ANALYSIS OF OXYGEN TRANSFER ENHANCEMENT BY OXYGEN CARRIER IN THE AUTOTROPHIC CULTIVATION OF *ALCALIGENES EUTROPHUS* UNDER LOW OXYGEN PARTIAL PRESSURE

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Growth rate of *Alcaligenes eutrophus* under non-explosive gas mixture of low oxygen partial pressure, where oxygen transfer was rate determining process, was enhanced by adding perfluorocarbon as an oxygen carrier. The trend of enhancement with increase of PFC addition was different across the phase inversion region where continuous phase changes from aqueous medium to PFC. The enhancement was analyzed quantitatively using a mass transfer model, which took into account two cases where either the medium or the PFC was continuous phase. An enhancement factor for the oxygen transfer rate, defined as the ratio of oxygen transfer rate in a medium mixed with PFC to that in a PFC-free medium, was used in evaluating the effect of PFC addition. The factor was a function of the oxygen transfer coefficient and the interfacial area between gas bubble and the medium, and the volume fraction of PFC when the medium was in continuous phase. Also the factor varied with the interfacial area and the oxygen transfer coefficient between the PFC and the medium, the volume fraction of PFC and the dissolved oxygen concentration when the PFC phase became continuous. The model revealed the factors affecting on the observed cell growth rate of *A. eutrophus*, such that the primary factor contributing to the enhancement of the cell growth in the latter case was the increase of the interfacial area between the PFC and the medium.

Introduction

Carbon dioxide fixation has become an urgent issue for global environmental protection. *Alcaligenes eutrophus* is considered as a candidate microbe for carbon dioxide fixation due to its rapid growth. However, a problem arises since its optimal substrate is an explosive gas mixture of hydrogen, oxygen and carbon dioxide^{3, 9)}.

To escape from the explosive composition region of the gaseous substrate, *A. eutrophus* was autotrophically cultivated under lowered oxygen partial pressure¹⁴⁾. In order to recover the decreased oxygen transfer rate due to the lowered oxygen partial pressure, biologically non-toxic perfluorotributylamine¹⁰⁾ was added to the medium as an oxygen carrier. Remarkable enhancement of the cell growth rate was observed when the aqueous medium was dispersed dropwise in a continuous PFC phase. Similar effect of oxygen carrier on microbial cultivation has been studied with scarce quantitative evaluation^{1, 2, 4-6, 8, 11, 12, 15)}.

In the present article, the enhancement of the oxygen transfer rate under the presence of PFC is discussed quantitatively using a mass transfer model.

1. Experimental

1.1 Cultivation conditions

Alcaligenes eutrophus H16 was cultured. The cultivation apparatus, medium composition, pH, temperature, agitational speed and gas feeding rate are described elsewhere¹⁴⁾ unless otherwise stated.

1.2 Perfluorocarbon

Perfluorotributylamine (FC-43, N (C₄F₉)₃) was used as a model oxygen carrier. Oxygen solubility in FC-43 is about 14 times of that in water under air saturation¹³⁾.

1.3 Analytical methods

Cell concentration, gas composition and dissolved oxygen concentration were measured by the same methods as described elsewhere¹⁴⁾.

1.4 Measurement of oxygen uptake rate

Oxygen uptake rate based on unit medium volume, OUR_M , was determined by measuring the inlet and outlet gas compositions in the fermenter and the gas flow rates into and from the fermenter.

1.5 Measurement of bubble and droplet sizes

The sizes of bubbles and droplets were measured via images recorded by a video camera equipped with a high speed shutter (CCD-V700, Sony Co., Tokyo, Japan). The video image was frozen on a monitor and the sizes were measured.

More than 150 samples of bubbles and droplets were

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(a) During cultivation.

(b) After agitation.

Fig. 1 Photographs of reactor under cultivation at 80 % PFC and 400 rpm (The lower part of the fermentor (marked H) was embedded in the heated casing)

measured respectively to determine their Sauter mean diameters, d_{BS} and d_{DS} .

1.6 Gas holdup measurement

Gas holdup, ε , was obtained by measuring the apparent mixed liquid volume under agitation in the reactor.

1.7 Determination of interfacial area

The specific gas-medium interfacial area based on unit medium volume, $(a_M)_{GM}$, was obtained by the following equation.

$$(a_M)_{GM} = \frac{6\varepsilon}{d_{BS}(1-\varepsilon)(1-\Phi)} \quad (1)$$

Similarly, the specific medium-PFC interfacial area based on unit medium volume, $(a_M)_{PM}$, is obtained by Eq. (2).

$$(a_M)_{PM} = 6/d_{DS} \quad (2)$$

2. Experimental Results

2.1 Cultivation results

Figure 1a shows a view of the reactor under cultivation using a medium mixed with PFC, where the PFC volume fraction Φ was 0.8. The medium phase was completely dispersed dropwise, while the PFC phase was continuous. **Figure 1b** shows a view of the reactor after the agitation was stopped. The clear lower layer was the PFC, while the opaque upper layer was the medium containing the microorganisms. All of the microbes

Fig. 2 Time course of growth of *Alcaligenes eutrophus* using non-explosive gas mixture in the presence of various amounts of PFC¹⁴⁾

Table 1. Cell growth rate per unit medium volume and dissolved oxygen concentration measured by a probe at steady state with various PFC volume fraction

Volume fraction of PFC, Φ [-]	Cell growth rate, dX/dt [g-cell/(L-medium-h)]	Dissolved oxygen concentration detected by the probe, DO [mg/L]
0	0.036	0*
0.3	0.027	0*
0.6	0.042	0*
0.7	0.16	0.15**
0.8	0.40	0.35**
0.9	0.64	0.50**

Continuous phases are aqueous medium (*) and PFC (**)

existed in the aqueous medium.

The mixture of aqueous medium and PFC shows a phase inversion at around $\Phi = 0.60 - 0.65$, i.e., a continuous phase changed from the aqueous medium to PFC¹⁴⁾.

Figure 2 shows examples of the growth of *A. eutrophus* in medium mixtures of various PFC volume fraction under oxygen partial pressure of 3 kPa. As long as the aqueous medium was in continuous phase, the cell grew linearly after the dissolved oxygen concentration in aqueous medium became zero. The linear growth rate is considered to be due to oxygen transfer limitation. Under the condition of the medium dispersed in continuous PFC, i.e., at Φ above 0.7 the linear cell growth was also observed, where the DO probe responded to the DO concentration in the continuous PFC phase. Although the DO measurement in the dispersed aqueous medium was difficult by the probe used in these experiments, it might

Fig. 3 Bubble size distribution

Fig. 4 Bubble size as a function of PFC volume fraction

have been depressed to zero considering the observed linear growth rate. The observed non-zero DO detected by the probe implied that PFC phase took a role of an oxygen pool. The cell growth rate at $\Phi = 0.9$ was about 18 times of that observed in PFC-free medium. The observed linear cell growth rates are summarized in Table 1.

2.2 Bubble size

An example of bubble size distribution in a microbe-free medium at $\Phi = 0.7$, is shown in Fig. 3. Bubbles of diameters below 0.45 mm may have existed under this condition, but the finer bubbles were difficult to be observed by the present method. However, the Sauter mean diameter of 1.7 mm calculated by the data will scarcely be changed even if the smaller bubbles existed.

In Fig. 4, the effect of PFC volume fraction Φ on the bubble mean size is shown. The bubble size was almost independent of Φ less than 0.6. On the other hand, Φ greater than 0.7, where PFC was in continuous phase, decreased with increase of Φ .

Effect of PFC addition on gas holdup, ϵ , is shown in Fig. 5. The gas holdup in a continuous medium phase,

Fig. 5 Gas holdup as a function of PFC volume fraction

Fig. 6 Droplet size distribution

where the PFC was dispersed, first increased with Φ and then gradually decreased. On the other hand, the gas holdup under the condition of continuous PFC tended to increase with Φ .

2.3 Droplet size

An example of droplet size distribution of aqueous medium at $\Phi = 0.7$ is shown in Fig. 6, which gives its Sauter mean diameter, d_{DS} , of 0.63 mm. Also, the Sauter mean diameter at $\Phi = 0.9$ was 0.35 mm (data not shown). Thus, the droplet diameter decreased with increase of the amount of PFC added.

2.4 OUR_M

Relationship between OUR_M and Φ is shown in Fig. 7. Since OUR_M at Φ less than 0.6 was constant and equal to that at PFC-free medium ($\Phi = 0$). On the contrary, it was enhanced significantly at Φ greater than 0.7. This behavior of OUR_M as a function of Φ is similar to that of the linear cell growth rate shown in Table 1.

Fig. 7 OUR_M as a function of Φ

Fig. 8 Effect of PFC volume fraction on the enhancement factor for oxygen transfer rate, E , and the relative cell growth rate, E_X

3. Discussion

3.1 Enhancement factor for oxygen transfer rate

Enhancement factor based on reactor volume, E , for oxygen transfer rate from gas bubble to aqueous medium under PFC addition is defined by Eq. (3).

$$E = OTR_M (1 - \Phi) / OTR_{M, \Phi=0} \quad (3)$$

where OTR_M and $OTR_{M, \Phi=0}$ are the oxygen transfer rates per unit medium volume under PFC addition and that under PFC-free medium, respectively. As shown in Fig. 2, the growth of *A. eutrophus* was linear after the oxygen supply was limited by zero-DO. Therefore, the oxygen transfer rate under this condition is equal to the oxygen uptake rate, resulting in the following modified definition of E .

$$E = OUR_M (1 - \Phi) / OUR_{M, \Phi=0} \quad (4)$$

where $OUR_{M, \Phi=0}$ is the oxygen uptake rate under PFC-free medium.

Fig. 9 Possible oxygen transfer pathways. (Arrows show the pathways, $(k_L a_{**})$ implies the volumetric oxygen transfer coefficient across the respective two phases)

The values of E obtained using the above equation are plotted in Fig. 8. With decrease of Φ , a little decrease of E was observed at low Φ region, while it increased significantly just across the phase inversion region and decreased passing a peak.

Relative cell growth rate, E_X , defined by Eq. (5), was also plotted in Fig. 8.

$$E_X = (dX/dt) (1 - \Phi) / (dX/dt)_{\Phi=0} \quad (5)$$

The values of E and E_X are very close to each other. This result implies that the cell growth rate is proportional to the oxygen uptake rate and the condition of Φ greater than 0.7 is very much effective in enhancing the cell growth rate. The relationship between the enhancement factor E and Φ shown in Fig. 8 suggests that oxygen transfer pathway would be different across the phase inversion region.

Since all of the microbes existed in the aqueous medium phase as shown in Fig. 1, the possible oxygen transfer pathways under the presence of PFC are as shown in Fig. 9. Since there exist four phases, i.e., gas bubble, PFC, medium and microbe, the oxygen balances among them are as follows:

$$V (1 - \Phi) dC_M / dt = (k_L a_M)_{GM} V (1 - \Phi) (C_M^* - C_M) + (k_L a_M)_{PM} V (1 - \Phi) (C'_M - C_M) - OUR_M V (1 - \Phi) \quad (6)$$

$$V \Phi dC_P / dt = (k_L a_P)_{GP} V \Phi (C_P^* - C_P) - (k_L a_P)_{PM} V \Phi (C_P - C'_P) \quad (7)$$

$$H = C_P / C'_M = C'_P / C_M = C_P^* / C_M^*$$

where C_M , C_P , Φ , V , C'_M , C'_P , C_M^* and C_P^* are concentrations of oxygen in the medium, in PFC, volume fraction of PFC, total liquid volume in the reactor, oxygen concentrations in the medium phase equilibrated with the PFC, in the PFC phase equilibrated with the medium phase, in the medium phase equilibrated with gas phase and in the PFC phase equilibrated with gas phase, respectively. The coefficients $(k_L a_M)_{GM}$ and $(k_L a_M)_{PM}$ are the volumetric oxygen

Fig. 10 Oxygen transfer pathways from gas bubble to medium

Fig. 11 Interfacial area between gas and mixed liquid as a function of PFC volume fraction

transfer coefficients based on unit medium volume between gas-medium and PFC-medium, respectively. Similarly, $(k_L a_P)_{GP}$ and $(k_L a_P)_{PM}$ are the corresponding volumetric oxygen transfer coefficients based on unit PFC volume. Parameter H is an oxygen partition coefficient between the PFC and medium.

The second terms in the right hand side of Eqs. (6) and (7) are the oxygen exchange rate between the aqueous medium phase and PFC phase.

From the observation of culture mixture shown in Fig. 1a, the following discussion is made under the assumption that either aqueous medium or PFC is always in a continuous phase while the other is dispersed as droplets and that all of the gas bubbles pass through the continuous phase and their contacting probability with the dispersed liquid phase is negligibly small.

The oxygen transfer pathways from gas bubble to aqueous medium are shown in Fig. 10. There are two cases, i.e., Case M and Case P where the continuous phases are aqueous medium and PFC, respectively.

3.2 Analysis of oxygen transfer on E in Case M

In Case M, oxygen is transferred from bubbles directly to the aqueous medium. Therefore, the PFC scarcely functions as an oxygen carrier.

Oxygen transfer coefficient, $(k_L a_P)_{GP}$ in Case M can be assumed to be negligibly small because oxygen in the bubble is directly transferred to the medium. From the experimental results of very small dissolved oxygen concentration at steady state shown in Fig. 2, C_M , C_P , C'_M

Fig. 12 Relative oxygen transfer coefficient as a function of PFC volume fraction

and C'_P can be equated to zero. Thus, from Eqs. (6) and (7) OUR_M is expressed by Eq. (8).

$$OUR_M = (k_L a_M)_{GM} C^*_M \quad (8)$$

By expressing $(k_L a_M)_{GM}$ as the product of $(k_L)_{GM}$ and $(a_M)_{GM}$, the enhancement factor E is written by Eq. (9).

$$E = \frac{(k_L)_{GM} (a_M)_{GM} (1 - \Phi)}{\{(k_L)_{GM} (a_M)_{GM}\}_{\Phi=0}} \quad (9)$$

Gas-liquid interfacial area $(a_M)_{GM}$ as a function of the PFC volume fraction in Case M is shown in Fig. 11. Since the bubble size was almost constant in this case, the variation of the area under PFC addition was similar to that of gas holdup.

The values of $(k_L)_{GM}/(k_L)_{GM, \Phi=0}$ obtained using the data in Figs. 8 and 11, were plotted in Fig. 12, which show a rapid decrease in the lower range of Φ and tend to be constant. Considering the positive spreading coefficient of the system PFC-medium-air, the PFC behaves like a surface active reagent. Therefore, in Case M, a thin layer of PFC must have been formed on the bubble surface, which gave an additional resistance to the transfer of oxygen from bubble to the medium. The rapid decrease of $(k_L)_{GM}/(k_L)_{GM, \Phi=0}$ shown in Fig. 12 may have been due to this reason, which is a similar phenomena observed in oleic acid-in-water system⁷.

3.3 Analysis of oxygen transfer on E in Case P

In Case P, oxygen is first transferred from bubble to continuous PFC, and then to the dispersed aqueous medium.

OUR_M for Case P is derived based on the similar assumptions made for Case M as:

$$OUR_M = (k_L a_M)_{PM} C'_M = (k_L)_{PM} (a_M)_{PM} C'_M \quad (10)$$

Thus, from Eqs. (4) and (10),

$$E = \frac{(k_L)_{PM} (a_M)_{PM} C_P / H (1 - \Phi)}{\{(k_L)_{GM} (a_M)_{GM} C^*_M\}_{\Phi=0}} \quad (11)$$

Eq. (11) shows that the enhancement factor E

depends on the dissolved oxygen concentration in the PFC phase, the oxygen transfer coefficient and the interfacial area between the medium and the PFC, and the volume fraction of PFC.

Interfacial area (a_M)_{PM} in Case *P* calculated by Eq. (2) using the measured droplet diameter was increased with Φ . The (k_L)_{PM} values at $\Phi = 0.7$ and 0.9 calculated by Eq. (11) using experimental data were 0.14×10^{-3} and $0.096 \times 10^{-3} \text{ m}\cdot\text{s}^{-1}$, respectively. The decrease in (k_L)_{PM} with increase of Φ from 0.7 to 0.9 is considered due to the decrease in droplet size from 0.63 to 0.35 . The slower fluid motion in a smaller droplet would have retarded the mass transfer rate. Thus, the peak in E shown in Fig. 8 was a resultant of the increase of interfacial area (a_M)_{PM} and the decrease of oxygen transfer coefficient (k_L)_{PM} with increase of Φ .

Conclusion

Enhancement of the oxygen transfer rate in an *Alcaligenes eutrophus* culture vessel containing aqueous medium mixed with PFC was discussed by taking mass balance in both the medium and PFC phases into account. The enhancement factor, defined as the ratio of oxygen uptake rate at PFC addition to that at PFC-free medium, was introduced. In the case where PFC was dispersed in continuous aqueous medium, enhancement of oxygen transfer rate was not observed, this being due to decreased oxygen transfer coefficient between gas and the medium. On the other hand, in the case where aqueous medium was dispersed in continuous PFC, the oxygen transfer rate was remarkably enhanced, showing a peak with increase of PFC volume fraction. The increase of the interfacial area between the medium and PFC contributed to the enhancement. The decrease in oxygen transfer coefficient due to the lowered inner fluid motion of the smaller droplet gave the decrease in the enhancement factor.

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Nomenclatures

a	= specific interfacial area	[m ⁻¹]
a_M	= specific interfacial area based on unit medium volume	[m ⁻¹]
a_P	= specific interfacial area based on unit PFC volume	[m ⁻¹]
C_M	= oxygen concentration in medium phase	[mg·L ⁻¹]
C'_M	= oxygen concentration in medium phase equilibrated with PFC phase	[mg·L ⁻¹]

C^*_M	= oxygen concentration in medium phase equilibrated with gas phase	[mg·L ⁻¹]
C_P	= oxygen concentration in PFC phase	[mg·L ⁻¹]
C'_P	= oxygen concentration in PFC phase equilibrated with the medium phase	[mg·L ⁻¹]
C^*_P	= oxygen concentration in PFC phase equilibrated with gas phase	[mg·L ⁻¹]
d_{BS}	= Sauter mean diameter of bubble	[mm]
d_{DS}	= Sauter mean diameter of droplet	[mm]
E	= enhancement factor	[-]
H	= partition coefficient of dissolved oxygen concentration in PFC and medium	[-]
k_L	= oxygen transfer coefficient	[m·s ⁻¹]
N	= agitational speed	[m ⁻¹]
OTR_M	= oxygen transfer rate per unit medium volume	[mg·L ⁻¹ ·medium ⁻¹ ·h ⁻¹]
OUR_M	= oxygen uptake rate per unit medium volume	[mg·medium ⁻¹ ·h ⁻¹]
t	= time	[h]
V	= reactor volume	[L]
X	= cell concentration	[g·L ⁻¹]
ε	= gas holdup	[-]
Φ	= volume fraction of PFC	[-]

<Subscripts>

B	= bubble
D	= droplet
G	= gas
M	= medium
P	= PFC
GM	= gas-medium interface
GP	= gas-PFC interface
PM	= PFC-medium interface

Literature Cited

- 1) Damiano, D. and S.S., Wang: *Biotechnol. Lett.*, **7**, 81-86 (1985)
- 2) Ho, C.S., L.K., Ju and R.F., Baddour: *Biotechnol. Bioeng.*, **36**, 1110-1118 (1990)
- 3) Ishizaki, A. and K., Tanaka: *J. Ferment. Bioeng.*, **69**, 170-174 (199)
- 4) Ju, L.K., J.F., Lee and W.B., Armiger: *Biotechnol. Prog.*, **7**, 323-329 (1991)
- 5) Junker, B.H., T.A. and Hatton, D.I.C., Wang: *Biotechnol. Bioeng.*, **35**, 578-585 (1990)
- 6) Junker, B.H., D.I.C., Wang and T.A., Hatton: *Biotechnol. Bioeng.*, **35**, 586-597 (1990)
- 7) Linek, V and P. Benes: *Chem. Eng. Sci.*, **31**, 1037-1046 (1976)
- 8) McMillan, J.D. and D.I.C., Wang: *Ann. N.Y. Acad. Sci.*, **589**, 283-300 (1990)
- 9) Morinaga, Y., S., Yamanaka, A., Ishizaki and Y., Hirose: *Agric. Biol. Chem.*, **42**, 439-444 (1978)
- 10) Riess, J.G. and M.L., Blanc: *Pure Appl. Chem.*, **54**, 2383-2406 (1982)
- 11) Rols, J.L. and G., Goma: *Biotechnol. Adv.*, **7**, 1-14 (1989)
- 12) Rols, J.L., J.S., Condoret, C., Fonade and G., Goma: *Biotechnol. Bioeng.*, **35**, 427-435 (1990)
- 13) Sargent, J.W. and R.J., Sefel: *Fed. Proc.*, **29**, 1699-1703 (1970)
- 14) Yamamoto, S., H., Honda, N., Shiragami and H., Unno: *Biotechnol. Lett.*, **14**, 733-736 (1992)
- 15) Yoshida, T., K., Yokoyama, K.C., Chen, T., Sunouchi, H., Taguchi: *J. Ferment. Technol.*, **55**, 76-83 (1977)