DROPWISE CULTIVATION METHOD—AN APPROACH TO OVERCOMING THE LIMITATION OF OXYGEN SUPPLY—

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Introduction

Aerobic bacterial cultivations are sometimes confronted with a limitation of oxygen supply. A general strategy for solving this limitation is to seek a method of increasing the volumetric oxygen transfer coefficient, k_{La} .

For safe cultivation of *Alcaligenes eutrophus*, whose optimal gaseous substrate mixture is explosive, a cultivation method utilizing oxygen carrier under a lowered oxygen partial pressure has been studied (Yamamoto *et al.*, 1992; 1994; 1995). Although the k_{La} was increased under the cultivation condition where the medium was dispersed in a continuous oxygen carrier phase, the cultivation was not necessarily economic because a large amount of oxygen carrier was required. Therefore, still more improved methods are required.

In the present paper, as an alternative approach to the above, a new cultivation method is proposed. The method is to increase the gas-liquid interfacial area by feeding the dispersed liquid medium into the gaseous substrate in the reactor. Its effectiveness is discussed by measuring k_{La} and culturing *A. eutrophus* under the lowered oxygen partial pressure.

1. Experimental

1.1 Bioreactor equipment

A schematic diagram of the bioreactor is shown in Fig. 1. The reactor consisted of two coaxial columns made of transparent polycarbonate resin, a medium distributor and a medium reservoir. The column was 1000 mm long and 54.5 mm in diameter. The reactor temperature was controlled by water flowing in the annular space.

The medium in the reservoir attached to the reactor bottom was pumped up to the top of the column, where the medium was dispersed into

Fig. 1 Schematic diagram of dropwise cultivation apparatus

droplets by the distributor, and then collected into the reservoir. The medium was recirculated through the bioreactor system. During falling of the medium drops, they absorbed gaseous substrate which was countercurrently fed from the bottom of the column. The distributor consisted of 144 plastic micropipes of 0.5 mm inner diameter.

1.2 Cultivation of *Alcaligenes eutrophus*

Alcaligenes eutrophus H16 was cultured using inorganic medium (Yamamoto *et al.*, 1992) and non-explosive gaseous substrate of $H_2:O_2:CO_2=81:3:16$.

The preculture for 40 hours was inoculated into the bioreactor containing 360 mL of the medium. Cultivation was conducted at 303 K and pH 7. The gaseous substrate was continuously fed into the reactor. The gas flow rate was $190 \text{ mL} \cdot \text{min}^{-1}$.

1.3 Determination of k_{La}

The gaseous substrate was absorbed in both the inner column and the reservoir. The volumetric oxygen transfer coefficient based on unit suspended liquid volume in the column was determined as follows. The gaseous mixtures in both the column

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and the reservoir were assumed homogeneous because the absorption amount of the gaseous mixture was small. Dissolved gaseous substrate concentration in the medium in the column was assumed to be homogeneous. Because the residence time of medium droplet in the column, which was estimated from the medium recirculation rate and diameter of micropipe of the distributor, was less than one-tenth of the time constant of gaseous substrate transfer obtained from the experiments.

The oxygen balances in the column and the reservoir are expressed as Eqs. (1) and (2), respectively.

$$V_R dC_R/dt = (k_L)_{RA} R (C_R^* - C_R) + R_M C_C - R_M C_R \quad (1)$$

$$V_C dC_R/dt = (k_L)_{CA} C (C_C^* - C_C) + R_M C_R - R_M C_C \quad (2)$$

Notations of all variables in the equations are listed in the Nomenclature.

Eqs. (1) and (2) are rearranged as follows:

$$dC_R/dt = (k_L)_{RA} R (C_R^* - C_R) + R_M/V_R (C_C - C_R) \quad (3)$$

$$dC_C/dt = (k_L)_{CA} C (C_C^* - C_C) + R_M/V_R (C_R - C_C) \quad (4)$$

where a_C and a_R are specific gas-liquid interfacial areas based on unit suspended medium volume in the column and unit medium volume in the reservoir, respectively.

Coefficients $(k_L)_{RA}$ and $(k_L)_{CA}$ were determined as follows.

When the medium is recirculated through the reservoir without passing the column, Eq. (3) is simplified as follows:

$$dC_R/dt = (k_L)_{RA} R (C_R^* - C_R) \quad (5)$$

The liquid medium deoxygenated by nitrogen gas was recirculated from the reservoir after starting the aeration. By monitoring the change in C_R using a DO electrode, $(k_L)_{RA}$ was evaluated based on Eq. (5) (Taguchi and Humphrey, 1966). In a similar manner, after the liquid medium was recirculated from the reservoir to the column by way of the distributor, C_C was estimated by Eq. (3) from the change in C_R and the liquid medium volume in the reservoir. From the changes in C_R and C_C , the measured $(k_L)_{RA}$ and suspended liquid medium volume in the column, $(k_L)_{CA}$ was determined by Eq. (4).

1.4 Measurements

The size of medium droplets suspended in the column was measured via images recorded by a video camera (CCD-V700, Sony, Tokyo). Sauter mean

diameters, d_{DS} , of more than 150 droplets in the video image frozen on the monitor were measured.

Cell concentration, gas composition and dissolved oxygen concentration were measured by the same methods as reported previously (Yamamoto *et al.*, 1992).

2. Results and Discussion

2.1 Volumetric oxygen transfer coefficient

Figure 2 shows a relationship between $(k_L)_{CA}$ and the medium recirculation rate, R_M . With an increase of R_M , $(k_L)_{CA}$ increased to a peak at R_M around $750 \text{ mL} \cdot \text{min}^{-1}$, and then decreased. To interpret this phenomenon, the specific gas-liquid interfacial area, a_C , was examined.

The estimated a_C by the suspended medium amount and the mean droplet diameter in the column increased with R_M as shown in Fig. 3. This resulted from the increase of the medium holdup in the column (Fig. 4) and the decrease of d_{DS} with increase of R_M (Fig. 3).

Figure 5 shows a relationship between R_M and $(k_L)_C$ calculated from the $(k_L)_{CA}$ and a_C , which was similar to that between R_M and $(k_L)_{CA}$. The increase and decrease of $(k_L)_C$ with increasing R_M are considered to be due to the extent of internal liquid circulation rate in the droplets (Slater *et al.*, 1988). The dominant factor for the decrease of $(k_L)_{CA}$ above $R_M = 750 \text{ mL} \cdot \text{min}^{-1}$ was considered to be the decrease of $(k_L)_C$.

2.2 Cultivation of *A. eutrophus*

Table 1 shows a comparison of maximum linear growth rate of *A. eutrophus* under various cultivation methods. All the cultivations listed in Table 1 manifested linear cell growth rate showing the rate-limiting of oxygen transfer by the depleted dissolved oxygen concentration.

The cell growth rates by the proposed method were more than 50 and 20 times greater than those respectively by the cultivations using the oxygen carrier and under the optimal condition in a jar fermentor.

The proposed method is promising to increase $k_L a$. However, increasing R_M in order to increase the interfacial area was not necessarily effective to increase $k_L a$ because $(k_L)_C$ would decrease with the medium droplet size as demonstrated in Section 2.1. Operational conditions, which allow medium droplet size to be more or less larger to avoid decreasing $(k_L)_C$ and to maintain higher suspended liquid medium holdup in the column, are required to obtain higher oxygen supplying rate.

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Table 1 Cell growth rates under various cultivation methods and conditions

Cultivation method	Conditions			Maximum cell growth rate [g·L ⁻¹ ·h ⁻¹]
	Gas composition H ₂ :O ₂ :CO ₂	Oxygen carrier addition ratio [%]	Medium recirculation rate [mL·min ⁻¹]	
Dropwise cultivation	81:3:16*	0	760	2.38***
			1420	0.800***
			1880	0.748***
Utilizing oxygen carrier in a jar fermentor (Yamamoto <i>et al.</i> , 1992)	81:3:16*	80	—	0.042
Optimal cultivation in a jar fermentor (Yamamoto <i>et al.</i> , 1994)	75:10:15*	0	—	0.104

* Non-explosive gas composition** Explosive gas composition which is optimal for cell growth

*** Calculated on suspended liquid medium volume basis

Fig. 2 Effect of medium recirculation rate on $(k_L)_C a_C$. Error bars represent standard deviation obtained from more than 3 experiments

Fig. 4 Effect of medium recirculation rate on medium holdup in the column

Fig. 3 Dependency of specific gas-liquid interfacial area and Sauter mean droplet diameter on medium recirculation rate

Fig. 5 Effect of medium recirculation rate on oxygen transfer coefficient

Nomenclature

A_C	= gas-liquid interfacial area in column	$[m^2]$
a_C	= specific gas-liquid interfacial area based on unit suspended liquid medium volume in the column	$[m^{-1}]$
A_R	= gas-liquid interfacial area in reservoir	$[m^2]$
a_R	= specific gas-liquid interfacial area based on unit medium volume in reservoir	$[m^{-1}]$
C_C	= dissolved oxygen concentration in medium in column	$[mg \cdot L^{-1}]$
C_C^*	= dissolved oxygen concentration in liquid medium equilibrated with gas phase in column	$[mg \cdot L^{-1}]$
C_R	= dissolved oxygen concentration in medium in the reservoir	$[mg \cdot L^{-1}]$
C_R^*	= dissolved oxygen concentration in liquid medium equilibrated with gas phase in reservoir	$[mg \cdot L^{-1}]$
d_{DS}	= Sauter mean diameter	$[mm]$
$(k_L)_C$	= oxygen transfer coefficient in column	$[m \cdot h^{-1}]$
$(k_L)_R$	= oxygen transfer coefficient in reservoir	$[m \cdot h^{-1}]$
V_C	= liquid medium volume in column	$[L]$
V_R	= liquid medium volume in reservoir	$[L]$
R_M	= medium recirculation rate	$[mL \cdot min^{-1}]$
t	= time	$[h]$

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