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Oxygen mass-transfer performance of low viscosity gas-liquid-solid system in a split-cylinder airlift bioreactor

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Abstract

The O_2 mass-transfer coefficient, k_La, decreased by 20% when the viscosity of a simulated broth increased from 1.38×10^{-3} to 3.43×10^{-3} Pa s in a split-cylinder airlift bioreactor with a broth volume of 41 l. When the paper pulp concentration was below 10 g l^{-1}, k_La hardly changed. While at 30 g l^{-1}, k_La decreased by 56%. $C_2O_4^{2-}$ and Na^+ were found to have some effect on the k_La value.

Nomenclature:
C – dissolved oxygen tension, %; C^* – saturated dissolved oxygen tension, %; u_g – superficial gas velocity based on the cross-area of the column, m s^{-1}; k_La – O_2 mass-transfer coefficient, s^{-1}; a – specific interfacial area, m^2 m^{-3}; ε – total gas hold-up based on the working volume of bioreactor, dimensionless; τ – average circulating time, s.

Introduction

Filamentous fungal fermentation has been used for many years to produce a variety of products. It is well known that such a fermentation system suffer from high broth viscosity that often leads to O_2 mass-transfer limitation. Both biomass and cell morphology contribute greatly to the high broth viscosity (Metz *et al.* 1979, Olsvik & Kristiansen 1992, 1994, Riley *et al.* 2000). Therefore, it is feasible to decrease the viscosity by controlling cell morphology during fermentation. By inducing individual mycelia to branch together and form pellets, the broth viscosity can be decreased considerably (Schügerl *et al.* 1983, Pazouki & Panda 2000). Our unpublished experimental data showed that, during the culture of a recombinant *Aspergillus niger* that secrete heterologous GFP (Green Fluorescent Protein), broth viscosity could be less than 2×10^{-3} Pa s when pellets instead of mycelia were formed. It is expected that the O_2 mass-transfer performance within the bioreactors can be improved as a result. However, as mycelia branch together to form larger pellets, the critical dissolved O_2 level required by their growth and metabolism increases correspondingly due to the intraparticle molecular diffusion limitation. This means the driving force for O_2 mass-transfer based on the concentration difference between the dissolved O_2 concentration and equilibrium O_2 concentration is decreased. The O_2 mass-transfer coefficient, k_La, even when decreased slightly as compared to the widely investigated air-water system, can make a significant difference in fungal fermentation outcomes.

Airlift bioreactors are showing great advantages in fungal fermentation because of their lower shear stress and excellent anti-contamination performance.

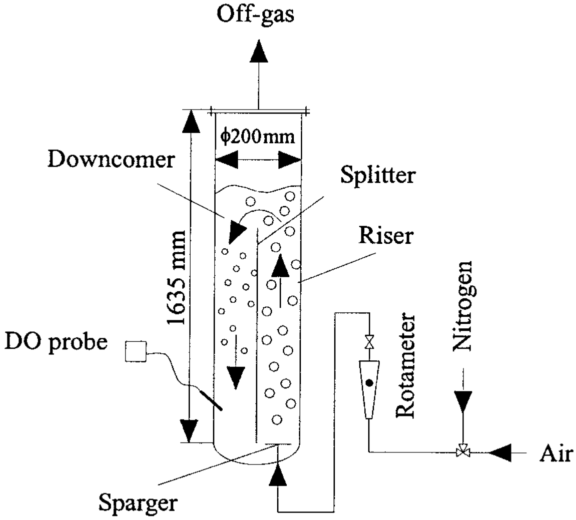

Fig. 1. Configuration of a split-cylinder airlift bioreactor.

However, their poor O_2 mass-transfer performance compared to traditional stirred tanks limits their commercial applications. Many efforts were made to improve their O_2 mass-transfer performance in the past (Chisti & Moo-Young 1987, 1993, Tung *et al.* 1998, Poulsen & Iversen 1999, Miyahara *et al.* 1999). This work used a split-cylinder airlift bioreactor with a broth volume of 41 l. Sodium carboxymethyl cellulose (CMC) and paper pulp were used to simulate the fungal fermentation broth. O_2 mass-transfer coefficient, k_La, gas hold-up, ε, and circulating time, τ, were studied. Experimental results indicated that compared with an air-water system, an average decrease of 30–50% for k_La was found within the normal operating parameter ranges. Therefore, the size distribution of pellets in the fermentation systems should be controlled properly by applying biological and engineering strategies such as controlling medium composition, inoculation level and spore age, etc. to prevent a possible O_2 mass-transfer limitation (van Suijdam & Metz 1981, Tucker & Thomas 1992, Cui *et al.* 1998, Xu *et al.* 2000).

Materials and method

Bioreactor

The custom-built split-cylinder airlift bioreactor with a working volume of 41 l used in this work is shown in Figure 1.

Simulated broth and biomass

CM-cellulose (ultra-low viscosity grade, Aldrich) solutions were used to simulate the various viscosities within the fermentation broth. Paper pulp (International Fibre Corporation, North Tonawanda, NY) with a fiber length of 0.3 mm was used to simulate fungal biomass. Sodium oxalate and sodium chloride were used to investigate the effect of ionic concentration on O_2 mass-transfer.

Viscosity of the simulated broth

Measured by using a Cannon–Fenske type kinematic viscometer tube (Fisher Scientific, Pittsburgh, PA).

Measurement of k_La, ϵ and τ

Experiments were carried out at 25 °C. The dynamic method was used to measure k_La (Dunn & Einsele 1975). An Ingold oxygen sensor (Mettler Toledo, Wilminton, MA) and the LabVIEW software (National Instruments, Austin, TX) were used to measure and record the change of dissolved O_2 tension with time. N_2 was used to de-oxygenate dissolved O_2 before k_La measurements. In the system without microbial growth, there exists:

$$\frac{dC}{dt} = k_La(C^* - C),$$
$$\text{at } t = 0, C = 0.$$

This gives

$$\ln \frac{C^* - C}{C^*} = -k_Lat.$$

Then k_La can be calculated according to the recorded $C-t$ values. The classic volume method (Chisti 1989) was used to measure total ϵ. Small fresh 5 × 5 mm carrot cubes, with a density of 1.03 g ml^{-1}, were used as particle tracers to measure the liquid circulating velocities. The time for a carrot cube to travel a certain distance in the reactor was measured by using a stopwatch to calculate its velocity. τ was calculated based on the liquid circulating velocities measured and the total length of the circulating channel within the bioreactor.

Results and discussion

Based on the fact that aeration rate range of 0.1–1 vvm is usually required for the culture of recombinant *Aspergillus niger* pellets, superficial gas velocity u_g was controlled between 0.4 × 10^{-2} and

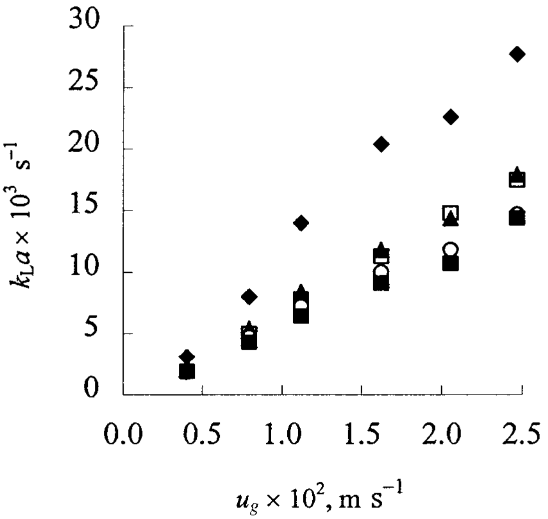

Fig. 2. The effects of the viscosity on k_La with 20 g l^{-1} paper pulp. ♦, Water without paper pulp; ●, 0.18% CMC; □, 0.4% CMC; ▲, 0.6% CMC; ○, 0.8% CMC; ■, 1.0% CMC.

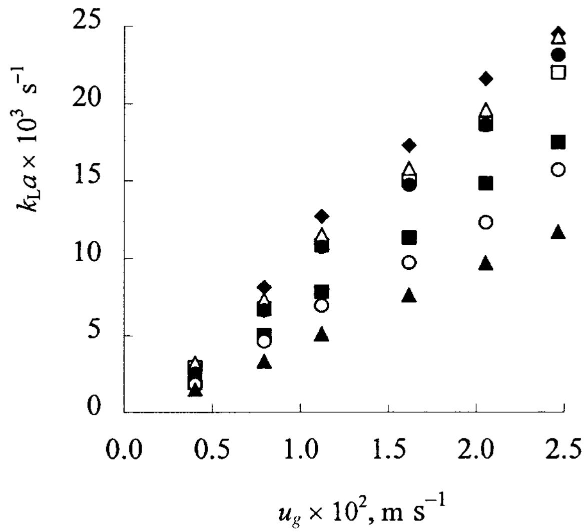

Fig. 3. The effect of paper pulp concentration on k_La with 0.18% CMC solution. ♦, No paper pulp; □, 5 g l^{-1}; △, 10 g l^{-1}; ●, 15 g l^{-1}; ■, 20 g l^{-1}; ○, 25 g l^{-1}; ▲, 30 g l^{-1}.

2.5×10^{-2} m s^{-1}. The viscosity of the simulated broth was 1.38×10^{-3}, 1.83×10^{-3}, 2.31×10^{-3}, 2.99×10^{-3} and 3.43×10^{-3} Pa s, respectively, corresponding to the CM-cellulose concentration of 0.18%, 0.4%, 0.6%, 0.8% and 1.0% (w/v). The effect of paper pulp concentration on k_La was tested at 6 concentration levels: 5, 10, 15, 20, 25 and 30 g l^{-1}.

O_2 mass-transfer coefficient k_La

The effect of the viscosity of the simulated broth on k_La was negligible when the viscosity was relatively low (Figure 2). The k_La values were nearly the same when the viscosity was increased from 1.38×10^3 Pa s to 1.83×10^{-3} Pa s. However, when the viscosity was increased from 1.83×10^{-3} Pa s to 3.43 $\times 10^3$ Pa s, k_La decreased by ca. 20% from 2.3×10^{-3} to 2.1×10^{-3} s^{-1} at low u_g (0.4×10^{-2} m s^{-1}) and from 1.79×10^{-2} s^{-1} to 1.25×10^{-2} s^{-1} at high u_g (2.5×10^{-2} m s^{-1}). Compared to the air-water system, this decrease was as high as 50%.

Paper pulp concentration also affected k_La (Figure 3). When the concentration varied between 0–10 g l^{-1}, k_La changed slightly from 2.8×10^{-3} to 3.2×10^{-3} at low u_g (0.4×10^{-2} m s^{-1}) and from 2.45×10^{-2} s^{-1} to 2.43×10^{-2} s^{-1} at high u_g (2.5×10^{-2} m s^{-1}). This phenomenon was also observed during the culture of recombinant *A. niger* when spores were used to inoculate the medium (unpublished data). This indicated that if the biomass was below 10 g l^{-1}, the biomass concentration itself does not affect k_La significantly. However, the total O_2 uptake rate was almost proportional to biomass concentration during this period of fermentation. Therefore, O_2 supply could still be the limiting factor for cell growth and metabolism. When paper pulp concentration was further increased, k_La decreased sharply. At 30 g l^{-1} paper pulp concentration, k_La value was 1.5×10^{-3} s^{-1} at low u_g (0.4×10^{-2} m s^{-1}) and 1.17×10^{-2} s^{-1} at high u_g (2.5×10^{-2} m s^{-1}), indicating a 56% decrease compared with the simulated broth without paper pulp. This suggested that it would be difficult for airlift bioreactors to support a high-density culture of fungal pellets. The desirable density of biomass should be limited to no more than 10 g l^{-1}.

Complex medium is widely used for fungal fermentation. Ions mainly come from the pH control operation which uses sodium hydroxide to neutralize the by-produced organic acids. Oxalic acid is one of the organic acids produced by *Aspergillus niger* cultured at pH 6–7 (Roehr *et al.* 1992). Therefore, sodium oxalate and other sodium salt of organic acids are present in real fermentation systems. The concentration of Na^+ is usually no more than 0.2 M at the end of fermentation. Owing to the poor solubility of sodium oxalate, sodium chloride was used together with sodium oxalate to increase the Na^+ concentration to 0.2 M. The pH value was maintained at 6.5 during the experiments. Figure 4 shows that the increase of ion concentration had no negative effect on k_La. k_La increased from 1.90×10^{-3} to 2.80×10^{-3} s^{-1} at

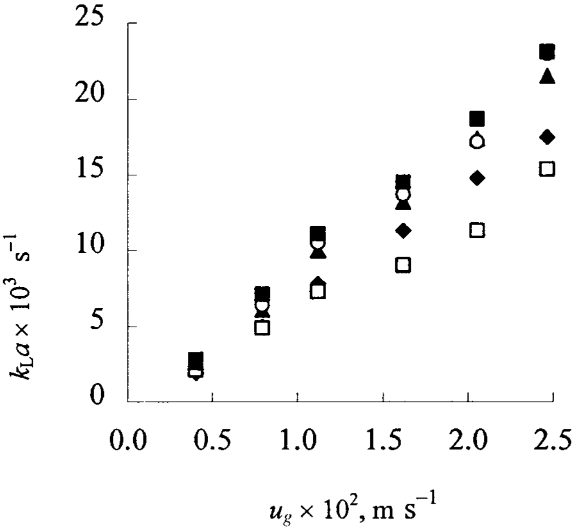

Fig. 4. The effect of ion concentration on k_La with 0.18% CMC plus 20 g l^{-1} paper pulp. ♦, No ions added; □, 0.025 M $Na_2C_2O_4$; ○, 0.05 M $Na_2C_2O_4$; ▲, 0.05 M $Na_2C_2O_4$ + 0.05 M NaCl; ■, 0.05 M $Na_2C_2O_4$ + 0.1 M NaCl.

low u_g (0.4×10^{-2} m s^{-1}) and from 1.75×10^{-2} to 2.31×10^{-2} s^{-1} at high u_g (2.5×10^{-2} m s^{-1}) when 0.2 M Na^+, 0.1 M Cl^-, and 0.1 M $C_2O_4^{2-}$ were added to the simulated broth. An average increase of 36% for k_La was observed. However, it is well known that electrolytes and other solutes decrease the equilibrium solubility of O_2 (Schumpe *et al.* 1978, Schumpe & Deckwer 1979). The increase of k_La resulted from dissolved ions will benefit O_2 supply in the fermentation of recombinant *Aspergillus niger* pellets.

Gas hold-up ε

Gas hold-up determines the fraction of gas in the bubble bed and thus, the residence time of phases in the bed, for a given gas flow rate. It is one of the key parameters that describe the quality of gas dispersion and the effectiveness of the gas-liquid mass transfer. When the simulated broth viscosity was increased from 1.38×10^{-3} to 3.43×10^{-3} Pa s, ϵ increased from 0.72×10^{-2} to 1.08×10^{-2} at low u_g and from 6.52×10^{-2} to 7.95×10^{-2} at high u_g, which represented an average increase of 30–50%. This suggested that the gas phase residence time in the bed was increased by the viscosity increase and thus, the gas hold-up was increased accordingly. However, the increase of viscosity also promotes the bubble coalescence, which leads to the formation of larger bubbles and thus decreases the surface area per unit volume of dispersion in the gas-liquid contactor. O_2 mass-transfer performance will therefore deteriorate. That was the reason why k_La decreased with the increase of broth viscosity. The appearance of solid particles in the bed decreased ε. When paper pulp concentration was increased from 0 to 30 g l^{-1}, ε was decreased from 1.01×10^{-2} to 0.51×10^{-2} at low u_g (0.4×10^{-2} m s^{-1}) and from 6.87×10^{-2} to 4.34×10^{-2} at high u_g (2.5×10^{-2} m s^{-1}), which reflect decrease of 30–40%. The effect of small solid particles on bubble behavior is complicated. When the difference of density between liquid and solid is small, many small solid particles are wrapped within bubble vortexes when bubbles rise, which increases the surface area per unit volume of dispersion in the gas-liquid contactor and thus enhances the O_2 mass-transfer. Ion concentrations affected ϵ only slightly.

Circulating time τ

τ is defined as the average time needed for particles to circulate one cycle in the bioreactor. It can be used to evaluate the mixing performance of bioreactors (Nagata 1975). Our experimental results showed that when the viscosity of the simulated broth reached 3.43×10^{-3} Pa s, a slight increase of τ was observed. Throughout our experiments, τ was no more than 30 s. This suggested a good mixing performance within this airlift bioreactor, which could guarantee an efficient transport of nutrients and metabolites. Other parameters, such as ion concentration, exhibited no significant effect on τ (usually less than 20% increase of τ compared to that of the air-water system).

Conclusions

Based on the experimental results presented above, the decrease of k_La caused by the increase of broth viscosity cannot be neglected even when the viscosity is much lower than that of the traditional mycelial fermentations. The final biomass concentration should be below 10 g l^{-1} to avoid O_2 limitation. Ions present within the culture system due to pH controlling do not have negative effects on k_La.

References

Chisti Y (1989) *Airlift Bioreactors*. New York: Elsevier.

Chisti Y, Moo-Young M (1987) Airlift reactors – characteristics, applications and design considerations. *Chem. Eng. Commun.* **60**: 195–242.

Chisti Y, Moo-Young M (1993) Improve the performance of airlift reactors. *Chem. Eng. Proc*. **89**: 38–45.

Cui YQ, Okkerse WJ, van der Lans RGJM, Luyben KCAM (1998) Modeling and measurements of fungal growth and morphology in submerged fermentations. *Biotechnol. Bioeng*. **60**: 216–229.

Dunn IJ, Einsele A (1975) Oxygen transfer coefficients by the dynamic method. *J. Appl. Chem. Biotechnol*. **25**: 707–720.

Metz B, Kossen WF, van Suijdam JC (1979) The rheology of mould suspension. *Adv. Biochem. Eng*. **11**: 103–156.

Miyahara T, Hamanaka H, Umeda T, Akagi Y (1999) Effect of plate geometry on characteristics of fluid flow and mass transfer in external-loop airlift bubble column. *J. Chem. Eng. (Japan)* **32**: 689–695.

Nagata S (1975) *Mixing: Principles and Applications*. Tokyo: Kodansha.

Olsvik ES, Kristiansen B (1992) Influence of oxygen tension, biomass concentration and specific growth rate on the rheological properties of a filamentous fermentation broth. *Biotechnol. Bioeng*. **40**: 1293–1299.

Olsvik ES, Kristiansen B (1994) Rheology of filamentous fermentations. *Biotechnol. Adv*. **12**: 1–39.

Pazouki M, Panda T (2000) Understanding the morphology of fungi. *Bioprocess. Eng*. **22**: 127–143.

Poulsen BR, Iversen JJL (1999) Membrane sparger in bubble column, airlift, and combined membrane-ring sparger bioreactors. *Biotechnol. Bioeng*. **64**: 452–458.

Riley GL, Tucker KG, Paul GC, Thomas CR (2000) Effect of biomass concentration and mycelial morphology on fermentation broth rheology. *Biotechnol. Bioeng*. **68**: 160–172.

Roehr M, Kubicek CP, Kominek J (1992) Industrial acids and other small molecules. In: Bennett JW, Klich MA, eds. *Aspergillus: Biology and Industrial Applications*. Boston: Butterworth-Heinemann, pp. 91–131.

Schügerl K, Wittler R, Lorenz T (1983) The use of molds in pellet form. *Trends Biotechnol*. **4**: 120–123.

Schumpe A, Adler I, Deckwer W-D (1978) Solubility of oxygen in electrolyte solutions. *Biotechnol. Bioeng*. **20**: 145–150.

Schumpe A, Deckwer W-D (1979) Estimation of O_2 and CO_2 solubilities in fermentation media. *Biotechnol. Bioeng*. **21**: 1075–1078.

Tucker KG, Thomas CR (1992) Mycelial morphology: the effect of spore inoculum level. *Biotechnol. Lett*. **14**: 1071–1074.

Tung HL, Tu CC, Chang YY, Wu WT (1998) Bubble characteristics and mass transfer in an airlift reactor with multiple net draft tubes. *Bioprocess. Eng*. **18**: 323–328.

van Suijdam JC, Metz B (1981) Influence of engineering variables upon the morphology of filamentous molds. *Biotechnol. Bioeng*. **23**: 111–148.

Xu J, Wang L, Ridgway D, Gu T, Moo-Young M (2000) Increased heterologous protein production in *Aspergillus niger* fermentation through extracellular protease inhibition by pelleted growth. *Biotechnol. Prog*. **16**: 222–227.