitoring the dissolved oxygen concentration after the start of the humidified air injection into the bioreactor. At this moment the oxygen transfer process to the medium begins and continues until oxygen concentration in the liquid reaches the saturation. Dissolved oxygen (DO) concentration values were measured online using an O_2 electrode (CellOx 325, WTW), and recorded directly in a PC, through a data acquisition board. The influence of oxygen electrode on k_La was considered negligible due to the low probe response time (6 s), which was significantly lower than $1/k_La$, corresponding to an experimental error lower than 6%.

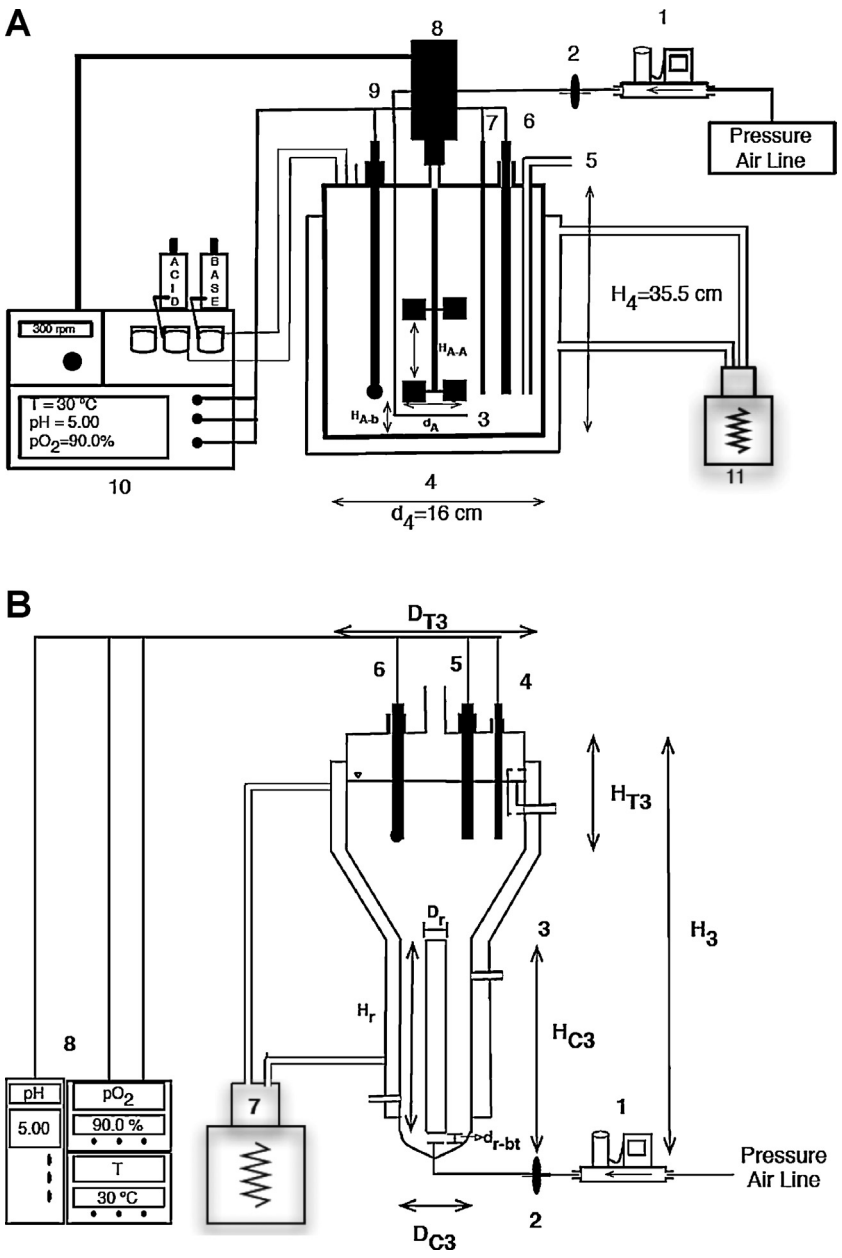

Fig. 1. Diagram of stirred tank and airlift bioreactors. STB: 1 – mass flow controller; 2 – air filter; 3 – gas distributor; 4 – stirred tank bioreactor; 5 – sample point; 6 – oxygen probe; 7 – thermocouple; 8 – stirrer; 9 – pH probe; 10 – monitoring unit with pH control; 11 – temperature controller; d_4 – reactor diameter (16.0 cm); H_4 – reactor height (35.5 cm); d_A – stirrer diameter (6.5 cm); H_{A-A} – distance between the stirrers (8.0 cm); H_{A-b} – distance between the stirrer and the bottom of the reactor (7.0 cm) (A). ALB: 1 – mass flow controller; 2 – air filter; 3 – airlift bioreactor; 4 – temperature probe; 5 – oxygen probe; 6 – pH probe; 7 – temperature controller unit; 8 – reading units; D_{C3} – column diameter (7.0 cm); D_{T3} – top diameter (19.0 cm); H_3 – reactor height (95.5 cm); H_{C3} – column height (67.0 cm); H_{T3} – top height (19.5 cm); D_r – riser diameter (3.0 cm); H_r – riser height (64.0 cm); $d_{r\text{-}bt}$ – distance between the bottom of the riser and the air distributor – 1.7 cm (B).

The dissolved oxygen concentration variation with time, t, was obtained, and k_La calculated according to the following equation:

$$\ln(C* - C) = \ln(C* - C_0) - k_La \cdot t \quad (1)$$

where C^* and C are, respectively, the saturation concentration of oxygen and oxygen concentration in the liquid. Assuming the liquid phase as homogeneous and being C_0 the oxygen concentration at $t = 0$, the volumetric mass transfer coefficient was determined by plotting $\ln(C^* - C)$ against time (t) using MATLAB (MathWorks, Natick, MA, USA), version 7.2.0.232 (R2006a).

The OTR was calculated using the k_La values obtained previously (experiments without biomass) and assuming its value remained constant trough each individual fermentation and the dissolved oxygen values measured at defined intervals, i, during the fermentation. The OTR values were determined using the following the equation:

$$\mathrm{OTR}_i = k_La(C* - C_i) \quad (2)$$

2.5. Viscosity measurement

The shear viscosity was determined at the beginning and at the end of the fermentations in both bioreactors, using a UK-Model ELV-8 viscometer equipped with a TL6 cone spindle at different shear rates for 10 min. All viscosity measurements were repeated three times and the temperature was controlled at 20 °C.

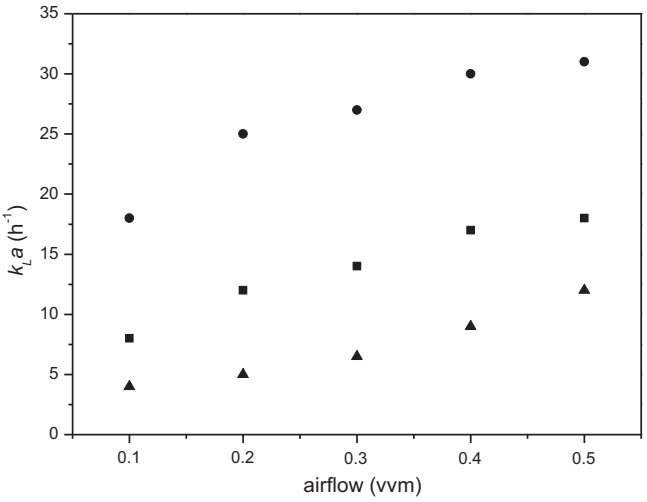

Fig. 2. k_La as a function of airflow volume obtained in Biostat (R) type A5 stirred tank bioreactor (STB) from Braun Biotech International and airlift bioreactor designed and constructed at the Department of Biological Engineering of UMINHO, at 30 °C. Symbols: -■- STB, 300 rpm; -●- STB, 400 rpm; -▲- ALB.

3. Results and discussion

3.1. k_La study in STB and ALB

In this work, k_La was calculated for similar conditions in the STB and ALB, using SR fermentation cell-free medium containing 1% (w/v) corncob. This is an important parameter to compare aerobic bioreactors, since these bioreactors present different configurations and k_La is affected by bioreactor design.

It can be observed in Fig. 2 that k_La values increased with increasing aeration rate and also with the agitation rate in STB. Besides, a higher k_La values can be observed for the STB than for the ALB, especially for the aeration rates tested at 400 rpm. The low k_La in ALB is related with its design composed by an enlarged top-degassing zone. The main function of top degassing part is to facilitate the gas release. However, it also promotes sedimentation, which is essential to maintain a high biomass amount inside the bioreactor if a continuous system is implemented [21]. Gaspar et al. [22] studying the influence of factors acting on k_La value during *Penicillium canescens* culture for xylanase production verified that k_La increased with agitation speed and airflow rate, in various turbine designs, including Rushton disk turbines with 6 blades.

Some of these k_La were selected for fermentations on studies of enzymatic production; but also the stirring and aeration effect were evaluated on the enzymatic production. The k_La selection was done considering similar k_La values in different stirring and aeration conditions.

A factor that influences oxygen transfer and has great importance in the productivity of a fermentation process is the viscosity of the broth. This is a consequence of biomass concentration and cell morphology, which causes changes in the broth characteristics and affects the bioreactor hydrodynamic properties, including mixing and mass transfer performances [23,24].

The viscosity of the culture broth was measured during the fermentation, being detected a light increase of the viscosity

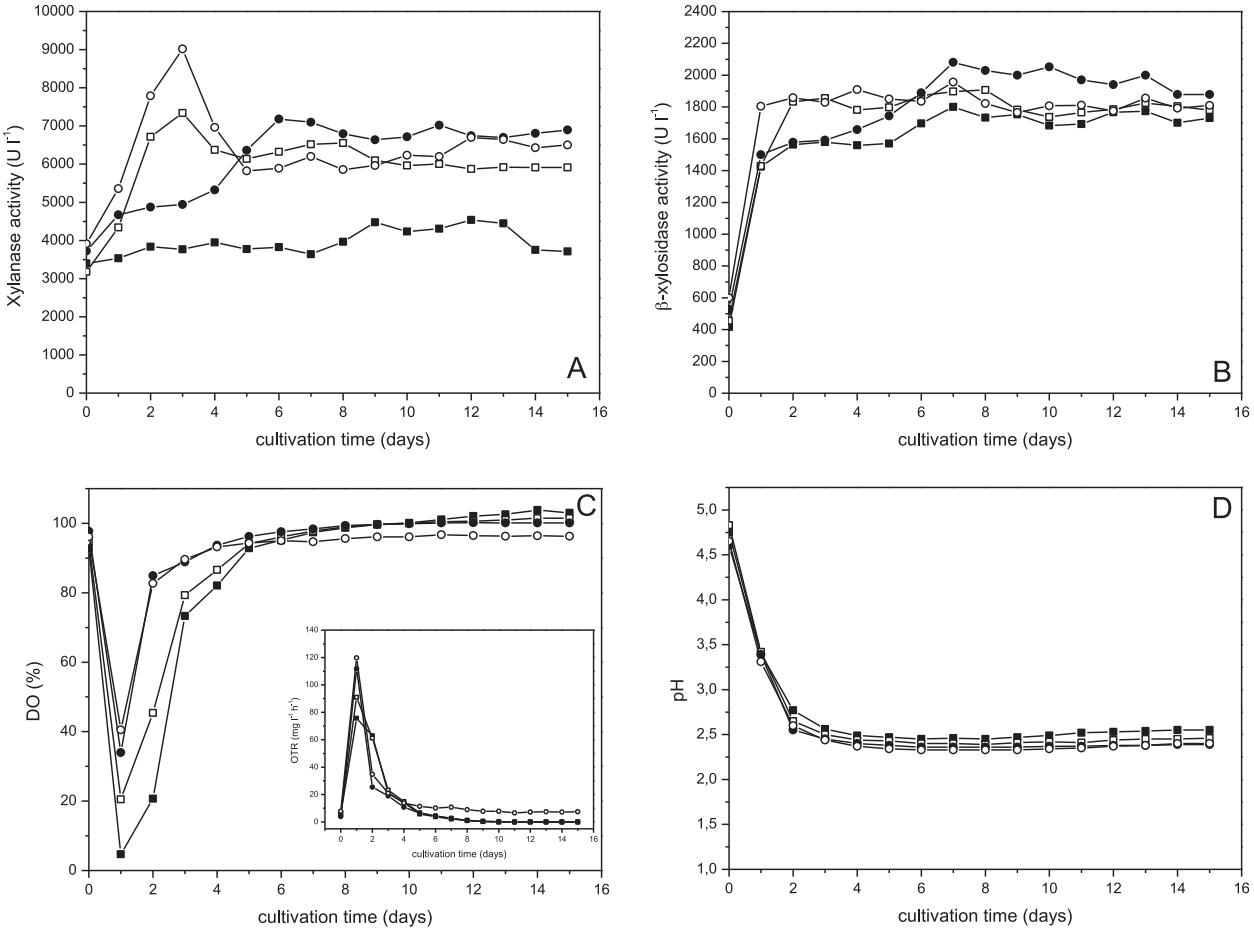

Fig. 3. Time course for xylanase activity (A), β-xylosidase activity (B), dissolved oxygen (C), oxygen transfer rate (insert C) and pH (D) in the stirred tank bioreactor, for different k_La. Symbols: -■- 12 h^{-1} (300 rpm; 0.2 vvm); -□- 17 h^{-1} (300 rpm; 0.4 vvm); -●- 25 h^{-1} (400 rpm; 0.2 vvm); -○- 30 h^{-1} (400 rpm; 0.4 vvm).

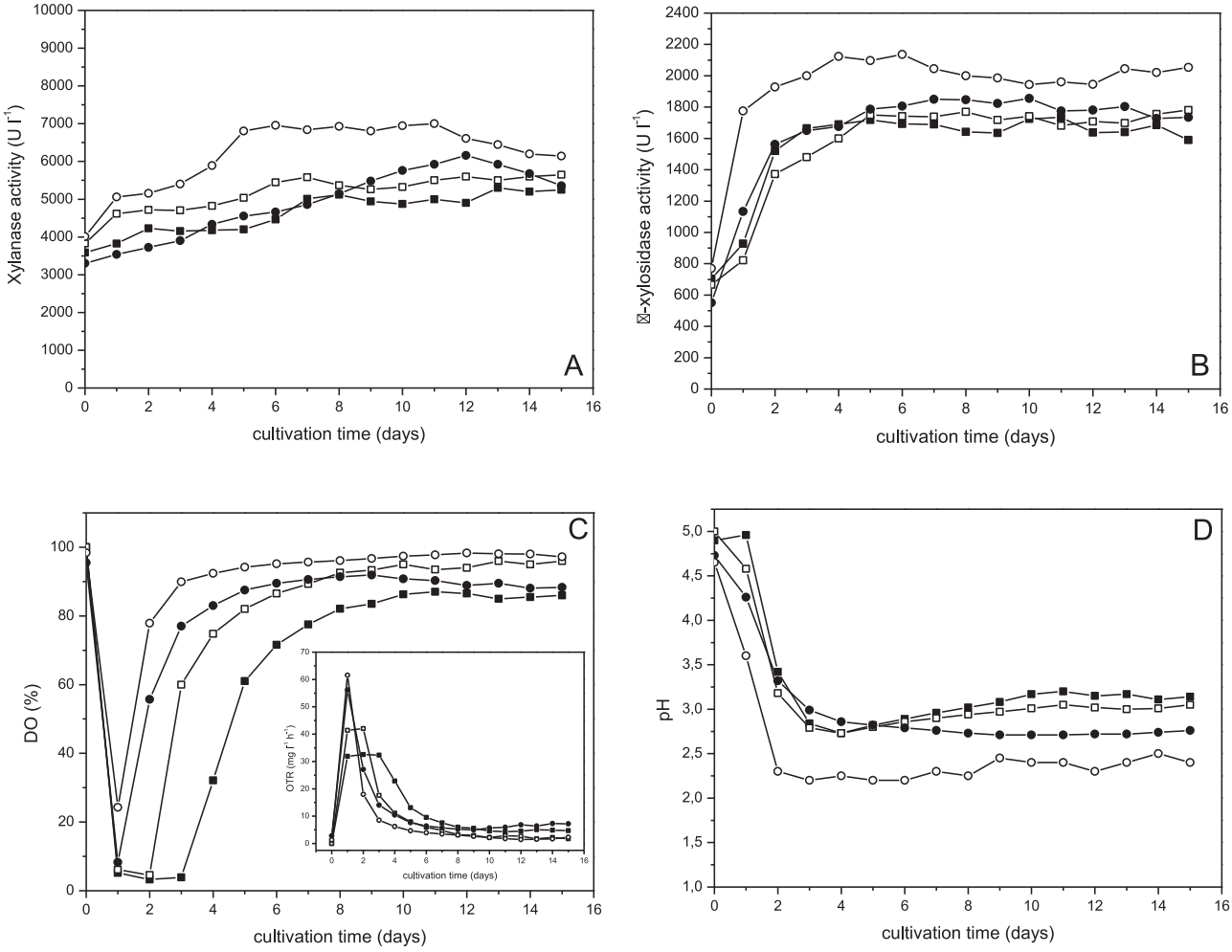

Fig. 4. Time course for xylanase activity (A), β-xylosidase activity (B), dissolved oxygen (C), oxygen transfer rate (insert C) and pH (D) in the airlift bioreactor, for different k_La. Symbols: -■- 5 h^{-1} (0.2 vvm); -□- 6.5 h^{-1} (0.3 vvm); -●- 9 h^{-1} (0.4 vvm); -○- 12 h^{-1} (0.5 vvm).

around the 5th day (38 mPa/s) in relation to the free-cell medium (28 mPa/s); however, this increase was similar for both bioreactors and therefore the calculated values of k_La for both bioreactors were considered comparable. Besides, for all fermentations *A. niger* van Tieghem grew in submerged culture as pellets. It is known that the formation of filamentous mycelia in a bioreactor may increase medium viscosity, fungal growth around the impellers, and a reduction in mass transfer, thereby reducing fungal growth, productivity, and reactor performance. In contrast, fungal growth in the form of pellets provides good mass and oxygen transfer, minimizes adverse effects on reactor performance, and does not increase fermentation broth viscosity [10,25,26]. Therefore, in general, the increase of the viscosity of the medium is more evidenced in filamentous growth than the pellet growth [27,28].

3.2. Influence of k_La on xylanases production in STB

In aerobic bioprocesses, oxygen is a key substrate employed for growth, maintenance and in other metabolic routes, including product synthesis. Therefore, the influence of oxygen transfer was evaluated on stirred tank bioreactor and, according to Fig. 3 the activities (U l^{-1}) of xylanase and β-xylosidase in filtrates from corncob grown culture were affected by k_La. The highest rates of xylanase production were obtained for the highest k_La value (30 h^{-1}), with a maximum activity of 9000 U l^{-1} obtained after 3 days of cultivation (Fig. 3A). It is important to highlight that aeration markedly affected xylanase productivity (U l^{-1} day^{-1}), independently of the agitation: 300 or 400 rpm. After 3 days of cultivation xylanase production was almost two times faster at 0.4 vvm (2445 U l^{-1} day^{-1} for 300 rpm and 3000 U l^{-1} day^{-1} for 400 rpm) than at 0.2 vvm (1255 U l^{-1} day^{-1} for 300 rpm and 1645 U l^{-1} day^{-1} for 400 rpm). It can also be seen that xylanase productivity is higher for all the cases when an agitation of 400 rpm is used.

Siedenberg et al. [29] observed that xylanase levels production from *Aspergillus awamori* were highest at 300 rpm, decreasing with increasing stirrer speed (500 and 750 rpm), where probably the k_La values are higher. This occurs because in submerged culture xylanase production by filamentous fungi is also affected by shear stress, which is related to the agitation rate. The harmful effect of the shear forces as a result of agitation intensity has been reported to cause a decrease of enzyme production in some filamentous fungi [30,31]. Considering this, it seems that the relation between shear stress and k_La is a decisive factor in order to achieve good xylanase levels in STB, where an agitation of 400 rpm and an aeration of 0.4 vvm are conditions that allow the production of the highest levels of xylanase.

β-Xylosidase production was good under the most tested conditions, being the higher production obtained (around 2000 U l^{-1}) for k_La of 25 h^{-1}, after 7 days of fermentation (Fig. 3B). β-xylosidase obtained by *A. niger* was higher than the obtained in other works by some strains of *Thermomyces lanuginosus* using xylan as carbon source [31–33]. This difference may be due to fact that

β-xylosidases are often intracellular or associated to the cell membrane and few microorganisms secrete this enzyme to culture medium [34].

Fig. 3C shows that DO concentration was maintained above 20% during all fermentations, except for k_La of 12 h^{-1}, in which case the DO dropped to a value near zero (5%), at the end of the first day of fermentation. The drop in DO concentration generally is attributed to an increase in oxygen consumption due to rapidly increase in cell concentration in the first hours of fermentation (exponential phase) [11]. At this phase the oxygen consumed per hour is equal or higher than the oxygen transfer rate (k_La) and the DO in the medium decreases. The low value of DO observed at the beginning of fermentation for the k_La of 12 h^{-1} may have been limiting for the xylanase production, where the levels of enzymatic production were lower that all the other conditions (Fig. 3A). These results show that fermentations using low values of k_La or agitation and aeration are not sufficient to supply the necessary oxygen for fungal growth. The insert of Fig. 3C shows the oxygen transfer rate during the fermentation process.

Amaral et al. [14] presents aeration as a critical factor in the scale-up of aerobic fermentative processes since growth and production can be limited by DO concentration. In many biosynthesis processes oxygen supply to the culture is not enough to meet the demand of the microorganisms and, therefore, it is one of the most limiting factors for successful operation of those fermentations.

After the second day it was also observed a drop on pH values (Fig. 3D), which is related with the release of acidic by-products into the medium [35]. This may be important in the control of bacterial contamination. Other important consideration is the stability of the enzymes in acid pH for a long time, since after 15 days of cultivation at 30 °C the enzymes remained active.

3.3. Influence of k_La on xylanases productions in ALB

Activities (U l^{-1}) of xylanase and β-xylosidase in filtrates from corncob grown culture were analyzed in the ALB and, as in the STR, xylanase and β-xylosidase productions were markedly influenced by k_La. Under these conditions, maximum xylanase synthesis was obtained for the highest values of k_La (12 h^{-1}), with a production of 7000 U l^{-1} between the 5th and the 11th day of fermentation (Fig. 4A). β-Xylosidase production also was higher for the highest k_La (2100 U l^{-1} in the period of 4–6 days) (Fig. 4B).

These results can be explained by the DO concentration during the fermentation process. For a k_La of 12 h^{-1} (Fig. 4C), DO concentration remained above 20% during all fermentations, explaining the higher level of enzymatic production. For the fermentations where other values of k_La were considered (less than 12 h^{-1}), DO dropped to values near of zero (3–8%) in the beginning of the fermentation, being also the OTR values lower in this period (insert Fig. 4C). Given the low DO concentration in the broth, the oxygen becomes a limiting nutrient suggesting the need of a higher input of oxygen for growth and enzymatic production. Pinches and Pallent [36] described the fall of DO concentration in broths due to the high demand during fast growth, then the microorganism reaches the stationary phase and DO concentration increases, because demand becomes smaller [37].

The pH value for k_La of 12 h^{-1} dropped to values near 2.0–2.5 after the first day of fermentation, and as occurred in STB, this drop has not caused enzyme inactivation, and it was less pronounced for lower k_La values (Fig. 4D). Fontana et al. [38] suggested that the production of these hydrolases is favored under stress conditions, since while studying polygalacturonase production by *Aspergillus oryzae* it was verified a highest enzymatic production in media with pH values below 3.0.

β-Xylosidase production by *A. niger* was significantly higher than that obtained by Michelin et al. [17], with the *Aspergillus terricola* fungus in the same ALB, using wheat bran as carbon source. This is in agreement with previous studies that revealed that *A. terricola* is not a good producer of extracellular β-xylosidase (data not shown).

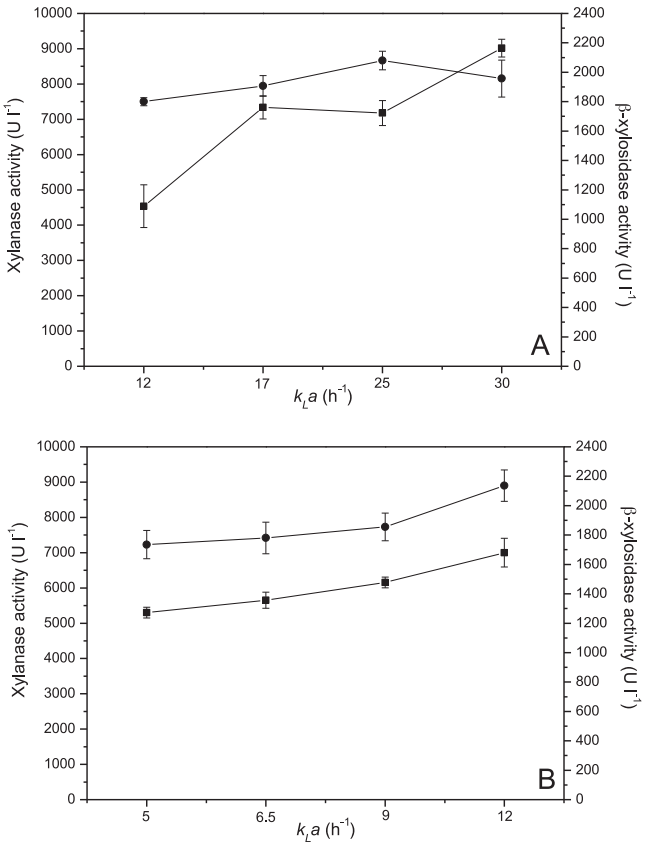

Fig. 5. Xylanase and β-xylosidase productions in STB (A) and ALB (B) as a function of k_La. Symbols: -■- xylanase; -●- β-xylosidase.

3.4. Relation between k_La and production of xylanolytic enzymes in STB and ALB

The oxygen supply, represented by the k_La values, showed to be an important factor affecting the production of xylanolytic enzymes by *A. niger* van Tieghem. Fig. 5 shows the relation between enzymatic production and k_La, considering the highest activities obtained during the cultivation period for each k_La.

The STB shows the highest xylanase production (9018 U l^{-1}) for a k_La of 30 h^{-1}; this production was almost two times higher than that obtained for a k_La of 12 h^{-1}. For β-xylosidase a value of 2080 U l^{-1} was obtained for a k_La of 25 h^{-1}; this value was 13.5% higher than that obtained for a k_La of 12 h^{-1} and 6% higher than that obtained for a k_La of 30 h^{-1} (Fig. 5A).

In relation to xylanase and β-xylosidase productions in ALB, it was verified that enzyme production increased for higher k_La values (Fig. 5B). This difference of behavior in the two bioreactors can be explained by the high agitation speed in STB, which promotes excessive shear stress resulting in a decrease in biomass concentration and, consequently, in enzyme production, even at high k_La values. Burkert et al. [35] observed that the biomass growth and enzyme production was lower in high agitation rate (500 rpm), probably due to the shear rate.

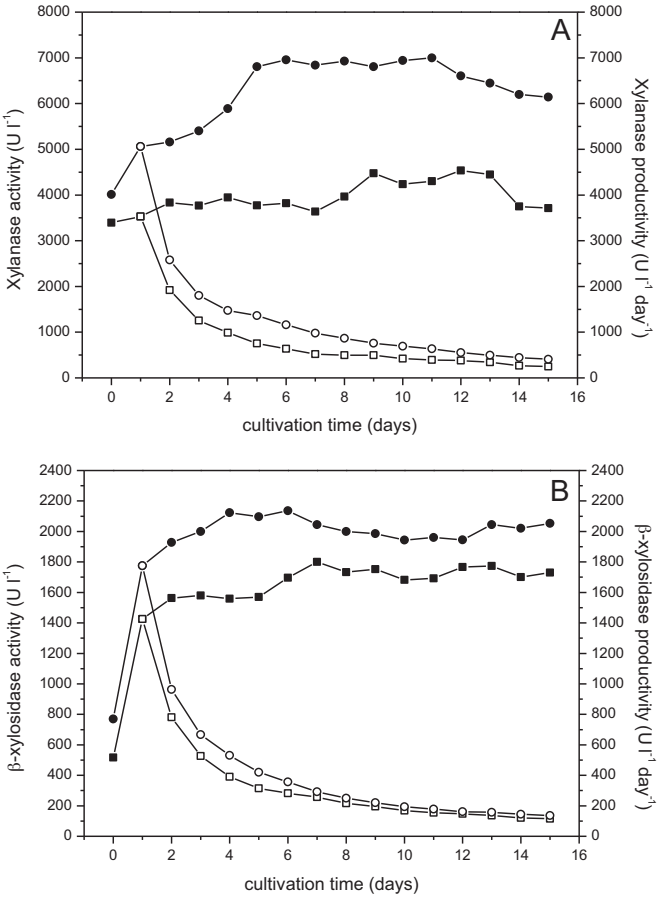

Fig. 6. Time course for xylanase activity (closed symbols) and productivity (open symbols) (A) and β-xylosidase activity (closed symbols) and productivity (open symbols) (B) in stirred tank (square) and airlift bioreactors (circle), for the same k_La (12 h^{-1}).

3.5. Comparison of enzymatic production in STB and ALB for the same k_La value

Stirred tank bioreactors (STB) and airlift bioreactors (ALB) are widely used in fermentations, and several studies evaluate and compare their behavior for similar biotechnological processes. For this study, and aiming the comparison of these two bioreactors in the enzyme production, the same k_La values (12 h^{-1}) were used (300 rpm and 0.2 vvm in the STB and 0.5 vvm in the ALB) for the production of xylanase and β-xylosidase during the fermentation process.

Despite of similar values of k_La in both bioreactors, the hidden effect of mixture intensity could play an important role and mask the results. However previous results (data not shown) of mixing intensity showed that at operating conditions of STB (300 rpm and 0.2 vvm) and ALB (0.5 vvm) the mixture time is similar, between 10 and 15 s.

Fig. 6A and B showed that the highest enzymatic production occurred in the ALB – 7000 U l^{-1} xylanase; 2100 U l^{-1} β-xylosidase – as compared to the STB – 4500 U l^{-1} xylanase; 1800 U l^{-1} β-xylosidase. The same behavior is observed in terms of productivity where the values were also higher for ALB.

Burkert et al. [35] also compared the production of lipase in STB and ALB showing that the maximum lipase activities were similar in both bioreactors, reaching to 21 U cm^{-3} in the stirred bioreactor at 300 rpm and 1 vvm (k_La, 21 h^{-1}) and approximately 20 U cm^{-3} in the airlift bioreactor at 2 vvm (k_La, 21 h^{-1}). However,

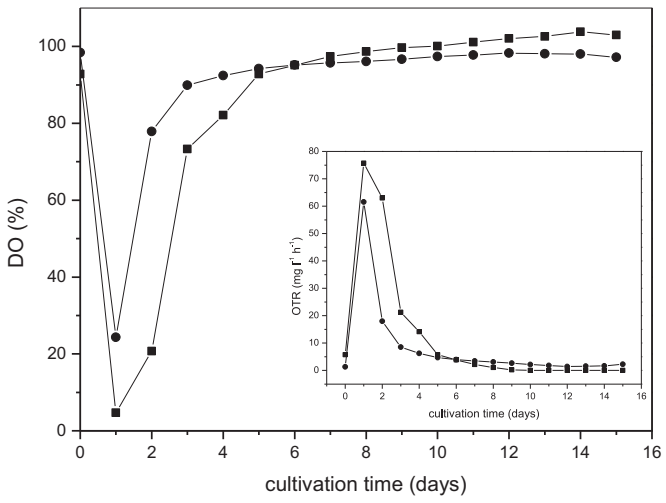

Fig. 7. Time course for dissolved oxygen concentration and oxygen transfer rate (insert) in stirred tank (-■-) and airlift (-●-) bioreactors, for the same k_La (12 h^{-1}).

maximum productivity was observed in ALB (0.57 U cm^{-3} h^{-1}) than STB (0.39 U cm^{-3} h^{-1}) at the same k_La.

The differences in enzymatic production between STB and ALB observed for the same k_La value can be related with the DO concentration. Despite the higher OTR in STB than ALB (insert Fig. 7), the DO concentration in STB was limiting, since a value near of zero was observed at the end of the first day of cultivation, while in the ALB the DO concentration remained higher than 20% (Fig. 7). Therefore, even using a same k_La value, the behavior in the two reactors was different, since the consumption of oxygen was higher in STB. A factor that may be influencing growth and enzymatic production is the negative impact of shear stress caused by the STB turbine on mycelia; as a consequence, a higher oxygen demand is required in this case to attain similar enzymatic production values to those observed in the ALB. Other possibility could be a higher microorganism growth in STB leading to higher oxygen consumption. The validation of this possibility requires the determination of biomass concentration; however, this was not possible due to its attachment to the corncobs (fungus and corncob mix and were impossible to separate in order to accurately measure the amount of consume corncob and biomass growth).

Other authors also observed that xylanase production was higher in ALB than in STB [17,29,39]. In all cases, the explanation provided was that airlift bioreactors have been widely used in fermentation with filamentous fungi due to low-shear environment and good mass transfer. However, results presented in this work indicate that the above mentioned advantages must take in account the effects of the mechanical stirring in STR that, on one hand, contribute to a significant enhancement in oxygen transfer, as seen for similar aeration rates and, on the other hand, to an increase in oxygen consumption as seen for similar k_La values, these effects being negative in the case of xylanases and β-xylosidase productions.

4. Conclusions

Oxygen transfer into microbial cells in aerobic bioprocesses strongly affects product formation by influencing metabolic pathways and changing metabolic fluxes. These bioreactors present different configurations: in STB there are two main factors (aeration and agitation) influencing oxygen transfer rate, while in ALB only aeration plays a major role. It was therefore important to consider one same parameter, such as k_La, in studies comparing bioprocess in different bioreactors. The results show that xylanase production, in two types of bioreactors evaluated, is influenced by oxygen

transfer rate, being the higher production of xylanases occurred on bioreactor which presented higher oxygen transfer rate (9000 U l^{-1}, 30 h^{-1} – STB; and 7000 U l^{-1}, 12 h^{-1} – ALB).

However, when enzymatic production was evaluated for the same values of k_La (12 h^{-1}), the xylanase and β-xylosidase productions were 35% and 16% superior, respectively, in the ALB. This because oxygen transfer in a filamentous fungi broth in these bioreactors resulted in complex interactions phenomena, where several other factors influenced oxygen transfer, such as bioreactor design and shear stress (in STB).

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