CHARACTERIZATION OF CHLORELLA CELL CULTURES IN BATCH AND CONTINUOUS OPERATIONS UNDER A PHOTOAUTOTROPHIC CONDITION

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In a microalgal culture under illuminated conditions, the production rate of cells depends on the distribution of light intensity and growth rate of cells. The relationship between light transmittance through culture liquid and cell concentration was estimated from experimental data using a green alga, Chlorella sp. UK001. In batch cultures of this alga, the optimum conditions for the growth were temperature of 30°C and pH of 5.7. Under these conditions, the value of specific growth rate of the alga increased with the increase in incident light intensity (Is) up to 130 W m⁻², and it decreased when Is was over 130 W m⁻². Continuous cultures of the alga in a rectangular reactor were performed at Is=55.8 and 71.4 W m⁻². The dilution rates maximizing the production rate of cells existed for the respective values of Is. The production rates of cells in these cultures were calculated considering the distribution of light intensity in the reactor. The calculated results could successfully describe the experimental data at different dilution rates, and when Is=55.8 and 71.4 W m⁻², the production rates of cells indicated maxima at dilution rates of 0.0218 and 0.0220 h⁻¹, respectively.

Introduction

Microalgal cultivations have been extensively investigated for the production of fine chemicals, health foods and foodstuffs (Benemann, 1990). In recent years, especially, the fixation of carbon dioxide using microalgae has become of interest to reduce carbon dioxide concentration in the atmosphere (Miyamoto and Benemann, 1991). Although some kinds of microalgae can grow even under heterotrophic conditions using organic compounds (Marquez et al., 1993), most microalgae grow under a photoautotrophic or mixotrophic condition associated with illumination. Photoautotrophs or mixotrophs, e.g. Chlorella, Spirulina and Dunaliella spp., have been cultivated on a large scale under sunshine irradiation (Goldman, 1979).

From a bioengineering point of view, the culture operation of microalgae differs from that of other microorganisms such as bacteria, yeasts and fungi because light is an important factor for cell growth and metabolite formation of microalgae (Lee et al., 1987; Akimoto et al., 1994). In microalgal cultivation using a photobioreactor, the distribution of light intensity in the culture occurs due to the absorption of light by algal cells. Therefore the estimation of light intensities in the culture is thought to be important under a photoautotrophic or mixotrophic condition. Yokota et al. (1994) performed the cultivation of Chlorella sp. using a rectangular reactor and proposed a kinetic model based on a relation between specific growth rate and specific light absorption rate. Ogbonna et al. (1995) presented a parameter to design a photobioreactor by considering the distribution of light intensity in a rectangular reactor.

In the present study, the growth characteristics of newly isolated microalga, Chlorella sp. UK001, under photoautotrophic conditions were first investigated, and the relation between specific growth rate and light intensity was clarified. The continuous cultures of the alga under various conditions were performed using a rectangular reactor with external light irradiation. The influence of dilution rate on production rate of the algal cells was investigated. Experimental results are described with the equations considering the attenuation of light in the reactor.

1. Experimental

1.1 Algal cells and medium

A unicellular freshwater green alga, Chlorella sp. UK001, was used in this study, which was isolated from a spring in Oh-ita prefecture, Japan and maintained in an axenic state (Murakami et al., 1995). The cells were spherical in shape with mean diameter of 4 μm. The algal strain was provided by the Research Institute of Innovated Technology for the
Earth, Kyoto. The medium for the algal culture was prepared by modifying Closterium medium (Ichimura, 1971). The phosphorus compound in the medium (0.05 kg m\(^{-3}\) β-glycerophosphate) was altered to 0.04 kg KH\(_2\)PO\(_4\) per m\(^3\) of medium. The medium was autoclaved at 121°C for 15 min prior to use unless otherwise noted. Preculture of the algal cells was carried out in a 1 dm\(^3\) Duran bottle containing 0.75 dm\(^3\) of the medium (pH 5.7) at 30°C, illuminated at a light intensity of 30 W m\(^{-2}\) with two 20-W white fluorescent lamps (FL20SW, Matsushita Electric Industrial Co.). CO\(_2\)-enriched air (\(\gamma_{c}=10\%\)) was sparged from a glass tubing of 1-mm bore at a constant flow rate of 0.5 dm\(^3\) min\(^{-1}\) through a filter (0.2 μm pore size, Millipore Corp.). The algal cells showing active growth were used as an inoculum for the subsequent cultures.

1.2 Culture methods

1) Batch culture A Roux flask was used to investigate the growth characteristics of the alga in batch culture. The medium of 0.2 dm\(^3\) was put in a 1 dm\(^3\) Roux flask placed horizontally on a rotary shaker (R-20, TAITEC Corp.) operating at 50 r.p.m. The depth of the medium was about 10 mm. A gaseous mixture composed of CO\(_2\), O\(_2\) and N\(_2\) was supplied to the surface of the medium at a constant flow rate of 0.25 dm\(^3\) min\(^{-1}\) through a filter (0.2 μm pore size, Corning Glass Works). The concentration of CO\(_2\) in the gaseous mixture was controlled at prescribed values. The flask was continuously illuminated by an upper side of the flask with 15 W white fluorescent lamps (FL15SW, Matsushita Electric Industrial Co.) or xenon lamps (UXL-500D-O, Ushio Inc.). The incident light intensity was regulated in the range of 0 - 365 W m\(^{-2}\) by alteration in the number and position of lamps. The irradiation area of the Roux flask was 178 cm\(^2\). The surface of the flask, except the irradiation face, was covered with black paper to avoid permeation and reflection of light. Initial pH of the medium was adjusted with 1 mol dm\(^{-3}\) HCl or NaOH aqueous solution at prescribed values before cultivation. The cultures were carried out with an inoculum of 0.01 kg m\(^{-3}\) in a chamber maintained at a prescribed temperature. To determine the specific growth rate of the alga, cell concentration was measured several times during the exponential growth phase in the cultures for 1 to 3 days.

2) Continuous culture Continuous culture in a rectangular reactor was conducted using the apparatus schematically shown in Fig. 1. The reactor was made of Plexiglas, except for the irradiation face, which was Pyrex glass. Surfaces of the Plexiglas were covered with black paper to shut out passage of light. The inner dimensions of the reactor were 26.3 cm in height, 4.8 cm in width and 15.6 cm in length, and the volume of the medium was 1.97 dm\(^3\).

For supply of carbon source and agitation of the culture, a gaseous mixture, CO\(_2\):O\(_2\):N\(_2\)=10:3:87 (v/v), was supplied through a dispersion plate with 24 holes (diameter of each hole=1 mm) at a constant flow rate of 1 dm\(^3\) min\(^{-1}\). Gas holdup in the medium could be neglected under the experimental conditions. The reactor was continuously illuminated from one side only with five 15-W white fluorescent lamps (FL15SW, Matsushita Electric Industrial Co.). The irradiation area was 108 cm\(^2\) (22.5 cm in height and 4.8 cm in width) and the incident light intensity at the irradiation surface was regulated at 55.8 or 71.4 Wm\(^{-2}\) by alteration of the distance between the fluorescent lamps and the irradiation face of the reactor. After batch culture for about 60 h, a fresh medium was fed to the culture for about 48 h at a constant flow rate using a peristaltic pump (CA-3, Tokyo Rikakikai Co.) and the volume of medium in the reactor was kept constant by means of overflow of culture broth from the reactor. When the cell concentration in the reactor became constant, cell concentration at a given dilution rate was measured several times for 24 h. Although cultivation was performed without sterilization, serious infection of the culture with contaminants did not occur during experiments.

1.3 Analytical methods

Cell concentration (X) in the culture was measured on a dry cell weight basis by the following procedure. An aliquot of culture was filtrated by a glass fiber filter (GC-50, Toyo Roshi Kaisha Ltd.) under reduced pressure. The harvested cells were rinsed with distilled water, dried at 105°C for 12 h and weighed. On occasions, the optical density at 680 nm of culture was measured with a spectrophotometer.
(UV-160A, Shimadzu Corp.) and the value of $X$ was determined by a correlative line.

Light intensity was measured with a quantum sensor (LI-190SB, LI-COR Inc.), which detected photosynthetically active light rays of wavelengths from 400 to 700 nm. The incident light intensity was the average of values measured at several points on the internal irradiance surface.

For the measurement of light transmittance through culture liquid, the cell suspension was put in a 1 dm$^3$ glass beaker and the light rays from a xenon lamp house (UI-501C, Ushio Inc.) were irradiated from the upper side of the beaker at an incident light intensity of 71.4 W m$^{-2}$. The intensities of transmitted light were measured with the quantum sensor placed under the beaker containing various volumes of cell suspension.

2. Results and Discussion

2.1 Light transmittance in suspension of the algal cells

In the culture of alga under light irradiation, the incident light is attenuated in cell suspension, and thus the distribution of light intensity in the culture existed. As the degree of light attenuation depends on cell concentration, it was first examined in the experiments using a cell suspension of Chlorella sp. UK001.

Figure 2 shows a semilogarithmic plot of the light transmittance ($I/I_0$) against light path length ($L$) at various concentrations of algal cells. At a given value of $X$, a linear relation was observed and yielded the following equation.

$$\ln(I/I_0) = -\alpha \cdot L$$  \hspace{1cm} (1)

where $\alpha$ is the effective absorption coefficient of light. The values of $\alpha$ were determined from the slopes of straight lines fitted by Eq. (1) to the experimental data, and then plotted against $X$ on a logarithmic scale as shown in Fig. 3. The following empirical equation for light absorption in cell suspension was obtained from a solid line shown in Fig. 3.

$$\alpha = 113X^{0.652}$$ \hspace{1cm} (2)

2.2 Effects of culture conditions of the algal cell growth

Optimum conditions for the growth of Chlorella sp. UK001 were investigated in batch cultures at a
fixed light intensity of 71.4 W m\(^{-2}\). Figure 4 shows the relationship between temperature (\(T\)) and specific growth rate (\(\mu\)) during the exponential growth phase in the cultures. The optimum temperature was 30\(^\circ\)C and the value of \(\mu\) at 30\(^\circ\)C was 0.20 h\(^{-1}\). The value of \(\mu\) was 0.01 h\(^{-1}\) at 10\(^\circ\)C and no growth was observed at 45\(^\circ\)C. The profile of \(T\) versus \(\mu\) of this alga was similar to that of a strain of *Chlorella pyrenoidosa* reported by Sorokin (1960). Figure 5 indicates the effect of medium pH on the algal growth. The optimum pH was about 5.7 and the alga grew between pH 4.0 and pH 9.0. Figure 6 represents a plot of \(\mu\) against CO\(_2\) concentration in the gaseous phase (\(y_c\)). In the range of \(y_c\) of 5 to 10\%, the value of \(\mu\) was about 0.20 h\(^{-1}\). At \(y_c=0.03\%\), the value of \(\mu\) was 0.15 h\(^{-1}\). In the gaseous mixture without CO\(_2\) (\(y_c=0\%\)), no growth was observed. The growth of the alga was stimulated by a 5 to 10\% concentration of CO\(_2\).

Figure 7 shows the effect of incident light intensity (\(I_0\)) on \(\mu\) in the cultures of the microalga under the conditions of pH 5.7, \(T=30\degree\)C and \(y_c=10\%\). The algal growth was not observed when \(I_0\) was less than 1.8 W m\(^{-2}\). The value of \(\mu\) increased with an increase in \(I_0\) from \(I_0=1.8\) to 130 W m\(^{-2}\), and reached 0.20 h\(^{-1}\) at \(I_0=130\) W m\(^{-2}\). Beyond this light intensity, however, a reduction of \(\mu\) was observed.

In the cultures shown in Fig. 7, the depth of the culture liquid was about 10 mm and the values of \(X\) in the exponential growth phase were less than 0.1 kg m\(^{-3}\). Therefore the distribution of light intensity in the culture could be neglected based on the data shown in Fig. 2, and the light intensity in the culture (\(I\)) was supposed to be equal to the incident light intensity (\(I_0\)).

From the data shown in Fig. 7, \(\mu\) could be expressed as a function of \(I\) by the following equation.

\[
\mu + m = f(I) \cdot g(I)
\]

where, \(f(I)\) is a function concerning the stimulating effect of light intensity, \(g(I)\) is a function concerning the inhibitory effect of light intensity and \(m\) is an empirical constant (y-intercept at \(I_0=0\) W m\(^{-2}\)). Assuming that light is a limiting factor for algal growth, a Monod type equation was adopted for \(f(I)\) as follows.

\[
f(I) = \frac{\mu_{max}}{(1 + \frac{I_0}{I})}
\]

To express the inhibitory effect of light on algal growth, the following equation presented by Bazua and Wilke (1977) was employed.

\[
g(I) = 1 - \frac{I}{I_m}
\]

Thus, the following equation was derived from Eqs. (3) to (5) (Taya et al., 1995).
Table 1 Values of constants in Eq. (6)

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \mu_{\text{max}} )</td>
<td>0.389 h(^{-1} )</td>
</tr>
<tr>
<td>( K_s )</td>
<td>56.3 W m(^{-2} )</td>
</tr>
<tr>
<td>( I_m )</td>
<td>711 W m(^{-2} )</td>
</tr>
<tr>
<td>( m )</td>
<td>1.19×10(^{-2} ) h(^{-1} )</td>
</tr>
</tbody>
</table>

\[
\mu + m = \frac{\mu_{\text{max}}}{1 + K_s / I} \left( 1 - I / I_m \right) \quad (0 \leq I \leq I_m) \quad (6)
\]

The experimental data shown in Fig. 7 were fitted to Eq. (6) using the nonlinear least squares method. A solid line indicated in Fig. 7 shows the fitted results. The estimated values of the constants in Eq. (6) are listed in Table 1.

Under photoautotrophic conditions, light is an essential factor for algal growth and it stimulates the growth rate. In this study, however, the growth of *Chlorella* sp. UK001 was inhibited at light intensity of more than 130 W m\(^{-2} \). Growth inhibition under very strong light intensity was reported for several microalgae (Sorokin and Krauss, 1958, 1962; Alba, 1982). The mechanism of this phenomenon may be attributed to the fact that toxic oxidants formed with strong illumination in a photosynthetic metabolism attack cellular components and important enzymes under a photoautotrophic condition (Devlin and Barker, 1971).

2.2 Continuous cultures using a rectangular reactor

1) Effect of operational conditions on production rate of algal cells

Continuous cultures of *Chlorella* sp. UK001 were conducted using a rectangular reactor at pH 5.7, \( T = 30^\circ \text{C} \), \( \gamma_e = 10\% \) and \( I_0 = 55.8 \) or 71.4 W m\(^{-2} \). Figure 8A shows the relationship between dilution rate \( (D) \) and production rate of cells \( (P) \) in several runs of the continuous cultures with the rectangular reactor. The value of \( P \) was defined by the following equation.

\[
P = DX
\]

When \( I_0 \) was 71.4 W m\(^{-2} \), the values of \( P \) were 5.8×10\(^{-3} \) to 6.6×10\(^{-3} \) kg m\(^{-3} \) h\(^{-1} \) at \( D = 0.019 \) to 0.021 h\(^{-1} \), and the value of \( P \) decreased at \( D = 0.043 \) and 0.056 h\(^{-1} \). When \( I_0 \) was 55.8 W m\(^{-2} \), \( P \) = 4.3×10\(^{-3} \) kg m\(^{-3} \) h\(^{-1} \) at \( D = 0.011 \) h\(^{-1} \), \( P \) = 5.4×10\(^{-3} \) kg m\(^{-3} \) h\(^{-1} \) at \( D = 0.027 \) h\(^{-1} \), and \( P \) = 4.2×10\(^{-3} \) kg m\(^{-3} \) h\(^{-1} \) at \( D = 0.047 \) h\(^{-1} \).

Figure 8B also shows the relationship between \( X \) and \( P \) in the continuous cultures shown in Fig. 8A. When \( I_0 \) was 71.4 W m\(^{-2} \), the value of \( P \) increased with increasing \( X \) and was 6.6×10\(^{-3} \) kg m\(^{-3} \) h\(^{-1} \) at \( X = 0.32 \) kg m\(^{-3} \). When \( X \) was more than 0.32 kg m\(^{-3} \), the value of \( P \) slightly decreased. In the case of \( I_0 = 55.8 \) W m\(^{-2} \), the value of \( P \) was 5.4×10\(^{-3} \) kg m\(^{-3} \) h\(^{-1} \) at \( X = 0.20 \) kg m\(^{-3} \).

These results indicate that there were optimum values of dilution rate and cell concentration in order to attain the highest production rate of algal cells in a continuous culture at a certain light intensity. Namely, in a continuous culture for the production of microalgal cells, the production rate can be maximized by regulating dilution rate at a given light intensity.

2) Estimation of production rate of the algal cells

In order to describe the distribution profile of light intensity in a rectangular reactor, the following assumptions were made. (i) Incident light rays strike uniformly over the entire irradiation face. (ii) Vertical distribution of light intensity does not exist in the reactor. (iii) Reflection and scattering of light rays by bubbles and insertions can be neglected. (iv) Cells are homogeneously suspended in the medium.

The light intensity at a distance \( L \) from the irradiation face \( (I(L)) \) was calculated by Eqs. (1) and (2) at a given value of \( X \). From the value of \( I(L) \), the apparent specific growth rate at a distance \( L \), \( (\mu(L)) \) was calculated by applying Eq. (6). The average value of \( \mu(L) \) in the reactor was estimated using the following equation.

\[
\bar{\mu} = \frac{\int_0^L \mu(L) dL}{\int_0^L dL}
\]

where \( L_e = 0.156 \) m.
At a steady state in a continuous culture, the following relation holds.

$$\bar{\mu} = D$$  \hfill (9)

The production rate of algal cells in the rectangular reactor was calculated by using Eqs. (1), (2) and (6) to (9), and the constant values shown in Table 1. The calculated curves to correlate $P$ with $D$, which are indicated in Fig. 8A, approximately coincided with the experimental data. At both light intensities of $I_0=55.8$ and 71.4 W m$^{-2}$, the maximum values of $P=4.8 \times 10^{-3}$ and $6.2 \times 10^{-3}$ kg m$^{-3}$ h$^{-1}$ were obtained at values of $D=0.0218$ and 0.0220 h$^{-1}$, respectively. The calculated results for the relationship between $P$ and $X$ are also indicated in Fig. 8B. These lines were in fair agreement with the experimental data. These results demonstrated that in a continuous culture with a reactor, the maximum value of $P$ could be determined from a value of $I_0$, and the maximum production of algal cells from a reactor was achieved by regulation of $D$ against $I_0$. The maximum values of $P$ were calculated by using Eqs. (1), (2) and (6) to (9), in terms of various values of $I_0$. In the range of $I_0=0$ to 400 W m$^{-2}$, it was found that the maximum value of $P$ increased with increasing value of $I_0$.

It is reasonable to regulate the dilution rate in response to changed light intensity in order to maximize the production rate of algal cells. The findings obtained from this research will provide a useful strategy to achieve favorable performance of algal culture with a photobioreactor.

**Conclusion**

Batch and continuous cultures of *Chlorella* sp. UK001 were carried out, and the following results were obtained.

1. The optimum conditions for algal growth were $T=30^\circ$C and pH 5.7. The light intensity exerted both stimulating and inhibitory effects on the algal growth. The equation expressing the relation between light intensity and specific growth rate was determined based on the experimental data.

2. In the continuous culture using the rectangular reactor, there was a dilution rate to maximize the production rate of algal cells at a given light intensity. The equations expressing light intensity inside the reactor were derived taking into account the attenuation of light intensities in the medium.

3. The calculated results could successfully describe the experimental data pertaining to the continuous culture with the reactor. When $I_0=55.8$ and 71.4 W m$^{-2}$, the maximum values of $P$ were determined to be $4.8 \times 10^{-3}$ and $6.2 \times 10^{-3}$ kg m$^{-3}$ h$^{-1}$, respectively, where at these values of $P$, the values of $D$ and $X$ were as follows: $D=0.0218$ h$^{-1}$, $X=0.22$ kg m$^{-3}$ and $D=0.0220$ h$^{-1}$, $X=0.28$ kg m$^{-3}$, respectively.

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**Nomenclature**

$D$ = dilution rate [h$^{-1}$]  
$f(l)$ = function concerning stimulating effect of light intensity [h$^{-1}$]  
$g(l)$ = function concerning inhibitory effect of light intensity [-]  
$I$ = light intensity in medium [W m$^{-2}$]  
$I_0$ = incident light intensity [W m$^{-2}$]  
$I(L)$ = light intensity in medium at a distance of $L$ from irradiation face of reactor [W m$^{-2}$]  
$I_m$ = maximum light intensity in Eq. (6) [W m$^{-2}$]  
$K_s$ = saturation constant [W m$^{-2}$]  
$L$ = length of light path [m]  
$L_e$ = horizontal length of reactor [m]  
$m$ = empirical constant [-]  
$P$ = production rate of algal cells [kg m$^{-3}$ h$^{-1}$]  
$T$ = culture temperature [$^\circ$C]  
$X$ = cell concentration [kg m$^{-3}$]  
$\gamma_c$ = concentration of carbon dioxide in gaseous mixture [%]  
$\alpha$ = effective absorbance coefficient [m$^{-1}$]  
$\mu$ = specific growth rate of algal cells [h$^{-1}$]  
$\bar{\mu}$ = average of specific growth rate of algal cells in reactor [h$^{-1}$]  
$\mu(L)$ = apparent specific growth rate of algal cells at a distance of $L$ from irradiation face of reactor [h$^{-1}$]  
$\mu_{max}$ = maximum of specific growth rate of algal cells [h$^{-1}$]

**Literature Cited**


