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Determination of Volumetric Oxygen Transfer Coefficient to Evaluate the Maximum Performance of Lab Fermenters

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The biomass productivity of some fermentation processes is limited by the oxygen availability in the medium. Consequently, it is necessary to know the microorganism's oxygen demand as well as the volumetric oxygen transfer rate of the fermenter. In this study, the oxygen transfer performance of two lab fermenters was evaluated from the volumetric oxygen transfer coefficient (K_La). Here the aeration system of these two fermenters was investigated by operating with two different impellers, i.e., the Rhuston turbine and the pitched-blade impeller, according to the stirring speed, the air flow rate, the broth-culture rheological properties, and the presence of antifoam agents in the medium. The experimental results show that the trends of K_La against stirring speed, obtained by using the two impellers, are different. Furthermore, the results highlight the strong lowering of K_La with increasing the viscosity of the medium. The formation of foam by using a typical rich medium, composed by peptone and yeast extract, reduces the transfer of oxygen. In these conditions the addition of antifoam into the medium is fundamental to improve the oxygen transfer and consequently the fermentation duration in the case of high agitation speed.

1. Introduction

In the fermentative processes the choice of the most suitable bioreactor is of fundamental importance. This choice is already made at the laboratory scale taking into account that the main function of a properly designed bioreactor is to provide a controlled environment in order to achieve the optimal growth and/or product formation (Falco et al., 2014; Landi et al., 2014, Paciello et al., 2013).
In aerobic fermentation processes, oxygen transfer is the key problem. As a matter of fact microorganisms growing in submerged cultures can use only dissolved oxygen. Therefore all oxygen must first be dissolved in the broth and then transported to the cell. In this perspective, no limitation should affect oxygen transfer from the gaseous phase to the microorganism.
The concentration of dissolved oxygen in a suspension of breathing microorganisms generally depends on the rate of oxygen transfer from the gas phase to the liquid phase, the rate of oxygen transfer to the site of utilization, and the rate of its consumption by the microorganism. In the conventional processes utilizing water soluble carbohydrate as substrate, it has frequently been found that the rate of oxygen transfer from dispersed air bubbles to liquid phase can become a limiting factor of the microbial growth rate (Enfors and Haggstrom, 1998). In the bioreactor, the aeration system supplies the oxygen and the agitation system maintains uniform conditions within the bioreactor. Altogether, the aeration and agitation are important in promoting effective oxygen transfer to the liquid medium in the bioreactor, but the oxygen transport is however strongly limited by various factors, for example the type of impeller, the aeration rate, the foam formation and the rheology of the broth-culture.To evaluate the performance of an aerobic bioreactor as a whole, the volumetric oxygen transfer coefficient (KLa) is the most important parameter because it represents the capacity of oxygen supply and transfer in the fermenter which is related to agitation speed, aeration rate, geometrical characteristic of fermenter and rheological character of the medium. In the scientific literature are present many works where correlations for kLa were proposed for the scale-up, design and performance optimization of agitated vessels (Labík et al., 2017). In this study, the oxygen transfer performance of two lab fermenters (Biostat® B - B.Braun Biotech Int., and Bioflo110 - New Brunswick Scientific) was evaluated from the determination of volumetric oxygen transfer coefficient (KLa). Aeration system of the two fermenters was investigated employing two

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different impellers, i.e., the Rhustun turbine and the pitched-blade impeller, according to the stirring speed, the air flow rate, the broth-culture rheological properties, and the presence of antifoam agents and cells in the medium. This experimental work is part of a wider work on heterologous protein production by engineered yeast cells. The main goal of this research is to know the maximum performance of fermenters in terms of oxygen transfer in order to exclude the engineering limitations as the cause of yeast growth arrest, a phenomenon frequently observed during fermentation runs in fed-batch reactor (Mazzoleni et al.,2015, Landi et al., 2011).

2. Materials and methods

2.1 The fermenters and impellers employed

Tests for the determination of K_La were carried out with two fermenters, the Biostat®B (B.Braun Biotech Int., Melsungen, Germany) and Bioflo110 (New Brunswick, Edison, NJ). A working volume of 1 litre was chosen in a 2-litres total volume bioreactor. Air was supplied to the fermenter with a standard sparger located at the base of the agitator shaft. The two-phase (liquid-gas) dispersion was agitated with one stirrer whereas four equally spaced baffles were used to enhance mixing. As regards Bioflo 110, the efficiency of two different stirrers i.e. a pitched blade propeller and a Rushton-style impeller (Figure 1) was investigated, whereas in the case of the Biostat®B-Braun only the Rushton-style impeller was taken into consideration (Figure1, right). The pitched blade propeller is constituted by three 45 degree inclined blades and the diameter and the height are 60 and 46 mm respectively. The design of Rushton-style impeller is based on cylinder with six flat blades vertically mounted. The impeller has a total diameter of 50 mm and a singular blade has dimensions of 18 x 12 mm.

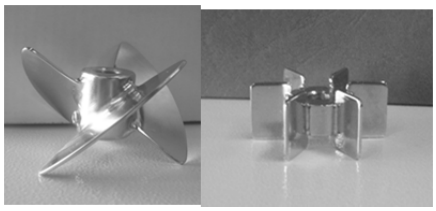

Figure 1: Pitched blade (left) and Rushton-style(right) stirrers

2.2 k_La determination

k_La (volume of oxygen per volume of liquid per time, time^{-1}) determination, at a fixed agitation speed, with the gassing out method (Enfors and Haggstrom 1998) was carried out without cells in the medium. Oxygen concentration in the medium was initially reduced to zero by flushing with nitrogen gas after which aeration was started and the increase in Dissolved Oxygen Tension (DOT) signal was monitored through the Clark electrode until air saturation was achieved (Figure 2).

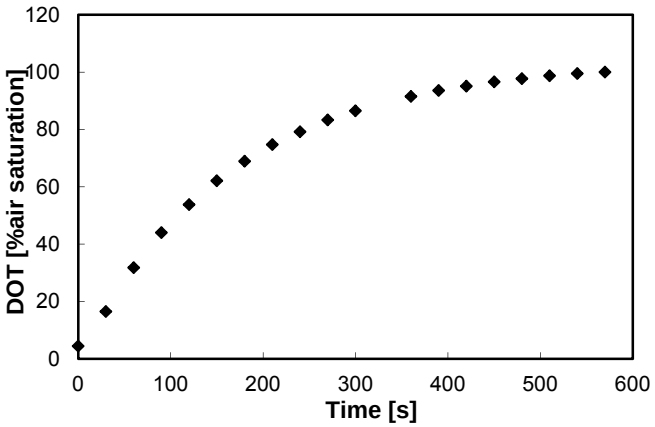

Figure 2: Dissolved Oxygen Tension (DOT) signal vs. time

The data so obtained have been manipulated to calculate the slope (-k_La) of curve ln(DOT*-DOT) *vs*. time where DOT* is the saturation value in the medium and DOT the current value at time t. K_La determination

tests have been made in duplicate for each examined stirring value. The examined media were the following: distilled water, YEP broth, a typical rich complex medium for yeast growth containing 1% Yeast Extract (BD^{TM}), 2 % Peptone (BD^{TM}) and 5% w/v Destrose (BD^{TM}), and YEP medium described above added with 1.4 ml antifoam C (diluted ten times) (Dow Corning). In the case of tests with broth-culture, yeast cells of *Saccharomyces cerevisiae* CENPK113.7D strain, subjected to thermal death (10 min at 80°C), were added to a final concentration of 20 $mg_{d.w}.ml^{-1}$.

2.3 Results

As regards Bioflo-110, first experimental results, shown in Figures.3 and 4 for pitched blade propeller and Rushton-style impeller, respectively, were obtained employing distilled water as liquid, varying stirrer speed in the range of 200-900 rotations per minute (rpm) and fixing, each time, the aeration rate at two different values, 1 and 2 air volumes per liquid volume per minute (vvm), that are the values most utilized during fermentation runs. In fact it is not possible to work over 2 vvm because if the air volume that passes through the liquid phase is more than twice that of the liquid phase the dragging phenomenon occurs.
For both the stirrers the k_La increases with increasing the aeration rate (Figures. 3 and 4). In fact, raising the aeration rate increases the parameter "a" of the product K_La, since the total volume of gas suspended in the liquid increases with the increase of aeration rate, and for high aeration rate the k_La values almost doubles.

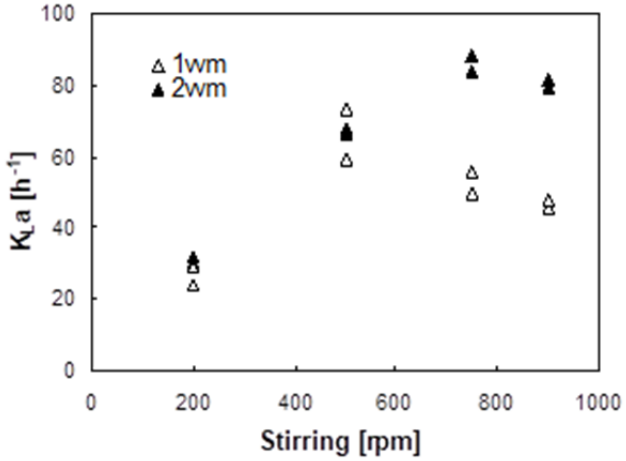

Figure 3: Pitched blade propeller: K_La values vs. stirrer speed using water as medium

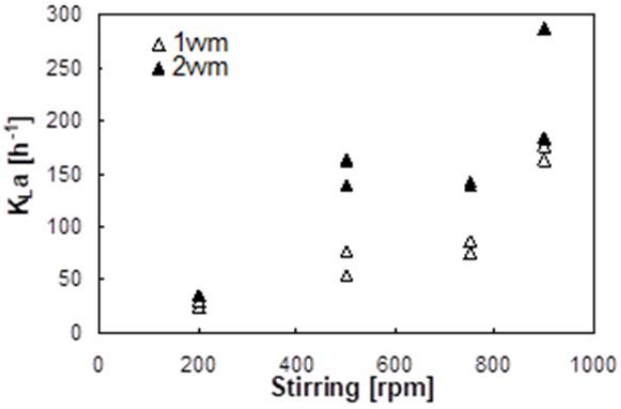

Figure 4: Rushton-style impeller: K_La values vs. stirrer speed for water as medium

When a pitched blade impeller was employed (Fig.3) K_La exhibited a maximum value at 500 and 800 rpm in correspondence with 1 and 2 vvm, respectively, then it decreased. This means that the pitched blade impeller gives a low contribution to turbulence and oxygen transfer at the highest stirrer speed. Consequently, it was not suitable for ensuring an optimal gas-liquid mass transfer rate required to satisfy the oxygen demand of respiratory metabolism during the bioprocess. On the contrary, by employing the Rushton-style impeller (Fig.4), K_La values increased as a monotone function of stirrer speed. The behavior exhibited by the two

stirrers is ascribable to the different flow patterns they promote. The propeller pumps the liquid axially and gives, as above mentioned, a low contribution to turbulence and oxygen transfer whereas the impeller pulls most of liquid to flow from the axis towards the periphery causing more turbulence and therefore a higher gas-liquid mass transfer rate.

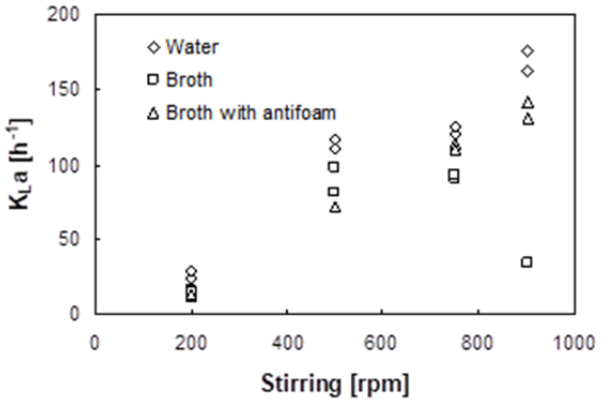

Figure 5: Effect of liquid composition in the vessel on K_La employing Rushton-style impeller (Bioflo110)

The study of K_La as a function of stirrer speed gave interesting results also when, employing a Rushton-style impeller, distilled water was replaced by rich medium (YEP) and YEP containing siliconic antifoam (Figs.5 and 6). As expected, K_La values of Bioflo 110, obtained with distilled water, drew higher values with respect to those obtained employing broth with antifoam (Fig.5). If the antifoam was not added, a maximum K_La value was achieved in correspondence of about 750 rpm agitation speed, then K_La diminished. The composition of YEP, especially the presence of proteins in the peptone made YEP a mixture similar to a colloidal solution which easily gave rise to foam bubbles capable of hampering oxygen transfer from gaseous into liquid phase. The addition of antifoam favored the coalescence of bubbles so that the dissolution rate of gas into the liquid phase raised up from 25 to 130 m^3 of oxygen per m^3 liquid volume per hour at 900 rpm. The addition of antifoam is a common procedure employed during the fermentation processes.

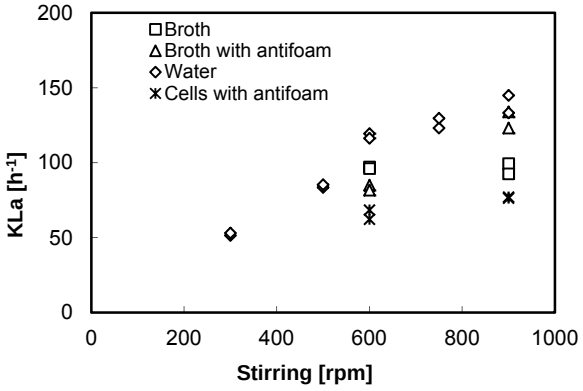

Figure 6: Effect of liquid composition in the vessel on K_La employing Rushton-style impeller (Biostat B)

The same behaviour was obtained with the Biostat® B (Fig.6), where K_La was evaluated employing the broth-culture as well. In this case, K_La values resulted the lowest encountered until then. As a matter of fact the viscosity of the broth-culture caused by dead cells in addition to the medium composition affected oxygen transfer further on. In the figure 7 it is possible to appreciate, through the pictures taken during the k_La determination tests for Bioflo110 bioreactor, the behavior of the different media at various stirring rate when the air bubbles come out from the sparger.

At low stirring speed, the oxygen bubbles exiting from the sparger go up almost undisturbed. In these conditions the oxygen transfer coefficient values are low because the permanence times in the liquid medium are low such as the “a” value, that is the amount of interfacial surface area per unit volume of oxygen.

With increasing the impeller speed, the oxygen permanence time in the liquid phase increases and, already in the case of distilled water, it is possible to see the little bubbles of air that are chopped by the impeller and the foam arises proving the large air volume trapped in the liquid phase.

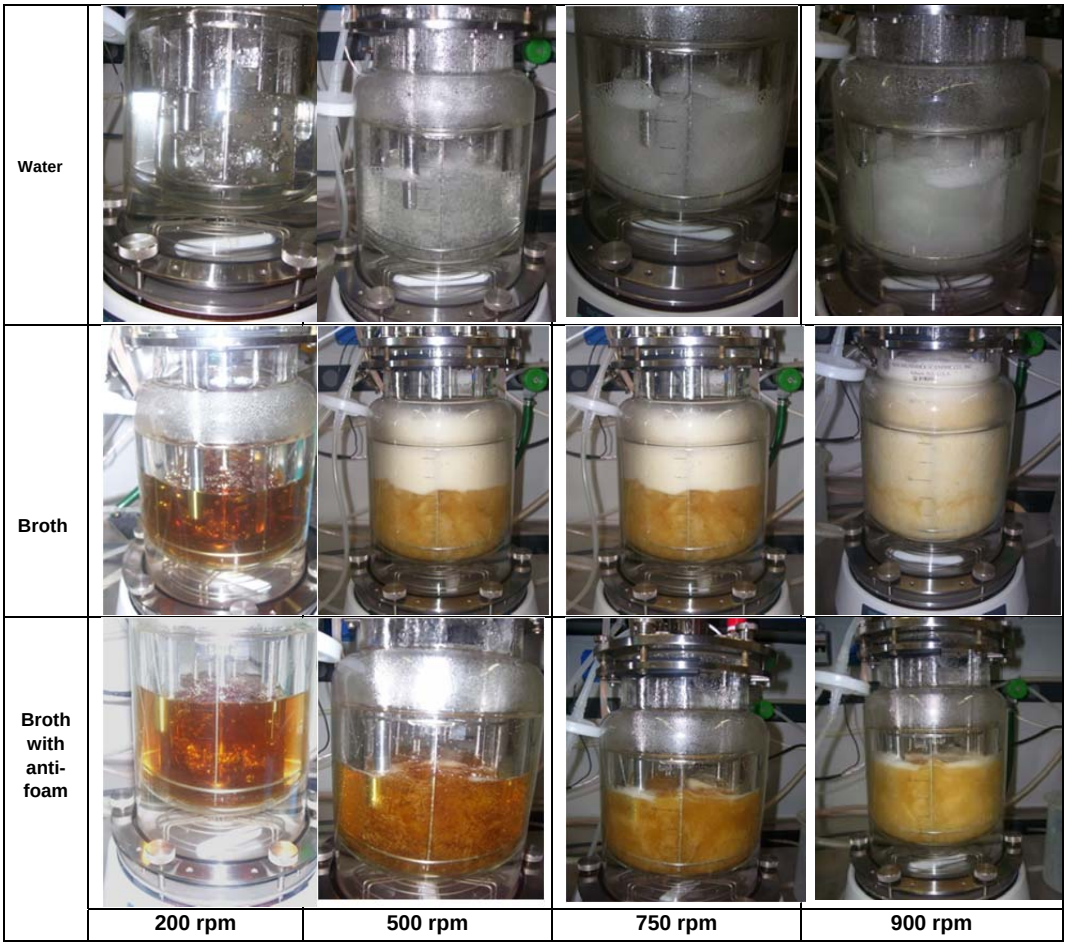

Figure 7: Effect of liquid composition in the vessel by using Rushton-style impeller (Bioflo110)

Interesting is the behavior of the rich medium without and with the addition of the siliconic antifoam. It is worth noting that the k_La value at low rpm is the same in both the media. However, when the stirring reaches the 500 rpm the foam formation is huge and it is clearly noticeable the separation between the foam volume and the volume with the gaseus and liquid phases. With the increasing of stirring the situation is getting worse and at 900 rpm stirring rate, the whole internal volume of bioreactor is filled with foam and the culture medium risks going outside: the medium goes up along the condenser to the filter at the extremity of the exhausted gas tube, leading to the wetting of the filter itself and system blocking due to overpressure.By antifoam addition, the foam is reduced even at high stirring speed, and the air volume is kept inside the liquid medium, resulting in longer permanence times of oxygen bubbles in the liquid phase and a more effective oxygen transfer to the liquid phase.

2.4 Determination of critical biomass concentration

Once K_La values are known together with that of the specific microorganism oxygen consumption rate,q_{O2} (h^{-1}) it is possible to calculate the critical biomass concentration, X (mg ml^{-1}), that is the biomass concentration in correspondence to which the oxygen consumption rate (r_{O2}) equals the maximum oxygen transfer rate (OTR_{max}) of the fermenter, that is the transfer rate, at a fixed stirring value, where the oxygen gradient is maximum (C* is the saturation concentration of oxygen in the liquid). From the following equation it is possible

$$K_L a C^* = \left(OTR_{\max}\right) = q_{O2} \cdot X = r_{O_2} \quad (1)$$

to determine the critical concentration value for each microorganism used for the aerobic bioprocess and therefore the maximum performance of bioreactors in terms of oxygen transfer. In these conditions, to avoid oxygen limitation which could determine a severe limitation of cell growth rate, the enrichment of air with pure

oxygen is required so that, C^* enhances and as a consequence OTR_{max} increases as well. A different way to by-pass the problem would be to reduce the feeding rate of carbon source so that the growth rate significantly diminishes so as to obtain a volumetric oxygen consumption rate again below $OTR_{max.}$

3. Discussion

To evaluate the maximum performance of the fermenters in terms of oxygen transfer it is necessary to evaluate the volumetric oxygen transfer coefficient, k_La, in the real conditions of microorganism growth. In fact the experimental evaluation *of* k_La must be made not by using distilled water as liquid medium but employing the same broth used for the microorganism growth because the presence in the culture medium of little amounts of salts, precursors, antifoam and cells lower the oxygen transfer efficiency. As expected, the agitation and aeration influence strongly the oxygen transfer in the fermenter. The agitation is the most influential parameter, affecting positively the oxygen mass transfer according to the experimental results obtained for the two lab fermenters. Also the aeration rate has a great influence on k_La. The choice of stirrer is the first step to set up the aerobic fermentation not only because it must guarantee the perfect mixing but also it must ensure a very good oxygen transfer. The stirrer frequently used in aerobic bioprocesses is the Rushton-style impeller for its vigorous stirring but for more gentle mixing, for example for mammalian cells growth, is often used the pitched-blade stirrer. The results indicate that trend of k_La versus agitation speed is a monotone increasing curve for the Rusthon impeller while for the pitched-blade impeller the curve have a maximum for central values of agitation rate range under investigation. The rheology of growth medium greatly influences the oxygen transport and for this reason it is fundamental to know the k_La value for each temporal value of bioprocess to allow to operate promptly to optimize the productivity and to reduce the bioprocess costs.

4. Conclusions

This work presents a valid method to evaluate the maximum performance of fermenters in terms of oxygen transfer. The results highlight the importance to evaluate the volumetric oxygen transfer coefficient (k_La) in the real bioprocess condition in order to not overestimate the maximum performance of lab fermenters.
The importance of this work compared to other experimental works is based on the possibility to know the maximum performance of the growth system in terms of oxygen transfer so that making possible a deeper investigation of other factors which can be responsible for the yeast growth arrest observed during fermentation runs in fed-batch reactor.

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