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ENHANCEMENT OF OXYGEN MASS TRANSFER IN PNEUMATICAL BIOREACTORS USING N-DODECANE AS OXYGEN-VECTOR

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Abstract

In biotechnology, oxygen mass transfer is a key parameter involved in the design and operation of bioreactors and it can be analyzed by means of the oxygen mass transfer coefficient (k_La). Due to the fact that oxygen has a very low solubility in an aqueous media (8–10 ppm at 20˚C), actively growing cells can consume all the dissolved oxygen very fast, therefore, it has to be supplied continuously into the broths. In conventionally aerated bioreactors, low oxygen solubility combined with slow oxygen transfer rates often results in reduced growth and culture productivity. Due to their higher oxygen solubility, non-toxicity to microbes, antifoaming action, oxygen-vectors addition is one of the most effective methods to improve oxygen mass transfer rate in aerobic fermentations. The aim of this study was to investigate the use of n-dodecane as oxygen-vector in bubble column and air-lift bioreactors, under different working conditions (air superficial velocity, volumetric fraction of the organic phase, medium temperature). The results show that volumetric fraction of oxygen-vector (φ) has a great influence on k_La; in the presence of low volumetric fraction (φ=0.005 (v/v)), the oxygen mass transfer coefficient's value in bubble column bioreactor was increased by almost 100% at 35˚C and for φ=0.02 (v/v) by 5% at 25˚C, while in air-lift bioreactor, at 25˚C and φ=0.005 (v/v), the k_La value was enhanced by approximately 50%.

Key words: air-lift, bubble column, n-dodecane, oxygen mass transfer, oxygen-vector

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1. Introduction

Pneumatically agitated bioreactors are frequently used in biological, chemical and petrochemical industry, mostly in biotechnology and wastewater treatment. The simplest one is the bubble column bioreactor, its main advantages being simple construction and excellent mass and heat transfer characteristic (Dhaouadi et al., 2008), low cost and maintenance (Kantarci et al., 2005) and ease of operation (Asgharpour et al., 2010). In these types of bioreactors, local flow, gas hold-up distribution and turbulence interrelated with the bioreactor's operating and design parameters (Dhaouadi et al., 2008) determine the efficiency and productivity of the system, so the gas–phase dispersion and the distribution of the bubbles are critical, as they define the gas-liquid interfacial area with a direct effect on the overall mass transfer (Chaumat et al., 2007). By introducing a draft tube in a bubble column bioreactor an increase in liquid circulation (in the same conditions) is achieved, the liquid circulates in a loop determined by the static pressure difference between the riser and the downcomer (resulting in an internal air-lift bioreactor). Due to a better liquid circulation, air-lift bioreactors are finding increasing applications

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in chemical industry, biochemical fermentation and biological wastewater treatment processes. Beside a better medium circulation, another advantage of the airlift, compared with others bioreactors, is that shear stress is mild (Vunjak-Novakovic et al., 2005) and relatively constant distributed throughout the bioreactor (van Baten and Krishna, 2003). Therefore, compared with mechanically stirred bioreactors, pneumatically agitated bioreactors are more suitable for stress-sensitive processes (which involves sensitive microorganisms), but they have a major disadvantage - the poor mixing inside (Fu et al., 2004).

The most important gaseous phase in biotechnology and wastewater treatment processes is oxygen, an important nutrient used by microorganisms for growth, maintenance and metabolite production. The oxygen mass transfer from bubble's swarm into liquid is the controlling step of the majority of aerobic biochemical processes.

Due to the fact that oxygen has a very low solubility in an aqueous medium (8 – 10 ppm at 20˚C), actively growing cells can consume all the dissolved oxygen very fast, so it has to be supplied continuously into the broths (Sauid and Murthy 2010). In conventionally aerated bioreactors, low oxygen solubility combined with slow oxygen transfer rates often result in reduced growth and culture productivity (Leung et al., 1997).

Hence, oxygen availability into the broths constitutes one of the critical parameters for aerobic bioprocesses and can play a very important role in the scale-up and economy of these systems (Suresh et al., 2009; Ntwampe et al., 2010; Galaction et al., 2005).

Oxygen transfer rate in a system is a function of the oxygen volumetric mass transfer coefficient (k_La) and the oxygen solubility in the medium. For a specific bioreactor and medium, it is possible to increase k_La using higher aeration rates; however, this causes high power consumption, significantly increasing operation costs (Gomes et al., 2008) and it may also stress the microorganisms with negative effects on the overall process.

To overcome this, many researchers studied the possibility of enhancing oxygen mass transfer by using some compounds, named oxygen-vectors or oxygen carriers, in witch oxygen solubility is higher than in aqueous solutions (15–20 times) (Amaral et al., 2008; Cascaval et al., 2006; Clarke and Correia, 2008; Crognale et al., 2007; Galaction et al., 2004; Galaction et al., 2005; Quijano et al., 2010). Oxygen-vectors have no toxicity against the cultivated microorganisms, and in some cases they could be used as supplementary source of carbon and energy or even as antifoaming agents (Cascaval et al., 2006; Galaction et al., 2004; Galaction et al., 2005). The main oxygen-vectors used in biotechnology are hydrocarbons, perfluorocarbons and oils: synthetic (silicone oil (Dumont et al., 2006; Leung et al., 1997; Narta et al., 2011)) and vegetable (palm oil (Sauid and Murthy 2010), castor oil (Pulido-Mayoral and Galindo, 2004), olive oil (Amaral et al., 2008), soybean oil (Zhao et al., 1999), among others). Therefore, addition of organic solvents (with higher oxygen solubility, non-toxicity to microbes and antifoaming action) is one of the most used and effective method for improving the oxygen transfer rate in aerobic fermentation (Narta et al., 2011).

The aim of organic phase addition, with a higher oxygen affinity, is to increase the oxygen mass transfer from the gaseous phase to the liquid one, mainly by increasing the overall gas solubility in the system. The present study has been done in order to evaluate the effect of n-dodecane addition, used as oxygen-vector, on oxygen mass transfer, in pneumatically agitated bioreactors (in bubble column and air-lift).

2. Experimental

The experiments were performed in a bubble column, represented in Fig. 1a, and in an air-lift bioreactor Fig. 1b. The air-lift was obtained by adding a draft tube inside the bubble column. The bubble column is a cylindrical column made of Perspex, covered by a Perspex rectangular box used for temperature control through water circulation.

The gas chamber, located at the bottom, is the place where the gas first enters and then passes through a sparger (Fig. 1c), consisting of uniformly spaced needles (the inner diameter is 0.29 mm) placed on a metallic perforated plate.

Because of the small diameter of the needles, the bubbles shape and size is very well defined. The distribution of the needles enables an uniform distribution of the bubbles along the column. The oxygen mass transfer experiments were performed in two and three-phase systems at different temperatures (25, 30 and 35˚C). Air and distillate water were used as gas and liquid phase, respectively. As oxygen-vector was used n-dodecane (Merck Chemicals) with the following properties: density 0.750 g L^{-1} at 20°C; molar mass 170.34 g mol^{-1}; practically insoluble in water at 25˚C; and oxygen solubility at 35°C of 54.90 mg L^{-1} at atmospheric pressure (Lai et al., 2012). n-Dodecane was used because it is a cheap solvent and it can also be utilized as a supplementary source of carbon and energy. The volumetric fraction of the hydrocarbon in the broth varied between 0.005 and 0.03 (v/v). The liquid height was 45.5 cm (6.5 L) for the bubble column and 40 cm (6.15 L) for the air-lift bioreactor.

For k_La value determination the static gassing-out method has been used, which is based on measuring the increase of dissolved oxygen concentration in broths, starting from a deoxygenated liquid (lowered by passing nitrogen gas through the system). At that point humidified air was fed into the bioreactor until saturation. Dissolved oxygen concentration values were measured on-line using an oxygen electrode (CellOx 325, WTW) positioned 0.2 m from the sparger, and all the data were recorded directly in a computer, through a data acquisition board.

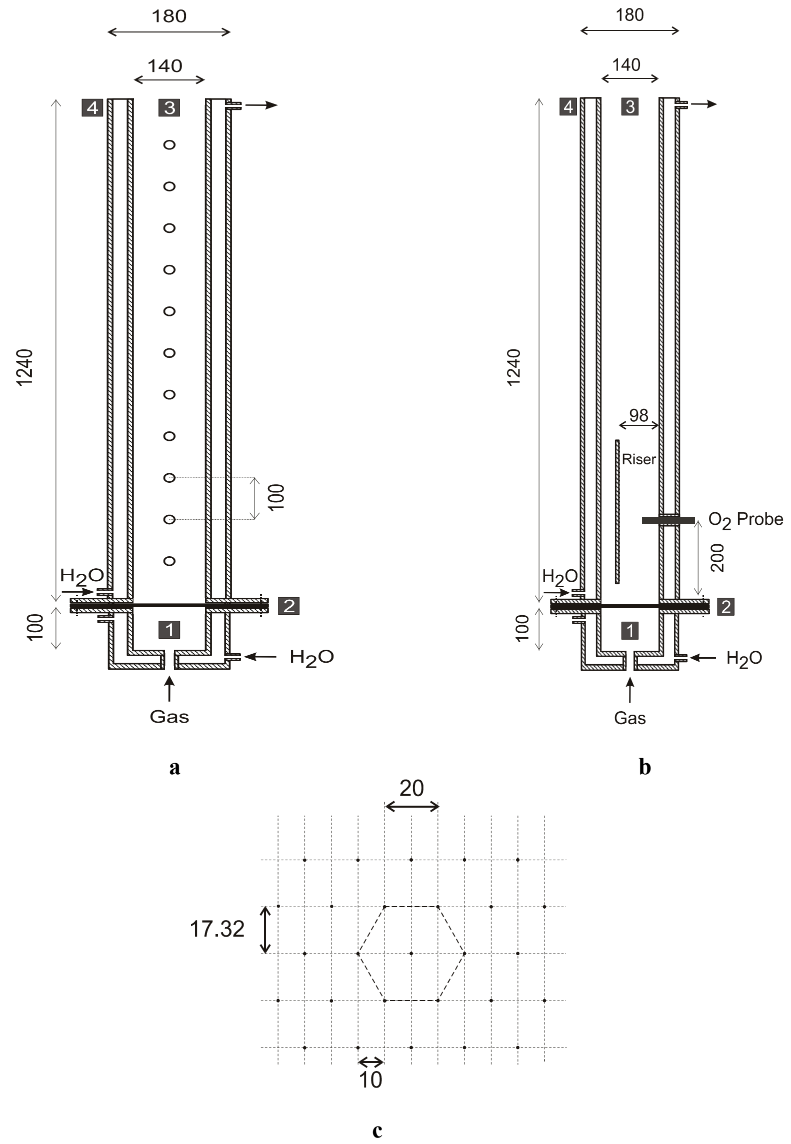


Fig. 1. a - Bubble column bioreactor (1-gas chamber, 2-sparger, 3-cylindrical column, 4-rectangular box); b– air-lift bioreactor; c- sparger (needles distribution on the plate) (all dimensions in mm)

This way, the oxygen concentration variation in time (*t*) was achieved and volumetric oxygen mass transfer coefficient calculated from the following equation (Eq. 1):

$$\frac{dC}{dt} = k_L a\left(C^* - C\right) \tag{1}$$

where C^* and C are, respectively, the oxygen solubility and oxygen concentration in the liquid. Assuming the liquid phase homogeneous and the oxygen concentration at $t=0$, C_0, the integration of the equation leads to (Eq. 2):

$$\ln\left(C^* - C\right) = \ln\left(C^* - C_0\right) - k_L a \cdot t \tag{2}$$

Plotting $ln(C^*-C)$ against time (*t*) the volumetric mass transfer coefficient can be determined. The experimental results are reproducible with an average relative error of ±6.3%.

The oxygen solubility in water (C^*) was experimentally taken for each run and the slope was calculated using the Test *F* method (this method consists in determining the optimum number of points for a linear regression of the experimental data) (Mena et al., 2011).

In order to have a better understanding of the air bubbles and n-dodecane drops, shape and dimensions, the image analysis technique developed by Ferreira et al. (2012) was used. The experimental image analysis set-up is presented in Fig. 2 and consists in a black and white high speed digital video camera (to grab images) connected to a computer.

For different conditions (temperature, gas superficial velocities and hydrocarbon volumetric fraction) different sets of images were recorded and analyzed.

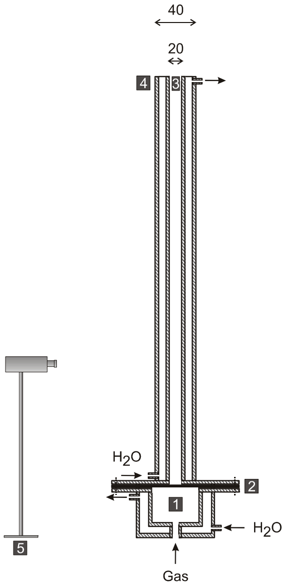


Fig. 2. Image analysis set-up (1-gas chamber, 2-sparger, 3-cylindrical column,4-rectangular box, 5-digital camera, 6-diffuser glass, 7-halogen lamp)

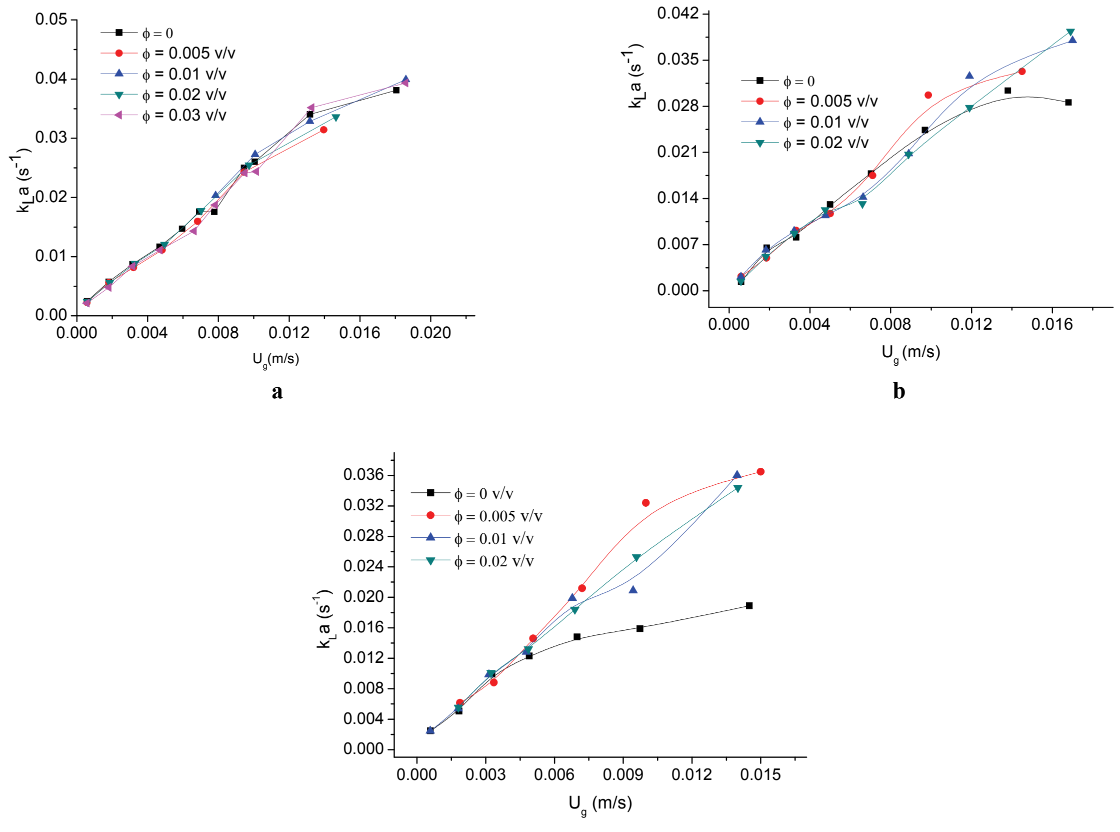
3. Results and discussion

In previous studies it was demonstrated that the addition of hydrocarbons as oxygen-vectors can increase the concentration of dissolved oxygen in simulated (Galaction et al., 2004) and real fermentation broths (Cascaval et al., 2006; Galaction et al., 2005) in stirred bioreactors; but the effect of an immiscible organic compound addition to enhance oxygen mass transfer in pneumatically agitated bioreactors was less studied.

Compared with mechanically stirred bioreactors, as Linek et al. (2005) observed, in dispersions with no mechanical agitation (bubble columns, air-lifts) the expansion of the gas fed into the system is the only source of energy, so there is a strong dependency between mass transfer and gas flow rate.

3.1. Bubble column bioreactor

As it can be seen from Fig. 3, higher flow rates produce an increment in k_La, no matter of the organic phase volumetric fraction, probably due to the gas hold-up increase with the gas flow (a higher number of bubbles generated), which leads to an increase of the interfacial gas-liquid area as Gómez-Díaz et al. (2010) also noticed. Increasing the temperature, the k_La value decreases due to a higher coalescence rate: larger bubbles determine smaller interfacial gas-liquid area.



a

b

c

Fig. 3. Influence of U_g (air superficial velocity) on k_La for different φ at different temperatures (a–25˚C, b- 30˚C, c- 35˚C) in the bubble column bioreactor

When n-dodecane is added to the system it seems that, globally, n-dodecane increases the mass transfer, especially, at higher temperatures and flow rates.

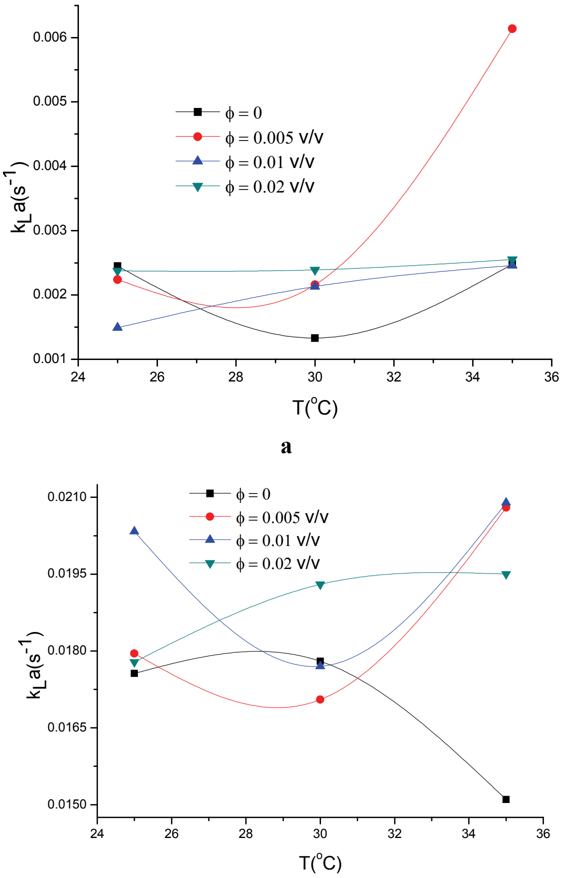
In these conditions n-dodecane seems to act in the coalescence process, reducing the frequency of bubble collisions and therefore, the small bubbles produced at the sparger maintain their size along the column, increasing, by this way, the interfacial area and the mass transfer.

As commonly accepted in the literature, in bubble columns bioreactors two main regimes can be distinguished depending on the gas flow rate and the liquid phase properties: homogeneous bubbly flow and turbulent flow (heterogeneous) regime (with a transition regime between them). The homogeneous flow characteristics are a narrow bubbles size distribution and an uniform spatial dispersion of gas hold-up, without interaction among bubbles (it occurs at low flow velocities and small holes sparger), while the second one appears at higher gas velocities and has large bubble size distribution and high concentration of large bubbles on column axis (this regime is governed by coalescence-break-up equilibrium).

By adding n-dodecane, as oxygen-vector, it was observed that the transition regime almost disappeared and the homogeneous one was enlarged, probably due to decay in coalescence. This effect is a result of the surface tension decrease, which leads to smaller bubbles and lower bubbles velocity, therefore higher gas hold-up and higher mass transfer coefficient can be achieved (Chaumat et al., 2007). The surface tension effect is particularly effective in homogeneous and transition regime and less in the heterogeneous one where the reduction in coalescence is overshadowed by the predominant effect of macro-scale turbulence.

From Fig. 4 it can be observed that at low gas flow rates, the addition of n-dodecane in the system has a low influence, since the air bubbles are already small and the coalescence low. The data shows that there is no linear and clear effect of the organic phase addition for bubble column bioreactors, this being in concordance with literature reports, where there are a lot of contrasting results on the effect of n-dodecane addition on oxygen mass transfer. According to Dumont et al. (2006) no agreement on the positive or negative effect of oxygen-vectors on mass transfer has been reached. On the other hand, in their review, Clarke and Correia (2008) stated that the effect, either positive or negative, of n-dodecane as oxygen-vector, depends on the percentage of vector used and the bioreactor design (Clarke and Correia, 2008).

One of the most important characteristics considered while evaluating the mechanism of oxygen mass transfer in three phase systems (gas-liquid-liquid) is the spreading coefficient of the organic phase. This property, quantifies its ability to either spread on (positive spreading coefficient) the air bubble surface and form a film around it or not spread (negative spreading coefficient) and form discrete droplets, attached or not to the bubbles.



a

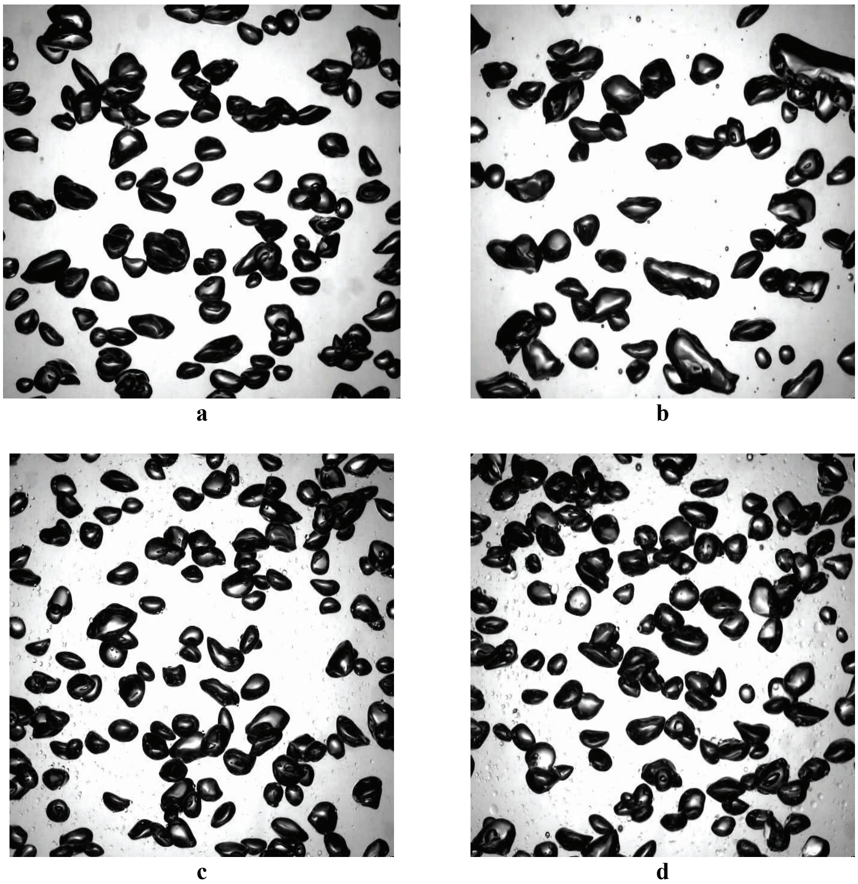
b

Fig. 4. Influence of temperature on k_La for different φ (n-dodecane volumetric fraction) at low U_g (0.0005 m s^{-1}) - a and high U_g (0.00704 m s^{-1}) – b in the bubble column bioreactor

According to some authors (Schaefer et al., 1999; Dumont et al., 2006) the spreading coefficient for n-dodecane is negative, while others, (Wei and Liu, 1998; Oliveira et al., 1999), calculated a positive value. Jia et al. (1997) and Jianlong (2000) stated that even if n-dodecane has a positive spreading coefficient, in the air-lift bioreactors is acting like a non-spreading liquid. According to our visual observations, (Fig. 5 c, d; Fig. 7 c, d), the n-dodecane is acting like a non-spreading liquid in pneumatically agitated bioreactors. Therefore, n-dodecane drops can be assimilated with rigid spheres, inducing an increased turbulence in the system and at the same time, they are an oxygen reservoir that transport the gas to the aqueous phase by diffusion (Amaral et al., 2008).

From Fig. 5 it can be observed that the bubbles shape is far from spherical; they do not have a very definite shape due to hydrodynamic processes such as detachment, collision, coalescence and break-up (Martín et al., 2010).

Also, it is commonly accepted that bubbles deform as they rise in the bioreactor.



a b c d

Fig. 5. Air bubbles and n-dodecane drops from the bubble column bioreactor (Ug=0.155 cm s^{-1}, without n-dodecane a- 25ºC, b – 35ºC, 2% n- dodecane c- 25ºC, d – 35ºC)

During the experiments, it was also notice that the bubble size distribution was affected by n-dodecane addition.

Analysing the data obtained by image analysis experiments (Fig. 8) we determined that the coalescence was less important in bubble column bioreactor when the organic phase was add.

3.2. Air-lift bioreactor

While in bubble columns, the liquid flow is low, in air-lifts the flow of gas and liquid is virtually a plug flow. At the same superficial gas velocities, the liquid circulation is much stronger in air-lifts, this leading to a much lower gas hold-up and consequently, to a lower k_La. Also, in air-lift bioreactors the interface between the organic phase and water is continuously renewed by the water circulation, therefore, the addition of oxygen-vectors leads to a clearer enhancement in k_La. In Fig. 6 the effect of n-dodecane addition for different superficial gas-velocities at 25˚C is plotted, and it can be observed that for low gas flow rates, the addition of a higher volumetric fraction of hydrocarbon has a positive effect on oxygen mass transfer coefficient, while for higher superficial gas velocities the best results were obtained for a low quantity of hydrocarbon. This is due to the fact that at low superficial velocities the liquid circulation is less important when compared with higher flow rates. Adding a bigger amount of organic phase to the system, the turbulence is amplified with a negative effect on the k_La.

In Fig. 7 it is shown that the bubbles shapes in air-lifts are also not spherical; they do not even have a very definite shape, but, by adding the oxygen-vector, the bubbles become more spherical than in pure system, for all the temperatures considered, and the coalescence is also reduced.

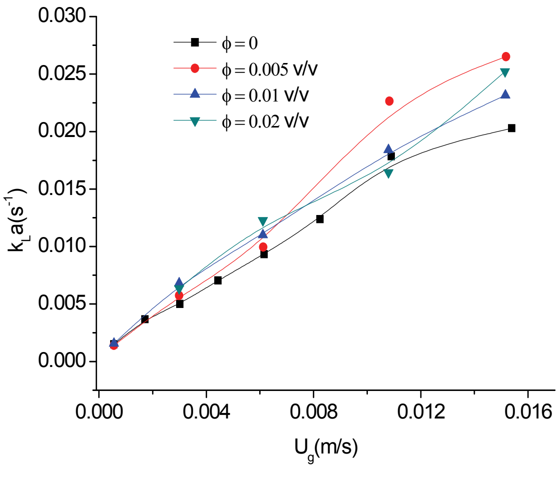
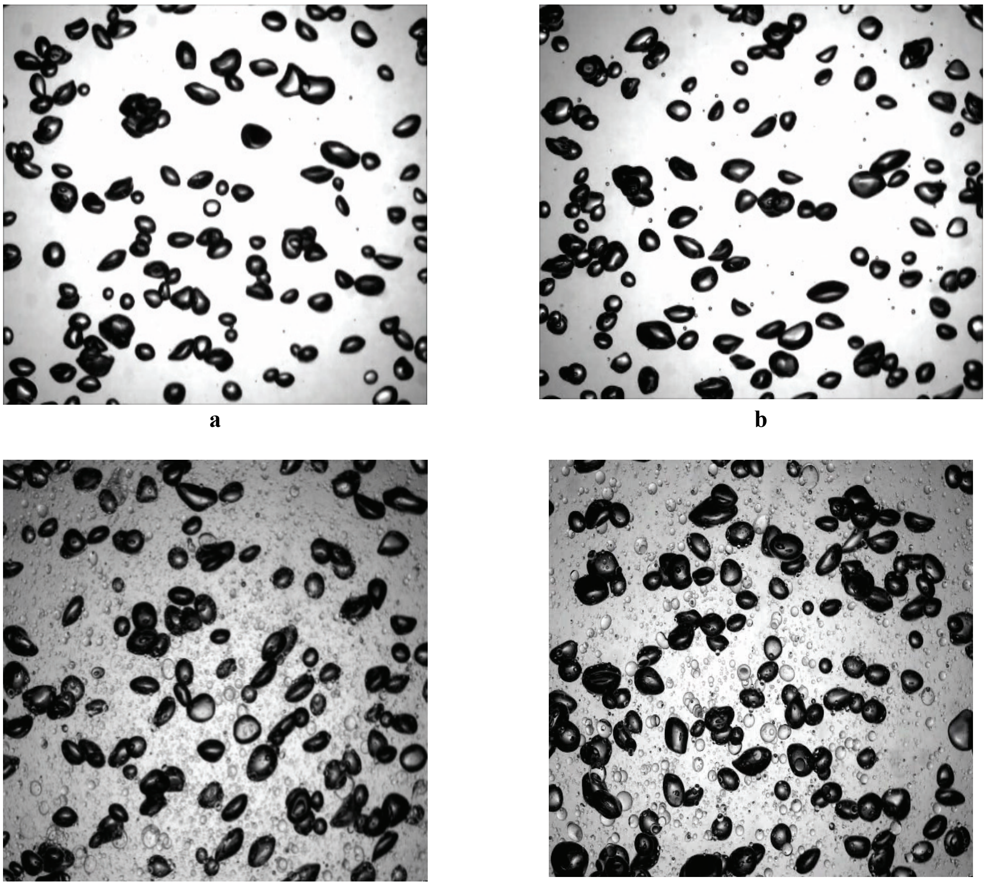


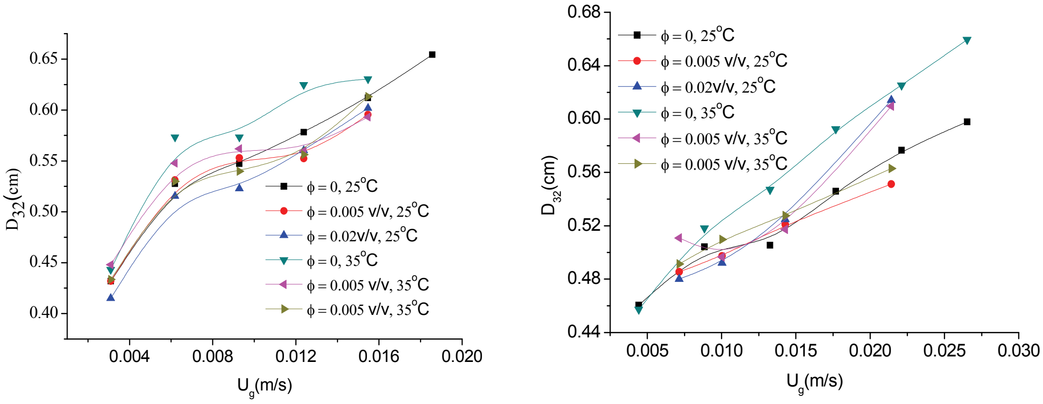
Fig. 6. Influence of U_g (air superficial velocity) on k_La for different φ (n-dodecane volumetric fraction) at 25˚C for air-lift bioreactor



a b

c d

Fig. 7. Air bubbles and n-dodecane drops from air-lift bioreactor (U_g=0.155 cm s^{-1}, without n-dodecane a- 25 ºC, b – 35ºC, 2% n- dodecane c- 25ºC, d – 35ºC)



a b

Fig. 8. Influence of U_g on D$_{32}$ for bubble column bioreactor (a) and air-lift bioreactor (b) at different volumetric fractions and different temperatures

3.3. Image analysis

Bubbles diameter and gas hold-up are two vital factors regarding mass transfer area available in the bioreactor. As it can be observed from Fig. 8, the Sauter mean diameter (D_{32}) increases with superficial gas velocity for all the range of considered data.

This can be explained by the increased bubble collision frequency due to the increased dispersion of small bubbles that leads to a higher coalescence rate and to a larger bubble diameter. This increase was even more visible at higher temperature.

The addition of n-dodecane to the system reduces bubble size; this can be attributed to the fact that the presence of the hydrocarbon molecules on the bubble surface can lead to a reduced tendency to coalescence, with a direct result on the bubbles size. Hence, this decrease can be attributed to the coalescence hindrance effect determined by the organic phase on the bubble surface.

For a better evaluation of hydrocarbons effect on oxygen mass transfer in this type of bioreactors, more experimental data are to be performed within a different study.

4. Conclusions

The objective of this paper was to present the utility of using n-dodecane as oxygen-vector in bubble column and air-lift bioreactors. For this purpose, the k_La value was determined at various aeration rates, with and without addition of n-dodecane and at three different temperatures. In the absence of the organic phase, the k_La values increased with the air flow rate to a maximum of 0.038 s^{-1} for 25ºC at a superficial gas velocity of 0.018 m s^{-1}. No clear pattern could be established when adding n-dodecane in the bubble column. However, k_La value was increased by almost 100% in the presence of low volumetric fraction of n-dodecane (φ=0.005 (v/v)) at 35˚C and by 5% at 25˚C for φ=0.02 (v/v). In the air-lift bioreactor, at 25˚C and φ=0.005 (v/v) the k_La value was enhanced by approximately 50%**.**

Therefore, the addition of n-dodecane can lead to an enhancement of oxygen mass transfer in pneumatically agitated bioreactors, at low volumetric fraction.

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