A NEW GROWTH KINETIC MODEL FOR PHOTO-AUTOTROPHIC MICROALGAE CULTURE

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In a photo-autotrophic microalgae culture, a linear functional relation between a specific growth rate and a light energy absorption rate has been adopted. However, this relation does not always hold for a wide range of variation of a specific light-absorption rate. Therefore, in this paper we have proposed a new model for growth kinetics as follows:

$$\mu = k (\xi - a)^{(1/n)}$$
$$k = \alpha \exp(-\beta I_0)$$

where μ is a specific growth rate, ξ is a specific light absorption rate and I_0 is an incident light intensity, and a , n , α , β are constants. The model was verified by experimental data obtained from a *Chlorella ellipsoidea* photo-autotrophic culture.

Introduction

Photosynthetic microorganisms—photosynthetic bacteria, blue-green and green algae, and so forth—have attracted interest recently. They have the potential of producing a diverse range of fine chemicals and biologically active compounds such as vitamins, carotenoid pigments, proteins, lipids, and polysaccharides, generating hydrogen from water, and recycling carbon dioxide in an artificial system modeled on a natural ecosystem.

Many references on specific growth rates of photosynthetic microorganisms have been published, but only a few papers have assessed the light energy absorbed by microorganisms^{1,6)} and described the incident light intensity^{5,9)}. Determination of the growth yield of photosynthetic microorganisms needs an appropriate value of light energy absorbed by suspended cells. Most estimations of light absorption were performed by applying Lambert-Beer's law to microorganism suspensions. However, Aiba²⁾ pointed out that the value of light absorption rate calculated from Lambert-Beer's law was overestimated because of the neglect of light scattering.

For the growth kinetics of photosynthetic microorganisms the specific growth rate is assumed to be a linear function of a specific light energy absorption rate with a coefficient of growth yield^{2,9)}. In this paper we propose a new functional relation between a specific growth rate and a specific light energy absorption rate. The model has been verified by experimental data from *Chlorella ellipsoidea* culture as well as by published data of *Oscillatoria agardhii*⁹⁾ and *Spirulina* sp.⁵⁾ cul-

tures.

1. Growth Kinetic Model

In a photo-autotrophic culture a functional relation of specific growth rate to specific light energy absorption rate is assumed by analogy to bio-energetic analysis of heterotrophic microorganism cultures.

$$\mu = (Y_{kl})_G (\xi - m) \quad (1)$$

Here the specific light energy absorption rate is the ratio of light energy absorption rate to cell concentration.

$$\xi = Q / X \quad (2)$$

The linear functional relation between μ and ξ of Eq. (1) is valid in a narrow range of ξ but is not always adequate for a wide-range change of light absorption. Van Liere and Mur's data⁹⁾ for *Oscillatoria agardhii* in a light-limited continuous culture are shown in Fig. 1. They estimated the light absorption rate by subtracting lost irradiance, the light energy that left the culture vessel, from incident irradiance. Plotted data of μ against ξ show convex curves. Iehana's data⁵⁾ for *Spirulina* sp. growth in batch and continuous cultures also indicate a non-linear relation between a specific growth rate and a specific absorption rate of light energy. Aiba²⁾ referred to this non-linearity earlier and mentioned that further experimental and theoretical consideration might be required.

Lee and others⁷⁾ pointed out the dependence of growth yield on light intensity. They obtained the data of

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Fig. 1 Relationship between specific growth rate and specific light absorption rate for *Oscillatoria agardhii* culture

the biomass energetic yield, Y_{kj} ($= \mu / \xi$), as a function of the average light intensity in a bioreactor for a *Spirulina platensis* culture. Their result is shown in **Fig. 2**, which suggests that growth yield is proportional to the exponent of light intensity multiplied by a negative constant.

Here we propose a new growth kinetic model for microalgae in a photo-autotrophic culture.

$$\mu = k (\xi - a)^{(1/n)} \quad (3)$$

$$k = \alpha \exp(-\beta I_0) \quad (4)$$

Equation (3) shows that the growth rate of photo-micro-organisms is proportional to the $(1/n)$ th power of the light absorption rate. The parameter a in the equation is a maintenance constant and the coefficient k corresponds to growth yield. Light intensity dependence of growth yield is specified by Eq. (4).

2. Experiments

The proposed growth kinetic model was experimentally verified by *Chlorella ellipsoidea* culture under autotrophic light-limited conditions. The experiments were undertaken in repeated fed-batch cultures with a variety of values of incident light intensity.

2.1 Materials and methods

Organism: *Chlorella ellipsoidea* was used throughout. The algae were obtained through a company from the culture collection of algae at the Institute of Molecular Cell Biology of the University of Tokyo.

Medium: The medium used was the chemically defined MC medium. The composition of the medium was the same as described elsewhere⁸⁾.

Preculture: Synchronous cultures were grown in 200-ml Erlenmeyer flasks for a week or more under a diurnal condition of 9 hours in the light and 15 hours in the dark. A portion of the synchronous preculture was inoculated into a main fermentor to attain an initial algae concentration (0.001 g l^{-1}).

Fermentor: The main rectangular fermentor used has a $10 \times 10 \text{ cm}^2$ irradiation surface of glass plate and a 5-cm optical length. It was submerged in a water bath

Fig. 2 Relationship between growth yield and light intensity for *Spirulina platensis* culture

Fig. 3 Light absorption fraction in rectangular fermentors with various optical lengths L

controlled at 30°C . A collimated light beam from a tungsten lamp (the effective wave length range used in the experiment: 370-700 nm, the maximum output wavelength: 640 nm) irradiated the fermentor. The light intensity was adjusted in a range of 2×10^{-3} - $1 \times 10^{-2} \text{ kJ cm}^{-2} \text{ h}^{-2}$ by changing the power to the lamp. The periodic lighting condition was the same in the precultivation. Air enriched to 5 % CO_2 was supplied at 1 vvm and no organic nutrient was added. Therefore, an autotrophic condition was maintained.

Culture: Repeated fed-batch cultures were grown in the fermentor. At the beginning and the end of every 9-hour illumination period, a 10 volume percent culture solution in the fermentor was withdrawn and the same volume of fresh medium was added. The cell concentration was determined by a spectrophotometric measurement of transmittance of the withdrawn sample at 680 nm. The specific growth rate and the mean cell concentration were obtained from the two concentrations measured in a day.

2.2 Assessment of light absorption

Lambert-Beer's law is commonly used to assess light energy absorption in a microalgae suspension^{3,4)}, but it disregards the effect of light scattering by algae cells. Therefore, it is acceptable only for a dilute suspension. We proposed a random-walk model which con-

Fig. 4 Growth curve of *Chlorella ellipsoidea* in a fermentor with 5-cm optical length

Fig. 5 Relationship between specific growth rate and specific light absorption rate for *Chlorella ellipsoidea* culture

siders light scattering as well as light absorption by cells to estimate light absorption in a microbe suspension. The validity of the model was experimentally confirmed¹⁰.

An actinometer of Reinecke's salt solution was used to assess incident light intensity, I_0 , of a visible light effective for photosynthesis. Once a value of incident light intensity is given, the light absorption rate becomes

$$Q = A I_0 q / V \quad (5)$$

where q is a light absorption fraction. The value of q can be evaluated by Monte Carlo simulation based on the random-walk model¹⁰. The results assessed for rectangular fermentors with several optical lengths are shown in **Fig. 3** as a function of cell concentration.

3. Experimental Results

Some typical growing curves in a fermentor with a 5-cm optical length are presented in **Fig. 4**. Pairs of cell concentrations measured in a day are plotted with time. When growth rate became equal to the cell mass withdrawn twice per day, the curves attained a pseudo-steady state.

Figure 5 illustrates plots of the specific growth rates determined from the growth curves as functions of

Fig. 6 Dependence of new kinetic model parameters on light intensity

specific light absorption rates evaluated at each cell concentration. The experimental data are represented as convex curves in the figure. Equation (3) was applied and a fitting technique was used for determining the parameters n , a and k in the equation. Dependence of parameters n , a and k on light intensity is shown in **Fig. 6**. Both parameters n and a are independent of light intensity, but k varies with induced light energy. Applying of Eq. (4) to the plot of k versus I_0 could determine the two parameters of α and β . The results of growth kinetics for *C. ellipsoidea* became

$$\mu = k (\xi - 0.89)^{(1/1.76)} \quad (6)$$

$$k = 0.067 \exp(-101 I_0) \quad (7)$$

All the experimental data of μ were compared with the specific growth rates calculated by Eqs. (6) and (7). These results are shown in **Fig. 7** and it can be concluded that the agreement between experimental and calculated data is fairly good. The experimental data were obtained from a scaled-up fermenter with a 10-cm optical length

Fig. 7 Comparison between experimental and estimated specific growth rates

and from a scaled-down fermenter with a 2.5-cm optical length. These data were compared with the values estimated by Eqs. (6) and (7), in which light absorption rates were determined by Eq. (5) replacing the right q for each fermenter. The results are shown in Fig. 8. The satisfactory correspondence verifies that the proposed model is acceptable.

4. Discussion

4.1 Growth rate variation with light intensity

Variation of a specific growth rate with incident light intensity is widely observed. Equations (3), (4) and (5) together offer a relation between specific growth rates and incident light intensities. Some computational results for *C. ellipsoidea* culture in a fermenter of $L = 5$ cm are shown in Fig. 9. The results are similar to those obtained experimentally by Lee⁷⁾, and those assessed by Aiba's model²⁾, which specifies the effect of growth inhibition above the saturation light intensity. The value of saturation light intensity is calculated by the following equation, which is derived to maintain a specific growth rate at a maximum from Eqs. (3), (4) and (5).

$$I_{\text{sat}} = \frac{1}{n\beta} + \frac{aX}{q(A/V)} \quad (8)$$

The value of the maximum μ_{Isat} is obtained from Eq. (3) with k evaluated at I_{sat} by Eq. (4)

4.2 Optimum conditions of continuous operation

When an energetic growth yield, which reflects the efficiency of energy transfer from light to biomass, is defined as

$$Y_{kj} = \frac{(dX/dt)}{Q} = \frac{\mu}{\xi} = \frac{\mu}{\{(\mu/k)^n + a\}} \quad (9)$$

Fig. 8 Comparison between experimental and estimated specific growth rates in scaled-up and scaled-down fermentor

it varies with light intensity and specific growth rate. Figure 10 shows the functional relation between Y_{kj} and μ for *C. ellipsoidea* cultures in a fermenter of $L = 5$ cm. The maximum growth yield is attained at the following $\mu_{Y_{\text{max}}}$ when n takes a value other than unity.

$$\mu_{Y_{\text{max}}} = k \left(\frac{a}{n-1} \right)^{(1/n)} \quad (10)$$

For $n = 1$, Y_{kj} approaches the maximum value of k as μ increases.

The value of $\mu_{Y_{\text{max}}}$ in Eq. (10) changes with light intensity because it includes the parameter k . On the other hand, μ_{Isat} is also a function of light intensity in Eq. (8). Therefore, defining $\mu_{Y_{\text{max}}}$ that is equal to μ_{Isat} gives an optimum operational condition of light intensity which can attain the maximum specific growth rate while keeping the growth yield at maximum. The optimum condition in *C. ellipsoidea* cultures for a fermenter of $L = 5$ cm becomes

$$\begin{aligned} I_{\text{sat}} &= 0.0099 \text{ kJ cm}^{-2} \text{h}^{-1} \\ \mu_{Y_{\text{max}}} &= \mu_{\text{Isat}} = 0.027 \text{ h}^{-1} \\ Y_{kj} &= 0.013 \text{ g kJ}^{-1} \\ X &= 0.65 \text{ g l}^{-1} \end{aligned}$$

In a continuous operation of biomass production, harvest yield is also an important factor. Biomass harvested from a fermenter per unit time becomes

$$FX = V\mu X \quad (11)$$

In Fig. 11, the value of μX for *C. ellipsoidea* cultures is shown as a function of light intensity and biomass concentration. The curving surface of μX exhibits a peak at which harvest yield is maximum. The optimum condition values at the maximum harvest yield are

Fig. 9 Variation of specific growth rate with light intensity

Fig. 10 Variation of growth yield with specific growth rate

$$I_{\text{opt}} = 0.0114 \text{ kJ cm}^{-2} \text{ h}^{-1}$$

$$X_{\text{opt}} = 0.937 \text{ g l}^{-1}$$

$$\mu X_{\text{opt}} = 0.0182 \text{ g l}^{-1} \text{ h}^{-1}$$

This condition is not very different from that where the growth yield is maximum.

Conclusions

For growth kinetics of photosynthetic microorganisms we have proposed a new model: a functional relation between a specific growth rate and a specific light absorption rate. The model was verified by experimental data of photo-autotrophic culture of *C. ellipsoidea*. The results show that it can predict growth rates observed in a scaled-up and a scaled-down fermenter. This new model can also make apparent the effect of growth inhibition under strong light irradiation. Based on this model, some optimum conditions for continuous operation of a photo-bioreactor have been studied.

Nomenclature

A	= irradiated area	$[\text{cm}^2]$
a	= maintenance coefficient given in Eq. (3)	$[\text{kJ g}^{-1} \text{ h}^{-1}]$
F	= flow rate of culture medium	$[\text{l h}^{-1}]$

Fig. 11 Variation of growth rate with light intensity and cell concentration

$-\Delta H_a$	= enthalpy change of cells	$[\text{kJ g}^{-1}]$
I_{av}	= average light intensity	$[\text{kJ cm}^{-2} \text{ h}^{-1}]$
I_o	= incident light intensity	$[\text{kJ cm}^{-2} \text{ h}^{-1}]$
I_{opt}	= optimum incident light intensity	$[\text{kJ cm}^{-2} \text{ h}^{-1}]$
I_{sat}	= saturation incident light intensity	$[\text{kJ cm}^{-2} \text{ h}^{-1}]$
k	= growth yield given in Eq. (3)	$[\text{kJ}^{-(1/n)} \text{ g}^{(1/n)} \text{ h}^{(1/n-1)}]$
m	= maintenance coefficient	$[\text{kJ g}^{-1} \text{ h}^{-1}]$
n	= power constant given in Eq. (3)	$[-]$
Q	= light absorption rate	$[\text{kJ l}^{-1} \text{ h}^{-1}]$
q	= light absorption fraction	$[-]$
V	= volume of culture medium	$[\text{l}]$
X	= cell concentration in culture medium	$[\text{g l}^{-1}]$
X_{opt}	= optimum cell concentration	$[\text{g l}^{-1}]$
Y_{KJ}	= growth yield	$[\text{g kJ}^{-1}]$
$(Y_{\text{KJ}})_G$	= true growth yield	$[\text{g kJ}^{-1}]$

α	= coefficient given in Eq. (4)	$[\text{kJ}^{-(1/n)} \text{ g}^{(1/n)} \text{ h}^{(1/n-1)}]$
β	= coefficient given in Eq. (4)	$[\text{kJ}^{-1} \text{ cm}^2 \text{ h}]$
μ	= specific growth rate	$[\text{h}^{-1}]$
μ_{exp}	= experimental specific growth rate	$[\text{h}^{-1}]$
μ_{cal}	= specific growth rate calculated by Eqs. (6),(7)	$[\text{h}^{-1}]$
μ_{Isat}	= specific growth rate at saturation light intensity	$[\text{h}^{-1}]$
$\mu_{Y_{\text{max}}}$	= specific growth rate at maximum growth yield	$[\text{h}^{-1}]$
ξ	= specific rate of light absorption	$[\text{kJ g}^{-1} \text{ h}^{-1}]$

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