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DISTRIBUTION OF OXYGEN TRANSFER RATES IN STIRRED BIOREACTOR FOR DIFFERENT FERMENTATION BROTHS - OXYGEN-VECTOR DISPERSIONS

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Abstract

The oxygen transfer into the fermentation broths could be improved in presence of oxygen-vectors, without intensification of mixing or aeration. The experimental results for simulated, *P. shermanii* and *S. cerevisiae* broths indicated the significant increase of k_La, by adding *n*-dodecane, but the magnitude of this effect depends especially on the cells affinity for hydrocarbon droplets. Therefore, due to the higher affinity of yeasts cells for hydrocarbon droplets during their entire growth cycle, the increase of oxygen mass transfer rate was lower and the influence of specific power input was different than those recorded for simulated or bacterial broths. By means of the experimental data, mathematical correlations describing the influences of the main parameters on k_La have been proposed for each studied fermentation systems at different positions on the broths height, with an average deviations varying between ±6.72% and ±6.93%.

Key words: bioreactor, *n*-dodecane, oxygen transfer, oxygen-vector

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1. Introduction

The oxygen supply into the broths constitutes one of the decisive factors of aerobic microorganisms' cultures, playing an important role in the scale-up and economy of large-scale fermentation systems. The aeration efficiency depends on the bioreactor capacity to generate high rate of oxygen diffusion from air to the broths, as well as of its transfer through the liquid phase to the microorganisms. Therefore, one of the priorities in designing and operating the aerobic bioreactor is to ensure the optimum oxygen transfer from the gaseous phase to the microbial cells (Dumont et al., 2006).

The biosynthesis processes of single-cell protein on various water insoluble hydrocarbon substrates indicated that the addition of the nonaqueous organic phase may induce significant increase of oxygen transfer rate from air to microorganisms, without needing a supplementary intensification of mixing (Caşcaval et al., 2006; Clarke and Correia 2008; Dumont et al., 2006; Rols et al., 1990). Thus, the compounds which added to fermentation media improve the oxygen transfer towards the microorganisms have been defined as the oxygen-vectors (Caşcaval et al., 2006; Rols et al., 1990). The oxygen solubility in these compounds is for several times higher than in water, the main oxygen-vectors tested in biotechnology being hydrocarbons, perfluorocarbons, and oils used as antifoam agents (Caşcaval et al., 2006; Clarke et al., 2006; Clarke and Correia, 2008; Da Silva et al.,

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2008; Dumont et al., 2006; Gomez et al., 2006; Lee et al., 2005; Li et al., 2012; Pilarek and Szewczyk, 2008; Rols et al., 1990; Xu et al., 2007). Oxygen-vectors exhibit no toxicity against the cultivated microorganisms, and could be used as supplementary sources of carbon and energy.

The addition of oxygen-vectors induces the appearance of four phases in the bioreactor: the gas phase (air), the aqueous phase, the liquid organic phase, and the solid phase (biomass), as well as the formation of new interfacial areas between the gas and liquid phases (Clarke and Correia, 2008; Li et al., 2012). In these systems, the oxygen transfer could occur directly to the microbial cells, or through oxygen-vectors adsorbed or not to the bubble surface (Caşcaval et al., 2006; Clarke and Correia, 2008; Rols et al., 1990). Moreover, the cells could be adsorbed to bubbles surface or to organic phase droplets. Among the possible mechanisms of oxygen transfer proposed in literature, the most plausible assumes that the hydrocarbon is adsorbed to bubbles surface, with or without formation of a continuous film, the oxygen diffusion from the gaseous phase to microorganisms occurring through oxygen-vector and then through aqueous phase or directly to the cells adsorbed to hydrocarbon droplets or film surface (Caşcaval et al., 2006; Clarke and Correia, 2008; Da Silva et al., 2008; Dumont et al., 2006; Li et al., 2012; Rols et al., 1990). Because the previous results indicated that the main resistance to oxygen transfer is due to the diffusion through aqueous boundary layer from hydrocarbon - aqueous phase interface, the oxygen mass transfer coefficient corresponding to aqueous film, k_La, can also be used to describe the oxygen transfer in these systems (Caşcaval et al., 2006; Clarke and Correia, 2008; Galaction et al., 2005). This diffusional resistance can be counteracted both by increasing the interfacial area of oxygen transfer, especially by organic phase droplets adsorption to the bubble surface, and by accumulation of oxygen in organic phase, which acts as an oxygen reservoir (Caşcaval et al., 2006; Clarke et al., 2006; Dumont et al., 2006; Galaction et al., 2005; Rols et al., 1990).

Although the literature offers numerous correlations for k_La, there are still many queries concerning the accuracy of k_La predictions, owing to the strong influence of bioreactor geometry and operating variable range for which the proposed models are adequate, as well as to the experimental methods used for k_La determination (Montes et al., 1999; Van't Riet and Tramper, 1991). Moreover, the applicability of these correlations is limited to certain microorganisms' cultures only, quantifying the system behavior for a given region without indicating the distribution of oxygen transfer rate in the whole bulk volume of the broth. These limitations become more important in the case of aerobic fermentation systems containing oxygen-vectors.

The literature underlined the possibility to characterize the performances of stirred bioreactors by means of the computation fluid dynamics method (CFD), which has been previously applied for analyzing the distribution of the flow streams and velocity, gas hold-up, air bubble size, interfacial area, bubble coalescence or formation, and oxygen transfer rate in stirred vessels (Hjertager, 1998; Kerdouss et al., 2006; Kerdouss et al., 2008). However, although the oxygen transfer is directly related to the processes analyzed and modeled by CFD or other techniques, there is no information concerning the distribution of oxygen transfer rate inside the polyphasic media containing oxygen-vectors for stirred bioreactors with multiple impellers.

For this reason, the aim of these experiments is to analyze the distribution of the oxygen transfer rate in broths - oxygen-vector dispersions, for a stirred bioreactor and different fermentation broths without biomass (simulated broths) and with microorganisms (bacteria, *Propionibacterium shermanii*, and yeasts, *Saccharomyces cerevisiae*), using a large domain of operating variables. n-Dodecane has been used as oxygen-vector. For quantifying the effects of the considered factors (apparent viscosity, biomass concentration, affinity of different microorganisms for the hydrocarbon phase, concentration of *n*-dodecane, specific power input, and superficial air velocity) on k_La value and distribution on broth height, some mathematical correlations have been established for each considered position inside the bioreactor. The proposed equations could be useful for mass transfer optimization or scaling-up in fermentation systems containing hydrocarbons as oxygen-vectors.

2. Experimental

The experiments were carried out in 5 L (4 L working volume, ellipsoidal bottom) laboratory bioreactor (Biostat A, B. Braun Biotech International), with computer-controlled and recorded parameters. The bioreactor and impeller characteristics have been presented in the previous papers (Caşcaval et al., 2006).

The bioreactor mixing system consists of two Rushton turbine impellers and three baffles. The impeller diameter was of 64 mm. The inferior stirrer was placed at 64 mm from the bioreactor bottom. The superior stirrer was placed on the shaft at the optimum distance from the inferior one according to the type of the studied broth, namely at 128 mm for the simulated broths, respectively at 64 mm for the bacterial and yeasts broths, as it was demonstrated in the previous works (Caşcaval et al., 2007). The rotation speed was maintained below 600 rpm, this level of rotation speed avoiding the cavity formation at the broths surface.

The sparging system consists of a single ring sparger with 64 mm diameter, placed at 15 mm from the vessel bottom, having 14 holes with 1 mm diameter. The air volumetric flow rate was varied from 75 to 450 L h^{-1}, corresponding to the superficial air velocity of 0.84 - $5x10^{-3}$ m s^{-1}.

In the experiments, simulated and real broths have been used. The simulated broths were carboxymethylcellulose sodium salt solutions with pseudoplastic behavior and apparent viscosity in the domain of 10 - 96 cP. The following real broths have been studied:

- bacteria (*P. shermanii*), C_X being of 30.5 - 120.5 g d.w. l^{-1}, and apparent viscosity of 1.8 - 5.7 cP
- yeasts (*S. cerevisiae*), C_X being of 30 - 110 g d.w. l^{-1}, and apparent viscosity of 2.2 - 7.8 cP.

Owing to the difficulty of *in-situ* measurement of viscosity during the experiments, the viscosity was measured before and after each experiment using a rotary viscometer of Haake Viscometer 6 Plus type. Both the experiments and viscosity measurements were carried out at a temperature of 30°C. Any viscosity or morphology change was recorded during the experiments. *n*-Dodecane (SIGMA Chemie GmbH) was used as oxygen-vector (oxygen solubility 54.9 10^{-3} g L^{-1} at 35°C and atmospheric air pressure (Rols et al., 1990). Its maximum volumetric fraction into the broth was 0.20.

For k_La values determination the static method has been used (Caşcaval et al., 2006; Montes et al., 1999; Van't Riet and Tramper, 1991). This method has the advantages that it can be applied for different media (for establishing the effect of media components on oxygen mass transfer) and does not involve chemical reactions that could affect the measurement accuracy. According to this method, the values of k_La were calculated from the slopes of the straight lines plotted by means of the dependence $\ln \frac{C_l^*}{C_l^* - C_l}$ *vs.* time (Van't Riet and Tramper, 1991).

The solved oxygen concentrations in broth were measured using an oxygen electrode of InPro 6000 Series type (Mettler Toledo). As it was underlined in literature, because the k_La values were in all cases less than 0.1 s^{-1}, it was assumed that the response of the oxygen electrode to the change in the oxygen concentration is sufficiently fast and does not affect the determination accuracy (Montes et al., 1999; Ozbek and Gayik, 2001).

Because the static method is adequate for non-respiring systems only, the respiratory activity of microorganisms was inhibited by suspending the biomass in a solution of 0.2% pyrogallic acid and 0.4% potassium hydroxide for about 20 min. Then, the biomass was filtered, washed with distillated water and used for the above mentioned suspensions preparation (Caşcaval et al., 2006).

For analyzing the distribution of oxygen transfer rate inside the broth, the oxygen electrode was introduced at four different positions, placed vertically from bioreactor bottom as follows:

- position 1: at 20 mm
- position 2: at 70 mm
- position 3: at 120 mm
- position 4: at 170 mm.

The variations of dissolved oxygen concentration were recorded by the bioreactor computer-recorded system and were analyzed for calculating k_La. The mathematical correlations, which describe the influences of considered factors on k_La for the four positions inside the broths, were developed using MATLAB software. For the experimental data, a multiregression analysis was performed, the difference between the experimental and modeled value being reduced to the minimum by least-square fit method. By means of the MATLAB program, the regression coefficients and standard deviations were calculated.

Each experiment has been carried out for three times, for identical conditions, the average value of k_La being considered. The maximum experimental errors have varied between 3.72 and 5.91%.

3. Results and discussion

The increase of broths viscosity, mainly as the result of the biomass accumulation, reduces significantly the rate of oxygen mass transfer, due to the reduction of turbulence in the system. Moreover, the microbial cells exhibit significant effect on oxygen mass transfer, the biomass influence being the results of (Galaction et al., 2004; Ozbek and Gayik, 2001):

- modification of rheological characteristics of broths during fermentation process, especially by increasing of the apparent viscosity, effect that is less pronounced for bacterial cultures; besides the reduction of turbulence, the increase of viscosity leads to the perturbation of bubbles dispersion-coalescence equilibrium
- obstruction of mass transfer, due to both of the reduction of oxygen solubility and to the barrier effect created by cells adsorption to the air bubbles surface; however, the adsorbed solid particles can promote the surface renewal or direct consumption of oxygen with favorable effect on oxygen transfer rate.

The previous works indicated that the addition of hydrocarbons could increase the concentration of solved oxygen into the broths (Caşcaval et al., 2006; Clarke and Correia. 2008; Da Silva et al., 2008; Dumont et al., 2006; Rols et al., 1990). The effect of oxygen-vectors has to be related to the broths characteristics, especially due to the above presented effect of biomass presence and accumulation on oxygen transfer. Furthermore, as the result of the different affinity of cells for hydrocarbon droplets, the cells could be absorbed to hydrocarbons droplets surface and the formed cells-droplets associations could be furthermore adsorbed to air bubbles surface. The formation and stability of the cells-droplets-air bubbles associations depends on biomass characteristics, especially cells affinity for hydrocarbon phase, mixing intensity, hydrocarbons droplets size, and tensides presence in broth (Caşcaval et al., 2006; Clarke et al., 2006; Clarke and Correia, 2008; Dumont et al., 2006). Although in these systems the oxygen could be directly consumed

from air bubbles by microorganisms included in these associations, the barrier effect could become dominant, its relative importance depending on microorganisms' type and morphology.

Also in the fermentation systems containing hydrocarbons as oxygen-vectors, the bubbles dispersion-coalescence equilibrium is supplementary affected by the cells presence, which could amplify or hinder the coalescence process, depending on their concentration and morphological conformation (Li et al., 2012). Therefore, the appearance of small bubbles is promoted, this leading to the increase of the interfacial aria for oxygen mass transfer. However, owing to the high retention time of these small bubbles into the broth, the oxygen concentration gradient between the gaseous and aqueous phases, or between the gaseous and hydrocarbon phases, and, implicitly, the oxygen mass flow are reduced (Galaction et al., 2004; Montes et al., 1999; Ozbek and Gayik, 2001). These phenomena induce the heterogeneous distribution of air bubbles, of gas-liquid interfacial area and, implicitly, of oxygen transfer rate inside the broths.

For these reasons, the use of a single mathematical model or of an average value for k_La corresponding to a given aerobic fermentation system does not offer the required accuracy for quantifying the effects of oxygen-vectors for operating the bioreactor at optimum conditions. Consequently, by means of the experimental data, it is necessary to plot "the map" of the distribution of oxygen transfer rate inside the microbial broths containing hydrocarbons.

4.1. Simulated broths

Although the shapes of the recorded curves are similar, Fig. 1 indicates different correlations between k_La and *n*-dodecane concentration depending on the broth apparent viscosity, specific power input, and position inside the bioreactor.

Therefore, regardless of the value of apparent viscosity of simulated broths, k_La is increased by increasing the volumetric fraction of oxygen-vector, but this effect has to be correlated with the viscosity of broths. At low apparent viscosity, 15 cP, two domains of k_La variation can be observed from Fig. 1.

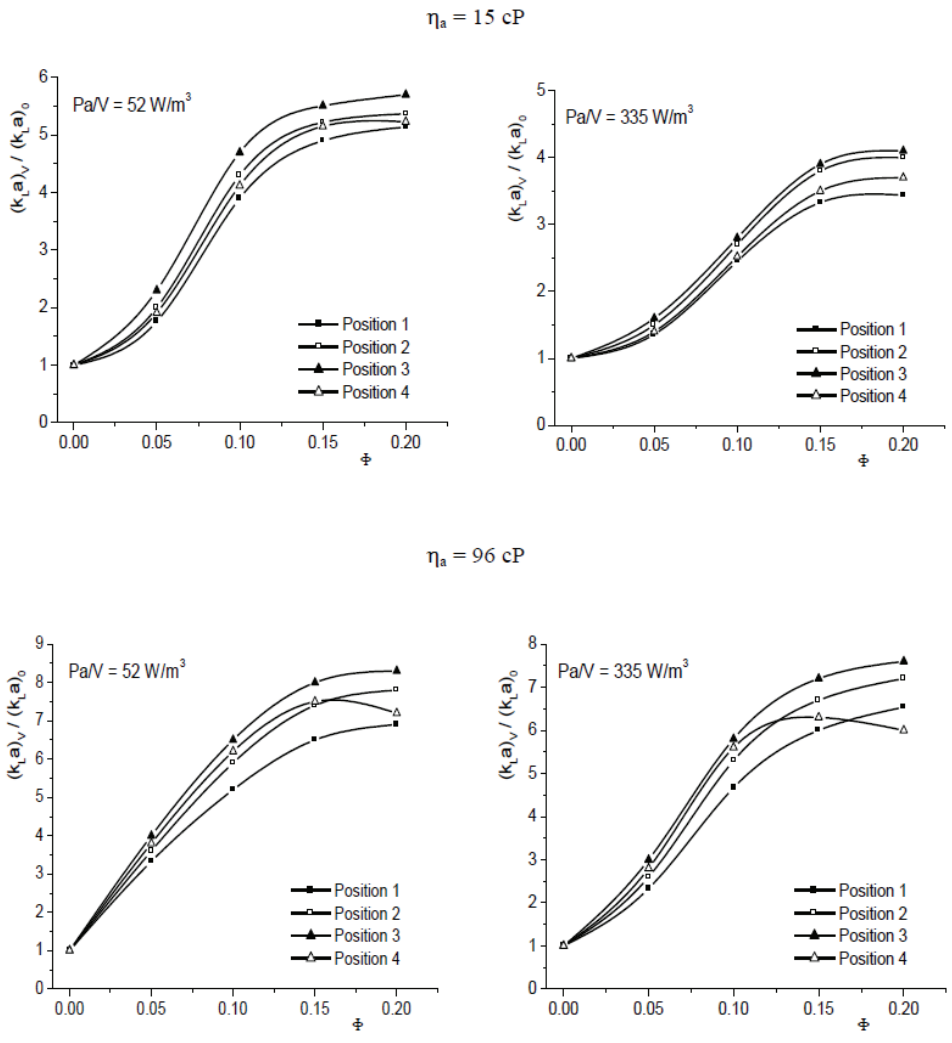

Fig. 1. Influence of *n*-dodecane concentration on oxygen mass transfer coefficient for different apparent viscosities of simulated broths and specific power inputs (v_S = 8.4x10^{-4} m s^{-1})

For oxygen-vector concentration up to Φ = 0.1 and the closest positions to the two impellers, corresponding to the bottom and top of the simulated broths, k_La initially increases with the increase of oxygen-vector concentration, reaching then a rather constant value. This dependence between the oxygen transfer rate and *n*-dodecane concentration is similar to those recorded for the intermediary positions of the oxygen electrode, but the value of volumetric fraction of oxygen-vector related to the constant level of k_La decreases from 0.15 to 0.10 by intensifying the mixing. For all considered positions inside the bioreactor, the analyzed variations are the consequence of the important influence of air - *n*-dodecane and *n*-dodecane - aqueous phase interfacial areas on oxygen transfer rate. The average size of hydrocarbon droplets is reduced by mixing intensification and, implicitly, the interfacial areas are strongly increased. Furthermore, the small droplets of hydrocarbon can be assimilated as rigid spheres which interact with the aqueous film surrounding the bubbles, amplifying the turbulence in this region and increasing supplementary the oxygen diffusion rate (Caşcaval et al., 2006; Clarke et al., 2006). The magnitude of these phenomena becomes more important in the regions with the highest turbulence, namely at the positions 1 and 4.

For more viscous broths, k_La increases continuously for the entire variation domain of *n*-dodecane concentration. Moreover, at higher specific power input and *n*-dodecane volumetric fraction over 0.05, the values of oxygen transfer rate recorded for position 2 exceeds those corresponding to position 4. This variation could be the result of the modification of the viscous broth flow, implicitly of the turbulence extent, in presence of air bubbles and hydrocarbon droplets in the region with intense mixing. Thus, at low rotation speed, the contribution of pneumatic mixing to the circulation of dispersion is important, the increase of rotation speed intensifying supplementary the broth agitation into the bioreactor. At higher rotation speed, the bubbles retention time increases, the gas-liquid dispersion or the two liquid phases flow becomes more complex and its circulation velocity is lower than that of the flow streams promoted by mechanical mixing in non-aerated media or in the regions placed between the impellers. Moreover, the retention of air diminishes the oxygen concentration gradient between the gaseous and liquid phases, this phenomenon affecting additionally the mass transfer.

The decisive influence of media hydrodynamics on oxygen transfer rate is underlined by the results obtained previously for the mixing time distribution into fermentation broths (Caşcaval et al., 2007). According to these studies, the maximum efficiency of mixing is reached for specific power input values close to those corresponding to maximum k_La, and the distribution of mixing intensity on the broth height is similar to that of oxygen transfer rate (Caşcaval et al., 2011). For higher apparent viscosity, the flow streams velocity is gradually reduced towards the bioreactor top, due to the diminution of the turbulence degree and to the increase of the distance from the sparger.

Compared to the system without oxygen-vectors, analyzed at similar experimental conditions, the addition of *n*-dodecane leads to the enhancement for several times of oxygen transfer rate. This intensification of mass transfer can be described by means of the *amplification factor*, which was defined as the ration between k_La in presence of oxygen-vector, $(k_La)_V$, and in absence of oxygen-vector, $(k_La)_0$, for similar experimental conditions (Galaction et al., 2004).

From Fig. 2 it can be seen that the positive effect of *n*-dodecane addition depends on the broth viscosity, mixing intensity, and position inside the bioreactor. For all cases, the reduction of apparent viscosity and the increase of specific power input lead to the diminution of the magnitude of hydrocarbon effect on oxygen mass transfer coefficient, due to the favorable influence of turbulence on oxygen solubilization and mass transfer. Thus, by varying the amount of dissipated energy by mixing from 52 to 335 W m^{-3}, the maximum value of amplification factor was reduced from 5.1 - 5.7 to 3.4 - 4.1 for simulated broths having the apparent viscosity of 15 cP, respectively from 6.9 - 8.3 to 6 - 7.6 for simulated broths with 96 cP apparent viscosity.

This reduction of $(k_La)_V/(k_La)_0$ ratio could be attributed to the two phenomena that occur with mixing intensification. On the one hand, even in absence of oxygen-vector, the enhancement of turbulence generates the increase of interfacial area between the gas and liquid phases and, consequently, high oxygen transfer rate (Galaction et al., 2004). On the other hand, according to the mechanism assumed for oxygen transfer in presence of hydrocarbons, an important role on oxygen diffusion is attributed to adsorption of hydrocarbon droplets to bubbles surface, with or without coalescing with superficial film formation (Rols et al., 1990). Although the volumetric fractions of air and oxygen-vector are generally in the same order of magnitude, the average bubble diameter is significant higher than that of the hydrocarbon droplets (literature indicates that the average value of bubble diameter is for 100 times that of the oxygen-vector droplets (Rols et al., 1990). Therefore, a large number of hydrocarbon droplets is adsorbed on bubble surface and coalesce forming a continuous film of oxygen-vector. The hydrodynamic instabilities, induced by turbulence increase and by collisions between bubbles and hydrocarbon droplets, lead to the disruption of oxygen-vector superficial film or to the removal of the oxygen-vector droplets from the bubble surface. Moreover, the increase of apparent viscosity cumulated with mixing intensification determines the increase of time needed for coalescence of the hydrocarbon droplets adsorbed to the bubble surface. In both cases, the

covering degree of the bubble surface by hydrocarbon droplets decreases, thus reducing the oxygen diffusion rate from air to aqueous phase.

Regardless of the apparent viscosity and specific power input, the analysis of amplification factor distribution inside the broth suggests that its highest values are reached for the intermediary positions, the *n*-dodecane addition counteracting the lowest turbulence corresponding to these regions. Obviously, for the regions with high turbulence, namely positions 1 and 4, the positive effect of oxygen-vector is diminished, the lowest amplification factor being recorded either for position 1 in less viscous broths, or for position 4 in viscous ones (Fig. 2).

As it was above discussed, for apparent viscosity of 96 cP, due to the more complex hydrodynamics of broth related to position 4, the effect of *n*-dodecane on k_La becomes the least important in this region, especially at hydrocarbon volumetric fraction over 0.10. These results underline once again the major role of the *n*-dodecane droplets size which controls the interfacial areas, according to the mechanism proposed for oxygen transfer in presence of oxygen-vectors.

Similar to the aerobic fermentation systems without oxygen-vectors, the increase of apparent viscosity of broth leads to the significant decrease of k_La, this effect being attenuated by increasing the hydrocarbon concentration, due to the favorable effect on oxygen solubilization and to the acceleration of diffusion. The apparent viscosity influences also the value and distribution inside the broth of the amplification factor, its effect depending mainly on the mixing intensity (Fig. 3).

Thus, for a specific power input of 52 W m^{-3}, by increasing the apparent viscosity from 1 to 96 cP, the amplification factor is reduced to a minimum value, corresponding to 15 cP, then increasing continuously. In this case, due to the less intense mixing and, consequently, lower turbulence, the negative influence of viscosity on amplification factor is more pronounced compared to systems containing water (1 cP).

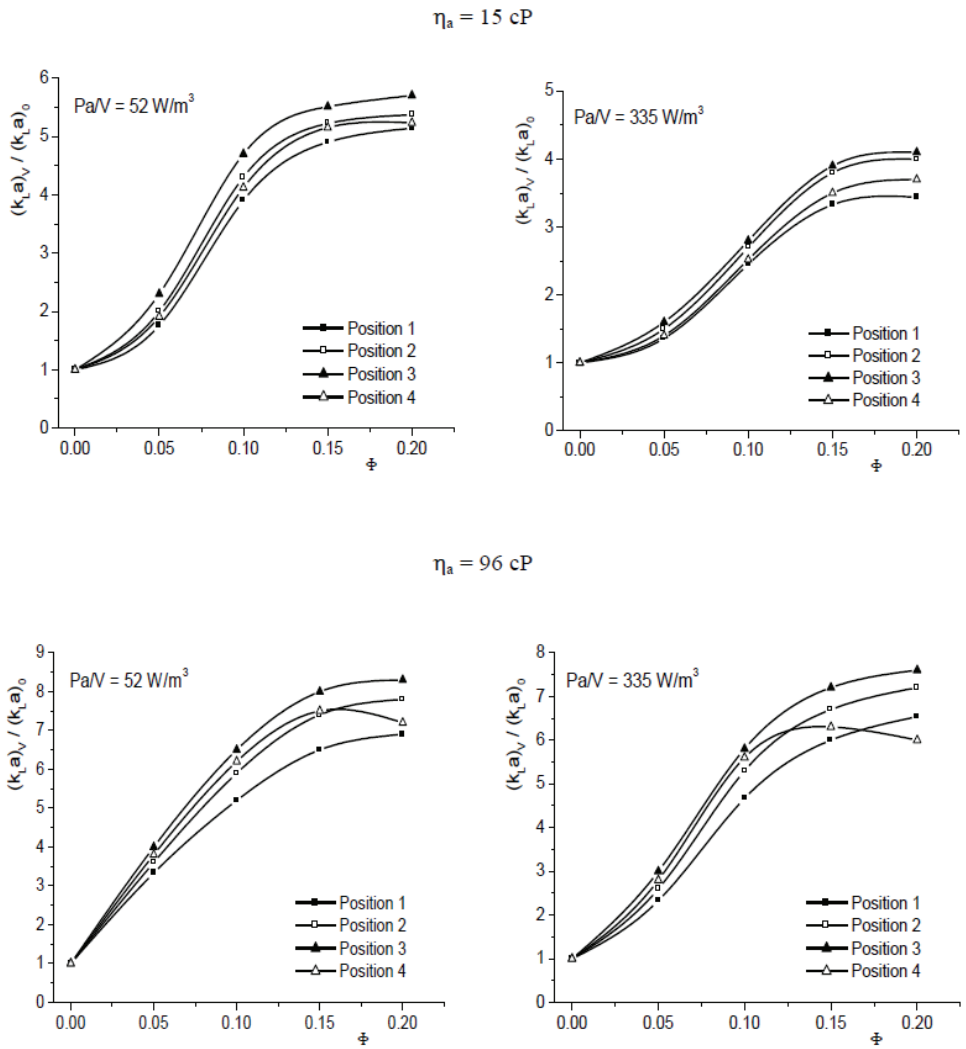

Fig. 2. Influence of *n*-dodecane concentration on amplification factor for different apparent viscosities of simulated broths and specific power inputs (v_S = 8.4x10^{-4} m s^{-1}, Φ = 0.20)

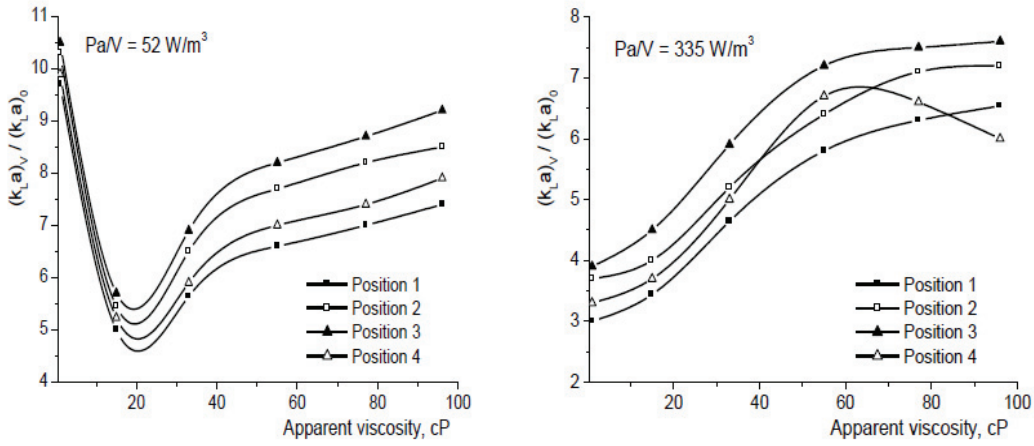

Fig. 3. Influence of apparent viscosity on amplification factor for different specific power inputs ($v_S = 8.4x10^{-4}$ m s^{-1}, $\Phi = 0.20$)

Due to the increase of the interfacial area between the gaseous and liquid phases in presence of *n*-dodecane, the magnitude of the positive influence of oxygen-vector becomes more important by increasing the apparent viscosity of the broths, but the values of $(k_La)_V/(k_La)_0$ ratio for the four positions remain below those obtained for water (Fig. 3).

Because the intensification of mixing induces the fine dispersion of gaseous and *n*-dodecane phases, with favorable effect on oxygen transfer rate, the increase of energy dissipated by mechanical agitation in more viscous media leads to the continuous increasing of amplification factor (Fig. 3). This variation is valid for the entire bulk volume of the broth, excepting the top region. Although at lower specific power input, the variation of oxygen transfer rate obtained for position 4 is similar to those recorded for the other considered positions, at higher mixing intensity and apparent viscosity over 50 cP, the amplification factor for position 4 is reduced and becomes the lowest one. This particular variation is the result of the apparent viscosity influence on the turbulence extent and interfacial area value. Thus, for low-viscous broths, Newtonian broths or for media containing electrolytes, tensides, polymeric compounds, the bubbles coalescence is avoided, the average bubbles size being small.

For more viscous broths or exhibiting non-Newtonian behavior, the equilibrium between the dispersion and the coalescence of bubbles is perturbed, large size bubbles are formed, this phenomenon inducing the decrease of gas-liquid interfacial area and the heterogeneous distribution of air into the broth (Mattiasson and Adlercreutz, 1987). In this case, the bubbles coalescence occurs especially around the impeller placed closer to the sparger, namely that related to position 1. Due to the air accumulation around the inferior impeller and to the nonuniform rising of larger bubbles, the bubbles distribution in region 4 is heterogeneous, and the volumetric fraction of air is low. Consequently, although the second impeller is placed in this region, the positive effect of *n*-dodecane addition is reduced and the amplification factor values recorded for position 4 become inferior to those for the other three positions.

The increase of aeration rate promotes the intensification of turbulence, the homogeneous distribution of air into the broth and the increase of oxygen concentration gradient between gaseous phase and media, thus exhibiting a favorable effect on oxygen mass transfer. As in the case of effect of energy dissipated by mechanical mixing, the intensification of aeration leads to the increase of k_La, even in absence of *n*-dodecane. Besides the increase of air - broth interfacial area, the increase of aeration rate induces the amplification of turbulence with similar effects to those of the intensification of mechanical agitation. Furthermore, by increasing the aeration, the air volumetric fraction in broth increases, thus diminishing the covering degree of bubbles surface by hydrocarbon droplets. This phenomena is more pronounced for high viscous broths, owing to the high bubbles retention time into the broth and, consequently, to the increase of air volumetric fraction.

4.2. Propionibacterium shermanii broths

P. shermanii is the main aerobic bacteria producer of vitamin B12 at industrial scale. Although the effects of cells on rheological characteristics of the broth and oxygen transfer rate are less significant compared to the other microorganisms types, they cannot be neglected. Moreover, the affinity of cells for the hydrocarbon droplets represents an important factor controlling the oxygen transfer in fermentation system containing oxygen-vectors.

Generally, from Fig. 4 it can be observed for all positions that the oxygen transfer rate increases with the increase of *n*-dodecane concentration and specific power input. However, the shapes of the plotted dependences between k_La and volumetric fraction of oxygen-vector are changed by increasing the biomass concentration or mixing intensity.

At low bacterial cells concentration and low specific power input, the increase of *n*-dodecane concentration inside the *P. shermanii* broth leads to the continuous improvement of oxygen transfer. At higher rotation speed, the contribution of the mechanical agitation to the oxygen transfer becomes more important and compensates the negative effect of bubbles surface blockage, by redistributing the adsorbed cells and renewing the gas-liquid interface. Consequently, at higher mixing intensity, the value of oxygen mass transfer coefficient remains at a rather constant level for *n*-dodecane volumetric fraction over 0.15, regardless of the considered position inside the bioreactor.

The bacterial cells accumulation exhibits a negative influence on k_La, especially due to the increase of apparent viscosity and of extent of the bubbles blocking effect by cells adsorption (Galaction et al., 2004). Moreover, Fig. 4 indicates that by increasing the oxygen-vector amount inside the broth, k_La increases, reaches a maximum value, then remaining at a constant level. The minimum value of *n*-dodecane volumetric fraction corresponding to the constant level of oxygen mass transfer rate is reduced from 0.15 to 0.10 by intensifying the mixing from 110 to 440 Wm^{-3}. This behavior of the system is the result of the modification of bacterial cells affinity for the hydrocarbon droplets during the growth cycle of *P. shermanii* in direct relation with its biomass concentration in the broth (Caşcaval et al., 2006; Rols et al., 1990). According to the previous studies, unlike the simulated broths without biomass, the bacterial cells are adsorbed on oxygen-vector droplets surface, this phenomenon diminishing the favorable effect of oxygen-vector addition (Caşcaval et al., 2006).

However, as it was reported in literature, the bacteria cells are known to be hydrophobic only at the beginning of their growth, at lower biomass concentrations, the microorganisms growth and accumulation inducing their desorption from droplets surface and dispersion into aqueous phase (Rols et al., 1990). In these circumstances, the coverage degree of *n*-dodecane droplets surface by cells decreases, the droplets are adsorbed on air bubbles surface, with or without the formation of a continuous film, thus avoiding the blockage of the bubbles free surface by cells. Consequently, the magnitude of the effect of oxygen-vector becomes more important than in fermentation broths with lower bacterial cells concentration, and, implicitly, the *n*-dodecane amount required for reaching the maximum value of k_La is reduced.

The above results are confirming by the dependences between the amplification factor and *P. shermanii* cells concentration plotted in Fig. 5. For both considered specific power inputs, regardless of the positions inside the broth, the bacterial cells accumulation leads initially to the reduction of $(k_La)_V/(k_La)_0$ ratio, which reaches a minimum value followed by its significant increase at higher biomass concentration. As it was above mentioned, the initial reduction of amplification factor is the result of the higher affinity of *P. shermanii* cells for the hydrocarbon droplets, especially at lower biomass concentration. For the studied fermentation system, the cells adsorption to the hydrocarbon droplets surface is more pronounced for cells concentration up to 10 - 30 g d.w. l^{-1}, depending on the mixing intensity. Therefore, the induced barrier effect hinders the oxygen transfer from the *n*-dodecane phase to the aqueous one, and reduces the amplification factor compared to the simulated broths without biomass. The growth of bacteria and, implicitly, the increase of the biomass amount in the broths, induce the cells desorption from the oxygen-vector droplets surface, thus exhibiting positive effect on oxygen transfer. The desorption process becomes more significant at higher bacterial cells concentration, being accelerated by increasing the specific power input. For this reason, the value of *P. shermanii* cells concentration related to the minimum $(k_La)_V/(k_La)_0$ ratio decreases by intensifying the mixing.

Furthermore, due to the nonuniform distribution on the broth height both of the biomass concentration, as the result of cells deposition at the bioreactor bottom, and of the mixing intensity, which is lower in the intermediary positions 2 and 3, the minimum values of amplification factor recorded for the positions 2 and 3 are below those for positions 1 and 4. For the same reasons, the $(k_La)_V/(k_La)_0$ ratio corresponding to the top position for biomass concentration over 96 g d.w. l^{-1} and specific power input of 440 W m^{-3} reaches a value inferior to those recorded for the other three positions.

The increase of specific power input induces the fine dispersion of *n*-dodecane with favorable effect on oxygen transfer rate. From Fig. 6 it can be observed that the positive effect of mixing intensification is recorded only for the values of specific power input up to 240 Wm^{-3}, the further increase of energy dissipated by mechanical agitation leading to the reduction of k_La.

The mechanism responsible for this variation is the same as in the case of simulated broths. However, for bacterial suspensions, the coverage of the bubbles surface by hydrocarbon droplets is partially hindered by mixing intensification and the free bubbles surface could be occupied by *P. shermanii* cells absorption, the oxygen diffusion rate from air to aqueous phase *via* hydrocarbon phase being reduced. Due to the above discussed aspects, this phenomenon is more pronounced for lower biomass concentration and specific power input over 240 Wm^{-3}. For this reason, the maximum value of the graphical dependence between k_La and the energy dissipated by mechanical agitation is not recorded for concentrated bacterial cell broths, the influence of specific power input over 240 Wm^{-3} on oxygen mass transfer rate becoming insignificant in these systems.

Consequently, the increase of power consumption for mechanical mixing induces the

continuous reduction of $(k_La)_V/(k_La)_0$ ratio, effect that is more pronounced for lower volumetric fractions of *n*-dodecane. The influence of superficial air velocity on amplification factor is similar to that of mixing intensity. By increasing the aeration rate, the supplementary induced turbulence promotes the diminution of the covering degree of bubbles surface by hydrocarbon droplets. In the case of *P. shermanii* broths, this phenomenon becomes more important at intense mixing for biomass concentration over 30 g d.w. l^{-1}.

Thus, due to the cumulated effect of mechanical and pneumatic mixing on the turbulence in the studied systems, by increasing the superficial air velocity from $8.4x10^{-4}$ to $5x10^{-3}$ m s^{-1} the amplification factor was reduced for about 1.5 - 2.5 times (the effect was more pronounced at higher level of specific power input).

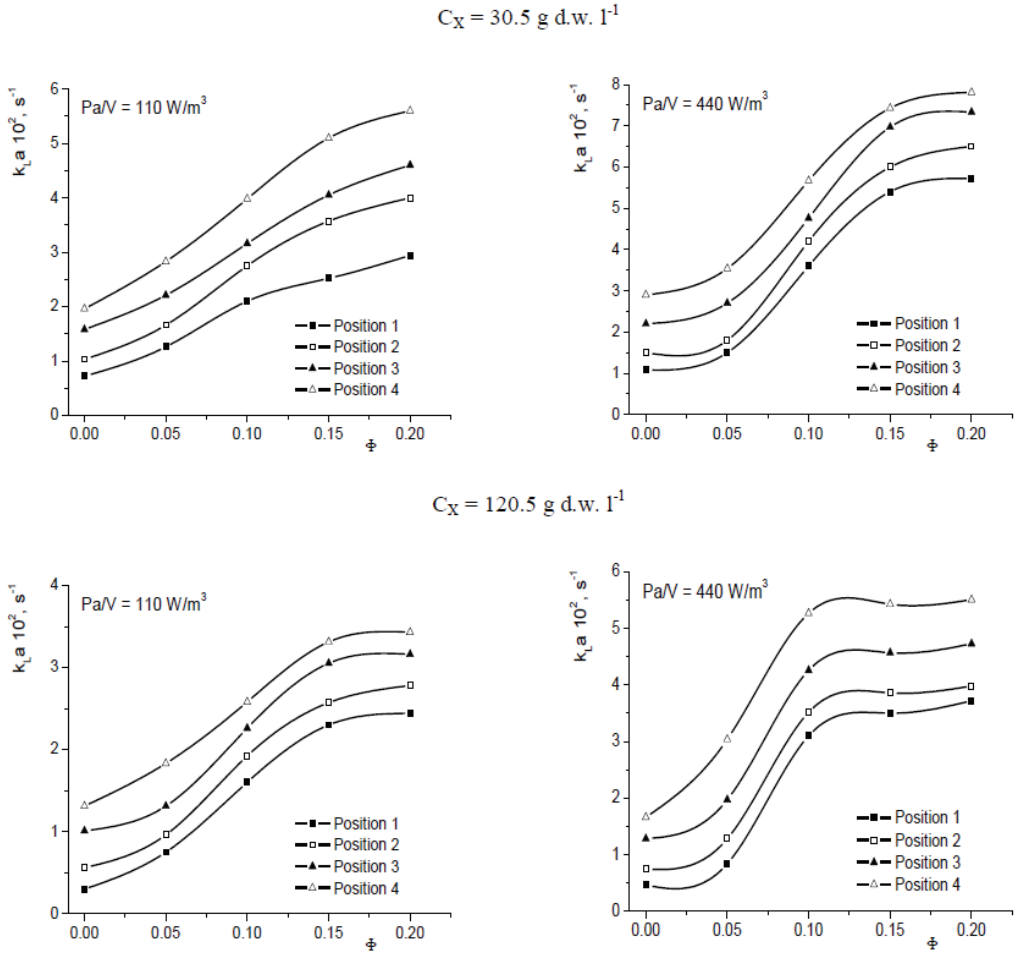

Fig. 4. Influence of *n*-dodecane concentration on oxygen mass transfer coefficient for different bacterial cells concentrations and specific power inputs ($v_S = 8.4x10^{-4}$ m s^{-1})

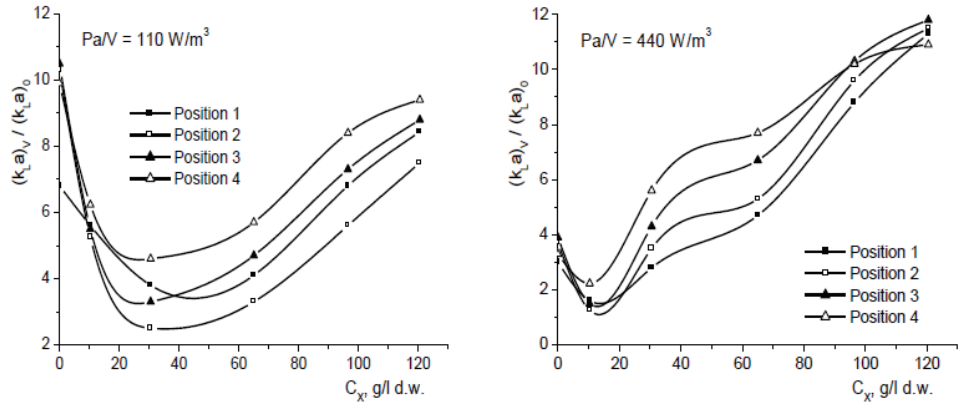

Fig. 5. Influence of bacterial cells concentration on amplification factor for different specific power inputs ($v_S = 8.4x10^{-4}$ m s^{-1}, $\Phi = 0.15$)

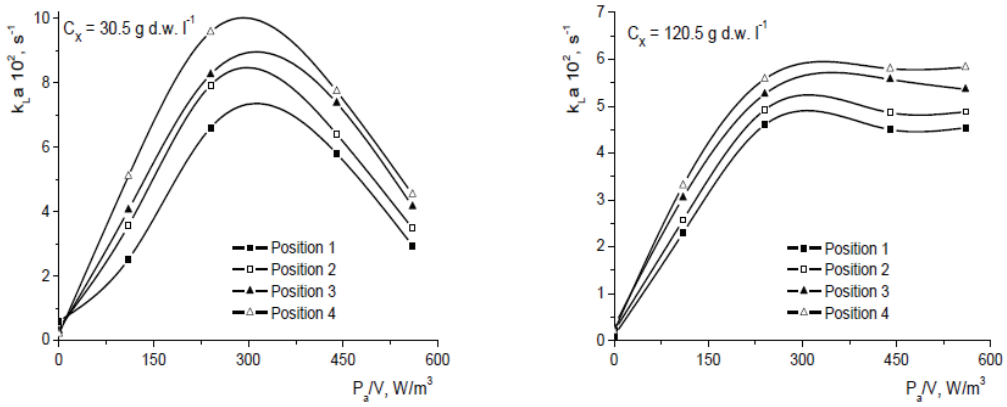

Fig. 6. Influence of specific power input on oxygen mass transfer coefficient for different bacterial cells concentrations ($v_S = 8.4x10^{-4}$ m s^{-1}, $\Phi = 0.15$)

4.3. Saccharomyces cerevisiae broths

In absence of *n*-dodecane, due to the closer viscosities of the bacterial and yeasts broths at similar biomass concentrations, the variations of k_La with bioreactor operating parameters, as well as the phenomena inducing these variations for *S. cerevisiae* cultures are similar to those recorded for *P. shermanii* ones (Galaction et al., 2004). However, the higher affinity for hydrocarbon phase of yeasts induces different behavior compared to that of bacteria, the yeasts cells being adsorbed to oxygen-vectors droplets during the entire fermentation cycle (Mimura et al., 1971; Rols et al., 1990).

Generally, from Fig. 7 it can be observed that the values of oxygen transfer rate in yeasts broths containing oxygen-vectors are lower than those recorded in bacterial broths, for identical experimental conditions. However, although the increase of *n*-dodecane concentration leads to the increase of k_La, the affinity of yeasts cells for oxygen-vector droplets and the mixing intensity control the shape of the dependence between the oxygen mass transfer rate and hydrocarbon volumetric fraction (Fig. 7). At lower biomass concentration (30 g d.w. l^{-1}) and less intense mixing, k_La is slowly enhanced by increasing the oxygen-vector concentration up to 10% vol., due to the yeasts cells adsorption to the hydrocarbon droplets surface. The further increase of the *n*-dodecane amount inside the broths partially counteracts the cells adsorption phenomenon, the oxygen mass transfer being accelerated for Φ over 0.10.

The mixing intensification induces the increase of turbulence and, implicitly, the cells desorption from the hydrocarbon droplets surface, generating an important positive effect on k_La for volumetric fraction of oxygen vector up to 0.10. For the same reasons as in the above discussed systems of bacterial fermentation, the supplementary addition of *n*-dodecane leads to the slower acceleration of oxygen transfer from the hydrocarbon to aqueous phase.

At higher *S. cerevisiae* concentration (110 g d.w. l^{-1}), its cells affinity for hydrocarbon phase exhibits a stronger negative effect. Thus, according to Fig. 7, for specific power input of 110 Wm^{-3}, the oxygen-vector effect on k_La is less important for its volumetric fraction below 0.10, becoming significant at higher concentrations. The increase of specific power input induces effects comparable to the case of lower biomass concentration.

As the result of the distribution of mixing intensity and biomass concentration on the broth height (Caşcaval et al., 2011), the highest values of oxygen mass transfer coefficient are recorded for positions 4 and 1, respectively, while the lowest ones correspond to the intermediary positions 2 and 3.

Similar to the oxygen mass transfer inside the bacterial broths, the yeasts concentration influences the k_La mainly by means of the broth apparent viscosity, and additionally by cells adsorption on the *n*-dodecane droplets surface. However, contrary to the effect of *P. shermanii* concentration, the strongest reduction of oxygen mass transfer coefficient with *S. cerevisiae* biomass accumulation from 30 to 110 g d.w. l^{-1} was recorded for higher amounts of *n*-dodecane (for ϕ = 0.20, k_La was reduced for about 1.15 to 1.3 times at specific power input of 110 W m^{-3}, and for about 1.2 to 1.33 times at 440 W m^{-3}, the most important reduction corresponding to positions 1 and 4). For the same experimental conditions, but without *n*-dodecane, k_La was reduced for 1.3 to 3.2 times by increasing the yeasts concentration in the mentioned domain. In this case, the most important diminution of the oxygen transfer rate was recorded for the intermediary positions.

The variation of amplification factor during the yeasts accumulation inside the broth is completely different from that previously observed for bacteria suspensions. Therefore, in the case of *S. cerevisiae* suspensions, $(k_La)_V/(k_La)_0$ ratio reaches a maximum value, decreasing then (Fig. 8). This variation is the consequence of two opposite phenomena induced by increasing the biomass concentration. On the one hand, the addition of *n*-

dodecane leads to the intensification of oxygen transfer from gaseous phase to the broth, effect which is more important in the regions with higher mixing intensity, namely positions 1 and 4. On the other hand, due to the affinity of yeasts cells for the hydrocarbons droplets, the biomass is adsorbed to the droplets surface and blocks the interface of oxygen transfer from aqueous to organic phase. Moreover, the cells-droplets associations could be adsorbed to the air bubbles surface, inducing an additional resistance to the oxygen diffusion.

On the basis of these considerations, according to Fig. 8, the maximum value of amplification factor becomes more obvious for the intermediary positions 2 and 3, due to the related poor broth circulation intensity (Caşcaval et al., 2007). Furthermore, the maximum values of $(k_La)_V/(k_La)_0$ ratio corresponding to positions 2 and 3 is recorded for lower biomass concentration compared to those related to positions 1 and 4, also as a result of lower intensity of mixing in the intermediary positions cumulated with the affinity of yeasts cells for hydrocarbon.

The influence of mixing intensity has to be correlated with the stronger affinity of yeasts cells for the hydrocarbon phase. As it can be observed from Fig. 9, the variation of oxygen transfer rate with the specific power input depends on the *S. cerevisiae* concentration and position inside the broth.

Regardless of the biomass concentration, the mixing intensification induces initially the enhancement of oxygen transfer. However, depending on the *S. cerevisiae* cells concentration and position inside the broth, the oxygen transfer rate decreases or increases slowly over a certain value of specific power input. Therefore, for less concentrated suspensions of *S. cerevisiae*, the oxygen transfer rate is continuously increased for positions 1 and 4 by intensifying the mixing, but this variation becomes less pronounced for specific power input over 240 W m^{-3}. In the case of the same suspensions of yeasts, the maximum value of k_La is reached at specific power input of 440 Wm^{-3}. For higher amount of yeast cells, the maximum k_La is reached at 440 Wm^{-3} for positions 1 and 4, while for the intermediary positions the value of specific power input corresponding to the maximum k_La is reduced to 240 Wm^{-3}. As it was above discussed, the intensification of mixing induces the fine dispersion of air and of *n*-dodecane, with the increase of interfacial area, as well as the disruption of cells-droplets and cells-droplets-air bubbles associations.

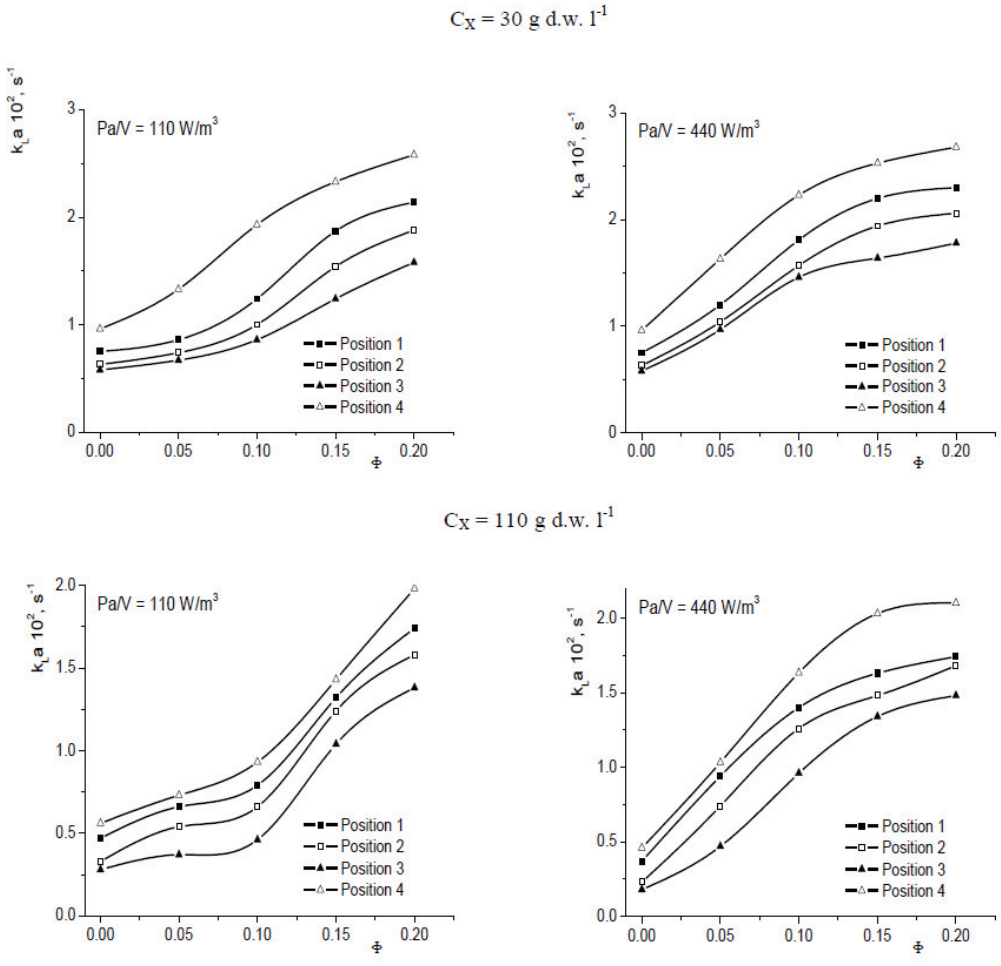

Fig. 7. Influence of *n*-dodecane concentration on oxygen mass transfer coefficient for different yeasts cells concentrations and specific power inputs ($v_S = 8.4x10^{-4}$ m s^{-1})

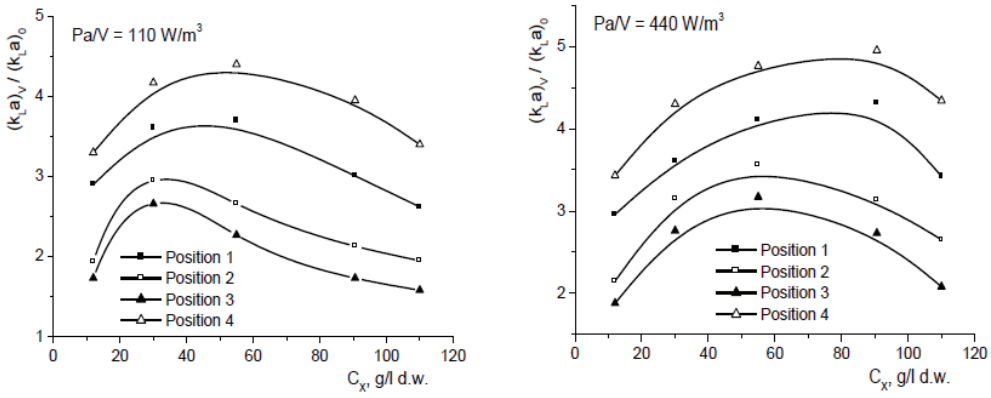

Fig. 8. Influence of yeasts cells concentration on amplification factor for different specific power inputs (v_S = 8.4x10^{-4} m s^{-1}, Φ = 0.15)

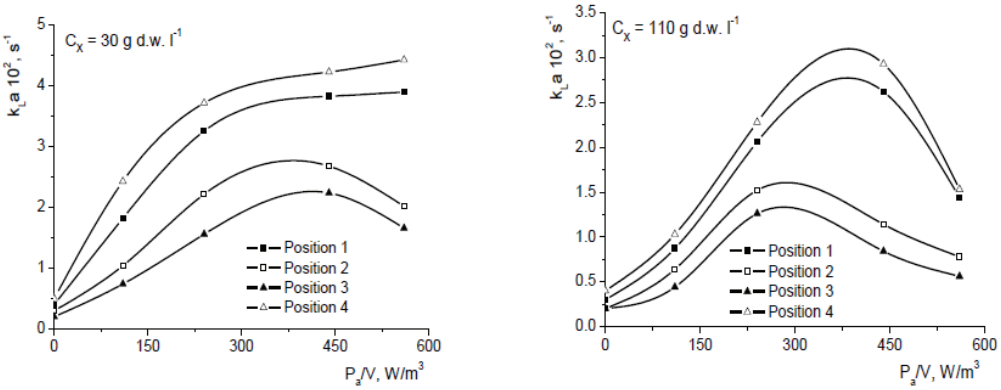

Fig. 9. Influence of specific power input on oxygen mass transfer coefficient for different yeast cells concentrations (v_S = 8.4x10^{-4} m s^{-1}, Φ = 0.15)

Both phenomena induce initially a positive effect on oxygen transfer rate. In the same time, due to the stronger affinity of yeast cells for the hydrocarbon droplets compared to that of bacterial ones, the surface of the finely dispersed bubbles and droplets can be easily occupied by *S. cerevisiae* cells adsorption, thus amplifying the resistance to the oxygen mass transfer and, consequently, reducing k_La. The negative effect of surface blockage is more important at higher biomass concentration and leads to the reduction of specific power input related to the maximum value of oxygen transfer rate for all considered positions in the bioreactor.

Although the intensification of aeration induces similar effect as the increase of specific power input, it also leads to the increase of the air volumetric fraction in broth and to the diminution of the covering degree of bubbles surface by hydrocarbon droplets. Consequently, the amplification factor is reduced. Owing to these opposite effects, the influence of aeration rate on amplification factor for *S. cerevisiae* suspensions is different to that recorded for simulated and bacterial broths. In this case, the maximum value of $(k_La)_V/(k_La)_0$ ratio is reached for the superficial air velocity of 3.35x10^{-3} m s^{-1} and decreases from 4.4 to 2.2 with *S. cerevisiae* biomass accumulation from 30 to 110 g d.w. l^{-1}.

4.4. Correlations for oxygen transfer

By means of the experimental data obtained for the studied simulated and real broths, mathematical correlations describing the influence of oxygen-vector concentration, apparent viscosity or biomass concentration, specific power input and air superficial velocity on k_La have been established for the four positions inside the broths. Depending on the studied system, two general expressions have been considered (Eqs. 1-2).

$$k_La = \alpha \cdot \eta_a^{\beta} \cdot \left(\frac{P_a}{V}\right)^{\gamma} \cdot v_S^{\delta} \quad (1)$$

for simulated broths, or

$$k_La = \alpha \cdot C_X^{\beta} \cdot \left(\frac{P_a}{V}\right)^{\gamma} \cdot v_S^{\delta} \quad (2)$$

for bacterial and yeasts broths. The coefficients β, γ and δ values are function on *n*-dodecane concentration.

The values of α, β, γ and δ coefficients were calculated by the multiregression method using MATLAB software. Thus, the following correlations have been obtained:

a. simulated broths (Eqs. 3-6):

• Position 1

$$k_L a = 1.18 \cdot \left[\frac{\eta_a^{1.27}}{v_S^{2.12} \cdot \left(\frac{P_a}{V} \right)^{3.11}} \right]^{\phi}, \text{ s}^{-1} \quad (3)$$

• Position 2

$$k_L a = 1.12 \cdot \left[\frac{\eta_a^{3.63}}{v_S^{0.85} \cdot \left(\frac{P_a}{V} \right)^{2.35}} \right]^{\phi}, \text{ s}^{-1} \quad (4)$$

• Position 3

$$k_L a = 1.72 \cdot \left[\frac{\eta_a^{1.91}}{v_S^{0.35} \cdot \left(\frac{P_a}{V} \right)^{3.10}} \right]^{\phi}, \text{ s}^{-1} \quad (5)$$

• Position 4

$$k_L a = 2.17 \cdot \left[\frac{\eta_a^{2.67}}{v_S^{0.31} \cdot \left(\frac{P_a}{V} \right)^{3.77}} \right]^{\phi}, \text{ s}^{-1} \quad (6)$$

b. bacteria (*P. shermanii*) (Eqs. 7-10):

• Position 1

$$k_L a = 0.76 \cdot \left[\frac{C_X^{1.61}}{v_S^{0.46} \cdot \left(\frac{P_a}{V} \right)^{3.33}} \right]^{\phi}, \text{ s}^{-1} \quad (7)$$

• Position 2

$$k_L a = 2.38 \cdot 10^{-2} \cdot \left[\frac{C_X^{2.27}}{v_S^{0.66} \cdot \left(\frac{P_a}{V} \right)^{8.19}} \right]^{\phi}, \text{ s}^{-1} \quad (8)$$

• Position 3

$$k_L a = 3.81 \cdot 10^{-2} \cdot \left[\frac{C_X^{0.31} \cdot \left(\frac{P_a}{V} \right)^{0.15}}{v_S^{0.66}} \right]^{\phi}, \text{ s}^{-1} \quad (9)$$

• Position 4

$$k_L a = 3.74 \cdot 10^{-2} \cdot \left[\frac{C_X^{0.15} \cdot \left(\frac{P_a}{V} \right)^{0.25}}{v_S^{8.72 \cdot 10^{-3}}} \right]^{\phi}, \text{ s}^{-1} \quad (10)$$

c. yeasts (*S. cerevisiae*) (Eqs. 7-10):

• Position 1

$$k_L a = 8.91 \cdot 10^{-2} \cdot \left[\frac{C_X^{8.46 \cdot 10^{-2}}}{v_S^{0.39} \cdot \left(\frac{P_a}{V} \right)^{0.17}} \right]^{\phi}, \text{ s}^{-1} \quad (11)$$

• Position 2

$$k_L a = 7.10 \cdot 10^{-2} \cdot \left[\frac{C_X^{1.95}}{v_S^{0.74} \cdot \left(\frac{P_a}{V} \right)^{3.81}} \right]^{\phi}, \text{ s}^{-1} \quad (12)$$

• Position 3

$$k_L a = 6.12 \cdot 10^{-3} \cdot \left[\frac{C_X^{0.81} \cdot \left(\frac{P_a}{V} \right)^{0.11}}{v_S^{1.60 \cdot 10^{-2}}} \right]^{\phi}, \text{ s}^{-1} \quad (13)$$

• Position 4

$$k_L a = 9.16 \cdot 10^{-3} \cdot \left[\frac{C_X^{0.33} \cdot \left(\frac{P_a}{V} \right)^{0.32}}{v_S^{6.26 \cdot 10^{-2}}} \right]^{\phi}, \text{ s}^{-1} \quad (14)$$

The proposed equations offer a good agreement with the experimental data, the maximum deviations being of 6.92% for simulated broths, 8.40% for *P. shermanii* broths, and 9.73% for *S. cerevisiae* broths.

The above discussed influences of the considered parameters and their importance are suggested by the sign and value of the corresponding exponents. Therefore, regardless of the position inside the bioreactor, the specific power input exhibits negative influence in the case of simulated broths. For broths containing biomass, the negative influence of mixing intensity is observed only for the positions placed at the bioreactor bottom, namely positions 1 and 2, due to the higher amount of solid phase in this region, which could block the hydrocarbon droplets or bubbles surface by adsorption. The influence of superficial air velocity is positive in all studied systems, but is more important in the absence of the blockage of the oxygen mass transfer surface by cells (simulated and bacterial broths).

4. Conclusions

The addition of oxygen-vector, namely *n*-dodecane, leads to the enhancement for several times of oxygen transfer rate compared to the conventional aerobic fermentations, without supplementary mixing or aeration intensification. However, the influence of oxygen-vector on $k_L a$ value and distribution inside the broths has to be analyzed in relation with the broth characteristics (apparent viscosity or biomass concentration), bioreactor operating parameters, and, most important, affinity of cells for hydrocarbon

droplets. Moreover, the amplitude of the considered factors influences differs from one region inside the broth to another. The oxygen mass transfer has been analyzed for a stirred bioreactor by means of k_La and amplification factor, $(k_La)_V/(k_La)_0$, for viscous simulated broths without biomass, *P. shermanii* and *S. cerevisiae* broths.

Generally, the increase of apparent viscosity or of biomass concentration induces the significant decrease of k_La, the magnitude of this influence being diminished by increasing the volumetric fraction of *n*-dodecane. The positive effect of *n*-dodecane addition was recorded for all four positions considered inside the bioreactor, being more important for simulated and *P. shermanii*, due to the absence or low affinity of cells for hydrocarbon droplets.

The different affinity of bacterial and yeasts cells for hydrocarbon phase induces different variations of amplification factor during the accumulation of biomass. Thus, for *P. shermanii* broths, the amplification factor initially decreases from the value reached in the absence of cells, increasing then strongly with the biomass accumulation. This phenomenon is the result of the modification of cells affinity for hydrocarbon droplets during the biomass growth and accumulation. The variation of amplification factor with the increase of *S. cerevisiae* concentration is completely different from that obtained for bacteria suspensions, due to the higher affinity of yeasts cells for organic phase. This parameter reached a maximum value, followed by its decrease. For positions 1 and 4, the maximum $(k_La)_V/(k_La)_0$ ratio corresponds to higher biomass concentration compared to the intermediary positions.

Although the shapes of the dependences between the oxygen transfer rate and specific power input are rather similar for the three studied fermentation systems, the influence of mixing intensification also depends on broths type. Therefore, the increase of specific power input influences positively the oxygen transfer rate for simulated broths and yeasts suspensions containing low cell concentrations, especially at the bottom and top regions of the bioreactor. For bacterial broths, regardless of the position inside the bioreactor, k_La increases with mixing intensification, reaches a maximum value followed by its decreasing. This variation is more pronounced for less concentrated suspensions.

In all studied cases, the influence of superficial air velocity on amplification factor was similar to that of mixing intensity. Thus, by increasing the aeration rate, the promoted turbulence leads to the diminution of the amplification factor, as the result of the reduction of covering degree of bubbles surface by hydrocarbon droplets.

The influences of the considered factors have been included in some mathematical correlations which allow predicting the oxygen mass transfer coefficient in different regions of the bioreactor for simulated, bacterial, and yeasts, and offer a good concordance with the experimental results (the average deviation varied between ±6.72% for simulated broths and ±6.93% for *P. shermanii* cultures).

Nomenclature

C_l - oxygen concentration in the broth, mol l^{-1}
C_l^* - maximum oxygen concentration in the broth, mol l^{-1}
C_X - biomass concentration, g d.w. l^{-1}
k_La - oxygen mass transfer coefficient, s^{-1}
$(k_La)_0$ - k_La in absence of oxygen-vector, s^{-1}
$(k_La)_V$ - k_La in presence of oxygen-vector, s^{-1}
$(k_La)_V/(k_La)_0$ - amplification factor,
P_a - power consumption for mixing of aerated broths, W
P_a/V - specific power input, W m^{-3}
v_S - superficial air velocity, m s^{-1}
Greek symbols
α, β, γ, δ - parameters of Eqs. (1) and (2)
ϕ - volumetric fraction of oxygen-vector
η_a - apparent viscosity, Pa·s

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