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Simple Monodimensional Model for Linear Growth Rate of Photosynthetic Microorganisms in Flat-Plate Photobioreactors

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Abstract The current study proposes a simple monodimensional model to estimate the linear growth rate of photosynthetic microorganisms in flat-plate photobioreactors (FPPBRs) during batch cultivation. As a model microorganism, *Chlorella kessleri* was cultivated photoautotrophically in FPPBRs using light-emitting diodes (LEDs) as the light sources to provide unidirectional irradiation in the photobioreactors. Various conditions were simulated by adjusting both the intensity of the light and the height of the culture. The validity of the proposed model was examined by comparing the linear growth rates measured with the predicted ones obtained from the proposed model. Accordingly, the value of $\frac{K \cdot \mu_m}{\alpha \cdot L}\log(I_0 \cdot I_s^{(\epsilon\ 1)} \cdot I_c^{-\epsilon})$ was proposed as an approximate index for strategies to obtain the maximal lightn yield under light-limiting conditions for high-density algal cultures and as a control parameter to improve the photosynthetic productivity and efficiency.

Key words: Linear growth rate, flat-plate photobioreactor, *Chlorella kessleri*, light yield, monodimensional model

About one-third of the world's photosynthetic biomass is produced by algae. The number of microalgal species has been estimated at between 22,000 and 26,000, among which the biochemistry and ecophysiology of only about fifty species have been studied in detail [30, 45, 49]. Today, it is widely recognized that microalgae can be used as feed for animals and foodstuff for humans, because their nutritional value is comparable to other conventional food products [4, 46]. In addition, many chemicals, biochemicals, and pharmaceuticals have been extracted from microalgae for use in the food, pharmaceutical, cosmetic, and chemical industries [2, 39]. The application of algae has also been expanded to environmental areas, such as wastewater treatment [27], heavy metal removal [19, 20], and CO_2 fixation [25, 26]. Furthermore, the advantages offered by the mass cultivation of microalgae over conventional crops include a short growth cycle, continuous production throughout a year, easier genetic engineering, and the possibility of growing microalgae in brackish water rather than freshwater, which is becoming a scarce resource [49].

Most microalgal culture systems in use today are open cultivation systems, particularly raceway ponds and circular ponds. However, over the last 50 years, great advances have been made in understanding the biology of algae and engineering requirements for large-scale algal culture systems. This has led to the development of several types of closed photobioreactors which will enable the commercialization of noble algal products within the next decade [3]. Flat-plate photobioreactors [15, 53, 58], which have been intensively studied in recent years, can be controlled with a short light path length, prevent O_2 build-ups, and can be easily cleaned [5].

In high-density algal cultures with optimized media, light is always limited despite the minimal thickness of the photobioreactors [22]. The growth rate of microalgae depends on the light energy absorbed by the cells. Thus, the growth rate is initially high when a small inoculum is used, the organisms exhibit their maximum growth rate, and an exponential phase ensues while light is not limited. However, as microalgae grow, mutual shading increases, thereby decreasing the specific (per unit of biomass) light-energy uptake rate so that the specific growth rate begins to fall. As the biomass continues to increase, the growth rate continues to decrease. An interesting feature of this phase is that the total biomass of the culture increases linearly with time, because the production per unit of time is proportional to the absorption of light by the culture [2, 29]. This linear growth phase in batch cultures is a characteristic of photosynthetic microorganisms during photoautotrophic growth. Recently, Ogbonna *et al.* [37] reported on the correlation between the linear growth rate and the final cell concentration in batch cultures using various types of reactor. The IEA Greenhouse Gas R&D Programme [17] also proposed the importance of an

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e tended linear growth phase with a high cell density for tl e conventional use of algal CO_2 fixation from flue gases. T ius, knowledge of the determining factors for the linear g owth rate can provide a strategy for optimizing algal c ltures and productivity.

To date, many models for microalgal growth kinetics h ve been proposed for batch and continuous cultures [6-8 24, 28, 33, 48]. In particular, Cornet *et al.* [7] and C gbonna *et al.* [37, 38] developed models for the linear g owth rate in batch cultures. However, their models differ f om each other with regards to the effect of the light i tensity and light path length of the photobioreactor on t e linear growth rate. No other studies have given any a tention to the linear growth rate of algal batch cultures d spite its importance.

Accordingly, the current study proposes a monodimensional m odel for interpreting batch growth kinetics under light-l mited and substrate saturated conditions. The proposed m odel was derived from a combination of light attenuation p henomena and a light response curve based on Beer-L ambert's law and a Monod-type equation, respectively. A fter a feasibility test, the proposed model was applied to i nterpret the efficiency and light yield of a photobioreactor.

MATHEMATICAL MODEL

Light Absorption and Penetration Depth

Light energy penetrates into a culture from the illuminating surface, and the intensity of the light energy dramatically decreases as the light travels into the culture, due to absorption by the light-harvesting pigments of algae and scattering by algal cells (Fig. 1). The simplest model for calculating the light absorption and penetration depth is Beer-Lambert's law. This law is widely used [14, 22, 44] as it is simple and easily available, despite its inaccuracy in dense algal cultures [1] and of the 'package effect' [10, 12].

$$\mathrm{Log}(I_0/I)=\alpha \cdot X \cdot x \qquad (1)$$

where, I_0, I, α, X, and x denote the incident light intensity, light intensity at depth x, specific light absorption coefficient, cell concentration, and distance from the illuminating surface, respectively.

Thus, the light penetration depth (x_c), depth to saturation point (x_s), and distance from the saturation point to the compensation point (x_{c-s}) can be calculated by introducing the compensation light intensity (I_c) and saturation light intensity (I_s) to Eq. (1) (see Fig. 1b)

$$x_c=\frac{\log(I_0/I_c)}{\alpha \cdot X} \quad x_s=\frac{\log(I_0/I_s)}{\alpha \cdot X} \quad x_{c-s}=\frac{\log(I_s/I_c)}{\alpha \cdot X} \qquad (2)$$

Specific Growth Rate Versus Light Intensity

Figure 2a represents the typical specific growth rate of microalgae as a function of the light intensity, when light is the only limiting substrate. The main features of this curve are: (i) the compensation light intensity (I_c), where the net growth rate is zero [14, 50]; (ii) the saturation light intensity (I_s), where the growth rate reaches the maximum possible ($\mu_{(I)}=\mu_m$) [44]; (iii) the photoinhibition light intensity (I_d), where the growth is inhibited by a high light intensity [14, 56].

When $I<I_d$, the curve can be described as follows:

$$\mu_{(I)}=\frac{\mu_m \cdot (I-I_c)}{K_I+(I-I_c)} \qquad (3)$$

K_I is the light intensity constant when the specific growth rate is half the maximal. In other words, $\mu_{(I)}=\mu_m/2$ at $I-I_c=K_I$.

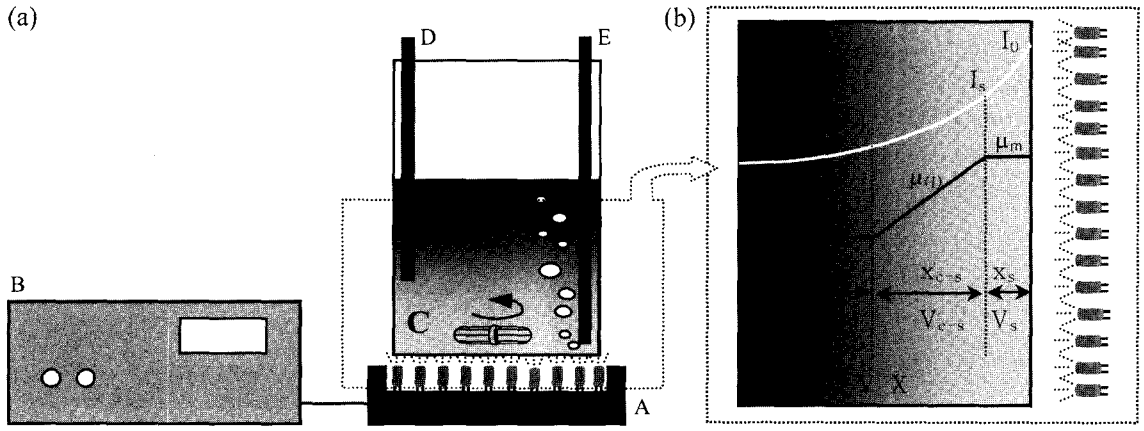

Fig. 1. (a) Schematic representation of flat-plate photobioreactors.
The red LEDs (A) were powered by DC power supplies (B) at a constant voltage depending on the light intensity. The algal suspension (C) in a photobioreactor with a sampling (D) and bubbling (E) tube was agitated by a magnetic bar. (b) Concept of model. The light intensity (I) was drawn based on Beer-Lambert's law, and the specific growth rate (μ) using Monod's equation. The culture volume was compared with the photic volume, separated by the volume (V_s) in $I_s<I$ and the volume (V_{c-s}) in $I_c<I<I_s$, and the dark volume (V_d) in $I<I_c$.

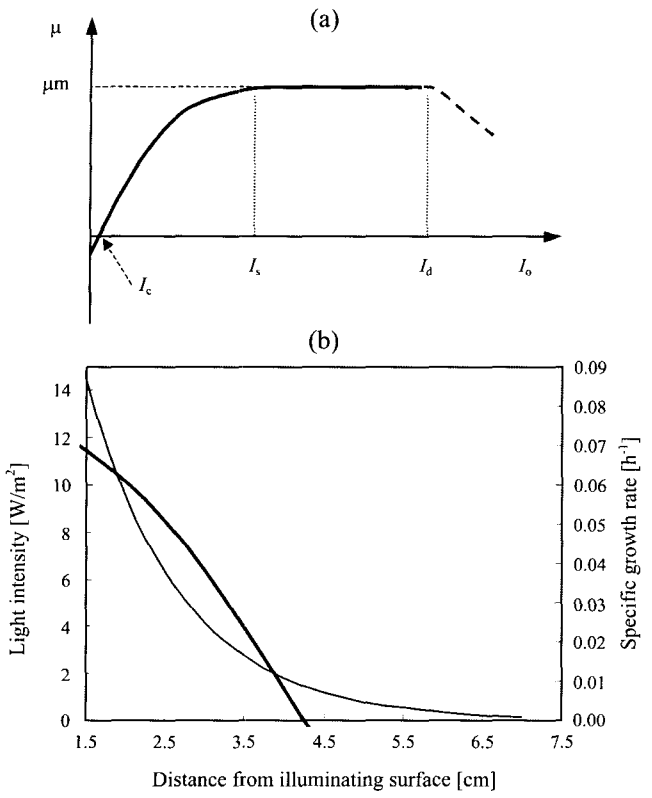

Fig. 2. (a) Graph of specific growth rate (μ) versus light intensity (I_0) and (b) plots of light intensity (—) and specific growth rate (—) calculated from Beer-Lambert's law and Monod's equation versus distance from illuminating surface.
The light intensity logarithmically decreased from the saturation point to the compensation point as it penetrated from the illuminating surface, while the specific growth rate decreased almost linearly. I_0=50 W/m², X= 1.2×10⁸ μm³ cell/ml, α=3.0×10⁻⁹ cm²/μm³ cell, μ_m=0.087 h⁻¹, K_1=3.44 W/m², I_c=1.456 W/m².

Although several models based on the light response curve of algae have already been proposed [13, 32], the Monod type model can be applied when the photoinhibition is relatively low, and is convenient for introducing the compensation irradiance.

To clarify the relation between the specific growth rate and the distance from the illuminating surface, Eq. (3) can be rewritten by introducing Eq. (1):

$$\mu_{(x)}=\frac{\mu_m \cdot (I_0 \cdot 10^{(-\alpha \cdot X \cdot x)} - I_c)}{K_1+(I_0 \cdot 10^{(-\alpha \cdot X \cdot x)} - I_c)} \quad (4)$$

As shown in Fig. 1(b), the specific growth rate in the zone between the illuminating surface and the light saturation point has the maximum value. Plus, in the adjacent zone where the light intensity of $I_c<I<I_s$, the mean specific growth rate, $\bar{\mu}_{(I)}$, can be defined as:

$$\bar{\mu}_{(I)}=\frac{\int_{x_s}^{x_c}\mu_{(x)}dx}{x_c-x_s}=\frac{\int_{x_s}^{x_c}\frac{\mu_m \cdot (I_0 \cdot 10^{(-\alpha \cdot X \cdot x)} - I_c)}{K_1+(I_0 \cdot 10^{(-\alpha \cdot X \cdot x)} - I_c)}dx}{x_{c-s}} \quad (5)$$

Equation (5) can be integrated using Eq. (2) as follows:

$$\bar{\mu}_{(I)}=\frac{\mu_m}{(I_c-K_1)}\left[I_c+\frac{K_1 \cdot \log\left(\frac{K_1}{K_1+I_s-I_c}\right)}{\log(I_s/I_c)}\right] \quad (6)$$

If a strain is selected, this equation can be simplified as:

$$\bar{\mu}_{(I)}=\varepsilon \cdot \mu_m \quad (7)$$

where ε is the dimensionless constant related to the inherent property of the cell that can be calculated with Eq. (6) using the specific growth rate and irradiance curve of the cell. These values for 24 strains are shown in Table 2, and the mean value was 0.43±0.07 (standard deviation). The value of the strain used in the current study was 0.44, which was calculated from the data in Table 1. If the incident light intensity was lower than the saturation irradiance, I_s should be replaced by I_0 in Eq. (6). Thus, ε becomes a function of the incident light intensity.

Consequently, for the strain tested in the current study, the specific growth rate was approximated to be μ_m when $I_s<I<I_d$, and $\varepsilon \cdot \mu_m$ when $I_c<I<I_s$. Figure 2(b) shows that the specific growth rate slowly decreased under the region of $I_c<I<I_s$ when the light intensity logarithmically decreased.

Differential Model Equation

If the light decreases, as shown in Fig. 1(b) ($I_s<I_0<I_d$), the differential cell concentration can be expressed as:

Table 1. Inherent properties of *C. kessleri* and operating conditions.

Inherent property	α, cm²/μm³ cell 3.0×10⁻⁹	I_c, W/m² 1.46	I_s, W/m² 13.7	I_d, W/m² 600	(K_1+I_c)* 4.9
Abbreviation	HL	HS	LL	LS	
Irradiance (W/m²)	35.2	35.2	17.6	17.6	
Light path length (cm)	10	5	10	5	
Agitation (rpm)	200 (with magnetic stirrer)				
Gas input rate (ml/min)	100 (5% CO_2 balanced with N_2)				

α: specific absorption coefficient; I_c: compensation irradiance; I_s: saturation irradiance; I_d: inhibition irradiance; HL: high light intensity and long light path; HS: high light intensity and short light path; LL: low light intensity and long light path; LS: low light intensity and short light path. *: this saturation constant was estimated from [43] at one-third of the saturation point (I_s).

Table 2. Light intensity and algal growth (data from [43]).

Species	Strain	Growth condition	Temp (°C)	Type of light	μ_m (dbl/day)	I_s (klx)	I_c (klx)	K_I+I_c (klx)	ε	K_I	I_{opt} (klx)
Cyanobacteria	*Anacystis nidulans*	-	30	T	4.8	3.5	0.30	1.3	0.41	1.00	3.51
	Nostic muscorum	-	24	F	-	3.1	0.11	1.4	0.30	1.29	3.10
Cryptophytes	*Chroomonas saline*	6L/18D	10	F	0.6	4.2	2.10	2.8	0.50	0.70	8.08
Dinoflagellates	*Cachonina niel*	-	23	F	0.95	6.4	0.53	2.1	0.43	1.57	$<I_s$
	Cryptomonas sp.	24L	18	T	0.5	15.0	1.50	3.7	0.54	2.2	$<I_s$
	Dissodium lunula	12L/12D	20	F	0.3	3.7	0.25	1.7	0.34	1.45	4.05
	Gonyaulax polyedra	12L/12D	21	F	0.3	3.2	0.81	1.9	0.40	1.09	5.05
	Gymnodium sp.	24L	23-26	F	1.2	6.4	0.32	2.1	0.39	1.78	$<I_s$
	Prorocentrum micans	24L	20	T	0.51	2.3	0.50	1.4	0.37	0.90	3.56
	Pyrocystis fusiformis	12L/12D	20	F	0.15	1.6	0.25	0.63	0.46	0.38	1.84
	Pyrocystis noctiluca	12L/12D	25	F	0.12	1.9	0.31	0.93	0.40	0.62	2.49
Diatoms	*Asterionella socialis*	-	13	F	1.1	4.0	0.75	2.0	0.41	1.25	5.45
	Biddulphia aurita	15L/9D	11	F	1.3	7.1	0.95	2.4	0.49	1.45	7.23
	"	9L/15D	11	F	1.1	7.1	0.95	2.4	0.49	1.45	7.23
	Chaetoceros sp.	-	23-26	F	6	6.5	0.11	2.2	0.31	2.09	$<I_s$
	Chaetoceros armatum	-	13	F	0.6	2.0	0.25	0.5	0.58	0.25	$<I_s$
	Coscinodiscus pavillardii	-	-	-	1.3	6.0	1.00	2.2	0.50	1.2	6.67
	Fragilaria striatula	9L/15D	11	F	0.9	9.5	1.90	4.8	0.42	2.9	13.18
	Melosira moniliformis	15L/9D	11	F	0.9	4.8	0.24	1.2	0.45	0.96	$<I_s$
	"	9L/15D	11	F	0.6	7.1	0.24	2.4	0.35	2.16	$<I_s$
	Phaeodactylum tricornutum	24L	17	F&T	2	3.3	0.026	0.5	0.39	0.474	$<I_s$
	Synedra tabulata	15L/9D	11	F	1.2	7.1	0.71	1.9	0.51	1.19	$<I_s$
	"	9L/15D	11	F	0.8	7.1	0.71	1.9	0.51	1.19	$<I_s$
	Thalassiosira pseudonana	14L/10D	17	F&T	2.5	5.68	0.057	1.17	0.36	1.113	$<I_s$
	"	24L	17	F&T	2.5	4.01	0.089	0.67	0.45	0.581	$<I_s$
Coccolithophores	*Coccolithus huxleyi*	6L/18D	2	T	0.8	9.6	0.60	3.8	0.36	3.2	$<I_s$
Green algae	*Chlorella kessleri* (UTEX 398)	-	26	680 nm	3	13.7*	1.46*	4.9*	0.44	3.44*	13.9*
Average±						5.5±	0.6±	1.9±	0.43±	1.3±	5.5±
standard deviation						3.0	0.5	1.0	0.07	0.7	3.0

* Light intensity measured in W/m^2 in the current study.

$$V\frac{dX}{dt}=\mu \cdot V_i \cdot X - k_d \cdot V_d \cdot X \quad (8)$$

$$V\frac{dX}{dt}=K \cdot (\mu_m \cdot V_s+\bar{\mu}_{(I)}V_{c-s}) \cdot X - k_d \cdot V_d \cdot X \quad (9)$$

where $\bar{\mu}_{(I)}$ denotes the mean specific growth rate in the area of $I_c<I<I_s$ and is approximated by $\varepsilon\cdot\mu_m$, as described in Eq. (7), k_d is the specific death rate, and K, which is introduced below, is the proportional constant for the mixing effect. The light/dark fluctuations in tubular photobioreactors, flat-panel reactors, and bubble columns are within a range of 100 to 0.25 Hz. In these systems, faster mixing of high-density cultures, resulting in light/dark cycles of 0.2-1 s (5-1 Hz), increases the biomass yield based on the light energy at high photon flux densities [16, 18, 21].

From Eq. (2) and the approximation of the specific growth rate using the light path, Eq. (9) can be rewritten as below:

$$\frac{dX}{dt} \approx K \cdot \left(\mu_m \cdot \frac{x_s}{L}+\varepsilon \cdot \mu_m \cdot \frac{x_{c-s}}{L}\right) \cdot X+k_d \cdot \left(1-\frac{x_c}{L}\right) \cdot X \quad (10)$$

If the specific death rate (k_d) is negligible and Eq. (2) is used, dX/dt in Eq. (10) is constant. Thus, the growth rate of photosynthetic cells is linear.

$$\frac{dX}{dt}=\frac{K \cdot \mu_m}{\alpha \cdot L} \cdot \log(I_0 \cdot I_s^{(\varepsilon-1)} \cdot I_c^{-\varepsilon}) \quad (11)$$

where K and I_0 are the operating parameters, μ_m, α, ε, I_s, and I_c are the inherent properties of the strain, and L represents the geometry of a flat-plate photobioreactor.

If ε is 0.44 for the strain tested in the current study, the linear growth rate (LGR) can be expressed as:

$$LGR=K \cdot \frac{\mu_m}{\alpha \cdot L}\log(I_0 \cdot I_s^{-0.56} \cdot I_c^{-0.44}) \quad (12)$$

When the incident light intensity is lower than the saturation irradiance, the linear growth rate can also be expressed by combining Eqs. (6) and (8).

Equation (11) is based on the following assumptions: (i) no substrate limitation or inhibition except light; (ii) the light absorption obeys Beer-Lambert's law, in other words, the

package effect can be ignored; (iii) no photoinhibition or the incident light intensity is lower than the photoinhibition point; (iv) the μ-I curve follows a Monod-type model in this light intensity regime; (v) the specific light absorption coefficient (α) is constant; and (vi) no background absorption by the culture broth.

Consideration of Mixing Effect

In a high-density culture, the mutual shading is so intense that the width of the photic zone, where the cells receive sufficient photons, is very thin (shallow). Turbulence causes a continuous shift in the relative positions of the cells with respect to the photic zone. In a deliberately designed mixing system with an optimal time scale, each cell can absorb the light energy while it is exposed to the light and then uses up the absorbed photons while it stays in the dark layer. From a microscopic point of view, depending on the mixing characteristics, algae are thus exposed to certain light/dark cycles. Moreover, the degree of mixing has already been shown to significantly influence the productivity [18]. Thus, correction for the mixing effect is necessary.

From Eqs. (9) through (12), K is introduced to consider the mixing effect. The value of K can be a function of the duty cycle and frequency of light that the algae sense in the culture medium [41]. Yet mixing can also have other positive and negative effects on the cells (for example, shear stress) [11]. These mixing effects also influence the linear growth rate (batch productivity), and thus the overall photosynthetic efficiency. For this reason, the proportionality constant of the mixing effect, K, is introduced from Eqs. (9) through (12), which has quite a complex meaning related to the mixing pattern of the system despite being a constant.

Materials and Methods

Strain and Culture Condition

A green unicellular alga, *Chlorella kessleri* (UTEX 398), was obtained from The Culture Collection of Algae at UTEX (Austin, TX, U.S.A.). The culture medium was modified N-8 medium. The original N-8 medium [55] consisted of six macro-nutrients (in g/l): $Na_2HPO_4 \cdot 2H_2O$, 0.26; KH_2PO_4, 0.74; $CaCl_2$, 0.01; Fe·EDTA, 0.01; $MgSO_4 \cdot 7H_2O$, 0.05; KNO_3, 1.00. The micronutrients were supplied by adding 1 ml of a trace element stock solution [per liter, 3.58 g $Al_2(SO_4)_3 \cdot 18H_2O$, 12.98 g $MnCl_2 \cdot 4H_2O$, 1.83 g $CuSO_4 \cdot 5H_2O$, and 3.2 g $ZnSO_4 \cdot 7H_2O$] to 1 l of the macronutrient solution. The modified N-8 media, N-8A, contained two times the concentration of those in N-8, except for $MgSO_4 \cdot 7H_2O$ where the concentration (0.15 g/l) was three times higher than that in N-8. The other modified N-8 media, N-8B, included enriched KNO_3 (2.00 g/l) and $MgSO_4 \cdot 7H_2O$ (0.15 g/l) compared to N-8. The culture temperature was maintained at 26±0.5°C. A mixed gas with the composition of 5% CO_2 and balanced with N_2 was introduced through a gas analyzer and refreshed, whenever either the O_2 or the CO_2 concentration changed by more than 1% from the pre-mixed initial concentration. The gas recycle rate was maintained by a peristaltic pump (model 7522-00, Cole-Parmer Instrument Co., Niles, IL, U.S.A.) at 100 ml/min. The detailed operating conditions are summarized in Table 1.

Photobioreactor and Light Supply

The photobioreactor was constructed using a glass bottle with a 5.55 cm inside diameter and 17 cm height that was covered with black paper and aluminum foil, except for the bottom to supply light in a dark room. An agitation rate of 200 rpm was applied using a magnetic bar on a multi-stirrer. Light was supplied through the bottom of the reactor using light-emitting diodes. Red DDH GaAlAs LEDs were obtained from Quantum Devices Inc. (Barneveld, WI, U.S.A.). The LEDs had narrow spectral outputs and a central wavelength of approximately 680 nm. The red LEDs were powered by a DC power supply (model GP-233, LG Precision, Seoul, Korea) at a constant voltage depending on the light intensity.

Analytical Methods

The cell concentration (in cell/ml), total cell volume (in μm^3 cell/ml), and average cell volume (in μm^3/cell) were measured by a Coulter Counter (model Z2, Coulter Electronics, Inc., Hialeah, FL, U.S.A.) using Coulter AccuComp® Software [27]. To obtain the specific absorption coefficient, the culture broth was sampled at various growth phases, and it was measured by the method previously described [22] using a spectrophotometer (model HP8453E, Hewlett Packard, Waldbronn, Germany). The light intensity of the LED unit was measured using a quantum sensor (model LI-190SA, LI-COR, Lincoln, NE, U.S.A.) with a DataLogger (model LI-1400, LI-COR, Lincoln, NE, U.S.A.).

Results and Discussion

Determination of Model Parameters

The compensation, saturation, and inhibition irradiance of *Chlorella* were calculated by averaging the intensity required to grow or inhibit the cells at various cell concentrations in flasks, as previously reported [22]. The compensation irradiance was calculated to be 17 pW/cell (pW stands for picowatt), and the approximate saturation irradiance was about 160 pW/cell. Since the flask with the lowest inoculation density and the highest light intensity did not show any growth, the per cell light intensity for photoinhibition was calculated from this point and was about 7,200 pW/cell.

The average cell size under the red light from the LEDs was reported to be 30 μm^3/cell [23], therefore, the average projection area per cell was 12 μm^2/cell. Thus, the

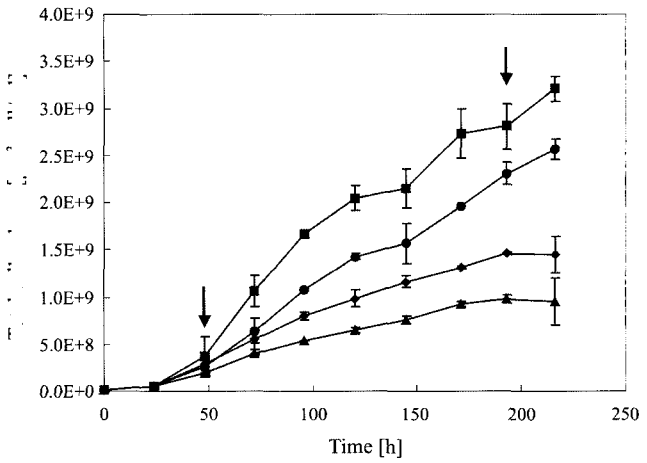

Fig. 3. Growth curves in flat-plate photobioreactors.
The experimental linear growth rate was calculated from point to point, as represented by the arrows. Curves represent HL (-◆-), HS (-■-), LL (-▲-), LS (-●-), respectively. Vertical lines indicate standard deviation.

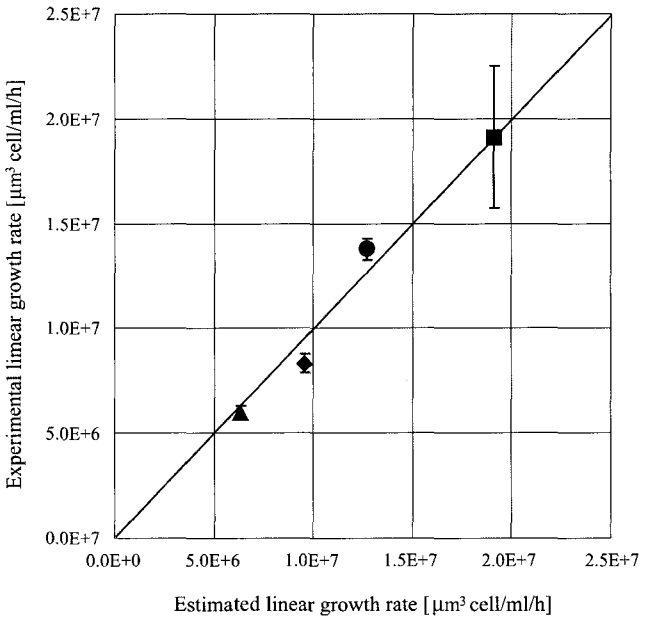

Fig. 4. Correlation of experimental and predicted linear growth rates.
R^2=0.99, HL (-◆-), HS (-■-), LL (-▲-), LS (-●-). Vertical lines indicate standard deviation.

compensation, saturation, and inhibition irradiance of *Chlorella* were rewritten as 1.46, 13.7, and 600 W/m^2, respectively. The cell specific absorption coefficient, α, was 3.0×10^{-9} m^2/μm^3 cell. The light intensities used in the cultures were higher than those of the saturation point (Table 1).

Comparison of Experimental Results with Model Prediction

To measure the linear growth rate under light-limiting conditions, the N-8 medium was modified to fortify other medium components: N-8A and N-8B. When *C. kessleri* was cultured in these fortified media, the growth rate and final cell concentration were almost identical (data not shown). Thus, N-8B was used in the following experiments.

It is known that microalgae mostly stay in a linear phase during a batch culture. Figure 3 shows the extended linear growth phase of *C. kessleri*. The linear growth rates calculated from the two points exhibited a good correlation with the calculated linear growth rates (period represented by two arrows in Fig. 3), as shown in Fig. 4 (the slope=1, R^2=0.99). The K value for the mixing effect in Eq. (12) was found to be 3.57.

Applications of the Monodimensional Model

Based on the above results, the linear growth rate [Eq. (12)] obtained in the current study could be used to estimate the photosynthetic productivity to achieve high-density algal cultures based on the strategies discussed below if the effect of the light distribution on productivity is negligible or small.

The K value in Eq. (12) represents the effect of mixing. Mixing can cause a dynamic light-dark pattern for a single alga as the algal cell moves through the different light intensity regimes. As the algal density increases, the light penetration depth dramatically decreases. Even though the light intensity is increased, the increase in the light penetration depth is minimal [22]. Thus, the higher the algal densities, the more the photosynthetic efficiency is enhanced by mixing. The effect of a flashing light increases with higher light gradients within the culture and with an increasing light intensity [31, 40, 41].

Judging from the studies of Märkl [31] and Persoone *et al.* [42], the range of variation for the K value can be predicted. They studied the effect of mixing on the photosynthetic productivity of algae under CO_2 supplemented conditions. Persoone *et al.* [42] obtained a 56% higher productivity in agitated *Chlorella saccharophila* cultures than without agitation after 18 days. Märkl [31] studied the effect of mixing on the photosynthetic CO_2 consumption rate with increased algal cell densities, and found that the productivity increased as the algal density increased. Doubling the stirring speed resulted in an increase of 20% in the CO_2 consumption rate of *Chlorella vulgaris* in a high density culture of 2.33 g/l, yet no or only a small increase in a low density of 0.7 g/l.

Based on the above discussions, the increase of the K value by mixing without increasing the shear stress on the cells would seem to be important to improve the productivity of high-density algal cultures operated under a high light intensity. Thus, the tolerance to high shear stress relative to the K value would appear to be an important factor affecting strains in high-density cultures, since more mixing is required as the cell density increases.

The common way to construct a strain with a faster growth rate (or μ_m) by genetic manipulation is mutagenesis, followed by many, preferably hundreds of, generations of competition in chemostat cultures or serial batch cultures [9]. Yet, if a high dilution rate is simply used, this selection method may be insufficient to identify a strain with a faster growth rate, because the specific absorption coefficient of the cell is also important for high-density algal cultures [see Eq. (11)]. To solve the mutual shading effect, an attempt to reduce the photosynthetic pigment content per cell [35] may also be unsuccessful, as the decrease in the growth rate of the cells and increase in the compensation irradiance can offset the deeper light penetration into the reactor due to the pigment reduction. Moreover, the challenge to raise the photosynthetic pigment content per cell to increase the specific growth rate may not be useful either, due to the increase in the specific absorption coefficient, resulting in a decrease of the photic zone.

The genetic engineering of the CO_2 fixation gene, RuBisCO (ribulosebisphosphate carboxylase/oxygenase, E.C. 4.1.39.) [52, 57], may be another alternative for reducing the photosynthetic pigment content, as it can help to increase the specific growth rate (μ_m) without changing the specific absorption coefficient (α).

Therefore, in order to achieve high-density algal cultures, the relation of the factors in Eq. (11) should be considered collectively from the above discussions.

Light Yield and Productivity

If the linear growth rate under the light limited growth conditions without a substrate limit can be used as a parameter for biomass productivity optimization in a batch culture, the light yield equation can be written as follows:

$$Y_{X/Q}=\frac{\beta \cdot P \cdot V}{\gamma \cdot I_0 \cdot A}=\frac{((t_c-t_l)/t_c)\cdot P \cdot L}{(t_l/t_c)\cdot I_0} \quad (13)$$

where $Y_{X/Q}$ denotes the biomass yield from the light energy, P is the parameter of productivity in μm^3 cell/l/h, which can be converted to g/l/h by a conversion factor, V, A, and L are the volume, illuminating surface, and light path of the reactor, respectively, and β and γ are the ratios of growth and illuminating time to culture time, respectively. Thus, Eq. (13) can be rewritten as below using Eq. (11):

$$Y_{X/Q}=\frac{(t_c-t_l)\cdot K \cdot \mu_m}{t_l \cdot \alpha}\left[\frac{\log(I_0 \cdot I_s^{\varepsilon-1} \cdot I_c^{-\varepsilon})}{I_0}\right] \quad (I_s<I_0<I_d) \quad (14)$$

Assuming $Y_{X/Q}$ is only a function of the incident light intensity, $Y_{X/Q}$ can be differentiated with respect to the incident light intensity:

$$Y'_{X/Q}=\frac{(t_c-t_l)\cdot K \cdot \mu_m}{t_l \cdot \alpha}\left[\frac{1/\ln 10-\log(I_0 \cdot I_s^{\varepsilon-1} \cdot I_c^{-\varepsilon})}{I_0^2}\right] \quad (15)$$

Therefore, at $I_0=10^{1/\ln 10}\cdot(I_s^{1-\varepsilon}\cdot I_c^{\varepsilon})$ or $2.72\cdot(I_s^{1-\varepsilon}\cdot I_c^{\varepsilon})$, the yield ($Y_{X/Q}$) will have a maximum value. Thus, this light intensity

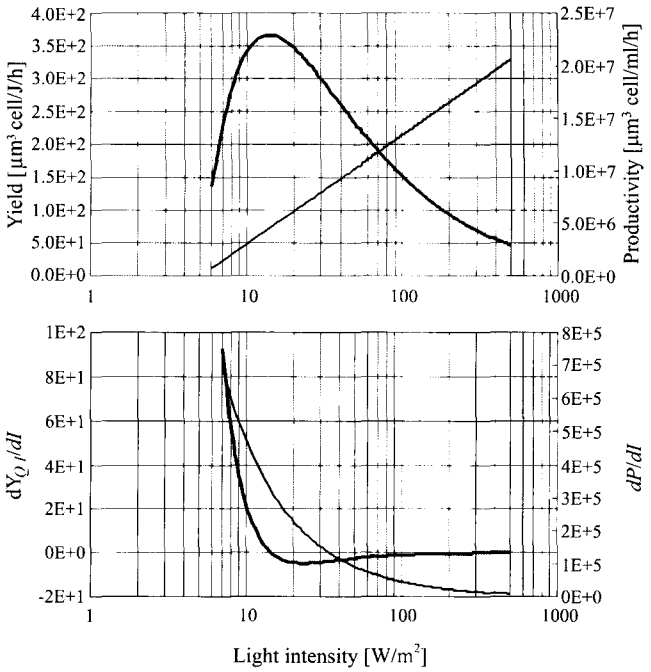

Fig. 5. (a) Simulation of productivity, (P, —) and light yield, ($Y_{Q/I}$, —).
About 12 W/m² was the optimal light intensity. (b) Simulation of differentiated productivity (P, —) and light yield ($Y_{Q/I}$, —) [Eq. (15)] by incident light intensity. The lower the light intensity, the better the difference between the productivity and the yield of photosynthesis. t_c=200 h, t_l=24 h, t_l=200 h, μ_m=0.087 h^{-1}, K_i=3.44 W/m², and the other values are shown in Table 1.

can be the optimum intensity for the light yield ($I_{0\,opt}$). Furthermore, if $I_c/I_s>10^{-1/(\varepsilon\cdot\ln 10)}$, $I_{0\,opt}$ is higher than the saturation irradiance (I_s); if not, $I_{0\,opt}$ is lower than I_s. Yet most algae are cultured under a light intensity above the saturation irradiance for high-density cultures.

Figure 5 shows the light yield and productivity as a function of the light intensity (Fig. 5a), and the differentiated productivity and light yield due to the incident light intensity (Fig. 5b). In this figure, $I_{0\,opt}$ is about 13.9 W/m², which is nearly the same as the saturation irradiance (13.7 W/m², see Table 1) for the strain used in the current study. $I_{0\,opt}$ calculated using the proposed model is shown in Table 2 and illustrated in Fig. 6, based on the correlation of $I_{0\,opt}$ to the half-saturation irradiance (K_i+I_c). An approximated result for the optimum light intensity can be obtained for most algae using the proposed modeling concept, as the incident light intensity is higher than the saturation point of the cell.

Microalgae have a typical saturation light intensity within a range of about 14-42 W/m² [47], and a compensation point within a range of about 0.2-4.0 W/m², based on an assumption of a compensation irradiance of 0.1 to 1% light reaching the water surface in the field [50, 51, 54] and maximum solar radiation of 395 W/m², the daily average in a desert area, and 155 W/m², the annual average in Japan [17].

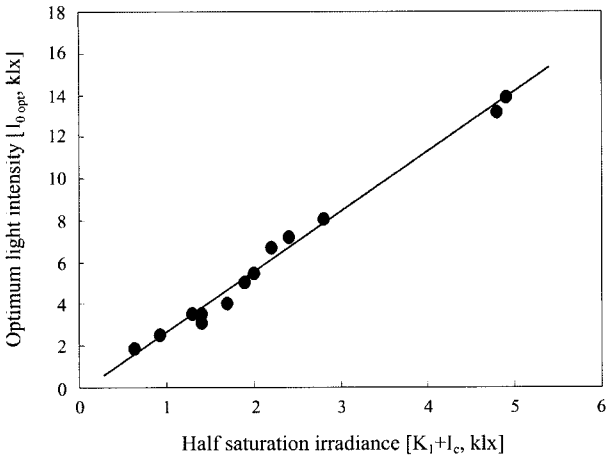

Fig. 6. Optimal light intensity for maximum light yield ($I_{0\,opt}$) and half saturation light intensity (K_I+I_c).
A linear correlation was exhibited when the light intensity was higher than the saturation irradiance. The data points were calculated by Eqs. (14) and (15), and shown in Table 2.

From Eqs. (14) and (15), the optimal light intensities for microalgae estimated to produce the maximal photosynthetic efficiency were lower than about 49 W/m^2, and within a range of 20–49 W/m^2 when the microalgae were cultured under a light intensity above the saturation irradiance. Thus, the idea to improve the photosynthetic efficiency through a combination of diffusing the incident light and diurnal cycle [34] would seem to be a good idea, as the incident light intensity was usually higher than the optimum value of the light intensity for the maximum light yield.

Ogbonna *et al.* [36, 38] stressed the linearity of the light supply coefficient versus the linear growth rate of batch cultures in various photobioreactor types. However, when their coefficient was applied in the current study, good correlation was not observed. Furthermore, their coefficient was different in dimensions from the currently proposed linear growth model. When a graph was drawn of the proposed model versus the light supply coefficient, a linear correlation was exhibited only at a low light supply coefficient. Actually, the data points [38] showed a linear correlation for a light supply coefficient only below 1. Cornet *et al.* [7] also referred to the linear growth of a batch culture and outlined briefly a model for such linear growth. Their model, which included a linear correlation with the incident light intensity and linear growth rate, was different in dimensions from the linear growth model proposed in the current study as well as in the model proposed by Ogbonna *et al.* Thus, further study is required to identify the determinant factors for the linear growth rate of a batch culture, especially with regard to the effect of mixing, pigment content per cell, and light intensity.

CONCLUSION

The current study derived a simple model to estimate the linear growth rate of photosynthetic microorganisms, based on a monodimensional approach. A feasibility test of the proposed model was conducted in flat-plate photobioreactors using LEDs as the light sources. The experimental results indicated that a model microorganism, *Chlorella kessleri*, exhibited clear linear growth when light was the sole limiting factor, and the linear growth rate could be successfully predicted using the simple model currently proposed. Accordingly, for high-density and large-scale cultures, when a simple estimate of the complex changes in FPPBRs is needed, the proposed model would appear to be useful for understanding the system of FPPBRs.

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NOMENCLATURE

α : specific absorption coefficient (cm^2/μm^3 cell)
ε : constant for mean of specific growth rate between x_s and x_c (-)
$\mu_{(I)}$: specific growth rate at given light intensity of I (h^{-1})
$\bar{\mu}_{(I)}$: mean of specific growth rate between x_s and x_c (h^{-1})
μ_m : maximal specific growth rate (h^{-1})
A : irradiating surface area (cm^2)
I : light intensity at depth x (W/m^2)
I_0 : incident light intensity (W/m^2)
$I_{0\,opt}$: optimal light intensity for maximum light yield (W/m^2)
I_c : compensation light intensity (W/m^2)
I_d : photoinhibition light intensity (W/m^2)
I_s : saturation light intensity (W/m^2)
K : proportionality constant for mixing effect (-)
K_I : light intensity constant for half of maximal specific growth rate (-)
k_d : specific death rate (h^{-1})
L : light path of reactor (cm)
P : photosynthetic productivity (μm^3 cell/L/h)
t : time (h)
t_c : culture time (h)
t_i : irradiation time (h)
t_l : lag time (h)
V : culture volume of reactor (ml)
V_{c-s} : culture volume when light intensity is between I_c and I_s (ml)
V_d : dark volume (below compensation point) (ml)
V_i : volume of photic zone (culture volume when light

intensity is over I_c) (ml)
V_s : culture volume when light intensity is over I_s (ml)
X : cell concentration (μm^3 cell/ml)
x : distance from irradiating surface (cm)
x_c : length of photic zone (cm)
x_{c-s} : distance between compensation point and saturation point (cm)
x_s : distance to saturation point from irradiating surface (cm)
$Y_{X/Q}$: photosynthetic yield relative to light energy (μm^3 cell/J)

List of Abbreviations

HL : high light intensity and long light path
HS : high light intensity and short light path
LL : low light intensity and long light path
LS : low light intensity and short light path
L/D : light/dark cycle
T : tungsten lamp
F : fluorescent lamp

REFERENCES

1. Acien Fernandez, F. G., F. Garcia Camacho, J. A. Sanchez Perez, J. M. Fernandez Sevilla, and E. Molina Grima. 1997. A model for light distribution and average solar irradiance inside outdoor tubular photobioreactors for the microalgal mass culture. *Biotechnol. Bioeng.* **55:** 701-714.
2. Becker, E. W. 1994. *Microalgae: Biotechnology and Microbiology.* Cambridge University Press, NY, U.S.A.
3. Borowitzka, M. A. 1999. Commercial production of microalgae: Ponds, tanks, tubes and fermenters. *J. Biotechnol.* **70:** 313-321.
4. Brown, M. R., S. W. Jeffrey, J. K. Volkman, and G. A. Dunstan. 1997. Nutritional properties of microalgae for mariculture. *Aquaculture* **151:** 315-331.
5. Cheng-Wu, Z., O. Zmora, R. Kopel, and A. Richmond. 2001. An industrial-size flat plate glass reactor for mass production of *Nannochloropsis* sp. (Eustigmatophyceae). *Aquaculture* **195:** 35-49.
6. Cornet, J.-F., C. G. Dussap, P. Cluzel, and G. Dubertret. 1992. A structured model for simulation of cultures of the cyanobacterium *Spirulina platensis* in photobioreactors: II. Identification of kinetic parameters under light and mineral limitations. *Biotechnol. Bioeng.* **40:** 826-834.
7. Cornet, J.-F., C. G. Dussap, and G. Dubertret. 1992. A structured model for simulation of cultures of the cyanobacterium *Spirulina platensis* in photobioreactors: I. Coupling between light transfer and growth kinetics. *Biotechnol. Bioeng.* **40:** 817-825.
8. Cornet, J.-F., C. G. Dussap, and J.-B. Gros. 1995. A simplified monodimensional approach for modeling coupling between radiant light transfer and growth kinetics in photobioreactors. *Chem. Eng. Sci.* **50:** 1489-1500.
9. Craig, R., B. Y. Reichelt, and J. L. Reichelt. 1989. Genetic engineering of micro-algae, pp. 415-455. *In* M. A. Borowitzka and L. J. Borowitzka (eds.) *Micro-algal Biotechnology.* Cambridge University Press, New York, U.S.A.
10. Duysens, L. N. M. 1956. The flattening of the absorption spectrum of suspensions, as compared to that of solutions. *Biochem. Biophys. Acta* **19:** 1-12.
11. Garcia Camacho, F., A. Contreras Gomez, T. Mazzuca Sobczuk, and E. Molina Grima. 2000. Effects of mechanical and hydrodynamic stress in agitated, sparged cultures of *Porphyridium cruentum. Process Biochem.* **35:** 1045-1050.
12. Geider, R. J. and B. A. Osborne. 1992. Chapter 6: Light utilization and optical properties of algae, pp. 122-155. In: *Algal Photosynthesis.* Chapman & Hall, Inc., New York, U.S.A.
13. Geider, R. J. and B. A. Osborne. 1992. Chapter 7: The photosynthesis-light response curve, pp. 156-191. In: *Algal Photosynthesis.* Chapman & Hall, Inc., New York, U.S.A.
14. Hader, D.-P. and M. Tevini. 1987. *General Photobiology,* 1st ed. Pergamon Press, New York, NY, U.S.A.
15. Hu, Q., H. Guterman, and A. Richmond. 1996. A flat inclined modular photobioreactor for outdoor mass cultivation of photoautotrophs. *Biotechnol. Bioeng.* **51:** 51-60.
16. Hu, Q., N. Kurano, M. Kawachi, and I. Iwasaki. 1998. Ultrahigh-cell-density culture of a marine green alga *Chlorococcum littorale* in a flat-plate photobioreactor. *Appl. Microbiol. Biotechnol.* **49:** 566-662.
17. IEAGHG. 1995. Carbon dioxide utilization. IEA Greenhouse Gas R&D Programme.
18. Janssen, M., M. Janssen, M. D. Winter, J. Tramper, L. R. Mur, J. Snel, and R. H. Wijffels. 2000. Efficiency of light utilization of *Chlamydomonas reinhardtii* under medium-duration light:dark cycles. *J. Biotechnol.* **78:** 123-137.
19. Jeon, C., J. Y. Park, and Y. J. Yoo. 2001. Biosorption model for binary adsorption sites. *J. Microbiol. Biotechnol.* **11:** 781-787.
20. Kim, M. K. and R. E. H. Smith. 2001. Effect of ionic copper toxicity on the growth of green alga, *Selenastrum capricornutum. J. Microbiol. Biotechnol.* **11:** 211-216.
21. Kim, N.-J. and C.-G. Lee. 2001. A theoretical consideration on oxygen production rate in microalgal cultures. *Biotechnol. Bioprocess Eng.* **6:** 352-358.
22. Lee, C.-G. 1999. Calculation of light penetration depth in photobioreactors. *Biotechnol. Bioprocess Eng.* **4:** 78-81.
23. Lee, C.-G. and B. O. Palsson. 1996. Photoacclimation of *Chlorella vulgaris* to red light from light-emitting diodes leads to autospore release following each cellular division. *Biotechnol. Prog.* **12:** 249-256.
24. Lee, H. Y., L. E. Erickson, and S. S. Yang. 1987. Kinetics and bioenergetics of light-limited photoautotrophic growth of *Spirulina platensis. Biotechnol. Bioeng.* **24:** 832-843.
25. Lee, J. H., J. S. Lee, C. S. Shin, S. C. Park, and S. W. Kim. 2000. Effects of NO and SO_2 on growth of highly-CO_2-tolerant microalgae. *J. Microbiol. Biotechnol.* **10:** 338-343.
26. Lee, J. S., D. K. Kim, J. P. Lee, S. C. Park, J. H. Koh, and S. J. Ohh. 2001. CO_2 fixation by *Chlorella* KR-1 using flue gas and its utilization as a feedstuff for chicks. *J. Microbiol. Biotechnol.* **11:** 772-775.
27. Lee, K. Y. and C.-G. Lee. 2001. Effect of light/dark cycles on wastewater treatments by microalgae. *Biotechnol. Bioprocess Eng.* **6:** 194-199.

3. Lehana, M. 1990. Kinetic analysis of the growth of *Chlorella vulgaris*. *Biotechnol. Bioeng.* **36:** 198-206.
9. Liere, L. V. and A. E. Walsby. 1982. Interactions of cyanobacteria with light, pp. 9-46. *In* N. G. Carr and B. A. Whitton (eds.) *The Biology of Cyanobacteria*. Blackwell Scientific Publications, Oxford, U.K.
0. Mallick, N., F. H. Mohn, L. Rai, and C. J. Soeder. 2000. Impact of physiological stresses on nitric oxide formation by green alga, *Scenedesmus obliquus*. *J. Microbiol. Biotechnol.* **10:** 300-306.
1. Märkl, H. 1980. Modeling of algal production systems, pp. 361-383. *In* G. Shelef and C. J. Soeder (eds.) *Algae Biomass; Production and Use*. Elsevier/North-Holland Biomedical Press, Amsterdam, Holland, The Netherlands.
2. Molina Grima, E., F. G. Acien Fernandez, F. Garcia Camacho, and Y. Chisti. 1999. Photobioreactors: Light regime, mass transfer, and scale up. *J. Biotechnol.* **70:** 231-247.
3. Molina Grima, E., F. Garcia Camacho, J. A. Sanchez Perez, J. M. Fernandez Sevilla, F. G. Acien Fernandez, and A. Contreras Gomez. 1994. A mathematical model of microalgal growth in light-limited chemostat culture. *J. Chem. Tech. Biotechnol.* **61:** 167-173.
4. Morita, M., Y. Watanabe, and H. Saiki. 2000. Investigation of photobioreactor design for enhancing the photosynthetic productivity of microalgae. *Biotechnol. Bioeng.* **69:** 693-698.
5. Nakajima, Y., M. Tsuzuki, and R. Ueda. 1999. Reduced photoinhibition of a phycocyanin-deficient mutant of *Synechocysitis* PCC 6714. *J. Appl. Phycol.* **10:** 445-452.
6. Ogbonna, J. C. and H. Tanaka. 2000. Light requirement and photosynthetic cell cultivation - Development of processes for efficient light utilization in photobioreactors. *J. Appl. Phycol.* **12:** 207-218.
7. Ogbonna, J. C., H. Yada, and H. Tanaka. 1995. Kinetic study on light-limited batch cultivation of photosynthetic cells. *J. Ferment. Bioeng.* **80:** 259-264.
8. Ogbonna, J. C., H. Yada, and H. Tanaka. 1995. Light supply coefficient: A new engineering parameter for photobioreactor design. *J. Ferment. Bioeng.* **80:** 369-376.
9. Park, E.-K. and C.-G. Lee. 2001. Astaxanthin production by *Haematococcus pluvialis* under various light intensities and wavelengths. *J. Microbiol. Biotechnol.* **11:** 1024-1030.
0. Park, K.-H., D.-I. Kim, and C.-G. Lee. 2000. Effect of flashing light on oxygen production rates in high-density algal cultures. *J. Microbiol. Biotechnol.* **10:** 817-822.
1. Park, K.-H. and C.-G. Lee. 2000. Optimization and algal photobioreactors using flashing lights. *Biotechnol. Bioprocess Eng.* **5:** 186-190.
2. Persoone, G., J. Morales, H. Verlet, and N. Depauw. 1980. Air-lift pumps and the effect of mixing on algal growth, pp. 505-522. *In* G. Shelef and C. J. Soeder (eds.) *Algae Biomass: Production and Use*. Elsevier/North-Holland Biomedical Press, Amsterdam, Holland, The Netherlands.
43. Philips, E. J. and A. Mitsui. 1982. Light intensity preference and tolerance of aquatic photosynthetic microorganisms, pp. 257-307. *In* O. R. Zaborsky, A. Mitsui, and C. C. Black (eds.) *CRC Handbook of Biosolar Resources*. CRC Press, Inc., Boca Raton, FL, U.S.A.
44. Prokop, A. and L. E. Erickson. 1995. Photobioreactors, pp. 441-477. *In* J. A. Asenjo and J. C. Merchuk (eds.) *Bioreactor System Design*. Marcel Dekker, Inc, New York, NY, U.S.A.
45. Rai, L. C., H. D. Kumar, F. H. Mohn, and C. J. Soeder. 2000. Services of algae to the environment. *J. Microbiol. Biotechnol.* **10:** 119-136.
46. Renaud, S. M., L.-V. Thinh, and D. L. Parry. 1999. The gross chemical composition and fatty acid composition of 18 species of tropical Australian microalgae for possible use in mariculture. *Aquaculture* **170:** 147-159.
47. Richmond, A. 1986. Outdoor mass cultures of microalgae, pp. 285-330. *In* A. Richmond (ed.) *Handbook of Microalgal Mass Culture*. CRC Press, Boca Raton, FL, U.S.A.
48. Ryland, J. R., P. M. Linzmaier, and J. M. Lee. 2000. Effect of gelatin on the stability of heavy chain monoclonal antibody production from plant suspension cultures. *J. Microbiol. Biotechnol.* **10:** 449-454.
49. Sasson, A. 1997. Foreword, pp. xi-xiii. *In* A. Vonshak (ed.) *Spirulina platensis* (*Arthrospira*): *Physiology, Cell-Biology and Biotechnology*. Taylor & Francis, London, U.K.
50. Sze, P. 1998. *A Biology of the Algae*, 3rd ed. WCB/McGraw-Hill, Boston, MA, U.S.A.
51. Szeligiewicz, W. 1999. Vertical mixing influence on the compensation depth. *J. Mar. Sys.* **21:** 169-177.
52. Tabita, F. R. 1999. Microbial ribulose 1,5-bisphosphate carboxylase/oxygenase: A different perspective. *Photosynth. Res.* **60:** 1-28.
53. Tredici, M. T. and G. C. Zittelli. 1998. Efficiency of sunlight utilization: Tubular versus flat photobioreactors. *Biotechnol. Bioeng.* **57:** 187-197.
54. UNEP. 1998. *Environmental Effects of Ozone Depletion: 1998, Assessment*. United Nations Environment Programme.
55. Vonshak, A. 1986. Laboratory techniques for the cultivation of microalgae, pp. 117-145. *In* A. Richmond (ed.) *Handbook of Microalgal Mass Culture*. CRC Press, Boca Raton, FL, U.S.A.
56. Vonshak, A. 1997. *Spirulina*: growth, physiology and biochemistry, pp. 43-65. *In* A. Vonshak (ed.) *Spirulina platensis* (*Arthrospira*): *Physiology, Cell-Biology and Biotechnology*. Taylor & Francis, London, U.K.
57. Watson, G. M. F. and F. R. Tabita. 1997. Microbial ribulose 1,5-bisphosphate carboxylase/oxygenase: A molecule for phylogenetic and enzymological investigation. *FEMS Microbiol. Lett.* **146:** 13-22.
58. Zou, N. and A. Richmond. 1999. Effect of light-path length in outdoor flat plate reactors on output rate of cell mass and of EPA in *Nannochloropsis* sp. *J. Biotechnol.* **70:** 351-356.