ARTICLE

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Optimization of Oxygen Mass Transfer in a Multiphase Bioreactor With Perfluorodecalin as a Second Liquid Phase

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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). The operational conditions of a stirred, submerged aerated 2-L bioreactor have been optimized by studying the influence of a second liquid phase with higher oxygen affinity (perfluorodecalin or olive oil) in the k_La. Using k_La measurements, the influence of the following parameters on the oxygen transfer rate was evaluated: the volume of working medium, the type of impellers and their position, the organic phase concentration, the aqueous phase composition, and the concentration of inactive biomass. This study shows that the best experimental conditions were achieved with a perfluorodecalin volume fraction of 0.20, mixing using two Rushton turbines with six vertical blades and in the presence of YPD medium as the aqueous phase, with a k_La value of 64.6 h^{-1}. The addition of 20% of perfluorodecalin in these conditions provided a k_La enhancement of 25% when pure water was the aqueous phase and a 230% enhancement when YPD medium was used in comparison to their respective controls (no perfluorodecalin). Furthermore it is shown that the presence of olive oil as a second liquid phase is not beneficial to the oxygen transfer rate enhancement, leading to a decrease in the k_La values for all the concentrations studied. It was also observed that the magnitude of the enhancement of the k_La values by perfluorodecalin depends on the biomass concentration present.

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KEYWORDS: multiphase bioreactors; aeration; oxygen mass transfer coefficient; *Yarrowia lipolytica*; perfluorodecalin; olive oil

Introduction

Aeration in industrial aerobic fermentations is a critical factor since growth and production can be limited by the dissolved oxygen concentration. In many biosynthesis processes the oxygen supply to the broths is not enough to meet the demand of the microorganisms and, therefore, it is one of the most limiting factors in the successful operation of those fermentations. The inclusion of a second liquid phase, in which oxygen has a greater solubility, such as hemoglobin, hydrocarbons, and perfluorocarbons (PFCs), is an alternative to overcome the problem of oxygen limitation in aqueous aerobic fermentations. The advantage of using these oxygen vectors in fermentations is that they increase the oxygen transfer rate from the gas phase to the microorganisms without the need of a large extra energy supply. PFCs have been previously studied in cultivations of various microorganisms including bacteria (Damiano and Wang, 1995), yeast (Elibol, 1996), animal (Hamamoto et al., 1987) and insect cells (Gotoh et al., 2001). The usefulness of PFCs as oxygen carriers to cultivation processes was demonstrated in these works. Since the efficiency of oxygen supply is directly proportional to the PFCs-medium interfacial area, some researchers used PFC-based emulsions (Elibol and Mavituna, 1996; Ju et al., 1991), but the emulsions have the inconvenience of recycling and re-oxygenation.

PFCs are petroleum-based compounds synthesized by replacing hydrogen by fluorine atoms in the analogues hydrocarbons. They are good candidates as oxygen carriers in fermentation media because they are non-toxic towards the cells, stable and chemically inert due to the presence of

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very strong carbon-fluorine bonds, and the oxygen solubility in these compounds is 10–20 times higher than in water (Dias et al., 2004; Freire et al., 2005a). Besides, they present very low solubilities in water and therefore they do not affect in a large extent the physical properties of the aqueous phase and can be easily recovered (Freire et al., 2005b). The ability to readily recover and recycle most of the PFC from aqueous-based culture systems overcomes the relatively high initial investment cost of these compounds making their routine use commercially feasible. This is of significance not only in basic research but, crucially, also in commercial laboratories that exploit large volume fermentation vessels (Lowe, 2002). Another important inherent property of PFCs is their very low surface tensions which is particularly important as it contributes to the mass-transfer enhancement in gas–liquid systems (Freire et al., 2006).

Reactions involving three-phase systems are frequent in the chemical process industry. Gas–liquid–liquid reactions are gaining importance due to the increase in this type of application in the bioprocess industry and homogeneous catalysis systems (Dumont and Delmas, 2003). The addition of dispersed liquid phases changes the transfer rate of the solute gas across the boundary layer and the gas–liquid characteristics can be changed due to the interfacial properties of the dispersed liquid. Nevertheless, the mechanisms involved in the mass-transfer in multiphase systems are complex and there are still many gaps to be fulfilled to a complete knowledge of this phenomenon (Dumont and Delmas, 2003).

The volumetric oxygen transfer coefficient, k_La, is one of the most important parameters in aerobic bioprocesses and depends on different factors, such as geometrical and operational characteristics of the vessels, media composition (notably ionic strength), type, concentration, and microorganisms morphology (Galaction et al., 2004a). This parameter has been assumed to be dependent on either the agitation power per unit volume (P/V) or the impeller rotation speed and on the gas superficial velocity (Hassan and Robinson, 1977). Scale-up is usually based on correlations of various kinds to predict k_La for specific vessel geometry. In the presence of a single interface, gas–liquid, the amount of oxygen transferred depends on the k_La value and on the difference between the dissolved oxygen concentration and its saturation. The introduction of an immiscible liquid phase, such as PFCs, in the medium, makes the system more complex from the oxygen transfer point of view. The transfer mechanism of oxygen has not yet been fully understood and an exhaustive study evaluating the effects of a number of design parameters on the overall values of k_La is required.

Preliminary shake-flask experiments showed that the use of perfluorodecalin as an oxygen carrier in the fermentation medium increased lipase production from *Yarrowia lipolytica* (Amaral et al., 2006a, 2007). Frost and Moss (1987) results indicate that improvements in aeration by agitation or air sparging are beneficial to lipase production by single cell organisms and filamentous moulds. Chen et al. (1999) showed that the intrinsic factor determining cell growth and lipase production was the oxygen transfer rate rather than the dissolved oxygen concentration, and Elibol and Ozer (2000) showed that the inclusion of an oxygen carrier further contributes to improve lipase production, beyond the increase due to the aeration and agitation rates. Microbial lipase production is also stimulated by the presence of triglycerides, especially olive oil, as a carbon source in the culture medium (Pereira-Meirelles et al., 1997). Olive oil also presents high oxygen solubility (Battino et al., 1983).

On this study, measurements of k_La in a 2-L stirred submerged aerated bioreactor were designed and conducted to identify the optimal operational conditions of an oxygen-dependent microorganism, *Y. lipolytica*, in the presence of a second immiscible liquid phase (perfluorodecalin or olive oil). The independent variables studied in order to determine the optimum levels and also the region that could satisfy the operating specifications were: the working volume in the bioreactor, the type and position of impellers, the concentration and type of added organic phase, the composition of the aqueous phase, and the concentration of inactive cells of *Y. lipolytica*. The mechanisms involved in the oxygen transfer from the gas phase to the water phase in the presence of the second liquid phase were studied through their impact on the k_La values measured.

Materials and Methods

Materials

The perfluorodecalin ($C_{10}F_{18}$) was acquired at Flutec with a purity verified by Gas Chromatography of 97.75 wt %. The relevant physical properties of perfluorodecalin at 25°C and atmospheric pressure are as follows: density of 1.917 $g \cdot cm^{-3}$, boiling point of 142°C, and oxygen solubility of 127.8 $mg \cdot dm^{-3}$ at 25°C (Dias et al., 2004). The olive oil used was a portuguese commercial available oil, ''Azeite Andorinha'', presenting a density of 0.9508 $g \cdot cm^{-3}$ at 29°C and atmospheric pressure. The volume fraction of the organic phase (Φ) in the studies carried ranged from 0 to 0.30.

The medium used in this study was the aqueous YPD medium composed of casein peptone (0.64% (w/v)), yeast extract (1.0% (w/v)), and glucose (2.0% (w/v)) that were obtained from Merck, Oxoid, and Vetec, respectively. After preparation, the medium was autoclaved at 121°C for 25 min before its use in the bioreactor.

A wild type strain of *Y. lipolytica* (IMUFRJ 50682) was selected from an estuary in the vicinity of Rio de Janeiro, Brazil (Hagler and Mendonça-Hagler, 1981). For measurements with inactive cells, *Y. lipolytica* were previously grown at 29°C in a rotary shaker (160 rpm), along 96 h, in flasks containing YPD medium. Afterwards the cells were inactivated with ethanol (30% (v/v)) and inoculated

(1, 5, and 10 g cells $\cdot$ dm^{-3} as described in the results section) in 1.5 dm^3 YPD medium inside the bioreactor.

The nitrogen used to degasify the system was provided by White Martins Praxair Inc (RJ, Brazil).

Methods

The volumetric oxygen transfer coefficient (k_La) was measured at 29°C in a 2 dm^3 bench bioreactor (New Brunswick Sci. Inc., Multigen F2000, Edison, NJ), using 1.0 or 1.5 dm^3 total volume of working medium. Stirring speeds of 100–350 rpm and airflow rates (Q) of 0.5–2.0 dm$^3 \cdot$ min^{-1} were employed. Three types of impellers were used in this study and they are shown in Figure 1. The bioreactor and impeller characteristics are given in Table I. The oxygen supply was carried out with atmospheric air by a submerged sparging system having 12 holes with 7 mm diameter located at 1.82 cm from the bottom of the bioreactor.

The volumetric coefficient of oxygen transfer was determined by the dynamic gassing-out method (Bandyopahyay and Humphrey, 1967). This method was performed by sparging nitrogen until the dissolved oxygen concentration falls to zero and then monitoring the dissolved oxygen concentration (C) after the start of the aeration with atmospheric air. Eq. (1) was then used to determine the k_La from the slope of the curve,

$$\ln\left(1 - \frac{C}{C^*}\right) = -k_La \times t \qquad (1)$$

where C^* is the equilibrium dissolved oxygen concentration and t the time.

The dissolved oxygen concentration was followed with a polarographic oxygen electrode, Lutron DO-5510 oxygen meter, fitted with a Teflon membrane and with and electrolytic solution of Na_3PO_4 in the cell. Since the k_La values were in all cases inferior to 0.03 s^{-1}, it was assumed that the response of the oxygen electrode to the variations in oxygen concentration is fast enough and does not affect the accuracy of the determination (Galaction et al., 2004b). Each experiment was carried at least three times in identical conditions, and the average and standard deviation for each k_La value were determined.

Results and Discussion

Volume of Working Medium

To determine the influence of the working medium volume in the oxygen transfer rate two working medium volumes of pure water were tested, 1.0 and 1.5 dm^3, agitated at 250 rpm using impellers type A and at aeration rates ranging from 0.5 to 2.0 dm$^3 \cdot$ min^{-1}. The results obtained are presented in Figure 2 and it can be seen that for the higher aeration rates the difference observed is not statistically significant and falls within the experimental uncertainty of the measurements. There is only a significant difference on k_La values for the lower aeration rate (0.5 dm$^3 \cdot$ min^{-1}) that could result from the higher residence time of the air bubbles in a higher volume. The size of the drops in a mixing vessel is largely dependent on the micro- and macro-scale turbulent motions and flow patterns in the vessel because of the mutual relation between the local energy dissipation rates, the residence time of the drops at a certain location in the vessel, and the local breakup or coalescence rates of the drops. For low aeration rates the turbulence is not enough to compensate the large volume tested and the k_La values consequently decreased. Besides, it was observed that with a 1.0 dm^3 working volume the gas bubbles blow at the oxygen electrode interface producing more incorrect results. Therefore based on the results obtained, a volume of 1.5 dm^3 was chosen for all the following measurements leading to a higher productive biomass system by working with higher volumes.

Impellers Position

The PFC, being denser than water, stays at the bottom of the bioreactor when no agitation is supplied. It was observed during the measurements that the agitation speeds studied were not enough to disperse all the PFC through the upper aqueous phase. Therefore, different positions for the impellers B were tested (changing h_1 and h_2), with a

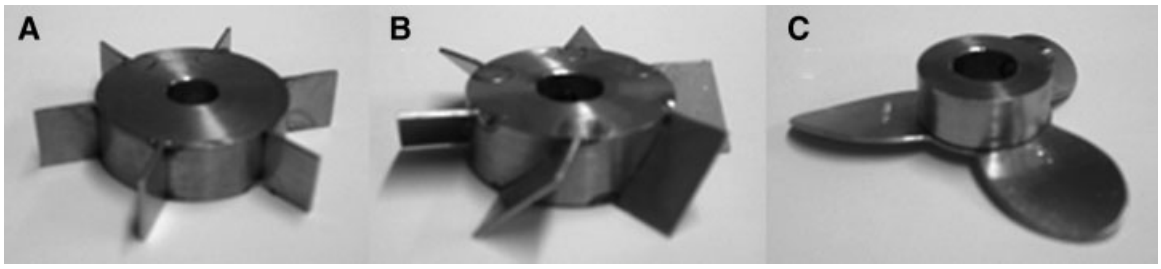

Figure 1. Impeller types used (**A:** Rushton turbine with six vertical blades; **B:** Pitched Blade (Axial Flow) Turbine with six blades; **C:** Marine type propeller with three blades).

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Table I. Characteristics of bioreactor and impeller.

d (mm)	47
d/D	0.42
H/D	4.89
w/d	0.19
l/d	0.28
h_1/d	0.74
h_2/d	0.64
Number of blades	6
Number of baffles	2
s/d	0.30
d'/d	0.15
l'/d	1.23

volume fraction of PFC of 0.20, to investigate whether it influenced the PFC dispersion and therefore the k_La values. Figure 3 presents the results obtained showing that the impeller position used previously (Position 1) was the best for the oxygen transfer rate of the overall system displaying the higher k_La values. Position 1 leads to an enhancement of 3.6- and 1.7-fold in the k_La values when compared to Position 2 and 3, respectively. Despite the fact that Positions 2 and 3 appear to perform a better dispersion of the PFC because the lower impeller achieved the PFC upper level, the worst performance observed for these positions can be due to the lower impeller being too close to the air outlet, interrupting the gas bubble's path. Further measurements were carried with the impellers in Position 1.

Impeller Type

To evaluate their effect on the oxygen transfer rate in the bioreactor three types of impellers (Fig. 1) were tested with 1.5 dm^3 total working volume of pure water. Aeration rates ranging from 0.5 to 2.0 $dm^3 \cdot min^{-1}$ and two agitation speeds (100 and 250 rpm) were tested. Figure 4 presents the results obtained showing that the impeller, which produces higher k_La values, is the type A for all the aeration rates and agitation speeds studied. The impeller type A presented an average enhancement on the k_La values of 1.5-fold and 2.2-fold with respect to impellers B and C, respectively. This impeller is more efficient in breaking the air bubbles because it has a higher transversal section area and, consequently, it increases the superficial area of the bubbles, enhancing the oxygen transfer rate. However at lower agitation speeds this difference in transversal section area is not so effective, and at 100 rpm, the k_La values do not present significant differences for the different types of impellers studied.

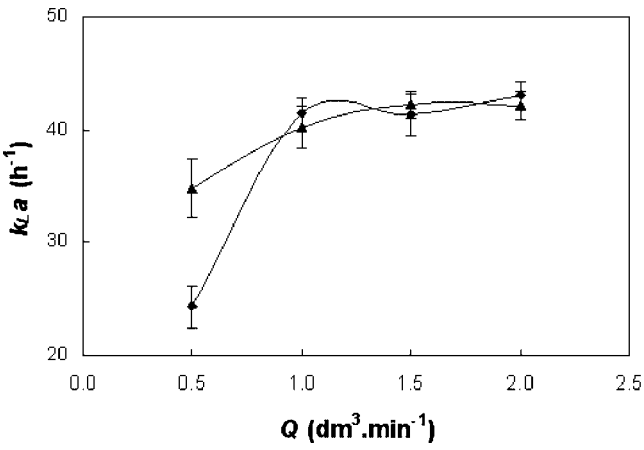

Figure 2. Influence of the working medium volume in the k_La values with pure water, at agitation rate of 250 rpm, and with impeller type A, as a function of the aeration rate. Working volume: (◆) 1.0 dm^3; (▲) 1.5 dm^3.

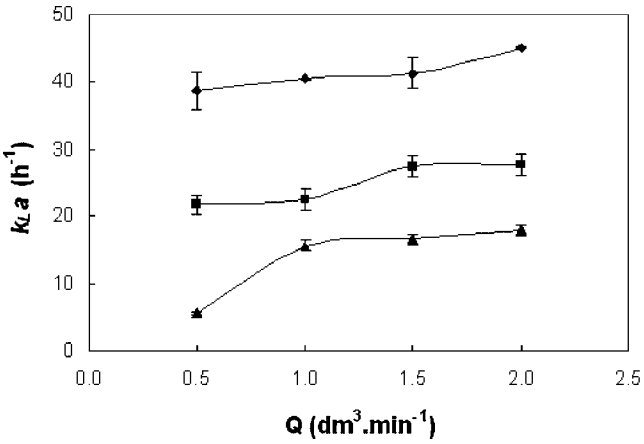

Figure 3. Influence of impeller position in the k_La values in a system with water and PFC with a volume fraction of 0.20, agitated at 250 rpm, and with impellers type B, as a function of the aeration rate: (◆) Position 1: $h_1 = 3.5$ cm, $h_2 = 3.0$ cm; (▲) Position 2: $h_1 = 6.5$ cm, $h_2 = 1.0$ cm; (■) Position 3: $h_1 = 3.5$ cm, $h_2 = 1.0$ cm.

Figure 4 also shows that for most of conditions studied the agitation proved to be more efficient in the k_La enhancement than the aeration. This behavior is in agreement with the results of Chen et al. (1999) that showed the overall productivity of lipase to depend more strongly on the agitation than aeration rates.

PFC Concentration

Besides the k_La measurements in pure water, studies with various perfluorodecalin volume fractions (from 0 to 0.30) as a second liquid immiscible phase were carried, with three types of impellers, and at different agitation (100 and 250 rpm) and aeration rates (0.5–2.0 $dm^3 \cdot min^{-1}$). Figure 5 presents an overview of the results obtained in terms of k_La values that show how the presence of a second liquid phase influences the oxygen mass transfer coefficient of the overall system.

For type A impellers, at both agitation speeds and with aeration rates above 1.0 $dm^3 \cdot min^{-1}$, the k_La reaches a maximum at approximately 0.20 volume fraction of PFC.

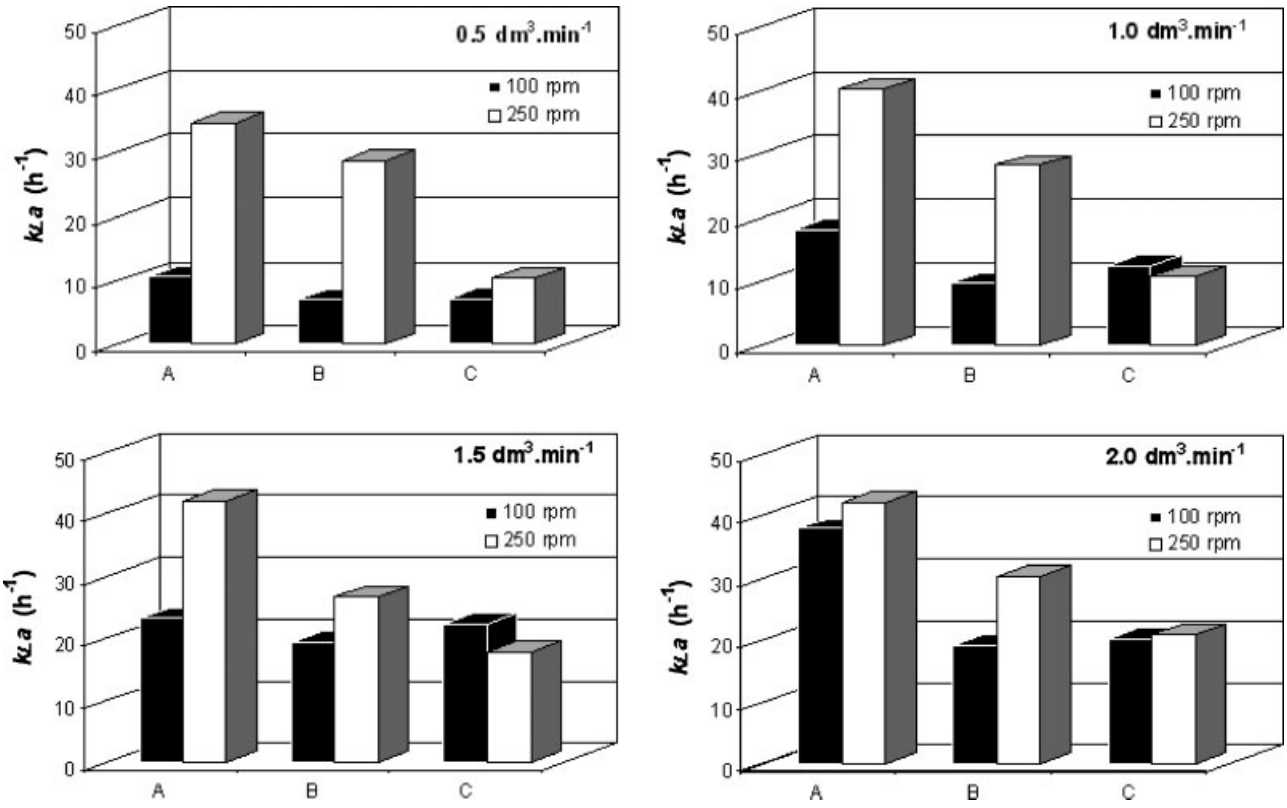

Figure 4. Influence of the impeller kind in the k_La values using pure water at 100 and 250 rpm agitation rate and at aeration rates from 0.5 to 2.0 $dm^3 \cdot min^{-1}$. (A: Rushton turbine with six vertical blades; B: Pitched Blade (Axial Flow) Turbine with six blades; C: Marine type propeller with three blades).

For impellers type *B*, the optimal PFC concentration in the k_La improvement depends on the agitation speed. For the higher agitation rate the maximum appears at 0.20 volume fraction of PFC and for the lower agitation rate it appears at a volume fraction of 0.25. From these results it seems that the k_La depends on the agitation and on the aeration rate, as well as the impeller capacity of adequately dispersing PFC through the aqueous medium. The impeller type *C* was found to be the less efficient in the oxygen transfer rate enhancement providing the lower k_La values, and it proved to be very dependent on both the aeration and agitation rates, where the others are primarily affected by the agitation speed rather than the aeration rate. This impeller presents a maximum efficiency with a volume fraction of PFC of 0.15, where the aeration proved to be the primary factor in dispersing the PFC drops. Also for impeller type *C* it was observed that this impeller produced a very low PFC dispersion compared with the others under study and leading to bubbles formation at the electrode interface, giving more imprecise results. For higher concentrations of organic phase the volume of the lower phase is larger and the aeration is not able to create small dispersive drops. As observed in pure water, the impellers of type *A* proved to be the more efficient in the k_La enhancement with values that are 1.2-fold and 1.9-fold with respect to impellers *B* and *C*, respectively, in their optimal k_La values achieved in the presence of different PFC concentrations. For all the impellers tested the k_La obtained was higher for higher agitation rates (independent of the PFC volume fraction), because the intensification of mixing induces the fine dispersion of air and PFC with the gas–liquid interfacial area increase.

Perfluorodecalin is thus shown to be beneficial for the oxygen transfer rate of the system, reaching a maximum in k_La values at a given PFC concentration and decreasing after that with the increase in PFC concentration. The presence of an oxygen carrier facilitates the oxygen transfer to water, but for higher concentrations the rheological behavior of the medium starts to be critical. At high PFC concentrations, the medium viscosity is increased decreasing the oxygen transfer rate. The observed peak in k_La is, therefore, a likely result of the competing influences of an increased oxygen transfer rate resulting from perfluorodecalin droplets acting as active oxygen transfer intermediates and an inhibition of convective oxygen transfer due to increased liquid viscosity.

There are some reports in literature (Dumont et al., 2006; Elibol and Mavituna, 1997; Elibol, 1999; Elibol and Mavituna, 1999) showing the k_La dependence on different kinds of systems as a function of PFC concentration. Obviously the k_La values depend on the concentration range studied, on the type of bioreactor used, and on the geometrical conditions being operated. Dumont et al.

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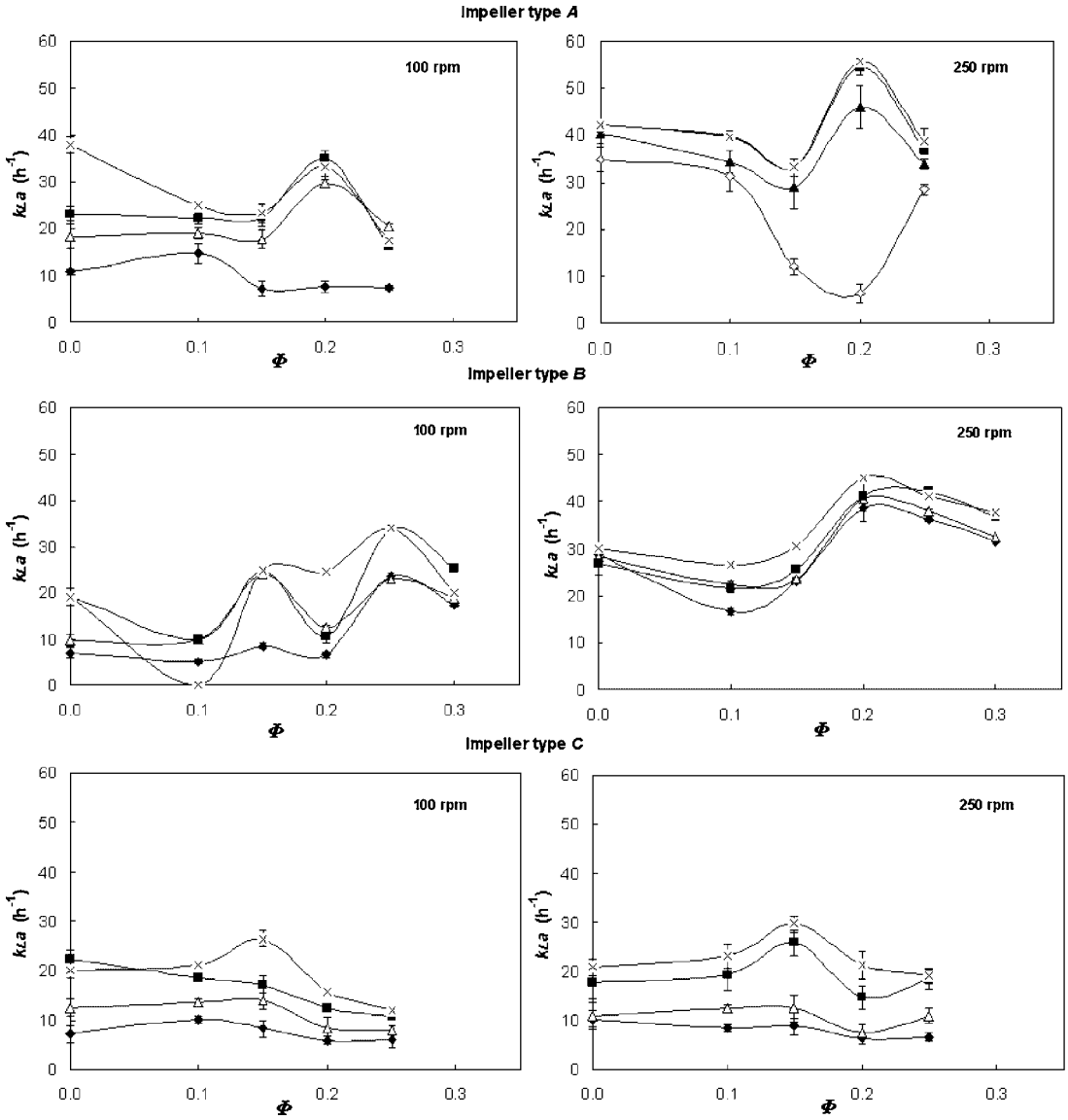

Figure 5. Influence of the PFC volume fraction (Φ) in the k_La values with water, with the three impellers type, with agitation rates of 100 and 250 rpm, and at different aeration rates, $dm^3 \cdot min^{-1}$: (◆) 0.5; (△) 1.0; (■) 1.5; (×) 2.0.

(2006) state that the organic phase addition has no significant influence on the k_La of the system, but they limited their study just to 4% (v/v) of PFC. On the other hand, Elibol and Mavituna (1997, 1999) showed a maximum in the k_La values with 20% (v/v) of perfluorodecalin and Elibol (1999) showed a maximum in the k_La values with 15% (v/v) of perfluorodecalin, but in the latter case this was the maximum organic phase concentration studied by the author.

Aqueous Phase

Inorganic electrolytes solutions are known to inhibit both gas bubble and oil droplet coalescence, affecting the k_La (Hassan and Robinson, 1977). The effect of using YPD medium instead of pure water in the overall k_La values was evaluated with two PFC volume fractions, 0.15 and 0.20, with the optimal geometrical conditions previously determined (1.5 dm^3 of working volume and impellers type *A* at Position 1), at two agitation rates (100 and 250 rpm) and at four aeration rates from 0.5 to 2.0 $dm^3 \cdot min^{-1}$. The results obtained are presented in Figure 6, where it can be observed that in the absence of PFC, there is a decrease in the k_La values when the YPD medium is used in comparison to water (Fig. 5, Impeller type *A*). In the presence of PFC similar profiles were obtained using YPD medium and water, with the optimal maximum k_La value at a Φ of 0.2, although the magnitude of the effect of the dispersed PFC on the k_La was different for water and YPD medium. The largest increase of k_La obtained in water was of 24.6% or 1.3-fold for the essay with 0.20 PFC volume fraction with pure water at

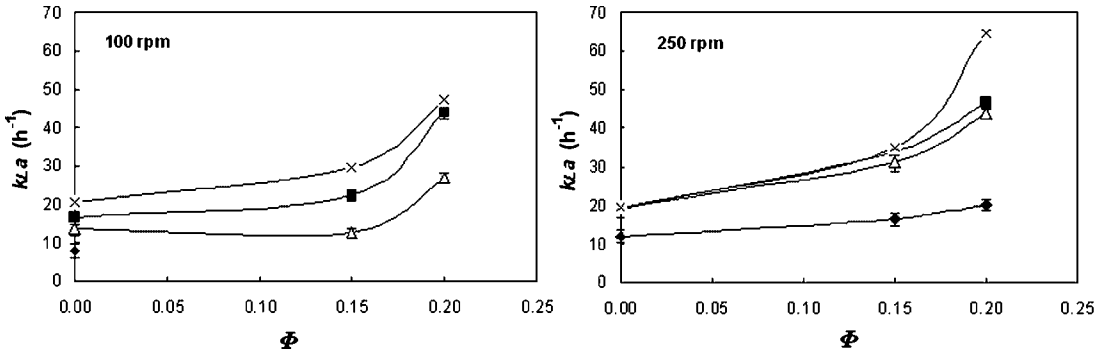

Figure 6. Influence of the PFC volume fraction (Φ) in the k_La values with YPD medium, with impellers type *A*, with agitation rates of 100 and 250 rpm, and at different aeration rates, $dm^3 \cdot min^{-1}$: (◆) 0.5; (△) 1.0; (■) 1.5; (×) 2.0.

an agitation rate of 250 rpm and at an air rate of 2.0 $dm^3 \cdot min^{-1}$. Under this same condition, with YPD medium, the presence of 0.20 PFC volume fraction promotes an increase in the k_La values of 227.9% or 3.3-fold.

The YPD medium is composed of casein peptone that has a slight emulsifier capacity, and therefore is more efficient in dispersing the PFC droplets through the aqueous medium, promoting lower size PFC droplets and a higher interfacial area. This was shown by preparing a 50% (w/v) of PFC and YPD medium mixture that was sonicated for 2 min, obtaining an emulsion stable for 48 h and with a mean particle size diameter of 0.36 μm, as determined by image analysis (Freire et al., 2005c).

Olive Oil Concentration

Olive oil is often used as an inducer for lipase production (Pereira-Meirelles et al., 1997) and also displays very high oxygen solubility (Battino et al., 1983). The attempt to use this organic phase to simultaneously induce the lipase production and enhance the oxygen transfer rate imposed itself. The k_Las measured for various olive oil fractions at the bioreactor are presented in Figure 7. It is possible to observe that, unlike what was observed with PFC, the presence of olive oil has caused a reduction in the k_La of the system for all the concentrations studied. The olive oil is more viscous than the PFC studied and less dense than water staying now at the top of the bioreactor. This situation makes the dispersion of the second phase more difficult requiring very high agitation rates to create a vortex that could effectively disperse the organic phase at the risk of cell inactivation due to high shear rates. Besides, the high viscosity of the olive oil leads to low diffusion coefficients for the oxygen in the organic phase and thus low permeabilities that may also contribute to the reduction of the k_Las observed.

The oxygen transfer rate enhancement promoted by the PFC addition is obtained not due to its spreading behavior, but via a relatively high diffusion coefficient and/or solubility in the organic dispersed phase, where organic droplets carry the oxygen from the gas/liquid interface to the bulk of the dispersion, the so-called "shuttle mechanism" (Dumont and Delmas, 2003). In the case of olive oil, an increase of the interfacial area takes place by reducing the gas/water interfacial tension. However, in general, the contribution of an interfacial area increase to the total enhancement is relatively small (Rols et al., 1990). Rols et al. (1990) concluded that the effect of organic-solvent droplets

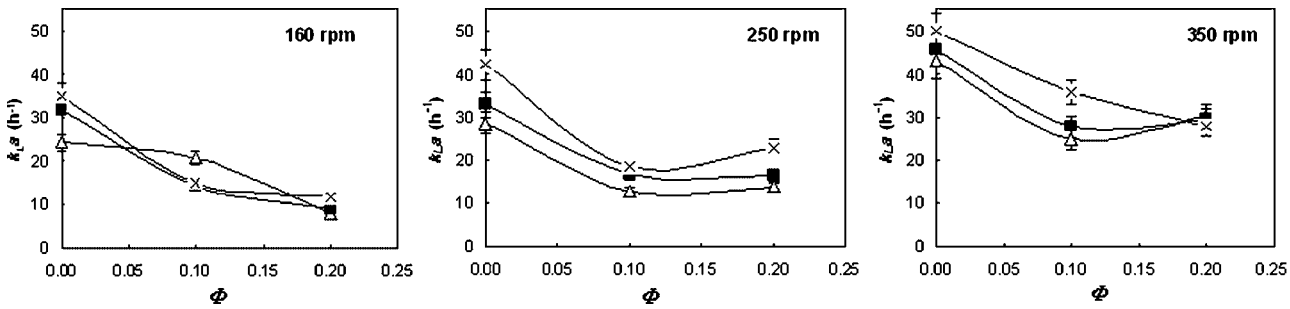

Figure 7. Influence of olive oil volume fraction (Φ) in the k_La values with water, with impellers type *A*, with agitation rates from 160 to 350 rpm, and at different aeration rates, $dm^3 \cdot min^{-1}$: (△) 1.0; (■) 1.5; (×) 2.0.

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on the specific gas-emulsion exchange area is usually of minor importance compared to the enhancement effect on the mass transfer coefficient. In fact, PFC dissolves oxygen to a larger extent when compared to olive oil, and thus promotes k_La enhancement.

Clarke et al. (2006) summarized the two main causes that have been proposed for the k_La enhancement in the presence of organic phases: positive spreading coefficient oils can spread as a thin film on the bubble, lowering the surface tension and increasing the interfacial area *per* unit volume of the bubble; the drops can act as rigid spheres increasing turbulence and it acts as an oxygen reservoir that transport the gas to the aqueous phase by simple diffusion. Also Dumont and Delmas (2003) have concluded that it is not possible to explain the mass transfer phenomena due to the effect of oil addition in a gas–liquid interface and boundary layer only by the spreading coefficient and further studies should be made. In fact, perfluorodecalin enhances the overall k_La by increasing the medium turbulence (since it stays at the bottom of the bioreactor due to its higher density and it is thrown to the aqueous phase essentially due to the air sparging system) and by acting as an oxygen carrier from the organic to the aqueous phase. The main reason governing the k_La enhancement of both systems is the sparging submerged aeration in combination with the physical properties of the second liquid organic phase.

Inactive Cells

The influence of inactive *Y. lipolytica* cells concentration in the oxygen transfer rate and in the k_La of the system was studied at different agitation speeds and aeration rates, and in the presence of different PFC concentrations. The influence of cells on the oxygen transfer rate results from the apparent viscosity increase of the medium due to biomass accumulation, oxygen solubility reduction, and blocking effect created by cells adsorption to the air bubbles surfaces (Cascaval et al., 2006). Figure 8 presents the k_La values of the system without cells and in the presence of 5 and 10 g cell dry weight $\cdot$ dm^{-3}, which was the cell concentration range for lipase production by *Y. lipolytica* in the presence of PFC in YPD medium (Amaral et al., 2006a). These results show a slight reduction in the k_La with the increase in cell concentration, reducing the magnitude of the beneficial effect of PFC in the oxygen transfer rate. The decrease in k_La values in the presence of cells is due to the change in the rheological properties of the medium. This decrease is very subtle because cell concentration is not high. Particles with a diameter somewhat greater than the thickness of the mass transfer layer enhance the gas absorption but the enhancement decreases with increasing particle diameter (Galaction et al., 2004a), where the inactive cells present an average diameter of 7 μm, the mass transfer coefficient decreases with increase in cell concentration. It can also be observed that the benefit of PFC in the presence of cells is only significant at the highest agitation speed (350 rpm). Cascaval et al. (2006) have shown the reduction of favorable effect of oxygen-vector addition as a result of cell's adsorption to the hydrocarbon droplets surface because of the hydrophobicity of the bacteria studied. As *Y. lipolytica* IMUFRJ has been proved to have high affinity to organic compounds (Amaral et al., 2006b), the adhesion and blocking effect of cells to the PFC surface might be leading to the oxygen transfer reduction. However, with the agitation speed increase, the PFC droplets become smaller and the cell blocking effect diminishes, allowing the PFC to transfer oxygen efficiently.

Correlations for Oxygen Mass Transfer Coefficients

In order to predict fermentation performance when using models that account for the effect of dissolved oxygen, an empirical correlation for the oxygen transfer rate in a multiphase bioreactor has been developed. Correlations that account for the presence of an immiscible, organic liquid phase (Hassan and Robinson, 1977; Nielsen et al., 2003) or the presence of microbial cells (Cascaval et al., 2006; Galaction et al., 2004a; Galaction et al., 2005) have been previously proposed. Generally these correlations were developed for hydrocarbons as organic phases while in the present work the correlation was developed for a perfluorocarbon, perfluorodecalin, in the presence of inactive yeast cells.

The specific power consumption is the parameter which indicates the turbulence degree and media circulation in bioreactor. For non-aerated systems and single turbine stirrer of Rusthon type, the calculation of power consumption for stirring uses the power number, Np (Galaction et al., 2005):

$$N_p = \frac{P}{\rho N^3 \mathrm{d}^5} = \frac{6}{\mathrm{Re}^{0.15}} \tag{2}$$

The power consumption for mechanical mixing of aerated media can be determined by means of the value obtained for non-aerated ones, using the following equation (Hughmark, 1980):

$$\frac{P_a}{P} = 0.10\left(\frac{gwV^{2/3}}{N\mathrm{d}^4}\right)^{0.2}\left(\frac{NV}{Q}\right)^{0.25} \tag{3}$$

Based on these concepts, a mathematical correlation which describes the influence of the studied parameters on the k_La has been established. This correlation was developed using EXCEL software and, for the experimental data, the difference between the experimental and modeled value being reduced to a minimum by the least-square fit method.

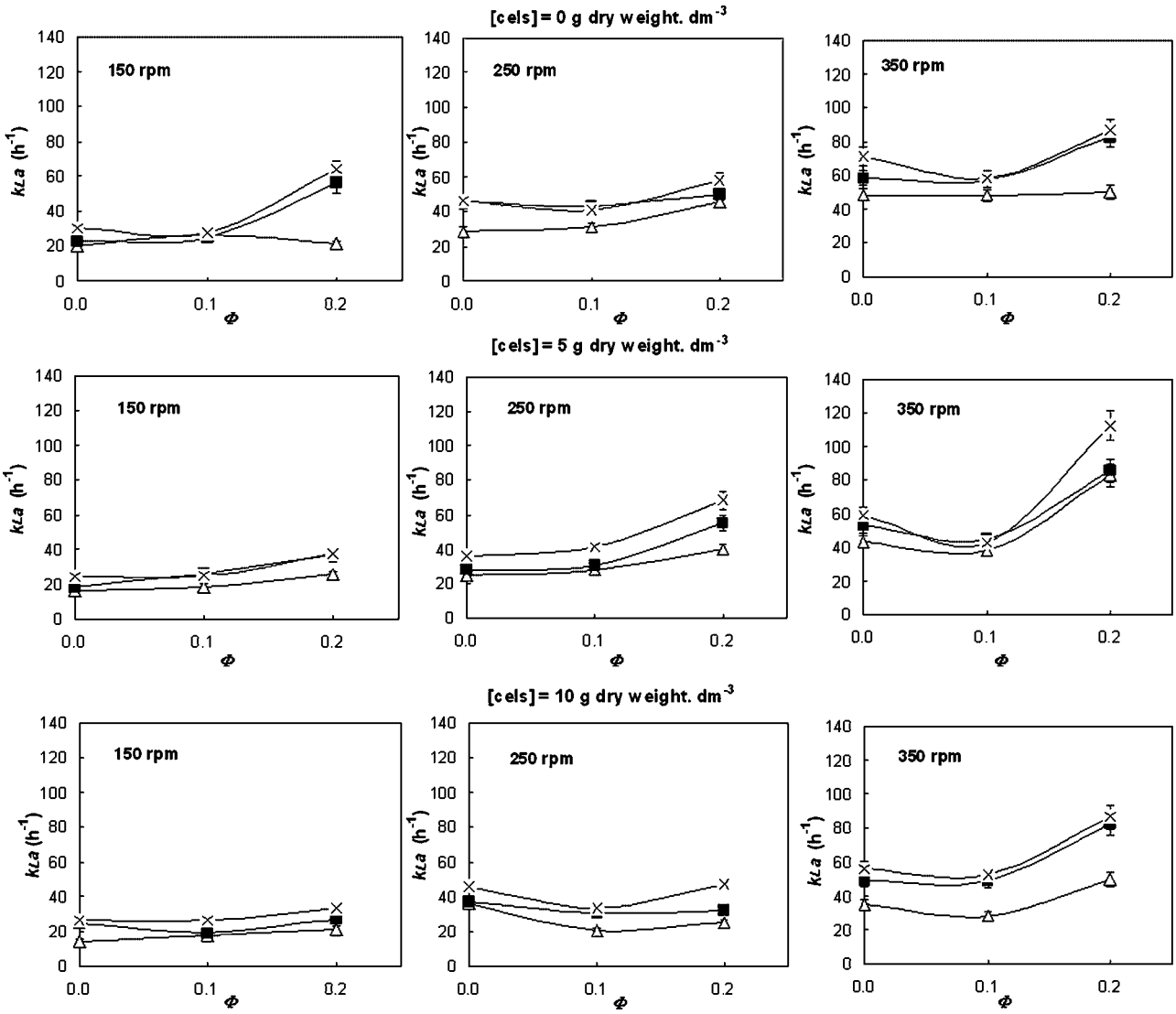

Figure 8. Influence of the PFC volume fraction (Φ) in the k_La values with YPD medium, with no cells or with two inactive cell concentrations, with impellers type *A*, agitation rates from 160 to 350 rpm, and at different aeration rates, $dm^3 \cdot min^{-1}$: (△) 1.0; (■) 1.5; (×) 2.0.

As shown by Nielsen and Villadsen (1994), a typical correlation for estimating k_La values is

$$k_La = \alpha\left(\frac{P_a}{V}\right)^{\beta} v_s^{\chi} \tag{4}$$

To account the influence of inactive cells concentration and PFC volume fraction, the general form of the proposed equations is

$$k_La = \alpha\left(\frac{P_a}{V}\right)^{\beta} v_s^{\chi}(1-\Phi)^{\delta} X^{\varepsilon} \tag{5}$$

Using the experimental data obtained for YPD medium, with inactive cells and PFC, the values of α, β, χ, δ and ε coefficients were estimated. The following correlation was obtained:

$$k_La = 0.153\frac{\left(\frac{P_a}{V}\right)^{0.302} v_s^{0.699} X^{0.068}}{(1-\Phi)^{1.378}} \tag{6}$$

with k_La given in s^{-1} units.

The proposed correlation offers a good agreement with the experimental data with an average deviation of ± 15.7%, as shown in Figure 9, which presents the predicted versus the experimental results with a line of slope equal to 1. The low value of ε (coefficient of X) translates the low dependence of k_La on this parameter. On the other hand, the large negative coefficient of $(1-\Phi)$ shows the influence of the PFC fraction in the oxygen transfer rate.

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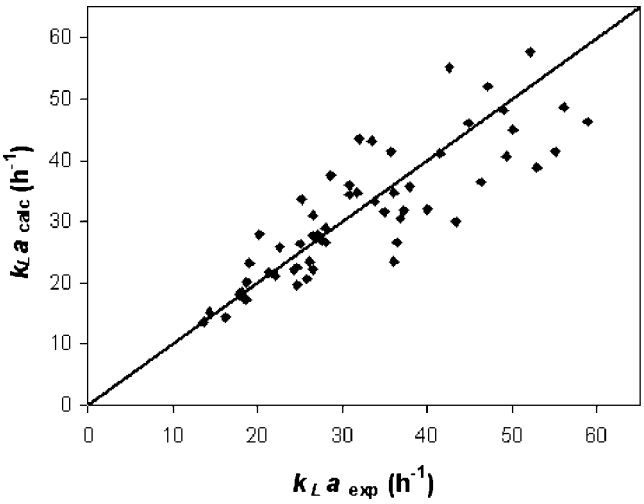

Figure 9. Correlation between the experimental and calculated values of k_La for YPD medium, inactive cells, with and without PFC, agitation rates from 160 to 350 rpm and aeration rates from 1.0 to 2.0 $dm^3 \cdot min^{-1}$ (k_La_{exp}, k_La experimental values), (k_La_{calc}, k_La calculated value).

Conclusions

The oxygen transfer into the microbial cell in aerobic bioprocesses strongly affects product formation by influencing metabolic pathways and changing metabolic fluxes. Thus, the optimization of the bioreactor performance in what concerns the oxygen transfer requirement needs to be clarified. In this work, the effects on the oxygen transfer rate were investigated in the presence of two immiscible liquid phases having different oxygen solubilities and opposite densities compared to water, and with two aqueous phases (pure water and YPD medium).

It was shown that the addition of perfluorodecalin promotes k_La enhancement while olive oil decreases the overall k_La of the multiphase reactor. The results obtained with perfluorodecalin show that the oxygen transfer rate is influenced by both the turbulence and oxygen diffusion to the aqueous phase and also the inhibition of convection due to the increased liquid viscosity, leading to an optimal PFC volume fraction. The change of the aqueous medium from pure water to YPD medium resulted in an increase in the k_La value to a maximum value of 64.6 h^{-1} with a volume fraction of perfluorodecalin of 0.20 and with two Rushton turbines with six vertical blades. The enhancement in the k_La obtained in pure water was of about 25% while for the same conditions with YPD medium in the presence of 0.20 PFC volume fraction an increase in the k_La value of 230% was observed.

The oxygen transfer in the three-phase system studied seems to result from complex interactions phenomena and from a combination of several factors, as agitation speed, aeration rate, organic phase volume fraction and kind of organic and aqueous phase that have shown to play an important role. The presence of cells as particles has shown to only marginally interfere in the oxygen transfer rate of the system.

Nomenclature

X	biomass concentration ($g \cdot l^{-1}$ dry weight)
d	stirrer diameter (mm)
d'	oxygen electrode diameter (mm)
D	bioreactor diameter (mm)
G	acceleration of gravity ($m\ s^{-2}$)
h_1	distance of the upper bioreactor stirrer to the shorter one (mm)
h_2	distance of the lower bioreactor stirrer to the bioreactor bottom (mm)
H	bioreactor height (mm)
k_La	oxygen mass transfer coefficient (s^{-1})
l	impeller blade length (mm)
l'	oxygen electrode immersed length (mm)
N	impeller rotation speed (rpm)
N_P	power number
P	power consumption for mixing of non-aerated broths (W)
P_a	power consumption for mixing of aerated broths (W)
Q	volumetric air flow rate ($m^3 \cdot s^{-1}$)
P_a/V	specific power input ($W \cdot m^{-3}$)
Re	Reynolds number
S	baffle width (mm)
v_S	superficial air velocity ($m \cdot s^{-1}$)
V	volume of medium (m^3)
w	impeller blade height (mm)

Greek Letters

ρ	density ($kg \cdot m^{-3}$)
Φ	volumetric fraction

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