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Semi-Continuous Cultivation of Photosynthetic Cells in a Flat Plate Photobioreactor

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Abstract

From an engineering point of view, the effect of light intensity distribution on the stability of growth rate should be taken into account in designing effective photobioreactors and sustaining stable growth rates. In the experiments described here, in order to keep operational parameters at an almost constant level, a semi-continuous culture method was developed for cultivation of photosynthetic cells under defined light intensity distributions. In the semi-continuous culture, a portion of culture broth containing grown cells was repeatedly replaced with the fresh medium at a predetermined time interval to maintain the cell concentration and the volume of the broth constant at their initial values.

Under illumination from one and both sides, photosynthetic cells were cultivated in a flat plate photobioreactor with various light path lengths. The results obtained showed that stability of the growth rate strongly depended on the distribution of light intensity and the ratio of light intensity in the illuminated to that in the dark zone inside a photobioreactor. These parameters should be taken into consideration for stable cultivation of photosynthetic cells.

Keywords: *Photobioreactor, Stability of growth rate, Light intensity distribution, Semi-continuous cultivation*

Introduction

In designing effective photobioreactors and sustaining stable growth of photosynthetic cells, optimization of light dependent variables in reactors is essential, as well as other variables, such as temperature, pH, DO, components of media and transport properties used in culture of general microorganisms. These light dependent variables are the incident light intensity, the distribution of emitted light wavelength, the light intensity distribution, light scattering and light-dark cycles in the photobioreactors. The challenge is the design of effective photobioreactors to increase the productivity and yield of photosynthetic cells or their valuable products. To achieve these goals, it is necessary to operate photobioreactors under relatively high cell concentrations and also higher light intensity. It is clear that the growth rate and productivity of photosynthetic cells can be estimated based on suitable models using these fermentation conditions and geometry of reactors. In most cases the average light intensity, which is calculated from model equations in various types of photobioreactor

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[2, 3, 6, and 7], is usually used as a light dependent variable, while the effects of the light intensity distribution and light scattering are rarely considered [9]. Furthermore, most of these kinetic data were determined at low cell concentrations in photobioreactors with short light path lengths to supply a constant intensity of light to cells [1]. Thus, illumination conditions in practical photobioreactors may be quite different from those in which the kinetic data were obtained. In batch cultures, for example, the average light intensity and distribution, as well as conditions in the culture media, change continuously with the cell concentration, complicating the results and reducing their reliability. To overcome these problems, kinetic data may be obtained by continuous culture, but this is a tedious approach. In continuous culture, special equipment must be used and longer times are necessary to attain steady state conditions. Thus, the approach is time and cost consuming, and sometimes, in addition, suffers from contamination problems.

In previous work [5], as an alternative to continuous culture, we proposed a semi-continuous cultivation method to obtain kinetic data under almost constant conditions. By this method the effect of distribution of light intensity on the stability of growth rate under various operational conditions with single side illumination in flat plate photobioreactors was studied. In the present work, different illumination conditions, which correspond to various types of light intensity distributions, were applied to semi-continuous cultivation of photosynthetic cells at relatively high cell concentrations. It was confirmed that the ratio of incident to transmitted light intensities in the flat plate photobioreactor could be used as a useful parameter to estimate conditions for stable growth, although there are several cases where this parameter failed to explain the stability of growth rate.

MATERIALS AND METHODS

M*aterials and microorganism*

Rhodobacter capsulatus ST-410 [8], a kind gift from Dr. S. Takakuwa at Kyoto Women's University, was used. This strain is a hydrogenase deficient mutant derived from *R. capsulatus* B-100, and can not assimilate carbon dioxide. In addition, it cannot assimilate nitrogen gas in the presence of ammonium ion. It was precultured anaerobically in RCV medium (25 cm^3; pH 6.8) containing 7.5 mM $(NH_4)_2$ SO_4 and 30 mM D, L-malate as sole nitrogen and carbon sources, respectively, and the following basal salts: 43 μM $FeSO_4$, 0.8 mM $MgSO_4$, 0.5 mM $CaCl_2$, 3.0 μM thiamine-HCl, 61 μM EDTA, 10 mM potassium phosphate buffer (pH 6.8), and trace elements (Mn, Borate, Cu, Zn, and Mo) [10]. A screw-capped test tube (25 cm^3) completely filled with the culture medium was inoculated with *R. capsulatus* and incubated in a glass-sided water bath at 32˚C under illumination with a halogen lamp (90 W, Toshiba, Japan) The incident intensity was ca.10 mW/cm^2. The cells were harvested by centrifugation at 10,000×g for 10 min and resuspended in the semi-continuous apparatus shown below containing the RCV medium.

Figure 1 shows the schematic diagram of the semi-continuous cultivation system used in the present work. A flat plate photobioreactor (height 9.8 cm; width 9.2 cm; thickness 20 mm; working volume 130 cm^3) was used for cultivation of *R. capsulatus* ST-410 under illumination with the halogen lamp from one side and both sides. The incident light intensity was measured with a thermopile photosensor [3] and the averaged value was used as the incident intensity on the illuminated surface. In semi-continuous culture, a part of the culture broth containing grown cells was repeatedly replaced with the fresh RCV medium at a predetermined time

interval (40 - 60 min) to keep the cell concentration and the volume of the broth constant at their initial values. At the end of each cycle of cultivation, the cell concentration was determined from the absorbance of the culture broth at 660 nm, and the volume of the medium to be replaced was calculated. For example, when the cell concentration increased x %, x/ (100 + x) of the culture broth was withdrawn and the same volume of the fresh medium was added. Before and after the replacement, the culture medium in the photobioreactor was mixed by bubbling N_2, and the cell concentration was measured. During cultivation cycles the medium was mixed only by natural convection and no sedimentation of cells was observed. To avoid penetration of O_2 into the culture broth, the reactor and the reservoir of the fresh medium were sealed by N_2.

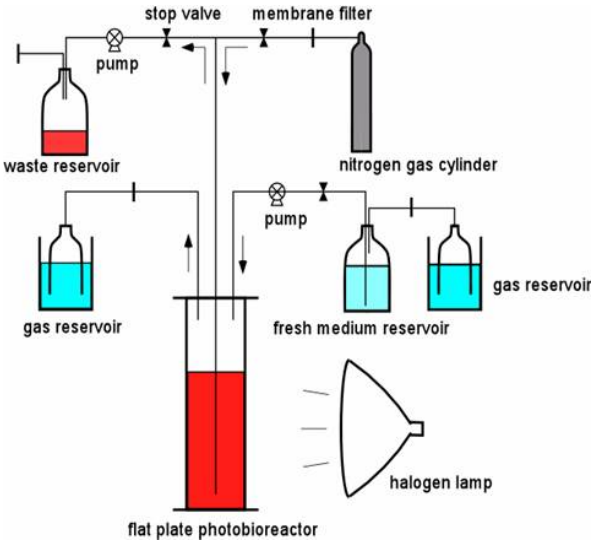

Figure 1. Schematic diagram of semi-continuous cultivation system

Estimation of light intensity distribution

The light intensity distribution was estimated from the following equation given in our previous work [4]. The attenuation of intensity of a polychromatic light source is given as the sum of the attenuation of each wavelength λ,

$$I = \frac{L_0^{\,2}}{(L+L_0)^2}\sum_{\lambda} I_{0,\lambda}\cdot 10^{-\varepsilon_{cell,\lambda} CL} \qquad (1)$$

where I is the light intensity of polychromatic light at a light path length L, $I_{0,\lambda}$ the intensity of incident light of the wavelength λ, L_0 the distance from the light source to the il-luminated surface, $\varepsilon_{cell,\lambda}$ the extinction coefficient of cell at λ and C the cell concentration. The predicted distributions showed good agreement with the measured ones under the experimental conditions employed [4]. The average light intensity was obtained by dividing the integrated value of the light intensity distribution along the light path length by the light path length of the photobioreactor.

RESULTS AND DISCUSSION

Semi-continuous cultivation method

From the increment of the cell concentration in each cycle, the specific growth rate can be calculated. The specific growth rate of the cell was obtained from the increase in the cell concentration during one cycle divided

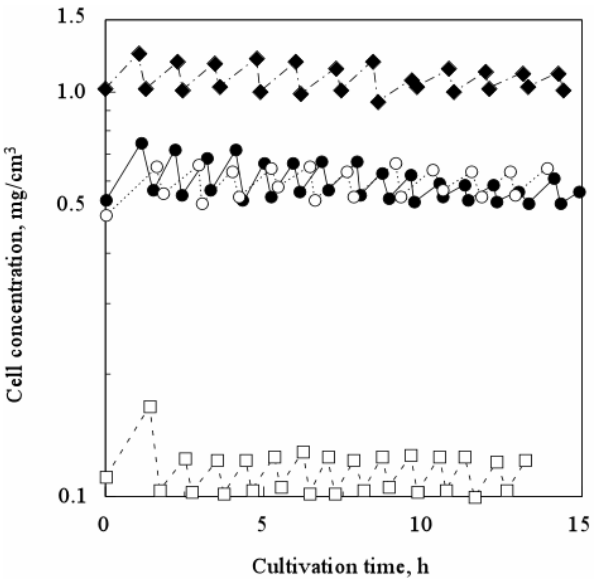

Figure 2. Time courses of cell concentration in semi-continuous cultivation

by the initial cell concentration and time for the one cycle. Figure 2 shows time courses of the cell concentration in semi-continuous cultures at the various average light intensities (I_{ave}).

As mentioned above, under some operational conditions using one side illumination, the specific growth rate was followed by rapid decrease. In the present work, various conditions of illumination were applied to ascertain which parameters are mainly responsible for the stability of growth rate.

Relationship between average light intensity and specific growth rate

Figure 3 shows the relationship between the average light intensity and the specific growth rate obtained in the flat plate photobioreactor with the light path length of 2.0 cm. Each bar shows the standard error of the experimental values. The open circles show the values of the specific growth rate, when the specific growth rate is kept stable over the entire period of cultivation. On the other hand, the closed circles show the values, when the unexpected decrease in the specific growth rate was observed.

The solid curve in Figure 3 shows a Monod-type equation obtained from the data shown as open symbols by the non-linear regression method

$$\mu = \frac{0.368 \cdot I_{ave}}{0.662 + I_{ave}} \qquad (2)$$

where μ and I_{ave} are the specific growth rate and the average light intensity, respectively. The experimental data agreed well with the curve obtained from Eq. (2) irrespective of the cell concentrations (0.1, 0.55 and 1.0 mg/cm^3) at least within the initial 5 hours. As mentioned by many researchers, the average light intensity is a useful and essential parameter for estimation of the specific growth rate in photobioreactor design, but it does not always assure that a stable growth rate is retained during a long period of cultivation. In Figure 3, open symbols show the specific growth rate, when constant growth rate was observed, and the closed symbols show those with the unexpected decrease after 5-6 hours.

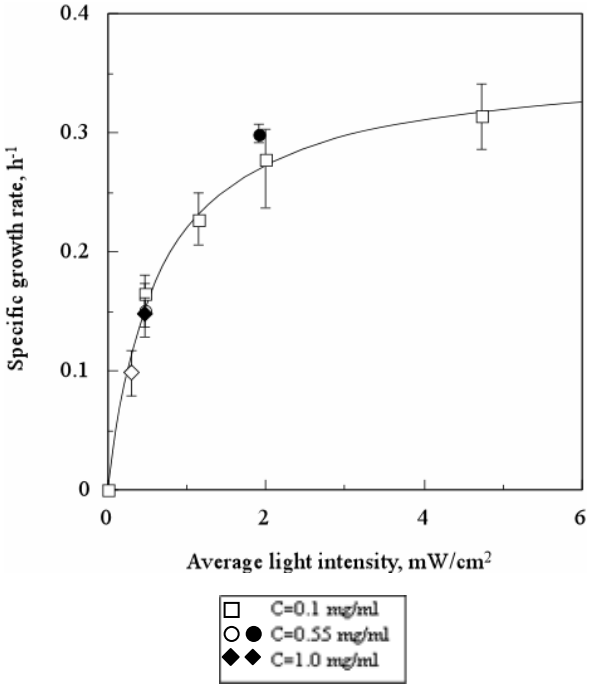

Figure 3. The relationship between the average light intensity and the specific growth rate

Illumination from one Side

As shown in Figure 4, under illumination of I_{ave} = 1.9 mW/cm^2 the specific growth rate was almost constant at about 0.28 h^{-1} over the entire period of cultivation at the cell concentration of 0.1 mg/cm^3. On the other hand, at a cell concentration of 0.55 mg/cm^3, the specific growth rate of about 0.28 h^{-1} was observed only for the initial 5 hours. Subsequently, a decrease in the specific growth rate with the cultivation time was noted. This unexpected decrease in the specific growth rate was also observed in the *R. capsulatus* ST-410 culture under the same conditions with agitation by a stirrer (data not shown). Even at the cell concentration of

0.55 mg/cm^3, the growth rate did not decrease under illumination of I_{ave} = 0.47 mW/cm^2 (incident light intensity I_0 = 2.1 mW/cm^2), while it decreased at the cell concentration of 1.0 mg/cm^3 (I_0 = 3.4 mW/cm^2). Generally, we expect that a stable specific growth rate can be maintained under constant light intensity with sufficient supply of nutrients and other essential materials. These results, however, suggest that cell growth may be inhibited by other factors. In order to discover the reason for the observed instability, a series of experiments with different operational conditions were carried out. In this work, we have focused on the distribution of the light intensity using various illumination geometries.

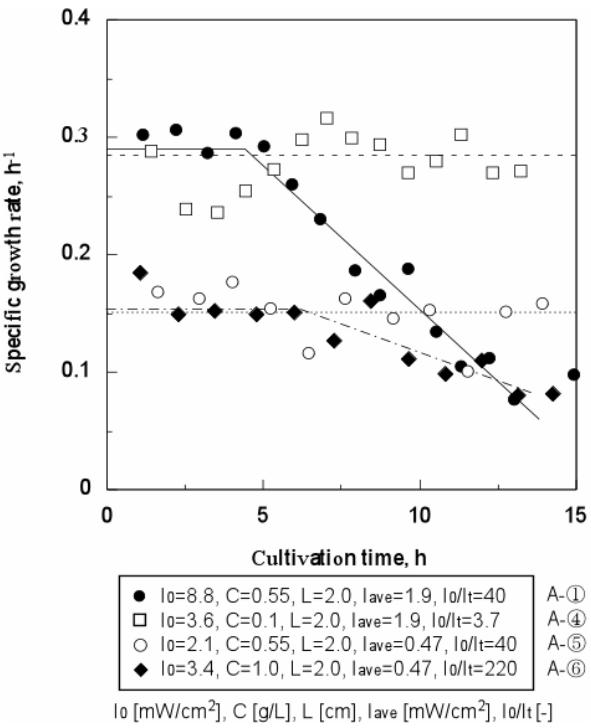

Figure 4. Time courses of specific growth rate in semi-continuous cultivation

Illumination from both Sides

In these series of experiments, the cultivation vessel was illuminated from both sides and the average light intensity inside the reactor was calculated by summing the average light intensity from each single light source.

As shown in Figure 5, with an average light intensity of I_{ave} = 4.9 and 0.75 mW/cm^2 provided by both sides the specific growth rate was almost constant at about 0.28 and 0.21 h^{-1} over the entire period of cultivation at cell concentrations of 0.1 and 0.55 mg/cm^3, respectively. On the other hand, at the cell concentration of 0.55 mg/cm^3 and I_{ave}=2.47 the specific growth rate of about 0.3 h^{-1} was observed only for the initial 5 hours, and again followed a decrease in the specific growth rate with the cultivation time.

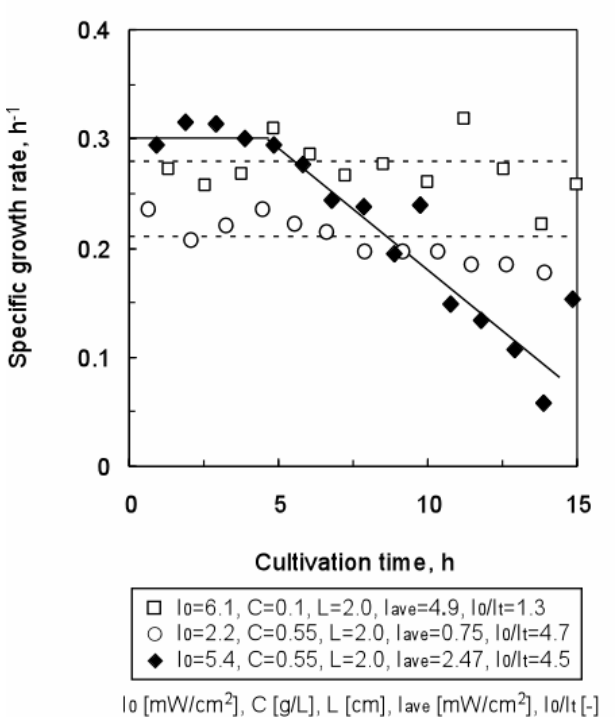

Figure 5. Time courses of specific growth rate in semi-continuous cultivation (Illumination: both sides)

Effect of ratio of incident to transmitted light intensities in various conditions of illumination

These results imply that the ratio of incident to transmitted light intensity can be used to estimate the stability of growth rate in of photosynthetic cell cultures. Figure 6 shows the light intensity distributions in single-

sided illumination calculated using Eq. (1) for the experimental conditions in this work.
In two cases in which a decrease in the specific growth rate was observed, the ratio of incident to transmitted light intensities in the flat plate photobioreactor was larger than 40 (solid lines). Under these conditions, cells pass through light and dark illuminated portions with large differences in the intensity. On the other hand, at the cell concentration of 0.1 mg/cm^3 this ratio is 3.7 irrespective the incident light intensity and decrease in the specific growth rate was not observed.

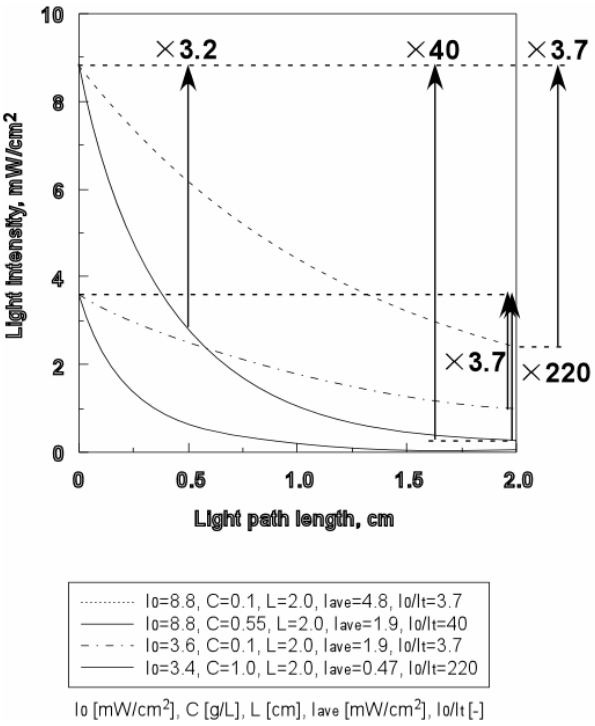

Figure 6. Light intensity distribution: illumination from one side

Thus, the ratio of the light intensity in the light portion to that in the dark portion, which reflects the light intensity distribution, seems to affect this phenomenon. Under the incident light intensity of 2.1 mW/cm^2, however, a decrease in the growth rate was not observed even at ratios >40, (at the cell concentration of 0.55 mg/cm^3), as shown in Figure 4, and thus there may be a specific threshold value of incident light intensity at which this phenomenon occurs.
However, in both side illumination in lower values of the ratio, an unstable growth rate was observed. This may be caused by the higher level of light intensity inside of the reactor. The physiological reason for this phenomenon is still unknown, but the conditions stated above will be often encountered in photobioreactors operated under high cell concentrations.
These results imply that we have to pay attention, not only to the effects of the average light intensity and distribution of light intensity, but also to the ratio of light intensities at light and dark positions to maintain stable growth of photosynthetic cells.

Effect of preculture conditions on the stability of growth rate

Effects of precultivation under various light intensities were studied by semi continuous cultivation in the flat plate photobioreactor.

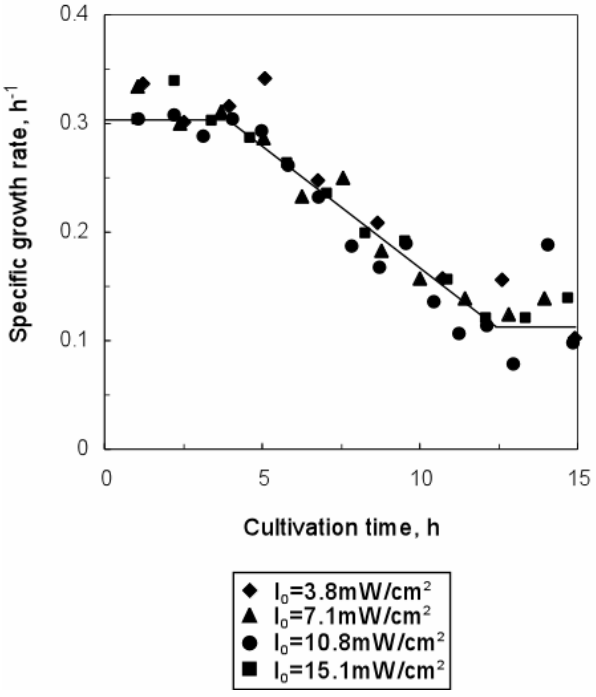

Figure 7. Time course of specific growth rate in semi-continuous cultivation (Operational condition in semi-continuous state: I_0 = 8.8 mW/cm2, C = 0.55 mg/ml, L= 20 mm, I_0/I_t= 40)

In all previous experiments, the supplied incident light intensity in precultivation was about 10 mW/cm2 but in this experiment the incident light intensity was varied from 3.8 to 15.1 mW/cm2 and after 20 hours, the cells were harvested by centrifugation and resuspended in a semi-continuous apparatus. The results, shown in Figure 7, show that the effect of different preculture conditions on the stability of growth rate is negligible.

CONCLUSION

In the present work, we developed a new semi-continuous cultivation method to easily observe growth characteristics of photosynthetic cells under defined distributions of light intensity. We found that the ratio of incident and transmitted light intensities in the flat plate photobioreactor affects the stability of cell growth of *R. capsulatus*, and this phenomenon must be considered for design of the stable batch and continuous cultivation of photosynthetic cells. However, under some other conditions, such as both side illuminations, this ratio cannot explain the instability of growth rate, and other operational parameters or physiological characteristics of photosynthetic cells must be considered for estimation of growth characteristics.

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