Analysis of distribution of oxygen transfer rate in stirred bioreactors for yeasts broths

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Abstract

The study on the distribution of oxygen transfer rate in a stirred bioreactor for S. cerevisiae broths underlined the major influence of the presence and concentration of biomass on the interphasic transfer of oxygen. Owing to the bubbles surface blockage by the bacterial cells, the accumulation of biomass from 40 to 150 g/l d.w. led to the decreasing of kla for about 1.8-3 times. Compared with the simulated broths without biomass having similar apparent viscosity, the oxygen transfer rate became of about 1.14-3.85 times lower in the yeast broths. The intensification of aeration promoted the acceleration of oxygen transfer for about 1.1-2 times, due to the intensification of turbulence and of the extent of free interfacial area needed for the oxygen transfer, this influence being similar to that recorded for the mixing efficiency. Indifferent of the operating parameters of the bioreactor, kla increased from the inferior region to the superior one, being non-uniformly distributed inside the broths.

Keywords: stirred bioreactor, mass transfer, mass transfer coefficient, kla, superficial air velocity, specific power input, Saccharomyces cerevisiae.

Introduction

Oxygen transfer from gaseous phase through microbial cells controls the most of aerated fermentation systems. The amount of dissolved oxygen into the broths is limited by its solubility and mass transfer rate, as well as by its consumption rate through the cells metabolic pathways. The oxygen mass transfer can be analyzed and described by means of mass transfer coefficient, kla. It represents the most important parameter implied on the design and operation of mixing-sparging equipment of the bioreactors. The kla values are affected by a lot of factors, such as geometrical and operational characteristics of the vessels, media composition, type, concentration and microorganism morphology, biocatalysts properties (particle size, porosity, etc.) [1-5].

Stirred bioreactors are widely used in biotechnology, because they provide high values of heat and mass transfer rate, due to the efficient mixing. But, an important challenge for the design and operation of these bioreactors is the non-uniform distribution of the energy dissipated inside the broths by mixing, with direct consequences on the distribution of mixing efficiency and mass/heat transfer rate [6].

Although the literature containing correlations for kla is extensive, there are still considerable problems concerning the accuracy and applicability of kla models, because these correlations can be applied for certain microorganism cultures only, and describe the system
behavior for a given region without indicating the distribution of oxygen transfer rate in the whole bulk volume of the broths [7,8].

The computation fluid dynamics method (CFD) has been recently used in the purpose to characterize the performances of stirred bioreactors. Therefore, the distribution of the flow streams and velocity, gas hold-up, air bubble size, interfacial area, bubble coalescence or formation, as well as the oxygen transfer in stirred vessels containing tap water have been analyzed [9-15]. But, although the oxygen transfer is directly related to the processes studied and modeled by CFD, there are no information concerning the distribution of oxygen transfer rate inside the stirred bioreactors with multiple impellers and real fermentation broths, probably due to the number and complexity of the involved factors.

For this reason, the aim of these experiments is to quantify the effects of the considered factors (biomass concentration, specific power input, superficial air velocity) on the distribution of oxygen mass transfer coefficient, $k_{la}$, for a stirred bioreactor containing \textit{Saccharomyces cerevisiae} broths, using a large domain of operating variables and bacteria concentration.

**Materials and method**

The experiments have been 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 [16].

The bioreactor mixing system consists of two turbine impellers and three baffles. The impeller diameter was of 64 mm. The inferior stirrer has been placed at 64 mm from the bioreactor bottom. The superior stirrer was placed on the shaft at the optimum distance from the inferior one, namely at 64 mm, as it was demonstrated in the previous works for bacterial broths [16]. The rotation speed was maintained below 600 rpm, domain which avoids 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, corresponding to the air superficial velocity domain of 0.84-5x10^{-3} m/s.

In the experiments non-respiring \textit{S. cerevisiae} suspensions have been used. The biomass concentration varied between 40 and 150 g/l d.w. The experiments have been carried out at a temperature of 25°C. Any morphology change was recorded during the experiments.

For $k_{la}$ values determination the static method has been used [2, 5, 17]. 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 precision. 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 30 min. Then, the biomass was filtered, washed with distilled water and used for the above mentioned suspension preparation [5].

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 [18, 19].

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 $k_{la}$. Each experiment has been carried out for three or four times, for identical conditions, the average value of $k_{la}$ being used. The maximum experimental errors were between ±4.48 and ±5.11%.

**Results and discussion**

Indifferent of the cultivated microorganism type, the biomass exhibits a significant effect on oxygen mass transfer. In principle, the influence of cells presence in broth is the result of [5, 20]:

- modification of rheological characteristics of broths during fermentation process, especially the increase of apparent viscosity due to biomass accumulation, effect that is less pronounced for bacterial cultures; the increase of viscosity induces two direct major effects on oxygen mass transfer: the reduction of turbulence and the perturbation of bubbles dispersion-coalescence equilibrium;

- obstruction of mass transfer, owing both to the reduction of oxygen solubility, and to the blocking effect created by cells adsorption to the air bubbles surface; but, the adsorbed solid particles can promote the surface renewal with favorable effect on oxygen mass transfer;

The bubbles dispersion-coalescence equilibrium is supplementary affected by the solid phase presence, which can amplify or diminish the coalescence process, function of the concentration and morphological characteristics of microorganism [4, 5]. Therefore, the appearance of small bubbles is promoted, these offering a higher interfacial area for oxygen mass transfer. But, owing to their high retention time into the broth, the gradient of oxygen concentration between the two phases and, consequently, the oxygen mass flow are reduced [5]. These phenomena lead to the heterogeneous distribution of air bubbles, of gas-liquid interfacial area and, implicitly, of oxygen transfer rate.

For these reasons, the use of a single mathematical model and of an unique/average value for $k_{la}$ for the whole bulk of fermentation broth do not offer the required accuracy 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.

For supporting this assertion, Figures 1 and 2 indicated differences between the values of $k_{la}$ on the bioreactor height. Thus, although the shapes of the plotted dependences are similar, the magnitude of the influence of specific power consumption differs significantly from one position to another.
Figure 1. Influence of specific power input on oxygen mass transfer coefficient for $v_S = 0.84 \times 10^{-3}$ m/s.

Indifferent of the considered position inside the broth, from these figures it can be observed that the oxygen transfer rate increases with the increase of specific power input, reaches a maximum value and then is decreasing. Because the apparent viscosity of the yeasts broths is rather low (for $C_X = 150$ g/l d.w. the apparent viscosity was of 7 cP [19]), this variation is less the effect of the modification of mixing mechanism with the increase of specific power input in presence of air bubbles, as in the case of simulated broths without solid phase [18]. For the systems containing $S. cerevisiae$ cells, the intensification of mixing initially compensates the negative effect of bubble surface blockage, by redistributing the adsorbed cells and thus renewing the gas-liquid interface. For higher specific power input, the bubbles coalescence is diminished, small bubbles are formed, consequently increasing the relative importance of blocking effect.

Figure 2. Influence of specific power input on oxygen mass transfer coefficient for $v_S = 5 \times 10^{-3}$ m/s.
The increase of superficial air velocity contributes to the supplementary dispersion of the bubbles by pneumatic mixing, owing to the intensification of turbulence and to the increase of air hold-up in the broth. Consequently, the cells adsorption to the bubble surface is diminished, phenomenon that is more pronounced at lower amounts of *S. cerevisiae*. For these reasons, for biomass concentration up to 75 g/l d.w. and superficial air velocity of 5x10^{-3} m/s, $k_{la}$ either increases continuously with the specific power input, or is slowly reduced over its maximum value (Figure 2). Moreover, for the superior region, the domain of the biomass concentration corresponding to the positive influence of mixing is extended from 75 g/l d.w., for 8.4x10^{-4} m/s, to 100 g/l d.w., for 5x10^{-3} m/s.

The maximum of oxygen mass transfer coefficient is more evident at lower aeration rate and higher biomass concentration. The favorable contribution of aeration intensification is also suggested by the increase of specific power consumption needed for the maximum level of oxygen transfer from 250 W/m^3, at 8.4x10^{-4} m/s (Figure 1), to 430 W/m^3, at 5x10^{-3} m/s (Figure 2).

These variations are similar to those previously recorded for bacterial cultures [20]. But, due to the more pronounced tendency of deposition of yeast cells to the bioreactor bottom, the biomass concentration in position 1 is higher compared with that of bacteria, the maximum of $k_{la}$ is less evident and the differentiation between the positions 1, 2 and 3, 4 becomes more important.

According with the studies on mixing distribution inside the aerated broths of *S. cerevisiae*, the values of specific power input which lead to the maximum oxygen transfer rates are those corresponding to the minimum levels of mixing time [21].

Contrary to the results obtained for the simulated fermentation broths without biomass and similar to those for *P. shermanii* broths, for yeasts suspensions the lowest values of oxygen mass transfer coefficients are reached for position 1, due to the highest concentration of biomass at the bioreactor bottom. The accumulation of *S. cerevisiae* cells by deposition diminishes the favorable effect of the presence of sparger and inferior impeller in this region.

$C_X = 40$ g/l d.w.  

$C_X = 75$ g/l d.w.  

$C_X = 100$ g/l d.w.  

$C_X = 150$ g/l d.w.

**Figure 3.** Effect of cells adsorption on bubbles surface on oxygen mass transfer coefficient for $v_S = 0.84 \times 10^{-3}$ m/s.
The conclusion that the oxygen transfer is controlled by the solid phase and not by the apparent viscosity is underlined by the progressive increasing of $k_l a$ from position 1 to 4, in direct relation with the reduction of biomass amount on the bioreactor height. Thus, the shapes of the curves describing the correlation between $k_l a$ and specific power consumption from Figures 1 and 2 are identical for the positions 1 and 2, being modified for the superior positions. Moreover, the maximum level of oxygen transfer rate is less evident for the positions 3 and 4.

As it can be seen from Figures 1 and 2, the biomass accumulation leads to the decreasing of oxygen transfer rate indifferent of the operational parameters of the bioreactor or position inside the broths. Thus, for 300 W/m$^3$ and cells accumulation from 40 to 150 g/l d.w., $k_l a$ has been reduced for about 1.8-3 times, the most important reduction being recorded for positions 1 and 2. Also in this case, the effect was more significant than for bacteria.

![Graphs showing the effect of cell adsorption on bubbles surface on oxygen mass transfer coefficient for $v_S = 5 \times 10^{-3}$ m/s.](image)

**Figure 4.** Effect of cells adsorption on bubbles surface on oxygen mass transfer coefficient for $v_S = 5 \times 10^{-3}$ m/s.

The experiments carried out for simulated broths, without biomass [18], and yeasts broths [21] with the same apparent viscosities and using identical operating conditions indicated that the oxygen mass transfer rate in biomass suspensions becomes inferior to that for simulated broths. The blocking effect due to the cells adsorption to the bubble surface can be described by means of the ratio between oxygen mass transfer coefficients for biomass suspensions, $(k_{l a})_C$, and for simulated broths without biomass, $(k_{l a})_0$, obtained for similar experimental conditions [5].

Besides the blocking effect, the adsorption of cells to gas-liquid interface, cumulated with the apparent viscosity of broths which is superior to the water one, promotes the bubbles coalescence, this leading to large bubbles formation and decrease of interfacial area. Moreover, the air is non-uniformly distributed into the broths [5, 22, 23].
From Figures 3 and 4 it can be observed that the presence of cells leads to the significant reduction of the oxygen mass transfer coefficient compared with the $k_{la}$ value recorded for simulated broths. In all cases, the increase of specific power input intensifies the effect of cells adsorption, because the air is finely dispersed and the free surface of small bubbles is easily occupied by cells adsorption. These results confirm those obtained by other authors [24, 25]. But this influence is more important for the superior regions, the ratio $(k_{la})/C/(k_{la})_0$ decreasing faster in the positions 3 and 4 compared with the positions 1 and 2. Due to the deposition of solid phase at the bioreactor bottom, the extent of the bubbles surface blockage by cells adsorption is maximum, thus the value of the ratio $(k_{la})/C/(k_{la})_0$ is lower and less affected by the modification of mixing intensity or aeration rate. Contrary, for the superior regions, the intensification of broth circulations generates the biomass dispersion also in these regions and, consequently, exhibits a strong blocking effect of bubbles surface. For this reason, for specific power consumption over a certain level, the ratios $(k_{la})/C/(k_{la})_0$ for positions 3 and 4 become lower than those for positions 1 and 2. The value of the specific power input that corresponds to the change of the relative magnitude of the blocking phenomenon on bioreactor height is defined as critical specific power and varies from 550 to 350 W/m$^3$ with the $S.\ cerevisiae$ cells accumulation.

Due to the inferior affinity for the bubble surface and to the superior deposition rate of yeast cells compared to the bacteria cells, the blocking effect is less important in the case of $S.\ cerevisiae$. This difference is suggested by the higher values of the ratio $(k_{la})/C/(k_{la})_0$ obtained for yeasts, as well as by the flattening of the curves describing the variation of this ratio in function of the power input.

The above discussed influences are attenuated by increasing the aeration rate, owing to the increase of air volumetric fraction inside the broths, but the shape of the dependence between the ratio $(k_{la})/C/(k_{la})_0$ and mixing intensity remains the same.

The distribution of oxygen transfer rate on the bioreactor height, indicated in the Figures 5 and 6, confirms the above conclusions. The minimum value of $k_{la}$ was recorded for the position 1 and its maximum for the position 4, the distribution of $k_{la}$ being non-uniform. These results underlie the decisive control of the yeasts biomass on the oxygen transfer from the gaseous phase to the liquid one. These data are in concordance with the variation of mixing efficiency inside the $S.\ cerevisiae$ fermentation broths [21].
The intensification of aeration exhibits a favorable effect on oxygen mass transfer by extending the turbulence, increasing the air hold-up, interfacial area and oxygen concentration gradient between the gaseous and liquid phases (Figures 1 and 2). For example, at 300 W/m³, the increase of superficial air velocity from 0.84 to 5x10⁻³ m/s leads to the increase of $k_{la}$ for 1.1-2 times, the effect being more important for lower concentration of biomass. For the above presented reason, although the influence of specific power input is similar indifferent of the superficial air velocity, the magnitude of its effect is diminished at higher aeration rate (the maximum of $k_{la}$ is less pronounced or is not recorded for lower biomass amount and superior positions, the differences between the plotted variations are attenuated).

**Figure 5.** Variation of oxygen mass transfer coefficient with position of oxygen electrode for $v_s = 0.84 \times 10^{-3}$ m/s.

**Figure 6.** Variation of oxygen mass transfer coefficient with position of oxygen electrode for $v_s = 5 \times 10^{-3}$ m/s.
Compared with the simulated broths [18], the influence of the aeration on \( k_{\text{La}} \) for yeast cultures is different. Therefore, Figures 7 and 8 indicate that the intensification of aeration exhibits a continuous favorable effect on oxygen transfer rate for all considered positions inside the broth. The biomass accumulation does not modify this influence (in the case of simulated broths, the increase of apparent viscosity led to the differentiation of the shapes of the variations of \( k_{\text{La}} \) with superficial air velocity plotted for the four positions).

\[ C_X = 40 \text{ g/l d.w.} \quad \text{CX = 75 g/l d.w.} \]

\[ C_X = 100 \text{ g/l d.w.} \quad \text{CX = 150 g/l d.w.} \]

**Figure 7.** Influence of air superficial velocity on oxygen mass transfer coefficient for \( P_j/V = 35 \text{ W/m}^3 \).

The comparative analysis of the dependences between mixing efficiency and aeration rate, on the one hand, and oxygen transfer rate and aeration rate, on the other hand, underlined their similitude. The variation of \( k_{\text{La}} \) with superficial air velocity can be clearly correlated with the variation of mixing time with superficial air velocity, both parameters increasing slowly with the aeration rate [21]. But, the strong acceleration of broths circulation, obtained in the previous studies does not induce the significant intensification of oxygen transfer, due to the supplementary dispersion of the cells inside the broths.

Although the intensification of mixing leads to the increase of oxygen mass transfer rate, the increase of \( k_{\text{La}} \) does not compensate the increase of power consumption demand for this purpose. Therefore, for better characterization of bioreactors performances from the viewpoint of oxygen mass transfer, the term of oxygen transfer efficiency, \( E_{O_2} \), was introduced and defined as [26]:

\[
E_{O_2} = \frac{k_{\text{La}}}{\frac{P}{V}} \tag{1}
\]
Figure 8. Influence of air superficial velocity on oxygen mass transfer coefficient for $P_a/V = 715 \text{ W/m}^3$.

As it can be observed from Figures 9 and 10, plotted for the two considered values of superficial air velocity, the variation of oxygen transfer efficiency with specific power input is contrary to that of $k_{la}$ with specific power input, this evolution suggesting that the oxygen mass transfer rate reaches high values in the stirred bioreactors, but with considerable energy consumption for mixing. The intensification of aeration from 0.84 to $5 \times 10^{-3} \text{ m/s}$ leads to the increase of oxygen transfer efficiency for about 1.2-3 times (the effect is more pronounced at lower cells concentration), as the result both of the intensification of turbulence by means of the supplementary contribution of pneumatic mixing to the broth circulation, and of the extent of the area of non-blocked interface needed for the interphasic transfer of oxygen.

$C_X = 40 \text{ g/l d.w.}$  
$C_X = 75 \text{ g/l d.w.}$
The increase of the superficial air velocity from 0.84 to 5x10^{-3} m/s led to the intensification of turbulence inside the broths, thus promoting the acceleration of oxygen transfer for 1.1-2 times. These results are similar to the variation of mixing efficiency previously studied [21].

The analysis of $k_{la}$ distribution indicated its heterogeneity on the bioreactor height, the oxygen transfer rate increasing from position 1 to 4.

The variation of oxygen transfer efficiency with specific power input is contrary to that of $k_{la}$ with specific power input. The increase of aeration rate led to the increase of...
oxygen transfer efficiency, due to the intensification of turbulence and of the extent of the free interfacial area needed for the oxygen transfer.

Notations

\[ \begin{align*}
  C_X & - \text{biomass concentration, g/l d.w.} \\
  E_{O2} & - \text{oxygen transfer efficiency, m}^3/\text{J} \\
  k\alpha & - \text{oxygen mass transfer coefficient, s}^{-1} \\
  P_a & - \text{power consumption for mixing of aerated broths, W} \\
  P_a/V & - \text{specific power input, W/m}^3 \\
  V & - \text{volume of broth, m}^3 \\
  v_S & - \text{superficial air velocity, m/s}
\end{align*} \]

Conclusions

The study on the distribution of oxygen transfer rate in stirred bioreactor for \textit{S. cerevisiae} broths indicated that the interphasic transfer of oxygen is controlled by the presence and concentration of biomass by means of the blocking effect of the bubbles surface. Therefore, the increase of the cell concentration from 40 to 150 g/l d.w. induced the reduction of \( k\alpha \) for about 1.8-3 times at 300 W/m\(^3\). Compared with the simulated broths without biomass having similar apparent viscosity, the oxygen transfer rate was for about 1.14-3.85 times lower in yeasts broths, this effect being more pronounced for low aeration rate and higher biomass amount.

References