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Comparison of growth properties of carrot hairy root in various bioreactors

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Summary. Growth properties of carrot hairy root cells in various bioreactors were investigated. A turbine-blade reactor and an immobilized rotating drum reactor were found to be advantageous for the hairy root culture because of a high oxygen transfer coefficient (k_La). After 30 days of culture, 10 g/l of dry hairy root cells were obtained in both bioreactors and maximum growth rates (V_m) were found to be 0.63 and 0.61 g/l per day for the turbine-blade reactor and immobilized rotating drum reactor, respectively. Specific growth rates (μ) at various cultivation times were observed to be linearly proportional to X/k_La for both bioreactor configurations where X is the cell concentration. The estimated specific oxygen uptake rate of 0.34 mmol O_2/g dry cells per hour compares fairly well with an experimental value of 0.3.

Introduction

Recently, plant hairy roots have become of interest because of their indefinite and active proliferation in phytohormone-free medium and their capacity to produce valuable materials accumulated at comparable levels to the original plant root (Flores et al. 1987). Recently, we induced Pakbung hairy roots and the amount of peroxidase produced was five times higher than that from the original plant root (Taya et al. 1989b).

The induction mechanism of hairy root caused by the infection of a soil bacterium, *Agrobacterium rhizogenes,* was studied in detail, and hairy root cell lines from approximately forty plant species were obtained (Mugnier 1988). However, there are only a few technological approaches with respect to cultivation of hairy roots, for example, suitable bioreactor design or development of culture systems for useful material production (Rhodes et al. 1986). A stirred-tank reactor is not suitable for hairy root cultures because root cells can be easily injured by shear stress or impeller rotation. We have previously reported that an airlift column reactor was superior for cultivation of horseradish hairy root cells (Taya et al. 1989a).

In this paper, the growth properties of carrot hairy root culture in various bioreactors are presented. A simple relationship between specific growth rate and the oxygen transfer coefficient (k_La) for these bioreactors is proposed as an important criterion for the development of further suitable bioreactors for hairy root culture.

Materials and methods

Plant materials and cultivation. Carrot hairy root cells induced by the leaf-disc method were used. The hairy root cells were maintained on hormone-free Murashige-Skoog (MS) medium containing 30 g/l sucrose and 10 g/l agar, and they were subcultured every month at 25° C in the dark. For preculture, a 200-ml Erlenmeyer flask with 40 ml MS medium was used as described previously (Taya et al. 1989c). For cultivation in bioreactors, about 0.2 g dry weight of carrot hairy root cells obtained from 15 days flask culture was inoculated, and the cells were cultivated in MS liquid medium containing 20 g/l sucrose at 25° C in the dark. The aeration rate was changed from about 3 to 40 l/h during cultivation according to the increase in cell concentration. For on-line monitoring of cell concentration during cultivation, estimation of the cell concentration was based on conductometry as reported previously (Taya et al. 1989a). At the end of a culture, dry cell weight was determined gravimetrically after drying the roots at 60° C for 1 day.

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Measurements of k_La and Q_{O_2}. Volumetric oxygen transfer coefficient (k_La) was measured by the sodium sulphite oxidation method. The hairy carrot cells, sterilized at 120° C for 10 min, were added to each bioreactor to analyse the effect of cell concentration on k_La values. The specific oxygen uptake rate (Q_{O_2}) was determined from the assay of outlet gas flow from the bioreactors using an exhaust gas analyser (Model EX-1562, ABLE Co., Tokyo, Japan).

Bioreactor configuration. The configuration of bioreactors used in this study is depicted in Fig. 1. In the turbine-blade reactor (Model TBR-2, Sakura Seiki Co., Tokyo, Japan) shown in Fig. 1A, a cultivation space (about 600 ml) was separated with an agitation space (400 ml) by a stainless steel mesh so that hairy root cells were not in contact with the impeller. An impeller with eight turbine blades was controlled at 200 rpm. Humidified air was introduced into the agitation space.

In a rotating-drum reactor shown in Fig. 1B, a cylindrical glass vessel (diameter × length: 20 cm × 33 cm) was used as a reactor. The drum reactor with 1 l medium was rotated at 5 rpm on a Cellrotator (Shibata Hario Glass Co., Tokyo, Japan). Humidified air was supplied to the medium through a submerged nozzle. In the case of the immobilized-cell system, a reticulate polyurethane foam sheet (0.8 × 60 × 40 cm, Ester type MF-18 with 18 pores per 2.5 cm, Inoue MTP Co., Anjo, Aichi, Japan) was fixed on an inner wall by stainless steel wires and used as a cell support. The hairy root cells obtained from the preculture were aseptically anchored to several positions on the polyurethane foam sheet.

As shown in Fig. 1C, a reverse teardrop-type glass vessel (working volume, 1 l, Shibata Hario Glass Co.) was used as an air-lift reactor. Humidified air was introduced into the bottom of the reactor through a sintered glass sparger.

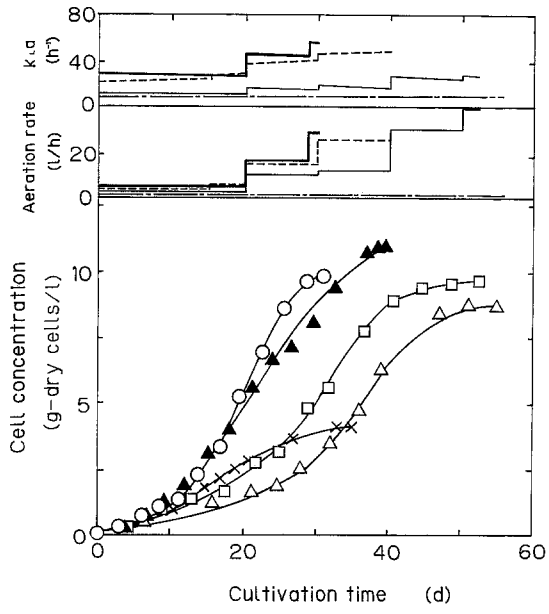

Fig. 2. Time courses of growth of hairy roots, aeration rate and estimated oxygen transfer coefficient (k_La) values in various reactor types. The time course of k_La values was estimated by using Eq. 1 (see text) and aeration rate: x, Erlenmeyer flask; ○, turbine-blade reactor; △, rotating-drum reactor; ▲, immobilized rotating-drum reactor; □, air-lift reactor. Aeration rate and k_La are shown by the following lines: ——, turbine-blade reactor; -·-·-, rotating-drum reactor; – – – –, immobilized rotating-drum reactor; ——, air-lift reactor

Results and discussion

The growth of hairy root and the transition of aeration rate in various bioreactors are shown in Fig. 2. The hairy root cells showed the most efficient growth in the turbine-blade reactor. The maximum growth rate was 0.63 g/l per day and 10 g/l of dry cell mass was obtained after 30 days, while only 4 g/l was obtained in flask cultures.

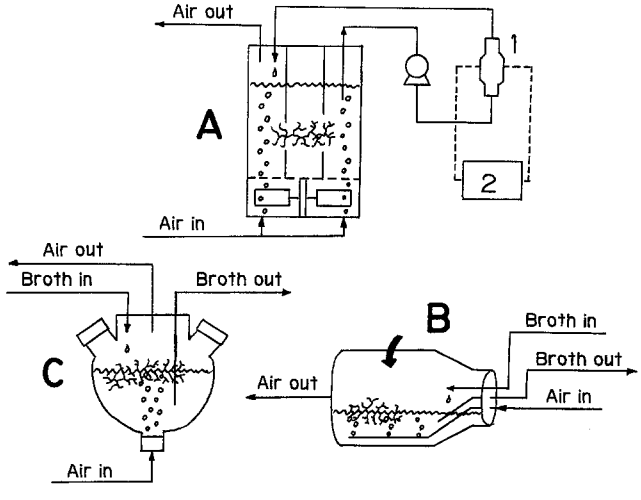

Fig. 1A–C. Experimental apparatuses. **A** Turbine-blade reactor: *1*, conductivity cell; *2*, conductivity meter. **B** Rotating-drum reactor. **C** Air-lift reactor

In the rotating-drum reactor, preliminary experiments showed that hairy root cells adhering to the wall of the reactor were lifted above the liquid medium and then dropped back into the medium as the drum rotated. Cell disruptions occurred due to these repeated drops and growth was slower. Most of cells did not become detached from wall of the reactor as the hairy roots grew, and the cell growth became active. Since the preculture cultivation time was the same as that in other reactor types, the slow growth seems characteristic in this reactor. To improve the slow growth, a pulyurethane foam sheet which served as a support for the cells was attached onto the wall of the reactor. The adhering hairy roots became entangled in the sheet and showed active proliferation from the beginning of cultivation without detachment from the support when lifted above the medium. With this modification, the growth rate was significantly improved and a maximum growth rate of 0.61 g/l per day as shown in Fig. 2 was

comparable to that obtained in the turbine-blade reactor.

Although 10 g/l of cell concentration could also be achieved in the air-lift reactor culture, longer cultivation time was required. The difference in growth rate in these bioreactors may be attributable to their difference in oxygen transfer rate. Therefore, the k_La in each reactor was measured against various aeration rates and cell concentrations. As shown in Fig. 3, the turbine-blade and immobilized rotating-drum reactors showed high k_La values. The k_La values were little affected by cell concentration in the turbine-blade reactor. In the immobilized rotating-drum reactor, a 50% increase in the k_La value was observed at 10 g/l of cell concentration compared with that without cell mass. This increase may be attributed to the increase in gas-liquid interfacial area (a) due to hairy root cells entangled on the polyurethane foam. On the other hand, the increase in cell concentration in the air-lift reactor decreased the k_La value due to the deterioration in fluidity.

The relationship between the k_La value, aeration rate (L) and cell concentration (X) was found to be expressed by the following equation:

$$k_La = \alpha L^{\beta}(1+\gamma X) \tag{1}$$

where it is assumed that k_La is proportional to cell concentration. The values of α, β and γ for different reactor types are presented in Table 1. In a conventional stirred tank reactor, a 0.5 β value has been generally used for scaling-up of a reactor. This is comparable to the values obtained in the turbine-blade and air-lift reactors. The smaller β value for the rotating-drum reactor may be due

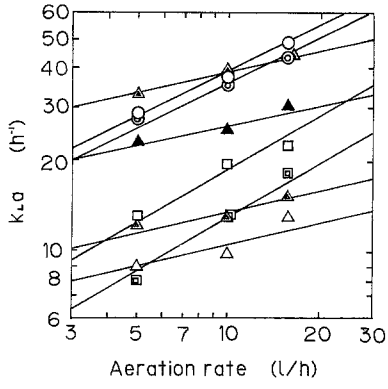

Fig. 3. Experimental results for k_La values in various reactor types: ○, ◎, turbine-blade reactor; △, ▲, rotating-drum reactor; ▲, ◬, immobilized rotating-drum reactor; □, ▣, air-lift reactor. Symbols, ○, △, ▲, □, and ◎, ▲, ◬, ▣, show the values at 0 and 10 g/l cell concentration, respectively

Table 1. Comparison of α, β and γ values for different reactor type

Reactor type	α (h^{-1})	β (—)	γ (l/g)
Turbine-blade	12.6	0.48	−0.007
Air-lift	5.1	0.56	−0.03
Rotating-drum	6.0	0.23	0.03
Immobilized rotating-drum	15.4	0.23	0.05

α, β and γ are parameters in Eq. 1 (see text)

to the shallow depth of the liquid phase. The very high k_La value (2.5 times higher) of the immobilized rotating-drum reactor was most probably the result of a large increase in gas-liquid interfacial area on the polyurethane foam film. We estimated the k_La values based on Eq. 1 during cultivation in various reactors. As shown in Fig. 2, the transition of k_La was in response to the growth curve in each reactor.

The oxygen uptake rate (OUR) is related to dissolved oxygen concentration (C) as follows:

$$\mathrm{OUR} = Q_{O_2}X = k_La(C^* - C) \tag{2}$$

where, X and C^* are hairy root concentration on dry weight basis and saturated dissolved oxygen concentration at 25° C, respectively. If hairy root growth is limited only by C and the saturation constant (K_m) is much larger than C, then the specific growth rate (μ) can be expressed as follows:

$$\mu = \frac{1}{X}\cdot\frac{dX}{dt} = \frac{\mu_m C}{K_m + C} = \frac{\mu_m C}{K_m} \tag{3}$$

From Eqs. 2 and 3, the specific growth rate is rewritten as:

$$\mu = \frac{\mu_m}{K_m}\left(C^* - Q_{O_2}\frac{X}{k_La}\right) \tag{4}$$

Equation 4 predicts that specific growth rate is a linear function of X/k_La if the specific oxygen uptake rate, Q_{O_2}, is constant. As shown in Fig. 2, the growth rate of the hairy root cells slowed down in all reactor types when the cell concentration exceeded 5 g/l. It appears that there may be other limiting factors such as sucrose depletion or accumulation of growth inhibitors. Therefore, Eq. 4 should be applied at cell concentrations lower than about 5 g/l in order to satisfy the assumptions mentioned above. From the cultivation curves shown in Fig. 2, specific growth rates were

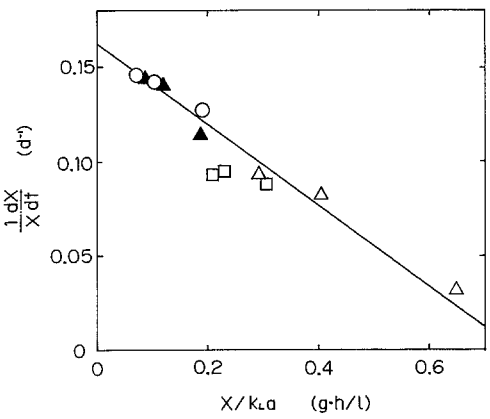

Fig. 4. Relationship between specific growth rate and X/k_La value where X = cell concentration. Symbols are the same as in Fig. 2

evaluated at the cell concentrations of 2, 3 and 5 g/l (these points were selected arbitrarily), and their values were plotted against X/k_La values. Here, the k_La value was estimated by Eq. 1 based on the aeration rate at each time and Q_{O_2} was assumed to be constant in the range of cell concentration 2–5 g/l.

As shown in Fig. 4, values of specific growth rate and X/k_La from various reactor cultures can be represented well by a straight line. The slope and intercept of the straight line correspond to $\mu_m Q_{O_2}/K_m$ and $\mu_m C^*/K_m$, respectively. Hence, the Q_{O_2} value was determined from Fig. 4 to be 0.34 mmol O_2/g dry cells per hour. This value agreed reasonably well with the observed Q_{O_2} value of 0.3 mmol O_2/g dry cells per hour, evaluated from the outlet gas analysis of the turbine-blade reactor culture. In the callus culture of *Catharanthus roseus*, Q_{O_2} was reported to be 0.45 mmol O_2/g dry cells per hour (Bond et al. 1988). The estimated and observed values are comparable to this value.

As illustrated in Fig. 4, the growth rate of the hairy root depended significantly on the k_La value. From these results, it is concluded that turbine-blade or immobilized rotating-drum types of reactor with a higher k_La value are more efficient for hairy root cultures. For bioreactor design in plant cell culture, the k_La value is one of the most important factors to be considered. The relationship proposed above may be used as a criterion of evaluation for bioreactor design in plant cell cultures.

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