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## THE EFFECT OF N-DODECANE ADDITION ON OXYGEN TRANSFER IN STIRRED BIOREACTORS FOR SACCHAROMYCES CEREVISIAE BROTHS

*The previous works on simulated and P. shermanii broths were continued and developed for Saccharomyces cerevisiae broths. The obtained results indicated the considerable increase of  $k_{La}$  in the presence of n-dodecane as an oxygen-vector, but the magnitude of this effect had to be correlated with the biomass characteristics, especially with hydrophobicity. Due to the higher affinity of yeast cells for hydrocarbon droplets, increase of the oxygen mass transfer rate was lower than that recorded for simulated broths or bacterial suspensions.*

*Key words: Stirred bioreactor, Oxygen-vectors, n-dodecane, S. cerevisiae, Yeast broths, Oxygen mass transfer coefficient, Submerged aeration, Surface aeration.*

The experiments carried out on biosynthesis systems of single-cell proteins using various water insoluble hydrocarbon substrates revealed that the addition of a non-aqueous organic phase might induce a significant increase in the oxygen transfer rate from air to the microorganisms, without any supplementary intensification of mixing [1]. Thus, compounds capable of enhancing oxygen transfer through microorganisms when added to the growth media, due to their higher oxygen solubilization capacity compared with water, were defined as *oxygen-vectors*. Oxygen-vectors have no toxicity against the cultivated microorganisms, and in some cases they could be used as a supplementary source of carbon and energy.

The main oxygen-vectors used in biotechnology are hydrocarbons [1–5] and perfluorocarbons [6–8], as well as oil added as an antifoam agent [9–11]. The oxygen solubility in these compounds is about 15 to 20 times higher than in water [12–15].

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), and the formation of a new interfacial areas between the gas and liquid phases. Among the mechanisms of oxygen transfer presented and analyzed in the literature for these systems, the most plausible one assumes that the hydrocarbon is adsorbed to the bubbles surface, with or without the formation of a continuous film, the oxygen diffusion from air to the microorganisms occurring through the oxygen-vector and then through the aqueous phase or directly to the cells adsorbed to the hydrocarbon droplets or film surface [2,3,7,16]. The experiments indicated that the main resistance to oxygen transfer

was due to the diffusion through the aqueous boundary layer from the hydrocarbon – aqueous phase interface, its negative influence being counteracted both by increasing the interfacial area of oxygen transfer and by the accumulation of the oxygen in the organic phase, which acts as an oxygen reservoir. For this reason, the oxygen mass transfer coefficient corresponding to aqueous film,  $k_{La}$ , can also be used to describe the oxygen transfer in these systems, a being the gas-liquid interfacial area [2,3,17].

Previous studies indicated that for simulated broths the oxygen mass transfer rate increased by about 5 times in the presence of n-dodecane, the magnitude of this effect depending on the apparent viscosity of the broth and hydrocarbon concentration [5]. The experiments carried out for *P. shermanii* broths in the same systems indicated an increase of  $k_{La}$  up to 9 times compared with bacterial broths without hydrocarbon, depending on the hydrocarbon concentration, biomass amount, mixing intensity and aeration rate [18]. These experiments were continued and developed for fermentation broths containing yeasts, namely *Saccharomyces cerevisiae*, because these suspensions exhibit different characteristics concerning the rheological properties and cell hydrophobicity compared with *P. shermanii* broths.

### MATERIALS AND METHOD

The experiments were carried out in a 5 l (4 l working volume, ellipsoidal bottom) laboratory bioreactor (Biostat A, B. Braun Biotech International), with computer-controlled and recorded parameters. The bioreactor characteristics and operating parameters have been presented in previous papers [5,18,19].

Non-respiring *S. cerevisiae* suspensions were used in the experiments. The biomass concentration was varied between 43 and 150 g/l d.w. For comparing the results with those obtained for simulated broths without biomass, the apparent viscosity of the yeast broths was measured, this varying between  $2.2 \cdot 10^{-3}$  –

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$5.7 \cdot 10^{-3}$  Pa·s for the considered biomass concentration domain. Owing to the difficulty of the *in-situ* measurement of viscosity during the experiments, the viscosity was measured before and after each experiment using a viscometer of the Ostwald type. Both the experiments and viscosity measurements were carried out at 21°C.

The respiratory activity of the microorganisms was inhibited by suspending the biomass in a solution of 0.2% pyrogallol acid and 0.4% potassium hydroxide for about 30 min. The biomass was then filtered, washed with distilled water and used for the mentioned suspension preparation [19].

n-Dodecane (SIGMA Chemie GmbH) was used as an oxygen-vector (density 750 g/l at 20°C, oxygen solubility  $54.9 \cdot 10^{-3}$  g/l at 35°C and atmospheric air pressure [3]). Its volumetric fraction in the broth varied between 0.05 and 0.20.

The static method was used to calculate  $k_{La}$  values [17,20,21]. The static method consists of the measurement of the rate of increase in the dissolved oxygen concentration in the broth after it was lowered by passing nitrogen gas through the system for about 20 min. The nitrogen gas flow was stopped when the oxygen concentration was nearly zero and it was followed by aeration at certain operating conditions (rotation speed, power input, aeration rate, etc.).

The solved oxygen concentrations in the broth were measured using an oxygen electrode, InPro 6000 Series type (Mettler Toledo), its response time being 5–20 s or even lower for higher mixing intensity. In this case, because the  $k_{La}$  values were in all cases less than or close to  $0.2 \text{ s}^{-1}$ , it was assumed that the response of the oxygen electrode to the change in the oxygen concentration was sufficiently fast and did not affect the experiment accuracy [21,22].

For non-aerated systems and a single Rushton type turbine stirrer, the calculation of the power consumption for stirring uses the power number,  $N_p$  [23,24]:

$$N_p = \frac{P}{\rho \cdot N^3 \cdot d^5} = \frac{6}{Re^{0.15}} \quad (1)$$

For multiple stirrers with a distance between the stirrers on the shaft of  $(1-2)d$ , the above calculated power number is multiplied by the number of stirrers [23,24].

The power consumption for mechanical mixing of aerated media can be determined by means of the value obtained for non-aerated ones, using the equation proposed by Hughmark [25]:

$$\frac{P_a}{P} = \left( \frac{g \cdot w \cdot V^{2/3}}{N \cdot d^4} \right)^{0.2} \cdot \left( \frac{N \cdot V}{D_a} \right)^{0.25} \quad (2)$$

Each experiment was carried out four times, under identical conditions using the average value of the

oxygen mass transfer coefficient. The maximum experimental error was  $\pm 5.39\%$ .

## RESULTS AND DISCUSSION

### Submerged aeration

Previous studies indicated that the addition of n-dodecane considerably increased the concentration of solved oxygen in the broth and counteracted the negative effect of the high apparent viscosity on the oxygen transfer rate [5,18].

The presence of biomass affects the oxygen transfer by modification of the rheological characteristics of the broth and by adsorption to the air bubble surface. In biomass suspensions containing oxygen-vectors, the cells could be adsorbed to the hydrocarbon droplet surface and the cell-droplet associations could furthermore be adsorbed to the air bubble surface. Although in these systems the oxygen could be directly consumed from air bubbles by microorganisms included in the associations, the blocking effect of the air-liquid phase was dominant, its relative importance depending on microorganism type and morphology.

The experiments carried out for bacteria suspensions demonstrated that bacteria cells are hydrophobic only at the beginning of their growth, at lower biomass concentrations, the microorganism accumulation inducing their desorption from the droplet surface and dispersion into the aqueous phase [3,18,26,27]. On the other hand, due to their higher affinity to hydrocarbon media compared with bacteria cells, yeast cells exhibit a completely different behavior, being adsorbed to the oxygen-vector droplets during the entire fermentation cycle [3,26,28].

As can be observed from Figure 1, the increase of *S. cerevisiae* biomass concentration leads to a decrease of  $k_{La}$ , this effect being independent of the volumetric fraction of the oxygen-vector and mixing intensity.

One of the main influences of the biomass concentration on  $k_{La}$  is due to the increase of the broth apparent viscosity by biomass accumulation. For the considered variation domain of the main parameters taken into account, respectively the superficial air velocity from  $8.36 \cdot 10^{-4}$  to  $5.02 \cdot 10^{-3}$  m/s and the specific power input from 100 to 500 W/m<sup>3</sup>, the strongest decrease of the oxygen mass transfer coefficient with *S. cerevisiae* concentration increase was recorded for higher amounts of n-dodecane ( $k_{La}$  was reduced by about 3 to 4 times for  $\phi = 0.15$  with a biomass concentration increase from 43 to 150 g/l d.w.). Contrary to the behavior of *P. shermanii*, the influence of yeast concentration on the oxygen transfer rate is diminished by decreasing the volumetric fraction of n-dodecane in the broth. Thus, under identical experimental conditions and  $\phi = 0$ ,  $k_{La}$  was reduced by 1.7 – 2.2 times for the mentioned domain of yeast concentrations (Figure 1).

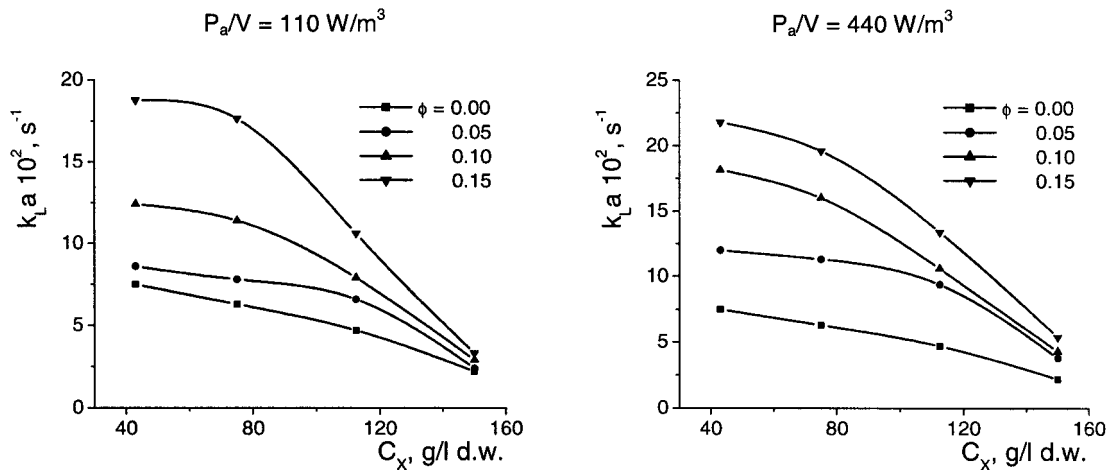


Figure 1. Influence of the biomass concentration on  $k_L a$  ( $v_s = 8.36 \cdot 10^{-4} \text{ m/s}$ )

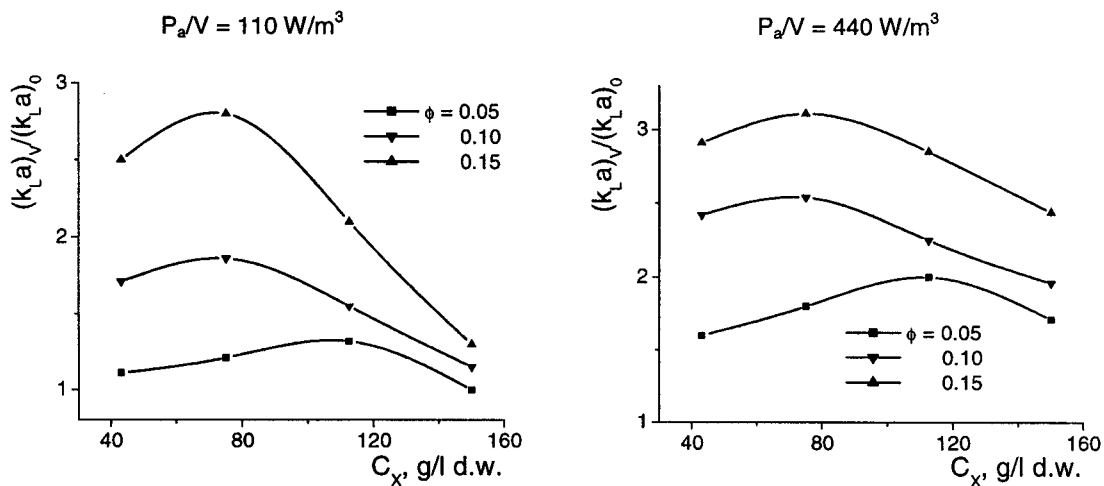


Figure 2. Influence of the biomass concentration on the amplification factor ( $v_s = 8.36 \cdot 10^{-4} \text{ m/s}$ )

Compared with a system without oxygen-vectors, under similar experimental conditions, the addition of n-dodecane leads to a multiple enhancement of the oxygen transfer rate [19]. This intensification of the mass transfer can be described by means of the *amplification factor*, defined by the ration between  $k_L a$  in the presence of the oxygen-vector,  $(k_L a)_v$ , and in the absence of the oxygen-vector,  $(k_L a)_0$ , under similar experimental conditions [5,18,29]. Figure 2 shows that the variation of the amplification factor with increasing biomass concentration reaches a maximum followed by decrease.

This evolution is the result of two opposite phenomena that occur with biomass accumulation. Thus, the addition of n-dodecane leads to the intensification of oxygen transfer from the gaseous phase to the broth, an effect which becomes more important at higher hydrocarbon concentrations. On the other hand, due to the affinity of yeasts cells toward hydrocarbon droplets, the biomass is adsorbed on the droplet surface, thus blocking the oxygen transfer interface between the organic and aqueous phases.

Furthermore, the cell-droplet associations could be adsorbed on the air bubble surface, inducing a supplementary resistance to oxygen diffusion. The stability of the formed cell-droplet-air bubble associations depends on the mixing intensity, hydrocarbon droplet size and tenside presence in the broth [17]. For this reason, the existence of a maximum value of the amplification factor becomes more pronounced at lower mixing intensity. The maximum value is shifted to lower biomass concentration with increasing oxygen-vector amount in the broth, also as a result of the high affinity of yeasts toward hydrocarbons.

The maximum value of the amplification factor indicates that it is possible to obtain an increase of the oxygen transfer rate up to 3.2 times compared to systems without oxygen-vectors, depending on the process parameters, the n-dodecane and biomass concentration.

Analysis of the influence of the hydrocarbon volumetric fraction on the oxygen transfer rate indicated two variation domains for  $k_L a$ , which are controlled by the *S. cerevisiae* concentration. According to the

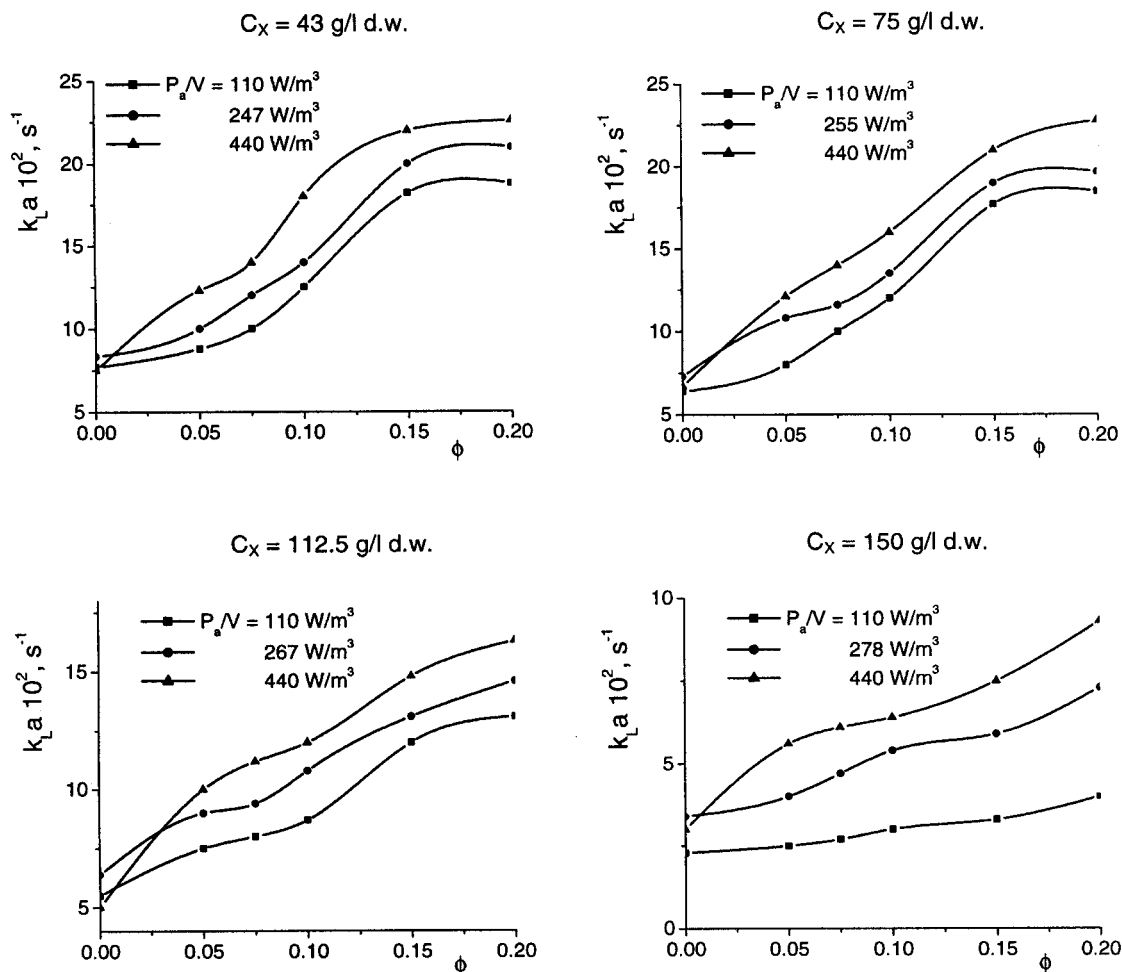


Figure 3. Influence of the n-dodecane concentration on  $k_{La}$  ( $v_s = 8.36 \cdot 10^{-4} \text{ m/s}$ )

experimental data plotted in Figure 3, for biomass concentrations lower than 43 g/l d.w. and regardless of the mixing intensity,  $k_{La}$  increases for n-dodecane concentrations up to 0.15, subsequently remaining at a constant level.

For higher values of biomass concentration, the continuous increase of  $k_{La}$  with increasing hydrocarbon concentration was recorded for the entire domain of n-dodecane volumetric fractions. These variations are the result of the reduction of the importance of the bubble blockage effect by cells with increasing n-dodecane concentration. The favorable effect of n-dodecane addition must be correlated with the influence of mixing intensity. Thus, regardless of n-dodecane amount, for lower mixing intensity, the highest values of the amplification factor are obtained for lower biomass concentration. At higher power consumption for mixing, the amplification of turbulence generates the disruption of cell-droplet and cell-droplet-air bubble associations, the favorable effect of n-dodecane becoming more pronounced at higher yeast and hydrocarbon concentrations. This conclusion is confirmed by the evolution of the amplification factor presented in Figure 4.

As suggested in Figures 1 and 3, the intensification of mixing induces a fine dispersion of air and n-dodecane, with an increase of the interfacial area, as well as the disruption of cell-droplet and cell-droplet-air bubble associations. Both phenomena induce a favorable effect on the oxygen transfer rate (Figure 5).

The amplification factor exhibits a particular evolution with increasing power consumption for mechanical agitation. Thus, its variation indicates a minimum value which corresponds to a specific power input of 270–300  $\text{W/m}^3$ . By intensifying the mixing up to 270–300  $\text{W/m}^3$ , the air bubbles and hydrocarbon droplets are finely dispersed in the broth, their surface being easily occupied by *S. cerevisiae* cell adsorption. For this domain of specific power consumption, blockage of the interfacial area between the air and the broth, respectively between the n-dodecane and the broth, represents the main resistance to oxygen transfer. At higher mixing intensity, the negative effect of surface blockage is diminished, due to the reduction of the covering degree of the bubble or hydrocarbon droplet surface by cell adsorption. Consequently, increase of the free interfacial area has the principal role in the increase of the oxygen diffusion rate (Figure 6).

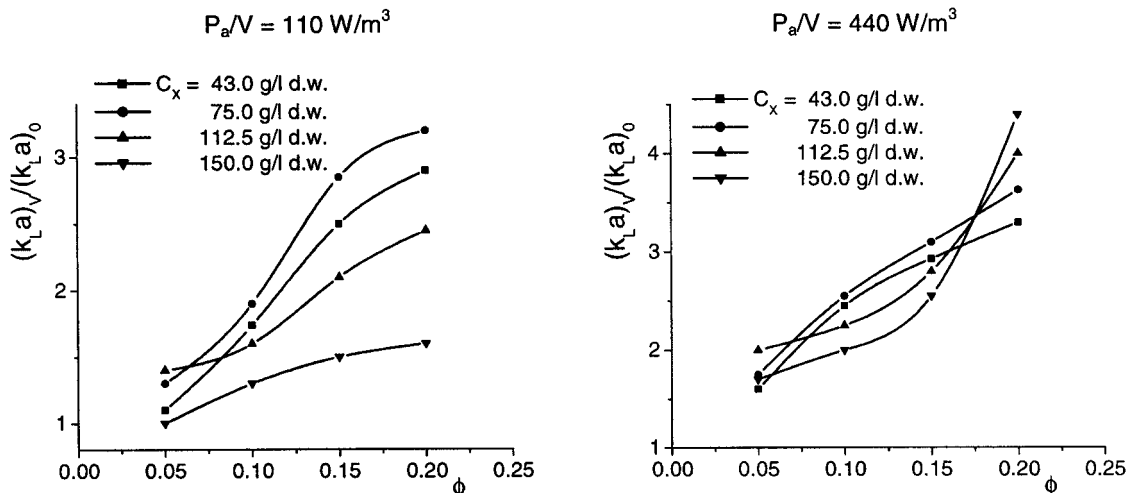


Figure 4. Influence of the n-dodecane concentration on the amplification factor ( $v_s = 8.36 \cdot 10^{-4} \text{ m/s}$ )

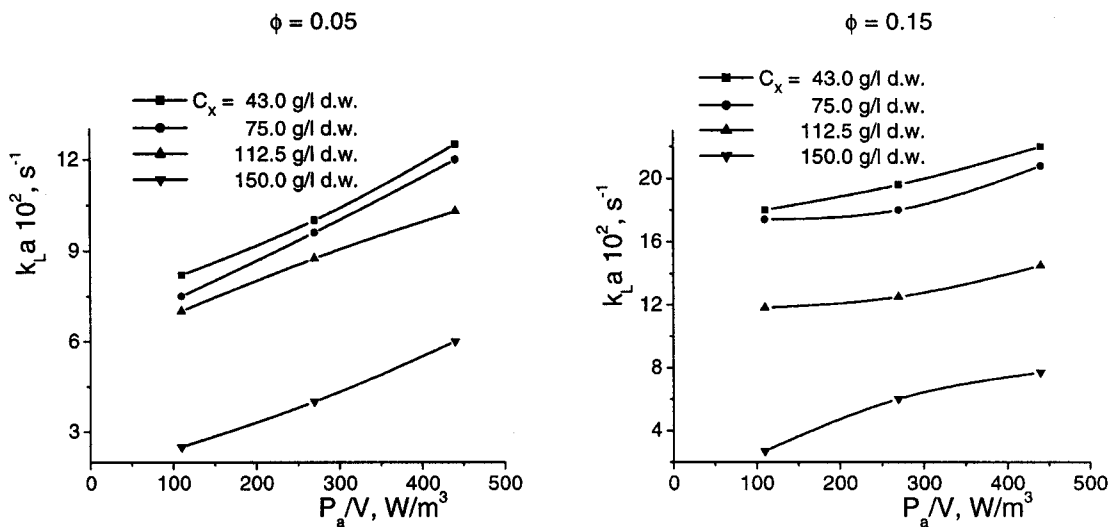


Figure 5. Influence of the specific power input on  $k_L a$  ( $v_s = 8.36 \cdot 10^{-4} \text{ m/s}$ )

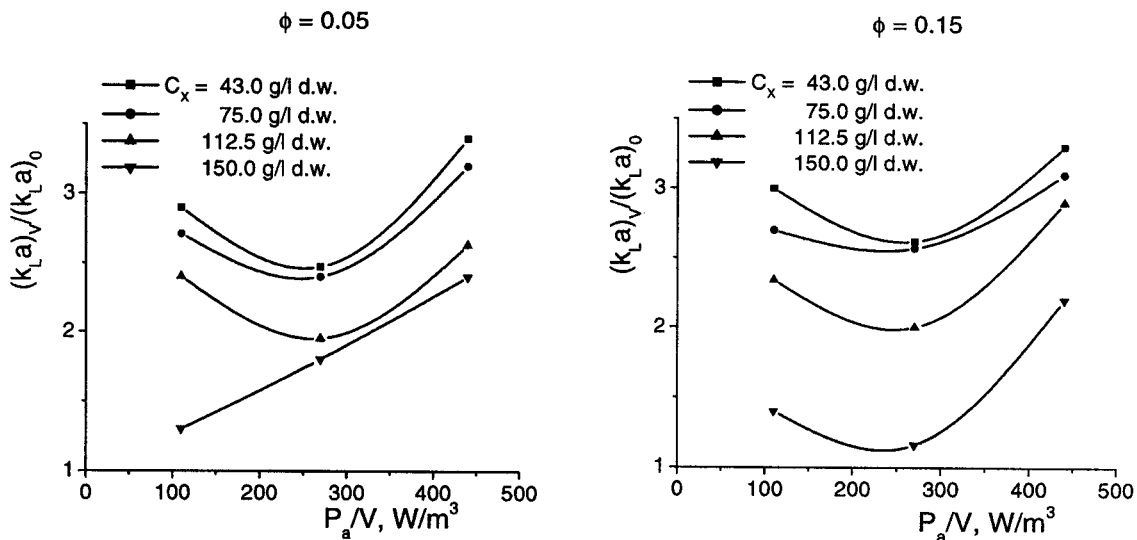


Figure 6. Influence of the specific power input on the amplification factor ( $v_s = 8.36 \cdot 10^{-4} \text{ m/s}$ )

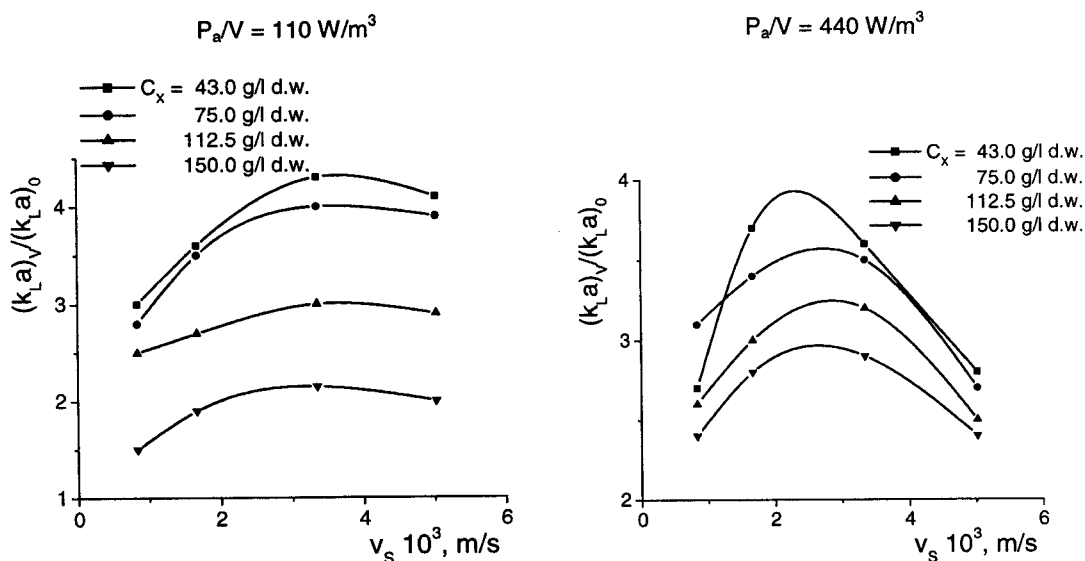


Figure 7. Influence of the superficial velocity on the amplification factor ( $\Phi = 0.10$ )

The variation of the  $(k_L a)_V / (k_L a)_0$  ratio obtained for low concentrations of n-dodecane and high amounts of biomass represents the exception from the above evolution of this factor. In this case, due to the significant blockage of the n-dodecane surface by cell adsorption, the intensification of mixing induces a favorable influence on oxygen transfer even for lower values of the power consumption.

As in the case of the effect of the energy dissipated by mechanical mixing, the intensification of aeration leads to an increase of  $k_L a$  in systems with or without n-dodecane [19,28,30]. This effect is the result of the increase of the interfacial area between the air and the broths, on the one hand, and of the increase of the driving force of oxygen transfer between the air and the broths, on the other hand. Increase of the aeration rate also induces amplification of the turbulence with similar effects on the intensification of mechanical agitation above  $270 - 300 \text{ W/m}^3$ . But, by increasing the aeration, the air volumetric fraction in the broth increases, thus diminishing the covering degree of the bubble surface by hydrocarbon droplets and, consequently, reducing the amplification factor. It can be seen from Figure 7 that this phenomenon becomes more significant for lower biomass concentrations at intense mixing.

Due to the opposite effects of the increase of the aeration rate, the amplification factor reaches a maximum value for a superficial air velocity of  $3.35 \cdot 10^{-3} \text{ m/s}$ , this value varying between 4.4 and 2.2 with *S. cerevisiae* biomass accumulation.

### Surface aeration

Oxygen diffusion from the gas to the liquid phase can simultaneously occur through the free surface of the broth. The relative magnitude of the surface aeration compared with the submerged aeration becomes more

important for small bioreactors or for vessels with a H/D ratio close to unity. The absorption of oxygen at the surface is controlled by liquid turbulence, as well as by the medium components, which can enhance or reduce the oxygen solubility. Furthermore, for broths containing biomass, the cell adsorption to the liquid free surface becomes an important limiting factor for surface aeration.

The previous conclusions about the oxygen transfer mechanism for systems containing oxygen-vectors, as well as about the effects of the considered factors on  $k_L a$  were confirmed by studying the surface aeration contribution to the overall oxygen transfer in the presence of n-dodecane. Thus, similar to systems without biomass [5,29], or containing *P. shermanii* broths [18], the dependence between the oxygen transfer rate for surface aeration and the n-dodecane concentration, plotted in Figure 8, indicates the increase of  $k_L a$  to a maximum value followed by its decrease.

The maximum of the oxygen mass transfer coefficient is more evident for suspensions with a low amount of yeast biomass at higher mixing intensity, due to the superior hydrophobicity of yeast cells compared with that of bacteria. This variation of  $k_L a$  is due to the interactions between n-dodecane droplets and the air-aqueous phase interface, leading to the formation of an oxygen-vector film at the free surface of the broth. This film thickness increases with increasing n-dodecane concentration and decreasing rate of oxygen diffusion through the film. The phenomenon becomes more important for *S. cerevisiae* suspensions as a result of the hydrophobic character of yeast cells, their adsorption to the hydrocarbon surface creating an additional resistance to oxygen diffusion. With time the cumulated effect of the film thickness increase and of cell adsorption becomes stronger than the increase of

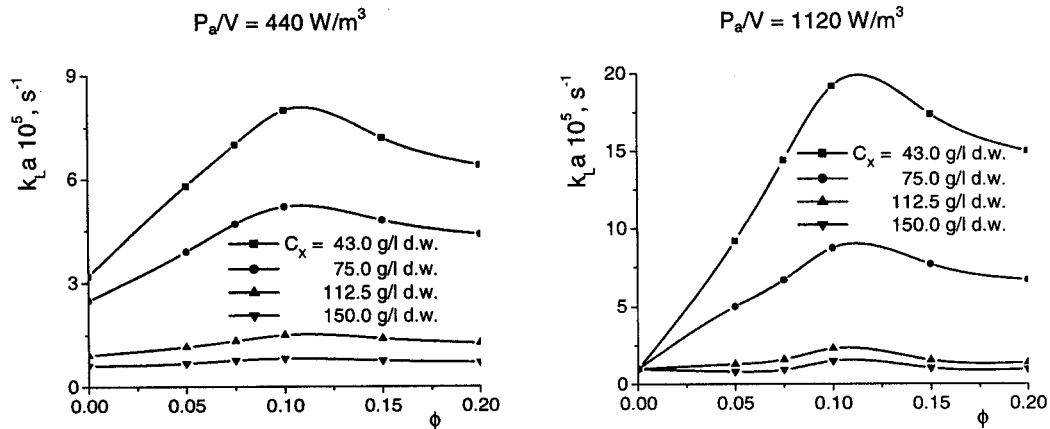


Figure 8. Influence of the n-dodecane concentration on  $k_L a$  for surface aeration

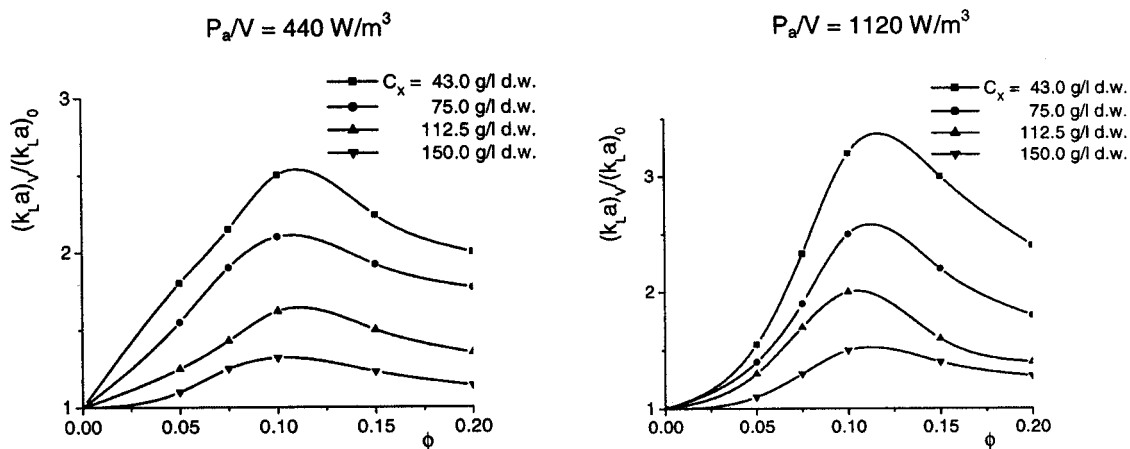


Figure 9. Influence of the n-dodecane concentration on the amplification factor for surface aeration

oxygen solubility in the hydrocarbon layer formed at the liquid free surface, thus reducing  $k_L a$ . For all the studied cases, the n-dodecane volumetric fraction corresponding to the maximum  $k_L a$  was 0.1. The relative importance of the resistance to oxygen diffusion induced by cell adsorption increases with biomass accumulation. Thus, at higher biomass concentrations, the oxygen transfer rate through the free surface is maintained at almost the level that was obtained for systems without oxygen-vectors, regardless of the n-dodecane concentration.

For the above mentioned reasons, the variation of the amplification factor with n-dodecane concentration is similar (Figure 9).

The intensification of mixing reduces the thickness of the hydrocarbon film, counteracting its negative effect on oxygen diffusion, and inducing the partial desorption of cells from the hydrocarbon film. Therefore, the increase of energy dissipated by mechanical agitation induces the acceleration of oxygen transfer through the free surface of broth, as may be seen in Figure 10.

This variation is the result both of the air-hydrocarbon and hydrocarbon-aqueous phase interfacial area renewal and of the decrease of the hydrocarbon film thickness formed at the free surface.

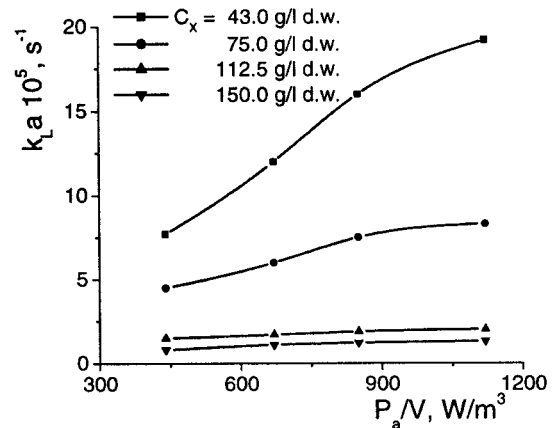


Figure 10. Influence of the specific power input on  $k_L a$  for surface aeration ( $\Phi = 0.10$ )

The analysis of the dependence between the amplification factor and mixing intensity, plotted in Figure 11, indicates the continuous increase of this parameter with mixing intensification.

The obtained evolution suggests that cell desorption from the hydrocarbon film surface together with surface renewal have a significant role on the

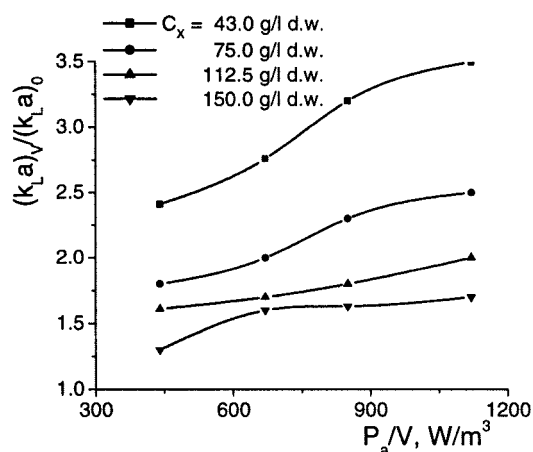


Figure 11. Influence of the specific power input on the amplification factor for surface aeration ( $\Phi = 0.10$ )

mechanism by which mechanical agitation controls oxygen transfer by surface aeration. Compared with simulated broths without biomass and with *P. shermanii* broths, in the case of *S. cerevisiae* broths the favorable effect of the increase of the specific power input is more important than the negative one due to hydrocarbon desorption from the free surface at higher mixing intensity.

Compared with *S. cerevisiae* broths without oxygen-vectors, the contribution of surface aeration to the overall oxygen transfer becomes more important in the presence of n-dodecane. Thus, for a hydrocarbon volumetric fraction of 0.1 and a specific power input of  $1120 \text{ W/m}^3$ , the contribution of surface aeration to the overall mass transfer of oxygen into yeast broths varied between 0.53 and 1.2% at a superficial air velocity of  $8.36 \cdot 10^{-4} \text{ m/s}$ , this variation domain corresponding to a decrease of biomass concentration from 150 to 43 g/l d.w. Due to the presence of the solid phase, namely biomass, and to its hydrophobic character, the contribution of surface aeration was about 5.7 times lower than that obtained for simulated fermentation broths without biomass, and about 1.8 times lower than that for *P. shermanii* broths, under identical experimental conditions and similar apparent viscosities [5,18,29].

## CONCLUSIONS

The addition of n-dodecane allows enhancement of the oxygen mass transfer rate, without requiring supplementary energy consumption for mixing intensification. By studying oxygen mass transfer by submerged and surface aeration in the presence of n-dodecane as an oxygen-vector for *S. cerevisiae* broths in a stirred bioreactor, the following conclusions could be drawn:

1. An increase of  $k_L a$  up to 4.4 times was recorded compared with yeast broths without hydrocarbons, depending on the hydrocarbon concentration, biomass amount, mixing intensity and aeration rate. Compared

with bacterial broths, the effect of n-dodecane addition was less important, especially due to the higher affinity of yeast cells toward hydrocarbon droplets.

2. By adding n-dodecane, the contribution of surface aeration to the overall mass transfer of oxygen into the broth was greater than for the systems without hydrocarbon, but inferior to that obtained for simulated or bacterial broths (the contribution of surface aeration was about 5.7 times lower compared to the case of simulated fermentation broths and about 1.8 lower than that obtained for *P. shermanii* broths, at the same viscosity and experimental conditions).

## NOTATIONS

$C_X$	– biomass concentration, g/l d.w.
$d$	– stirrer diameter, mm
$D_a$	– volumetric air flow rate, $\text{m}^3/\text{s}$
$H$	– bioreactor height, mm
$k_{L,a}$	– oxygen mass transfer coefficient, $\text{s}^{-1}$
$N$	– 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
$(P_a/V)$	– specific power input, $\text{W/m}^3$
$Re$	– Reynolds number
$v_s$	– superficial air velocity, $\text{m/s}$
$V$	– volume of medium, $\text{m}^3$
$w$	– impeller blade height, mm
$\phi$	– volumetric fraction of oxygen-vector
$\rho$	– density, $\text{kg/m}^3$

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## IZVOD

### EFEKAT DODATKA N-DODEKANA NA PRENOS MASE KISEONIKA U BIOREAKTORU SA MEŠANJEM SUSPENZIJE SACCHAROMYCES CEREVISIAE

(Naučni rad)

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Prethodna istraživanja, koja su se bavila simulacijom P. shermanii suspenzije nastavljena su i u ovom radu primenjena na suspenziji *Saccharomyces cerevisia*. Dobijeni rezultati ukazuju da je moguće značajno uvećanje zapreminskog koeficijenta prenosa mase  $k_{La}$  u prisustvu n-dodekana kao "kiseoničnog vektora", ali se intenzitet uvećanja mora korelirati sa karakteristikama biomase, posebno njenom hidrofobnošću. Zbog velikog afiniteta ćelija kvasca ka kapima ugljovodonika, povećanje prenosa mase kiseonika je nešto manje nego što je zabeleženo u slučaju simulacionih fermentacionih medijuma ili suspenzija bakterija.

Ključne reči: Bioreaktor sa mešanjem, Kiseonični vektor, n-dodekan, *S. cerevisiae*, Kvasci, Koeficijent prenosa mase kiseonika, Aeracija, Površinska aeracija.