OXYGEN TRANSFER IN BIOREACTOR FOR CULTURE OF PLANT HAIRY ROOTS

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Introduction

Most notably, plant hairy root, which exhibits active propagation in a phytohormone-free medium and has a metabolite content comparable to the original plant root, has become an object of great interest as a material for the production of plant-derived metabolites (Toivonen, 1993). In developing an appropriate type of fermentor for hairy root culture, many researchers have reported that the hairy roots could be well cultivated in a fermentor where they were anchored to supporters and were supplied with nutrients by means of medium flowing (Kondo *et al.*, 1989; Hilton and Rhodes, 1990; Rodriguez-Mendiola *et al.*, 1992; Ramakrishnan and Curtis, 1994).

Because of numerous problems, achievement of high concentrations of hairy root cells in fermentors was difficult because sufficient oxygen supply could not be realized due to the low rate of oxygen transfer. Therefore, a fermentor where the oxygen transfer can be analyzed in the hairy root culture needs to be designed.

In the present study, using the bioreactor where the interfacial oxygen transfer between liquid and solid will be separated from that between gas and liquid, the effect of liquid flow on the distribution in dissolved oxygen (DO) concentration will be examined. Moreover by analyzing oxygen transfer distributions in the bioreactor will be expressed.

1. Experimental

The hairy roots of red beet (*Beta vulgaris* L. cv. Detroit dark red) were used and the culture conditions as the same as in the previous report (Taya *et al.*, 1992). The measurements of DO concentrations were conducted at 25°C using a single column reactor (SCR) depicted in Fig. 1. The SCR consisted of a column for the oxygen uptake of hairy roots and a stirred vessel (TBR-2, Sakura Seiki Co., Tokyo) for the saturation of gaseous air. The hairy roots were

Fig. 1 Schematic diagram of single column reactor

packed into the compartment between two glass filters in the column, and the liquid medium subjected to O₂ enrichment in the stirred vessel was made to flow from the top to the bottom of the column. The flow rate of the medium was kept in the range from 0.1 to 0.6 dm³/min. The total volume of medium in this system was 1.0 dm³ (0.3 dm³ in the column and 0.7 dm³ in the stirred vessel). DO concentration in the bulk liquid at the inlet (C_{O1}) or the outlet (C_{O2}) of the column was measured with a DO electrode (Model DO-1, Sakura Seiki Co.). The value of ϵ was controlled at 7.5 × 10⁻³ kg/m³ by changing the agitation speed (150–450 r.p.m.) and air flow rate (0.1–0.4 dm³/min) in the stirred vessel. The variations in DO concentrations in the bulk liquid in the column (C_O) were obtained by measuring C_{O2} at various heights of hairy roots packed in the column. The hold-up of hairy roots in the compartment (ϵ) was also varied by changing the mass of the hairy roots inoculated into the column. Here, by measuring the volume of hairy roots, ϵ was calculated as follows.

$$\epsilon = \frac{(AZ - V_H)}{AZ} \quad (1)$$

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The dry weight of hairy roots was determined gravimetrically after the harvested hairy roots were rinsed with water followed by drying at 80°C for 2 days.

The apparent specific oxygen uptake rate in the column at a given superficial velocity was expressed as,

$$Q^{app} = (C_{O1} - C_{O2})A u_s / X \quad (2)$$

The specific oxygen uptake rate of red beet hairy roots at the cell surface was measured by a dynamic method (Shirai *et al.*, 1988; Yamane, 1991). To obtain a suspension of the hairy roots, they were cut into about 5 mm segments and introduced into a closed vessel (volume: 34 cm³) containing a medium saturated in advance with air and a magnetic stirrer bar coated by glass. The change in DO concentration was measured using a DO electrode and the specific oxygen uptake rate versus the DO concentration was obtained. Here, the film resistance to oxygen transfer between the medium and hairy roots was considered to be negligible by vigorous stirring in the vessel (500 r.p.m.), that is, the measured DO concentration was regarded as the DO concentrations at cell surface.

2. Results and Discussion

2.1 Distribution in DO concentration in SCR

The variations in C_O in the column in the range of $Z = 0.0 - 6.0 \times 10^{-2}$ m are shown in Fig. 2. With increasing Z at $u_s = 3.6$ m/h, C_O decreased. The value of C_O at $Z = 6.0 \times 10^{-2}$ m reached one-third the value at the inlet of the column. At $u_s = 18.5$ m/h, C_O values in the range of $Z = 0.0 - 6.0 \times 10^{-2}$ m were maintained over 6×10^{-3} kg/m³ due to the efficient oxygen supply.

The values of Q^{app} in the column at $Z = 6.0 \times 10^{-3}$ m which were obtained from Eq. (2), were $8.1 \times 10^{-3} \text{ h}^{-1}$ and $12.3 \times 10^{-3} \text{ h}^{-1}$ at $u_s = 3.6$ m/h and 18.5 m/h, respectively.

On the other hand, the specific oxygen uptake rate at the cell surface was evaluated by measuring the change in DO concentration. Concerning the specific oxygen uptake rate (Q) in suspension cultures of plant cells and microorganisms, the variation in Q values is often expressed as the function of DO concentration at cell surface (Pareilleux, 1988; Atkinson and Mavituna, 1991) as,

$$Q = Q_m C_{Os} / (K_O + C_{Os}) \quad (3)$$

Using Eq. (3), the values of Q_m and K_O of the hairy roots used in Fig. 2 were estimated as $32.6 \times 10^{-3} \text{ h}^{-1}$ and $3.11 \times 10^{-3} \text{ kg/m}^3$, respectively. The Q_m value was 2.6–4 times that of Q^{app} . Ramakrishnan

Fig. 2 Distribution in dissolved oxygen concentration in single column reactor

Fig. 3 Conceptual drawing of oxygen transfer at cell surface

and Curtis (1995) suggested the existence of large film resistance between the bulk liquid and the cell surface in the culture of *Hyoscyamus muticus* hairy roots and proposed the necessity of the analysis of oxygen transfer in the fermentor on the basis of the oxygen uptake rate at the cell surface and not on the apparent oxygen uptake rate in the bulk liquid.

2.2 Analysis of oxygen transfer between medium and hairy roots

In the column for oxygen uptake of hairy roots in the SCR, as shown in Fig. 3, a difference in DO concentrations between bulk liquid and cell surface occurs. The oxygen transfer rate on a reactor volume basis is expressed as,

$$N_{LS} = k_{LS} a_s (C_O - C_{Os}) \quad (4)$$

When the medium moves along a vertical direction of the column in a plug flow manner and the oxygen diffusion in the horizontal direction is negligible, one obtains a mass balance yielded by Eq. (5) in

key	ε [-]	Z [10^{-2} m]	key	ε [-]	Z [10^{-2} m]
○	0.60	1.0	□	0.60	3.0
×	0.50	1.0	△	0.60	4.5
+	0.40	1.0	▽	0.60	6.0

Fig. 4 Correlation of volumetric mass transfer coefficient of oxygen between liquid and solid against superficial velocity

the vertical direction.

$$u \cdot dC_o/dZ = -N_{LS}/\varepsilon \quad (5)$$

And hence,

$$u = u_s/\varepsilon \quad (6)$$

N_{LS} and differential dry weight in differential height are expressed by the following equations, respectively.

$$N_{LS} = (1 - \varepsilon)QX/V_H \quad (7)$$

$$\Delta X = (1 - \varepsilon)AX\Delta Z/V_H \quad (8)$$

Using Eqs. (3) – (8), k_{LSa_s} values were obtained at various values of u_s , Z and ε by measuring the C_o values at the inlet and outlet of the column. Furthermore, to see the effect of liquid flow on oxygen transfer in the column, $k_{LSa_s} \cdot \varepsilon / (1 - \varepsilon)$ was plotted against u_s/ε as shown in **Fig. 4**. k_{LSa_s} increased with increasing u_s and the following experimental equation was obtained.

$$k_{LSa_s} \cdot \varepsilon / (1 - \varepsilon) = 75.5(u_s/\varepsilon)^{0.27} \quad (9)$$

The average error in estimating k_{LSa_s} by Eq. (9) was 26% for 41 data. Wilson and Geankoplis (1966) indicated that k_{LS} was proportional to $(u_s/\varepsilon)^{1/3}$ in the column with packed beds of spheres and the power of (u_s/ε) in Eq. (9) was found to be close to their data.

As shown in Fig. 2, the variations in C_o and C_{os} were calculated by Eqs. (3) – (9). Here, the values of k_{LSa_s} were calculated in Eq. (9) as 81.7 h^{-1} and 127 h^{-1} at $u_s = 3.6$ and 18.5 m/h, respectively. C_o values

agreed well with those observed experimentally. At $u_s = 3.6$ m/h, C_{os} was much lower than C_o and increased with increasing u_s due to the improvement of the oxygen transfer between medium and hairy roots.

From these results, the oxygen transfer between bulk liquid and hairy roots is significantly influenced by the film resistance, whose extent is affected by the liquid flow rate. Thus, the liquid-solid interfacial oxygen transfer is an important factor for the design of fermentors suitable to hairy root cultures.

In conclusion, a distribution of DO concentration in medium in the column was found to exist. By the analysis of oxygen transfer between medium and hairy roots, the correlation between the superficial velocity of the medium and the volumetric oxygen transfer coefficient was obtained, and it indicated that the film resistance between medium and hairy roots governed the oxygen transfer in the column of SCR.

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Nomenclature

A	= sectional area of column	[m ²]
C_o	= concentration of dissolved oxygen in bulk liquid	[kg/m ³]
C_{o1}	= concentration of dissolved oxygen in bulk liquid at inlet of column	[kg/m ³]
C_{o2}	= concentration of dissolved oxygen in bulk liquid at outlet of column	[kg/m ³]
C_{os}	= concentration of dissolved oxygen at cell surface	[kg/m ³]
DW	= dry weight of hairy roots	[kg]
k_{LS}	= mass transfer coefficient of oxygen between liquid and solid	[m/h]
k_{LSa_s}	= volumetric mass transfer coefficient of oxygen between liquid and solid	[h ⁻¹]
K_o	= oxygen saturation constant	[kg/m ³]
N_{LS}	= oxygen transfer rate between liquid and solid on reactor volume basis	[kg/(m ³ ·h)]
Q	= specific oxygen uptake rate of hairy root at cell surface	[h ⁻¹]
Q^{app}	= apparent specific oxygen uptake rate of hairy root in column	[h ⁻¹]
Q_m	= maximum specific oxygen uptake rate of hairy root	[h ⁻¹]
u	= velocity of liquid fluid in column	[m/h]
u_s	= superficial velocity of liquid fluid in column	[m/h]
V_H	= volume of hairy roots packed in column	[m ³]
X	= dry weight of hairy roots in column	[kg-DW]
Z	= height of hairy roots packed in column	[m]
ΔZ	= differential height of hairy roots in column	[m]
ΔX	= differential dry weight of hairy roots in column	[kg-DW]
ε	= hold-up of hairy roots in column	[-]
ϕ	= diameter of column	[m]

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