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Influence of Operating Conditions and Physical Properties of Liquid Medium on Volumetric Oxygen Transfer Coefficient in a Dual Impeller Bioreactor

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Abstract

The objective of this research was to study the effects of process variables on the volumetric mass transfer coefficient of oxygen, K_La, in a stirred bioreactor using the static gassing-out method. In this study, various process conditions were chosen, including 3 parameters, namely, concentration of glucose in medium (10, 15 and 20 g/l), air flow rate (1, 1.25, 1.5 and 1.75 vvm), and agitation rate (300, 400, 500 and 600 rpm). From the results, it was found that the K_La increased with increasing air flow rate and/or speed of agitation, but decreased with increasing concentration of glucose in medium. The maximum K_La occurred when the concentration of glucose in medium was the least (10 g/l), with an air flow rate of 1.75 vvm, and an agitation rate of 600 rpm. Correlations have been developed for the estimation of volumetric mass transfer coefficients at various process conditions for medium with different glucose concentrations. The exponent values representing dependence of K_La on the process conditions were then compared with literature values.

Keywords: Stirred bioreactor, agitation, air flow rate, dissolved oxygen, glucose concentration, volumetric mass transfer coefficient of oxygen

Introduction

In aerobic fermentations, dissolved oxygen is one of the most important substrates consumed by microorganisms, and may be a limiting substrate because of the low solubility of oxygen in liquid medium. Naturally, mass transfer of oxygen from gas phase to bulk liquid is very low, while demand for the growth of aerobic microorganisms is high, especially at high cell concentration, so adequately dissolved oxygen in the culture medium is often a critical factor in aerobic fermentation.

It is well-known that the mechanically stirred tank bioreactor in which gaseous oxygen is introduced into the liquid medium by sparging air is widely used for aerobic fermentation and waste treatment [1]. The effects of agitation and aeration increase the gas-liquid interfacial areas, and also enhance both the rate of oxygen mass transfer and mixing in the medium. In general, the basic parameter describing the oxygen transfer rate is the volumetric mass transfer coefficient of oxygen (K_La), which is an important variable and a critical parameter in the design procedure of bioreactors due to the low solubility of oxygen. The magnitude of K_La depends on various process variables, such as the geometry of the bioreactor, agitation, aeration, carbon nutrient and also medium composition [2]. However, at a fixed geometry of bioreactor, the most important variables influencing volumetric mass transfer coefficient are the agitation and aeration rates and the medium composition used.

The influence of oxygen on the control of metabolic pathways of microorganisms by nutritional regulation is an important concern in bioprocess engineering. For example, *Saccharomyces cerevisiae* is widely known as a microorganism which increases amounts of cell masses under aerobic condition and can produce ethanol under anaerobic conditions. Moreover, even under aerobic conditions at high glucose concentrations, some ethanol formation occurs, which shows the regulation metabolism of yeast not only by oxygen but also by glucose concentration [3]. Carbon nutrient affects oxygen demand in a major way. Especially, glucose as a carbon source is generally metabolized more rapidly than other carbohydrate substances such as sucrose or lactose [4]. Although several studies have reported the effects of operating conditions on the value of the volumetric oxygen transfer coefficient in liquid, few researchers have reported the value changes of the volumetric mass transfer coefficient at different glucose concentrations in YPG (Yeast extract Peptone Glucose) medium, which is a medium used for liquid culture of the *S. cerevisiae* yeast.

The purpose of this work is to evaluate the volumetric oxygen transfer coefficient in YPG medium with different glucose concentrations at various operating conditions, such as air flow rates, and speed of agitation. The effects of process variables and glucose concentration in medium on the K_La value were investigated by using a modular bioreactor (laboratory-scale) with a dual impeller system and a capacity of 2.0 l (total volume). Finally, correlations representing the dependence of K_La on process variables have been developed by using the experimental data. The parameters of the correlations obtained were compared with the literature values.

Materials and methods

Preparation of material

In various glucose concentrations of synthesis, YPG mediums were used to investigate the effect on the K_La value. Three aqueous mediums of known concentrations were prepared by dissolving analytical grade yeast extracted and peptone in distilled water. Each medium contained glucose concentrations of 10, 15 and 20 g/l, the remainder being yeast extract 2 g/l and peptone 3.33 g/l. The pH of each medium was adjusted to 4.5 and then sterilized at 121 °C for 20 min by autoclave.

Experimental method

The experimental apparatus comprised of a 2.0 l baffled bioreactor (Model No: BioFlo 2000 Modular Benchtop bioreactor, manufactured by New Brunswick Scientific Co. Inc., USA) which was constructed with a borosilicate glass vessel and a stainless steel headplate. This bioreactor was used for the determination of the volumetric oxygen transfer coefficient (K_La) values. The controls and measurements of various parameters such as impeller speed, air flow rate, temperature, dissolved oxygen and pH were performed by its modules. The working volume was 1.7 l. The stirrer consisted of dual six flat-bladed disk turbines on one axle. The sparging ring with 3 holes was situated below the bottom impeller. The rate of the air inlet that passed through a ring sparger was measured with an air flow meter. The K_La was determined by the static gassing-out technique. Three types of medium with different glucose concentrations (10, 15 and 20 g/l) were used to study the effects of aeration rate (1, 1.25, 1.5 and 1.75 vvm) and agitation rates (300, 400, 500 and 600 rpm). The temperature and pH of the medium were controlled at 28 °C and pH 4.5, respectively. The dissolved oxygen in the medium was stripped by passing nitrogen gas until the dissolved oxygen was zero for about 15 min before the start of the experiment, to achieve the steady state. Then, the agitation rate and the air flow rate were adjusted to selected values. Air as a source of oxygen was passed through the air sparger at the bottom of the bioreactor instead of nitrogen gas in the medium, and the dissolved oxygen in the medium was recorded every 15 s during the experiment. The experiment was stopped when the dissolved oxygen increased to the saturation value. All experiments were repeated 3 times and all the readings (the dissolved oxygen) were producible with errors of measurement being less than 2 %. Average values were used. In the absence of biomass, the oxygen transfer rate from the gas to the liquid (OTR) is obtained by multiplying the gradient between the saturation and the actual dissolved oxygen concentration ($C^* - C$) with K_La, according to Eq. (1);

$$OTR = \frac{dC}{dt} = K_L a.(C^* - C) \quad (1)$$

where dC/dt is the oxygen transfer rate per unit of medium volume, C is the actual concentration of oxygen at any time, and C^* is the saturation concentration of oxygen. Integration of Eq. (1) yields;

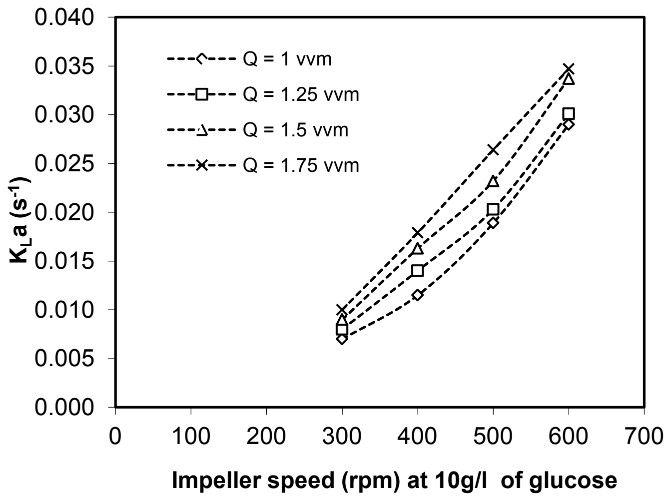
$$\ln(C^* - C) = -(K_L a).t + \ln C^* \quad (2)$$

The $K_L a$ value was evaluated, using Eq. (2), from the slope of the straight portion of the curve between $\ln(C^* - C)$ versus time.

Results and discussion

Effect of impeller speed on the K_La values

Agitation is an important factor that affects the $K_L a$ values of a stirred bioreactor. In this study the effect of agitation on the $K_L a$ value was evaluated at 4 impeller speeds, namely 300, 400, 500 and 600 rpm, at each of aeration rate and glucose concentrations in YPG medium. It was found that the $K_L a$ values increased with increasing impeller speed for all tests. For the medium with glucose a concentration of 10 g/l, at air flow rate of 1 vvm, the $K_L a$ values increased from 0.007 to 0.029 s^{-1} when the impeller speeds were 300 to 600 rpm (**Figure 1a**). Similarly, at air flow rates of 1.25, 1.5 and 1.75 vvm, the $K_L a$ values increased from 0.008 to 0.0301 s^{-1}, from 0.009 to 0.0337 s^{-1}, and from 0.01 to 0.0347 s^{-1}, respectively, over the aforementioned impeller speeds. Similar trends were obtained with other glucose concentrations in the medium (**Figure 1b** and **1c**). The results showed that higher impeller speeds produced a rapid breakage of air bubbles into a smaller size, and thus enhanced the gas-liquid interfacial area per unit volume for oxygen mass transfer in the medium.



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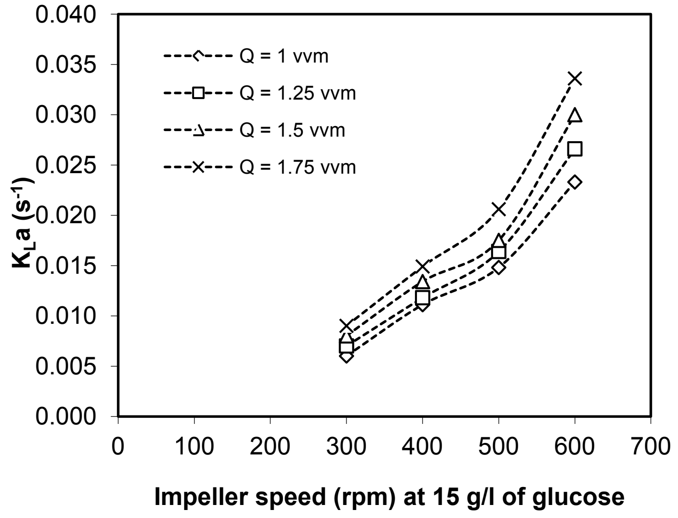

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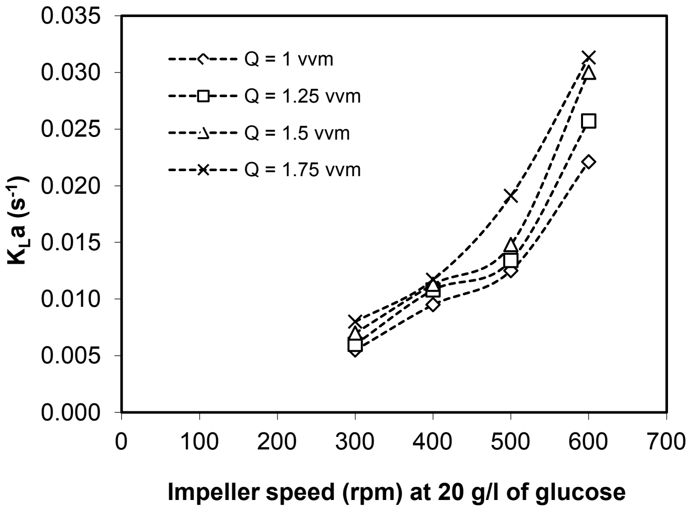

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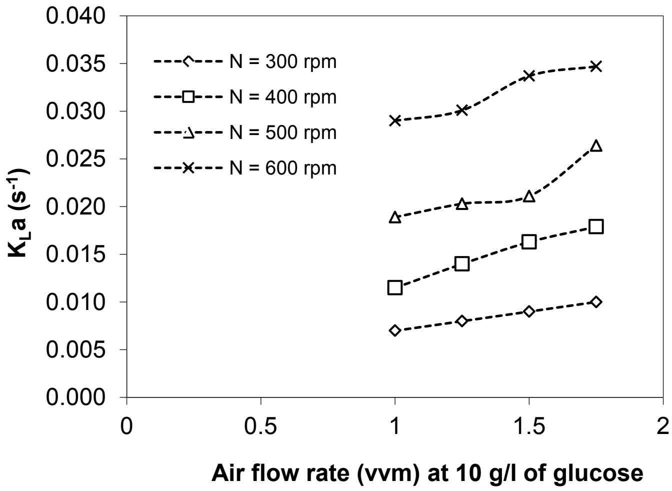
Figure 1 The K_La values versus impeller speeds in medium at each glucose concentration: (a) 10 g/l of glucose, (b) 15 g/l of glucose, (c) 20 g/l of glucose.

Effect of aeration rate on the K_La values

The change of aeration rate is also one of the most important factors which affect the K_La values. Generally, efficient utilization of sparged gas will be reflected by increased fractional gas hold-up, which enhances the gas-liquid interfacial area for the oxygen mass transfer. From the experimental results, it was found that the K_La values increased with an increase in aeration rates. At an impeller speed of 300 rpm, K_La values obtained for medium with glucose concentration of 10 g/l increased from 0.007 to 0.01 s^{-1} over the range of air flow rate of 1 - 1.75 vvm, as shown in **Figure 2a**. The K_La values increased similarly at other impeller speeds of 400, 500 and 600 rpm over the aforementioned air flow rates. Similar trends were obtained with other glucose concentrations in the medium, as shown in **Figure 2b** and **2c**. The main reason for the above results is that at higher air flow rates, the total volume of gas bubbles in the bioreactor increase, leading to high surface area between gas and liquid, which increases the K_La values.



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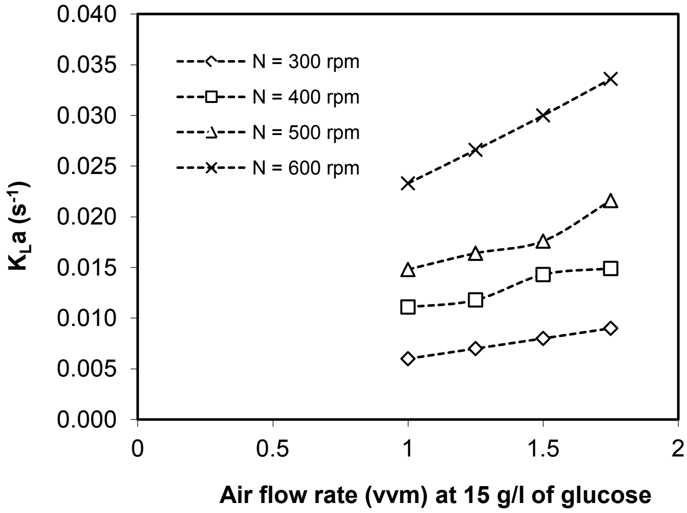

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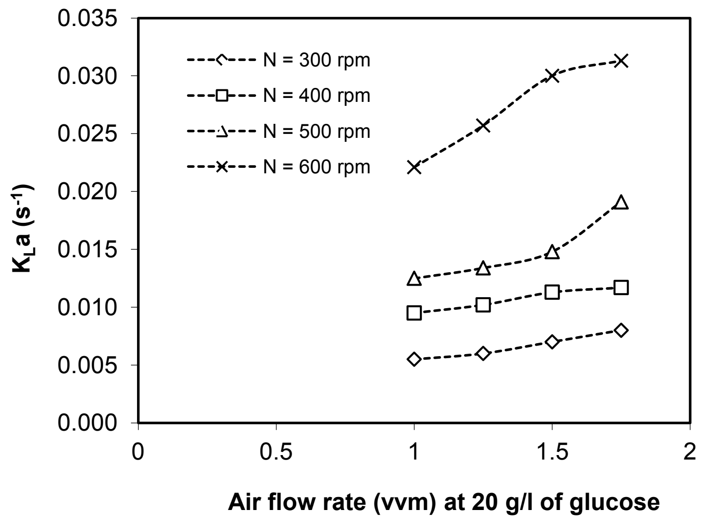


(c)

Figure 2 The K_La values versus air flow rates in medium at each glucose concentration: (a) 10 g/l of glucose, (b) 15 g/l of glucose, (c) 20 g/l of glucose.

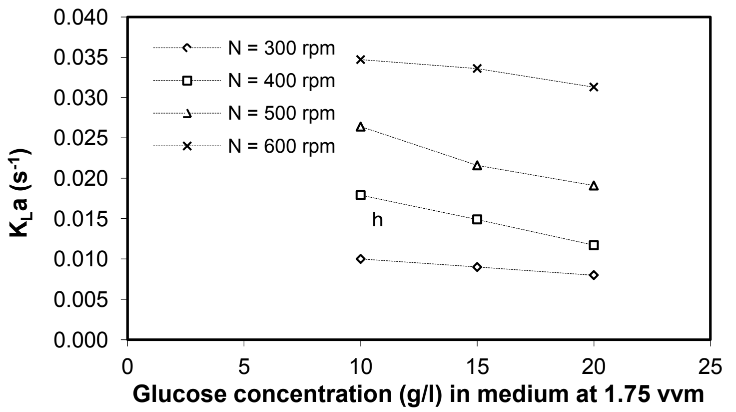
Effect of glucose concentration in medium on the K_La values

Another factor known to affect the mass transfer in a bioreactor is the viscosity of the liquid. In this study, mediums at glucose concentrations of 10, 15 and 20 g/l were prepared. The effect of viscosity on the K_La value was investigated using impeller speeds at 300 - 600 rpm and aeration rates in the range of 1 - 1.75 vvm.

The K_La values decreased with increasing glucose concentration to 20 g/l at each agitation rate. The influence of glucose concentration over this range of agitation rates, shown at 1.75 vvm (**Figure 3a**), is a typical of the trends observed at other aeration rates.

The K_La values also decreased with an increase in glucose concentration to 20 g/l at each aeration rate. The influence of glucose concentration over this range of aeration rates, shown at 500 rpm (**Figure 3b**), is a typical of the trends observed at other aeration rates.

The reduction of K_La value with increasing glucose concentration is a likely consequence of reduced turbulence or an increase in liquid viscosity, thereby increasing liquid film resistance. These results highlight the importance of process conditions on the reduction of K_La through glucose addition.



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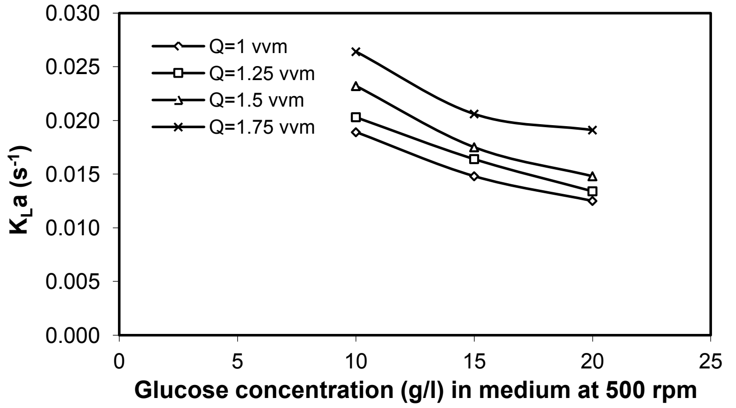

(b)

Figure 3 The K_La values versus glucose concentrations in medium at: (a) various impeller speeds (b) various aeration rates.

Correlations for mass transfer coefficient

Generally, the volumetric mass transfer coefficient (K_La) is a function of physical properties and vessel geometry. Different types of correlations are available for the prediction of volumetric oxygen transfer coefficients in stirred vessels. In these correlations [1,5-13], K_La is related to 2 major coefficients: the mechanical agitation power consumption per unit volume (P_g/V) and the gas sparging rate, expressed as the superficial gas velocity (v_g). Numerous studies for the correlations of K_La have been reported and their results have a general form as;

$$K_La = k\left(\frac{P_g}{V_L}\right)^a \left(v_g\right)^b \tag{3}$$

where k is the constant; V_L is the liquid volume; a and b are exponents which vary considerably depending on the geometry of the system, the range of variables covered, and the experimental method used. These values were determined by plotting on the logarithmic coordinates. As reported by Warren L. McCabe and others [12], superficial air velocity is the average speed of bubble velocity when rising from the bottom of bioreactor to the liquid surface. It is strongly affected by liquid dispersion and an important factor in bioreactor design. Ungassed power consumption (P_0) was obtained through a graph of power numbers (N_p) as a function of a modified Reynold number (Re), as proposed for non-viscous liquids as following.

$$N_P = \frac{P_0}{\rho N^3 D_i^3} \tag{4}$$

$$\mathrm{Re} = \frac{\rho N D_i^2}{\mu} \tag{5}$$

where ρ is the liquid density; μ is the liquid viscosity; N is the impeller speed; and D_i is the vessel diameter.

For non-viscous liquids, a good estimate of gassed power consumption (Pg) can be obtained using the following relationship [7,10,12-13].

$$P_g = m\left(\frac{NP_o^2 D_i^3}{Q^{0.56}}\right)^{\beta} \tag{6}$$

where Q is the air flow rate. The constants (m and β) depend on the impeller geometry. For dual 6 flat-bladed turbines, the values of m and β are 0.832 and 0.45, respectively.

For this study, correlations for K_La, P_g/V and v_g obtained were developed by regression analysis of the experimental data. The exponential relationship between the volumetric oxygen transfer coefficient and the power input per volume and the superficial gas velocity for the various glucose concentrations are given in Eq. (7) - (9). The statistical values of R^2 obtained using the data were 0.985, 0.956 and 0.918, respectively.

- Medium at glucose concentration of 10 g/l.

$$K_La = 0.0135\left(\frac{P_g}{V_L}\right)^{0.58}\left(v_g\right)^{0.58} \tag{7}$$

- Medium at glucose concentration of 15 g/l.

$$K_La = 0.0119\left(\frac{P_g}{V_L}\right)^{0.59}\left(v_g\right)^{0.58} \tag{8}$$

- Medium at glucose concentration of 20 g/l.

$$K_L a = 0.0107\left(\frac{P_g}{V_L}\right)^{0.61}\left(v_g\right)^{0.60} \tag{9}$$

For stirred tank bioreactors, the values of the exponents (a and b) in Eq. (3), representing the dependence of the $K_L a$ on P_g / V and v_g, are compared with literature values (**Table 1**). It can be seen that the exponent values show a wide variation range in this correlation proposed by different authors: $0.4 \le a \le 1$; $0.3 \le b \le 0.7$. The exponents obtained from this study are in the range obtained from the literature. The model makes it possible to predict the effects of operational conditions and properties of liquid on the volumetric mass transfer coefficient, $K_L a$, in a stirred bioreactor.

Table 1 Exponent values of $K_L a$ for each variable in the dimensional correlations proposed in the literature, found by several authors.

Data from literature	System	P_g / V	v_g
Gagnon *et al*. [14]	Water	0.6 - 0.8	0.5
Vant't Riet [15]	Water	0.4	0.5
Yagi and Yoshida [16]	Water+glycerol	0.8	0.3
Kawase and Moo-Young [17]	Water	1	0.5
Garcia-Ochoa and Gomez [18]	Water/Water+xantan	0.6	0.5 - 0.67
Pedersen *et al*. [19]	Water+xantan	-	0.5 - 0.7
This study	YPG medium	0.58 - 0.61	0.58 - 0.6

Conclusion

The general agitated tank bioreactors operated at various conditions, such as agitation speed, aeration rate and glucose concentration, influenced the volumetric oxygen transfer coefficient in liquid medium. The experimental results found that at each glucose concentration in medium the $K_L a$ values increased with increasing agitation speeds and aeration flow rates, but the $K_L a$ values decreased with increasing glucose concentration in medium. The best correlations for predicting the $K_L a$ values in this study have been reported in a general form as $K_L a = k\left(\frac{P_g}{V_L}\right)^{a}\left(v_g\right)^{b}$ for each glucose concentration in medium, especially for medium with low glucose concentration.

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