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A Theoretical Consideration on Oxygen Production Rate in Microalgal Cultures

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Abstract Because algal cells are so efficient at absorbing incoming light energy, providing more light energy to photobioreactors would simply decrease energy conversion efficiency. Furthermore, the algal biomass productivity in photobioreactor is always proportional to the total photosynthetic rate. In order to optimize the productivity of algal photobioreactors (PBRs), the oxygen production rate should be estimated. Based on a simple model of light penetration depth and algal photosynthesis, the oxygen production rate in high-density microalgal cultures could be calculated. The estimated values and profiles of oxygen production rate by this model were found to be in accordance with the experimental data. Optimal parameters for PBR operations were also calculated using the model.

Keywords: light penetration depth, limiting factor, oxygen production rate, photobioreactor (PBR)

INTRODUCTION

The effect of light is probably the most important design consideration for successful photobioreactors. Two properties of light energy are important for algal growth and metabolism: spectral quality and intensity (irradiance or illumination). Spectral quality is defined by the absorption spectrum of the chlorophylls and other photosynthetically active pigments. A detailed review on the spectral efficiency of photosynthesis can be found elsewhere [1]. The effect of light intensity has been studied in greatest detail in connection with photosynthesis, and is usually measured as O_2 evolved or as CO_2 consumed [2]. However, algal growth is the ultimate measure of the algal photosynthetic unit, either from the point of view of gas exchange or food production. Furthermore, growth is the most valid measure of gas exchange in the long term. A series of experiments were performed to grow algae in cultures at various densities using different light intensities [3]. The total amount of organic material that can be produced from light energy by growing plant cells increases with the intensity of light up to a certain point (saturation intensity), but beyond that point the amount produced per unit of light energy decreases rapidly when the intensity of light increases. Light of high intensity apparently stimulates a process of photooxidation, which partially offsets some of photosynthesis [4]. As a result, the growth rate shows the expected drop at higher optical densities and increasing the light intensity does not increase the growth rate. Further, higher light intensity beyond a certain threshold normally damages the photosystems and thus the cells. The term 'photoinhibition' is frequently used to describe the damage caused by excess light [5]. Photoinhibition is a function of temperature and shows a coupled response with photorespiratory carbon metabolism. Due to the dramatic decrease in light intensity penetrating the *Chlorella* cultures [6], the light penetration depth will be shorter than 1.0 cm at a cell concentration of as low as 1 g/L. In fact, more than 90% of 680 and 440 nm light will be absorbed by the cells within 1.0 cm of culture near the illumination surface. This mutual shading will decrease the portion of the cells exposed to the light. Thus, only the cells close to the illuminated surface are exposed to meaningful light levels at high cell densities regardless of the supplied light intensity [6]. As a result, the apparent oxygen production rate (OPR) will decrease after the cell concentration reaches a certain density, as observed earlier [7]. The models for estimating OPRs are reported here with calculated values.

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MATERIALS AND METHODS

Cell Line and Culture Conditions

Chlorella kessleri (UTEX 398) was obtained from the Culture Collection of Algae at UTEX (Austin, TX, USA) on proteose agar. The N-8 culture medium was used without modification [8]. The PBR described earlier [7] was used for this study. The LED units had 90 LEDs each on a 2" × 4" printed circuit board. All experiments were performed at a constant temperature of 25°C and an initial pH of 6.2. A peristaltic pump was used to circulate the culture medium and to keep the cells in suspension. Pre-mixed gas with the composition of 10% CO_2 and balanced with N_2 was introduced through the

gas analyzer and refreshed whenever either the O_2 or CO_2 concentration had changed more than 1% from the pre-mixed initial concentration.

Light Source and Supply

Red DDH GaAlAs LEDs, obtained from Quantum Devices Inc. (Barneveld, WI, USA), were powered by DC power supplies (GP-105, LG Precision, Seoul, Korea). For flashing light effect, a switching power supply (AVR-3-PW-C-P-UMB Pulser, Avtech Electrosystems Ltd., Ogdensburg, NY, USA) was used. The spectral outputs of the LEDs were peaked at 680 nm. Light intensities of the LED units were measured using a silicon photo cell (Model 0560.0500, Testoterm GmbH & Co., Germany).

Data Analysis

The cell concentration was determined using a Coulter Counter model Z2 (Coulter Electronics, Inc., Miami, FL, USA) and occasionally confirming with a hemacytometer. Since the average cell size was highly variable (about 100 μm^3/cell while the cells were actively growing and about 30 μm^3/cell during the stationary phase), the total cell volume (= cell concentration (cells/mL) × average cell volume (μm^3/cell)) was used to calculate the volumetric specific growth rate and thus the doubling time. The gas concentrations were measured using a Micro-Oxymax gas analyzer (Columbus Instruments, Columbus, OH, USA) as described previously [7].

LIGHT AS A DESIGNING FACTOR OF PBRS

Photosynthetic Yield

The net photosynthetic reaction can be thermodynamically expressed by the following equation [9].

$$CO_2 + H_2O + 4.7 \times 10^5\ J \rightarrow 1/6\ C_6H_{12}O_6 + O_2 \quad (1)$$

However, the stoichiometric mass balance of photosynthetic growth can be very different from the above equation [10-12]. The energy captured by the photosynthetic process is dependent on various factors, such as the nitrogen source used and the chemical composition of the biomass, which both affect photosynthetic efficiency.

A mole of photons (= 1 Einstein or 1 E) at a wavelength of λ (expressed in nm) has energy of

$$\begin{aligned} 1\ E \text{ at } \lambda &= N h \nu \\ &= 6.022\times10^{23}\ mol^{-1} \times 6.626\times10^{-34}\ J/sec \times \frac{2.998\times10^{8}\ m/sec}{\lambda\times10^{-9}\ m} \\ &= \frac{1.2\times10^{8}\ J}{\lambda} \end{aligned} \quad (2)$$

where N is Avogadro's number, h is Planck's constant and ν is the frequency of vibration. Photosynthetically active radiation (PAR, λ = 400-700 nm) is commonly used for calculating the theoretical photosynthetic efficiency.

According to the *Z*-scheme [13,14], eight photons (four in each photosystem) are theoretically required to transfer all four available electrons in two molecules of water [15]. Thus, it is the number of photons that matters in photosynthesis and photosynthetic efficiency is a function of the wavelength of the photons. As a result, red light, whose wavelength is longer (and thus the energy per photon is lower) than blue light, will have a higher photosynthetic efficiency. The theoretical photosynthetic yield for red light (680 nm) and for blue light (480 nm) is 33.3% and 23.5%, respectively, according to equation (3).

$$Y_{max} = \frac{4.7\times10^{5}\ J}{\frac{1.2\times10^{8}\ J}{\lambda}} \quad (3)$$

In reality, however, 4 to 16 quanta are needed to generate one molecule of O_2 in *Chlorella* cells [12,16,17]. The minimum quantum demand per molecule of O_2 evolution becomes larger as the CO_2 partial pressure decreases and is likely to be 5-6 at the optimal condition [12]. This wide range of quantum number resulted in the wide range of photosynthetic efficiency of *Chlorella*. The reported values of photosynthetic yield range from 20% to 54% [10,11,17,18].

Estimation of Oxygen Production Rate

In a previous experiment [7], each *C. kessleri* cell was found to be able to produce up to 200 fmol O_2 $cell^{-1}$ hr^{-1} at saturation light intensity. For 680 nm, one mole of photons per hour is equivalent to about 50 W by equation (2). As a result, a PBR operated at a cell concentration of 10^9 cells/mL has a potential of producing 200 mmol O_2 L^{-1} hr^{-1} if sufficient photons are provided without mutual shading effect. The theoretically required energy of photons for 10^9 cells (in 1 mL) to undergo photosynthesis at their maximum rate would be 80 mW/mL, if all the photons are provided at 680 nm (see equation (4)).

$$\begin{aligned} &\text{Photon Requirement} \\ &= 200\ fmol\ O_2\ cell^{-1}\ hr^{-1} \times 10^9\ cells/mL \\ &= 200\ \mu mol\ O_2\ mL^{-1}\ hr^{-1} \\ &= 1.6\ mmol\ 680\ nm\ photon\ mL^{-1}\ hr^{-1} \\ &= 80\ mW/mL \end{aligned} \quad (4)$$

Package Effect

A consequence of the localization of light-absorbing pigments within cells or organelles is to reduce their absorption per unit pigment in comparison to the same quantity of pigment dispersed in solution. This effect is referred to as the 'package effect' (also known as the sieve effect) and results in a reduced absorption at the absorption maxima of the pigments, while there is little reduction in absorption at those wavelengths that are

weakly absorbed by the pigments [19]. For unicellular algae, the magnitude of the package effect depends on cell size and intracellular pigment content [20]. Large cells and cells with a high pigment content have greater package effect than small cells or those with a lower pigment content.

Light Attenuation in Photosynthetic Cultures

Since the antenna pigments of the reaction centers of algal cells are very efficient in terms of light absorption, and light intensity dramatically decreases as the light penetrates into the algal culture. Optical measurements of light intensity distribution within the photosynthetic culture are extremely difficult due to the complicated nature of scattering, such as reflection, refraction, and diffraction, which occur in addition to cellular absorption of photons. Beer-Lambert's law is usually used to estimate light intensity within a culture medium;

$$\log\frac{I_0}{I_x}=\alpha X x \tag{5}$$

where, I_0 and I_x are the light intensities at the surface and at penetration depth of x (distance from the illuminating surface), respectively, and X is the particle (or cell) concentration. α is the specific absorption coefficient.

In fact, PBRs have been conventionally designed based on this law to estimate the attenuation of light energy along the depth of liquid column [21]. However, Beer-Lambert's law assesses light intensity distribution in a photosystem without considering the light scattering by the cells nor the package effect. Thus, it only holds for pigment solutions and is not strictly applicable to cell suspensions that scatter light, where most pigments reside inside the cells. Some sophisticated models on the angular dependence of the scattered light for homogeneous spheres [22] and a simpler anomalous diffraction [23] can be found in the literature. These models don't account for light absorption by the particles. Numerous attempts have been made to formulate a numerical model for predicting light intensities within the culture by considering both scattering and absorption [20,24-27].

Scattering by unicellular algae tends to conform to these predictions [20], although there is some evidence for a disproportionably high percentages of wide-angle scattering in some algal cells [28].

Flashing Light Effect

When much more light is absorbed by the cells than can be handled by the photosynthetic reaction, only a fraction of the light will actually be used. As a result, the portion of light energy fixed to organic material will decrease rapidly as the intensity of light increases. However, if this same high intensity is given in short flashes, with each flash short enough to prevent the overexposure of available enzyme molecules in the photosystems, and a dark period is interposed of sufficient duration to allow these molecules to discharge completely, no light will be wasted.

Photosynthesis is hypothesized to be as a discontinuous, linear, four-step process [29]. As a result, it cannot use the photons that are captured between processes. The existence of a longer dark period between the short flashes of the light can increase photosynthetic efficiency, especially for high intensity illumination [30-34]. Thus, by using a flashing light, algae can assimilate the same amount of light as they can by using the same average intensity of steady light. A flashing light has advantages over a steady light of same net intensity, since the total amount of photosynthesis will be the same in either case [35]. The efficiency with which light is utilized by the algae tends to be greater in intermittent light than it is in steady light of intensity equal to that of the light flashes [36]. However, the rate of photosynthesis in flashing light cannot be greater than the rate in continuous illumination of equal average intensity nor than the rate in steady light of the same intensity.

RESULTS AND DISCUSSION

Calculation of Light Penetration Depth

Penetration depth is defined as the distance from the illumination surface at which the light intensity reaches the compensation intensity per cell. Beer-Lambert's law can be used to determine light penetration depth in *C. kessleri* culture [6]. In addition, more complex models would make the estimation of OPR impractical.

Under the assumptions of perfect light distribution and a uniform light attenuation within the culture, the depth of the photic zone can be calculated as a function of incident light intensity (I_0) and the cell concentration (X) using equation (5) [6]. The penetration depth calculated based on this value will give the portion of culture which can be exposed to enough light to undergo photosynthesis at its maximum rate.

According to the calculation, no matter what the maximum intensity of light source used is or what the geometry of the photobioreactor is, supplying enough photons to each cell in a 1 cm thick layer of culture containing 2×10^9 cells/mL is virtually impossible. However, the actual fraction of the culture that can be exposed to sufficient light will be significantly increased by mixing.

Estimation of Volumetric Oxygen Production Rate (OPR)

The culture chamber of the PBR reported upon earlier was of the rectangular plate type and of dimension 2″ (L) × 4″ (H) with variable thickness (W) [7]. The PBR has two closely packed LED units on each side, spaced either 1 or 1.55 cm apart. The detailed specification for the two types of PBRs is listed in Table 1. Each LED unit could give a light intensity as high as 25 mW/cm^2

Table 1. Detailed dimensions and numbers for the PBRs used.

	PBR-S	PBR-L
Total culture volume ($V_l + V_d$)	70 cm^3	100 cm^3
Illumination chamber dimension	2 in × 4 in × 1 cm	2 in × 4 in × 1.55 cm
Thickness of chamber (L)	1.00 cm	1.55 cm
Chamber volume (V_l)	52 cm^3	80 cm^3
Illumination area per mL	1 cm^2	0.65 cm^2

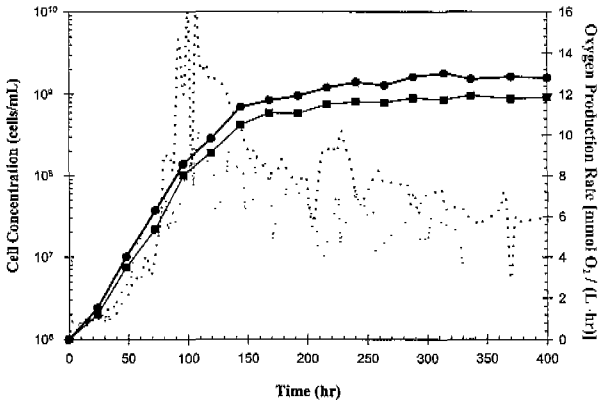

Fig. 1. Typical growth curves (solid lines) and corresponding OPRs (dotted lines) in two different photobioreactors: PBR-S (thick lines) and PBR-L (thin lines).

on each illuminating surface of the PBR. However, the PBRs were normally operated at 12 mW/cm^2 since operating at the maximum intensity could damage the LEDs in long-term culture. At this LED output level the expected OPR would be 30 mmol O_2 L^{-1} hr^{-1} (= 12 mW/cm^2 × (1 E hr^{-1}/50 W) × (1 mol O_2/8 E) × 1 cm^2/mL) for PBR-S (PBR operated at a thickness of 1 cm) or 20 mmol O_2 L^{-1} hr^{-1} for PBR-L (PBR operated at a thickness of 1.55 cm). However, a typical OPR peaked at *ca.* 10-15 mmol O_2 L^{-1} hr^{-1} and then decreased (dotted lines in Fig. 1). The reason for the fluctuations in OPR measurements can be found elsewhere [7]. Fig. 1 also shows typical growth curves (solid lines in Fig. 1) in the two different illumination chambers: PBR-S (thick lines) and PBR-L (thin lines).

Using the simple model based on equation (5) as reported previously [6], the penetration depths to either compensation point or to saturation point can be calculated as shown in Fig. 2. The saturation penetration (x_s; dotted lines in Fig. 2) represents the distance from the light source within which all cells are photosaturated (I_s = 160 pW/cell [6] ÷ 12 μm^2/cell = 13 mW/cm^2, where 12 μm^2 is the projection area of a typical 30 μm^3-cell). The penetration distance at which the light intensity reaches the compensation point (I_c = 17 pW/cell ÷ 12 μm^2/cell = 1.4 mW/cm^2) is denoted as compensation penetration (x_c; dashed lines in Fig. 2). As expected, the penetration depth decreases exponentially as the cell concentration increases.

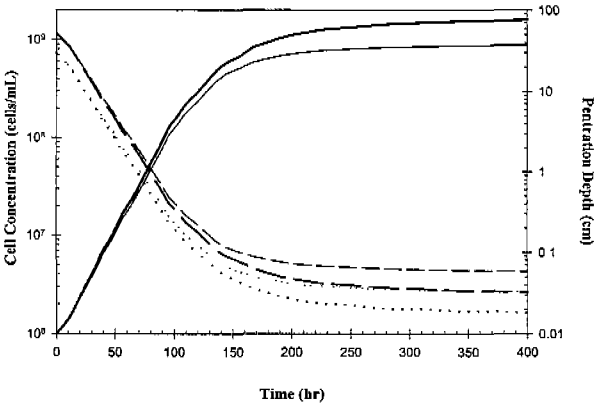

Fig. 2. Simulated growth curves (solid lines) and the corresponding light saturation (dotted lines) and compensation (dashed lines) penetration depths in the PBR-S (thick lines) and the PBR-L (thin lines).

In order to quantify the effect of the photic fraction, let the portion (or fraction) of the cells that can experience the light as θ. The θ fraction can be calculated for the two different illumination chambers using the penetration depths. Since the illumination chamber has two illumination surfaces, θ can be expressed as follows:

$$\theta = \frac{V_l}{V_l + V_d} = \begin{cases} \dfrac{52\,cm^3}{70\,cm^3} = 0.74 \; or \; \dfrac{80\,cm^3}{100 cm^3} = 0.80 & \text{if } x \geq \dfrac{L}{2} \\ \dfrac{52\,cm^3 \left(\dfrac{2x\,cm}{1.00\,cm}\right)}{70\,cm^3} \; or \; \dfrac{80\,cm^3 \left(\dfrac{2x\,cm}{1.55\,cm}\right)}{100\,cm^3} & \text{if } x < \dfrac{L}{2} \end{cases} \quad (7)$$

where V_l is the portion of the illumination chamber volume where the local light intensity is higher than either saturation level or compensation level, V_d is the volume where no light is available (mainly the volume inside the tubings), x is the penetration depth to either the saturation point (x_s) or the compensation point (x_c), and L is the thickness of the illumination chamber. Thus, θ_s is the volume fraction in which the cells can undergo photosynthesis at their maximum level (= $P_{O_2,max}$, the maximum specific oxygen production rate or maximum SOPR) and θ_c is the fraction where the light intensity is higher than the compensation intensity. Now, assuming that the cells between the two penetration depths ($x_s < x < x_c$), or in the fraction of θ_c - θ_s (= θ_{c-s}), produce oxygen at half the maximum rate (= $P_{O_2,max}/2$) on average, which is a reasonable assumption since the net OPRs at the compensation point and the saturation point are zero and $P_{O_2,max}$, respectively. The plots of the simulated θ_s (solid lines) and θ_{c-s} (dotted lines) are shown in Fig. 3 for both PBR-S (thick lines in Fig. 3) and PBR-L (thin lines).

Then the volumetric oxygen production rate (OPR) can be calculated by equation (8).

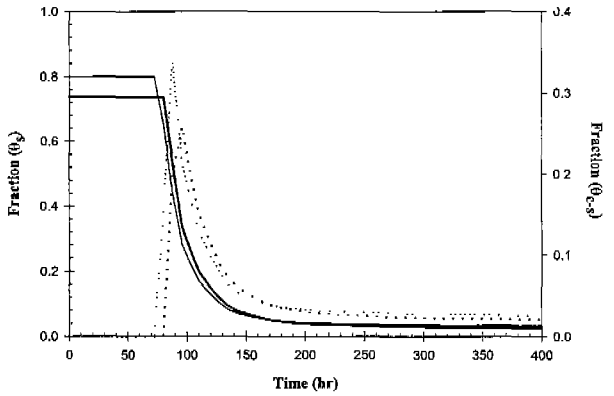

Fig. 3. Plots of illuminated fractions, θ. The profiles of the volume fractions where the light intensity is higher than the saturation light intensity (θ_s) are shown by the solid lines, while the profile of the volume fraction with a light intensity intermediate between the compensation intensity and the saturation intensity (θ_{c-s}) is shown by dotted lines. Thick and thin lines represent the simulation data from the proposed model for PBR-S and PBR-L, respectively.

$$OPR = X \cdot (V_l + V_d)\left[\theta_s \cdot P_{O_2,max} + \theta_{c-s} \cdot \frac{P_{O_2,max}}{2} - (1-\theta_c) \cdot Q_{O_2}\right] \quad (8)$$

where X is the cell concentration and Q_{O_2} is the intrinsic specific oxygen consumption rate in the dark.

The salient feature of equation (8) is that it can predict the net oxygen production rate as shown in Fig. 4. It can also successfully estimate time profiles of net oxygen production rate as well as the highest oxygen production rate obtained experimentally [7,37,38].

The difference between the actual data and the simulated curves is attributed to enhancement by mixing. In a high-density culture, the mutual shading is so intense as to permit only a very shallow photic zone in which the cells receive sufficient photons. Mixing causes a continuous shift in the relative position of the cells with respect to the photic zone. An individual cell will experience an alternating light and dark period as it travels within the turbulent liquid. In a deliberately designed mixing system on the optimal time scale, each cell will absorb light energy while it is exposed to the light and use the absorbed photons while in the dark layer. From a microscopic point of view, each cell will experience a flashing light, while it undergoes the same amount of photosynthesis and other metabolism. Thus, mixing, in effect, mimics the effect of the flashing light, and thus, increases the actual penetration depth, which causes the incident light energy to be distributed more evenly to every algal cell in the culture. This far-reaching effect increases the efficiency of photosynthesis by about 30%, as shown in Fig. 4.

Productivity Considerations

The estimation model suggested here can be applied to calculate the economy of oxygen production or the cell growth rate. For a given light intensity, the concentration was back calculated to maximize θ_s for the PBR-S. Then the oxygen production rate was calculated from equation (8). Dividing the production rate by the energy provided gives the oxygen yield in the unit of mmol O_2 L^{-1} J^{-1}. The profiles of oxygen yield (thick solid line), OPR (dotted line) and cell concentration (thin solid line) are shown in Fig. 5. From the figure, the most economic operating point for oxygen production from the PBR-S was determined to be at 2×10^7 cells/mL with 3.5 mW/cm^2 of light.

From this information, one can conclude that simply increasing light intensity will not increase the performance of the PBR. It will rather result in poorer energy conversion. As a result, to optimize the performance of a PBR, the geometry of the illumination chamber as well as the fluid flow pattern inside the PBR need to be taken into consideration. It is hoped that data presented here will play a fundamental role in future PBR designs and operations.

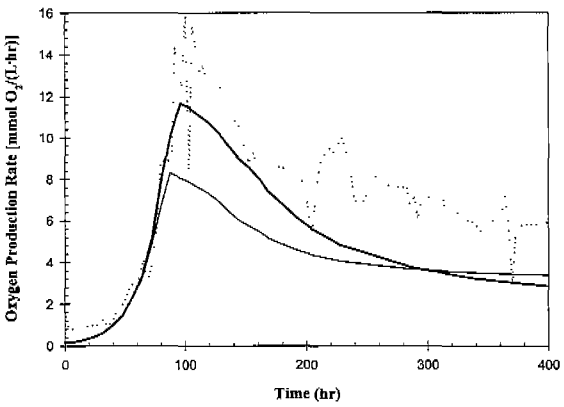

Fig. 4. Comparison of the net oxygen production rate obtained by experiment (dotted line) and those calculated using the proposed model for PBR-S (thick line) and PBR-L (thin line).

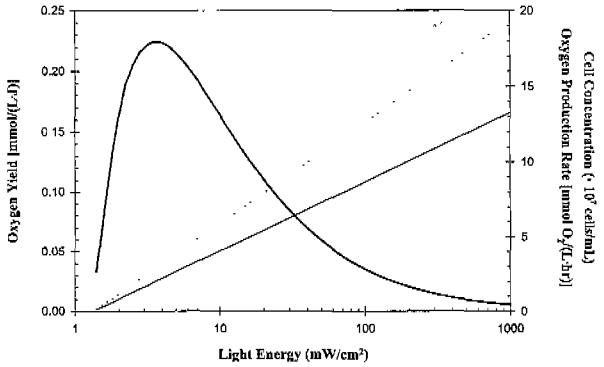

Fig. 5. Oxygen production rate (dotted line) and oxygen yield (thick solid line) as functions of incident light intensity and maximum cell concentration (thin solid line) for the light intensity in the PBR-S.

CONCLUSION

For high-density microalgal cultures, which absorb incident light efficiently, performance is determined by light distribution and penetration rather than light intensity. Simply increasing the light intensity does not produce corresponding increases in photosynthesis. The oxygen production rate may be successfully estimated by light penetration depth based on the Beer-Lambert Law. Results obtained under flashing light and light penetration depth calculations suggested that the geometry of the illumination chamber and fluid flow pattern inside the PBR need to be optimized to maximize energy conversion.

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REFERENCES

[1] Rabinowitch, E. (1961) Spectral efficiency of photosynthesis. pp. 274-298. In: P. A. Campbell (ed.). *Medical and Biological Aspects of the Energies of Space*). Columbia University Press, New York, NY, USA.
[2] Rabinowitch, E. I. (1945) *Chemistry of Photosynthesis, Chemosynthesis and Related Processes in Vitro and in Vivo*. Interscience Publishers, New York, NY, USA.
[3] Krauss, R. W. and A. Osretkar (1961) *Minimum and Maximum Tolerances of Algae to Temperature and Light Intensity*. Columbia University Press, New York, NY, USA.
[4] Thacker, D. R. and H. Babcock (1957) The mass culture of algae. *J. Sol. Energy Science. Eng.* 1: 37-49.
[5] Kyle, D. J., C. B. Osmond, and C. J. Arntzen (1987) *Photoinhibition*. Elsevier, Amsterdam, Netherlands.
[6] Lee, C.-G. (1999) Calculation of light penetration depth in photobioreactors. *Biotechnol. Bioprocess Eng.* 4: 78-81.
[7] Lee, C.-G. and B. O. Palsson (1994) High-density algal photobioreactors using light-emitting diodes. *Biotechnol. Bioeng.* 44: 1161-1167.
[8] 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, USA.
[9] Hoover, J. K. (1984) *Chloroplasts*. Plenum Press, New York, NY, USA.
[10] Kok, B. (1952) On the efficiency of *Chlorella* growth. *Acta Bot. Neerl.* 1: 445-467.
[11] Myers, J. (1980) On the algae: thoughts about physiology and measurements of efficiency. pp. 1-16. In: P. G. Falkowski (ed.). *Primary Productivity in the Sea*). Plenum Press, New York, NY, USA.
[12] Pirt, S. J. (1986) The thermodynamic efficiency (quantum demand) and dynamics of photosynthetic growth. *New Phytol.* 102: 3-37.
[13] Lehninger, A. L. (1982) *Principles of Biochemistry*. Worth Publisher, New York, NY, USA.
[14] Stryer, L. (1995) *Biochemistry*. W. H. Freeman and Company, New York, NY, USA.
[15] Hill, R. and F. Bendall (1960) Function of the two cytochrome components in chloroplasts: A working hypothesis. *Nature* 186: 136-137.
[16] Emerson, R. and C. M. Lewis (1943) The dependence of quantum yield of *Chlorella* photosynthesis on wave length of light. *Amer. J. Bot.* 30: 165-178.
[17] Pirt, S. J., Y.-K. Lee, A. Richmond, and M. W. Pirt (1980) The photosynthetic efficiency of *Chlorella* biomass growth with reference to solar energy utilization. *J. Chem. Tech. Biotechnol.* 30: 25-34.
[18] Kok, B. (1960) Efficiency of photosynthesis. pp. 566-633. In: W. Ruhland (ed.). *Encyclopedia of Plant Physiology*). Springer, Berlin, Germany.
[19] 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.
[20] Morel, A. and A. Bricaud (1981) Theoretical results concerning light absorption in a discrete nedium, and application to specific absorption of phytoplankton. *Deep-Sea Reseach* 28A: 1375-1393.
[21] Aiba, S. (1982) Growth kinetics of photosynthetic microorganisms. pp. 85-156. In: A. Fiechter (ed.). *Microbial Reactions* (Adv. Biochem. Eng., vol. 23). Springer-Verlag, Berlin, Germany.
[22] Mie, G. (1908) Beitrage zur optik truber medien, speziell kolloidaler metallosungen. *Ann. Phys.* 25: 377-445.
[23] Van de Hulst, H. C. (1957) *Light Scattering by Small Particles*. John Wiley, New York, NY, USA.
[24] 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.* 38: 817-882.
[25] 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.* 38: 826-834.
[26] Frohlich, B. T., I. A. Webster, M. M. Ataai, and M. L. Shuler (1983) Photobioreactors: models for interaction of light intensity, reactor design, and algal physiology. *Biotechnol. Bioeng. Symp.* 13: 331-350.
[27] Geider, R. J. and B. A. Osborne (1992) Light utilization and optical properties of algae. pp. 122-155. In: R. J. Geider and B. A. Osborne (eds.). *Algal Photosynthesis* (Current Physiology). Chapman and Hall, New York, NY, USA.
[28] Geider, R. J. and B. A. Osborne (1987) Light absorption by a marine diatom: experimental observation and theoretical calculations of the package effect in a small *Thalassiosira* species. *Mar. Biol.* 96: 299-308.
[29] Kok, B., B. Forbush, and M. McGloin (1970) Cooperation of charges in photosynthetic O_2 evolution - I. A linear four step mechanism. *Photochem. Photobiol.* 11: 457-475.
[30] Kok, B. (1953) Experiments on photosynthesis by *Chlorella* in flashing light. pp. 63-75. In: J. S. Burlew (ed.). *Algal Culture from Laboratory to Pilot Plant*). Carnegie Institution of Washington Publication, Washington, DC, USA.
[31] Kok, B. (1956) Photosynthesis in flashing light. *Acta* 21:

245-258.
[32] Phillips, J. N. and J. Myers (1954) Growth rate of *Chlorella* in flashing light. *Plant Physiol.* 29: 152-161.
[33] Weller, S. and J. Franck (1941) Photosynthesis in flashing light. *J. Phys. Chem.* 45: 1359-1373.
[34] 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.
[35] Park, K.-H. and C.-G. Lee (2000) Optimization of algal photobioreactors using flashing lights. *Biotechnol. Bioprocess Eng.* 5: 186-190.
[36] Fredrickson, A. G. and H. M. Tsuchiya (1970) *Utilization of the Effects of Intermittent Illumination on Photosynthetic microorganisms*. Center for Agricultural Publication and Documentation, Wageningen, The Netherlands.
[37] Lee, C.-G. and B. O. Palsson (1995) Light-emitting diode-based algal photobioreactor with external gas exchange. *J. Ferment. Bioeng.* 79: 257-263.
[38] Lee, C.-G. and B. O. Palsson (1995) Continuous medium perfusion leads to long-term cell viability and oxygen production in high-density photobioreactors. *Biotechnol. Lett.* 17: 1149-1154.

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