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Calculation of Light Penetration Depth in Photobioreactors

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Light penetration depth in high-density *Chlorella* cultures can be successfully estimated by Beer-Lambert's law. The efficiency of light energy absorption by algal cultures was so high that algal cells near the illuminating surface shade the cells deep in the culture. To exploit the potential of high-density algal cultures, this mutual shading should be eliminated or minimized. However, providing more light energy will not ease the situation and it will simply drop the overall light utilization efficiency.

Key words: photobioreactor, *Chlorella*, light penetration depth, Beer-Lambert's law

The major factors that must be considered in the design of a high-density photobioreactor (PBR) are light, gas transfer into (CO_2) and out of (O_2) the reactor, balancing medium components, and removal of the potentially harmful secondary metabolites. Among these, light is undoubtedly the key parameter in designing a successful PBR. Two properties of light energy are important for algal growth and metabolism: spectral quality and intensity. Spectral quality is defined by the absorption spectrum for the chlorophyll and other photosynthetically active pigments, and the photosynthetic efficiency is a function of spectral quality [1]. The spectrum of emitted light from the light source, the delivery and the distribution of the light into the culture, and the light scattering and attenuation in the culture must be considered.

Supplying and delivering adequate amount of photosynthetically active radiation (PAR) into a photobioreactor offers many challenges. However, distributing the delivered photons evenly over the illuminating surface and penetrating the photons deep into the culture are another problem. Antenna structures of microalgae are so efficient that they can absorb all the photons that hit them even though the cell cannot use all the photons in photosynthesis. This will cause mutual shading: the cells shielded from light by other cells cannot see the light, and thus consume oxygen by photorespiration. However, the photosynthetic photon flux (PPF) cannot be increased to infinity to overcome this mutual shading. The effect of light intensities has been studied in the greatest detail in connection with photosynthesis. A series of experiments was performed to grow algae at various high densities under varying light intensities [2]. The total amount of organic material which can be produced by growing plant cells from light energy increases as the light intensity increases up to a certain point (saturation intensity), but the amount produced per unit of light decreases rapidly as the intensity of light increases beyond that point. Light of high intensity apparently stimulates a process of photooxidation, which partially offsets some of photosynthesis [3]. As a result, the growth rate shows the expected drop at higher optical densities and increasing the light intensity doesn't increase the growth rate. Further, higher light intensity normally damages the cells. As the cell concentration in the culture increases, the color of cells become lighter. The term 'photoinhibition' is frequently used for the damage caused by excess light [4]. Photoinhibition is also a function of temperature and shows a coupled response with photorespiratory carbon metabolism. This mutual shading will decrease the portion of the cells exposed to the light. Thus only the cells close to the illuminated surface 'see' the light at high cell densities regardless of supplied light intensity. As a result, apparent oxygen production rate (OPR) will decrease after the cell concentration reached a certain density.

A high-density algal photobioreactor has been designed and reported previously [5]. The PBR uses light-emitting diodes (LEDs), which is one of the most efficient light sources invented to date. The emitted spectrum from the LEDs overlaps with the photosynthetic absorption spectrum of chlorophylls, maximizing the overall light utilization efficiency. The delivery and the distribution of the photons from the LEDs into the illuminating chamber are also satisfactory. However, the oxygen production rate was significantly lower than the expected value. The net oxygen production rate in the PBR was 10 mmol O_2/L/hr or about 20 fmol O_2/cell/hr considering the algal concentration of 5×10^8 cells/mL at that point (fmol stands for femtomol) [5]. If there was no mutual shading in the PBR, the specific oxygen production rate would increase at least 5 fold. This work is on the theoretical consideration on light penetration into high-density algal cultures to explain the observed data.

Chlorella kessleri (UTEX 398) was obtained from The Culture Collection of Algae at UTEX (Austin, TX, U.S.A.) on proteose agar. The N-8 culture medium was used without modification [6]. Red DDH GaAlAs LEDs, obtained from Quantum Devices Inc. (Barneveld, WI, U.S.A.), were powered by DC power supplies (GP-4303D, LG Precision Co. Ltd., Cerritos, CA, U.S.A.). The spectral outputs of LEDs were peaked at 680 nm. The light intensity of the LED units was measured by a silicon photocell (Model 0560.0500, Testoterm GmbH & Co., Germany). The cell concentration and average

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cell size were determined by Coulter Counter (model Z2, Coulter Electronics, Inc., Hialeah, FL, U.S.A.). Light absorbance of the culture at various cell concentrations was measured by Spectrophotometer (model UV-160, Shimadzu, Japan).

The minimum light required to initiate the growth (= compensation irradiance or compensation point) of *Chlorella* was calculated by averaging the minimum intensity required at various cell concentration. Three sets of flasks were prepared for three different inoculation density: 1×10^5, 1×10^6, and 1×10^7 cells/mL. Every set consisted of four flasks with different intensities: 0.073, 1.482, 5.802, and 39.65 mW per flask (or 2.58, 52.4, 205, and 1400 μmW/cm^2). Each flask was wrapped with heavy-duty aluminum foil and was illuminated by three LEDs. The culture volume of each flask was 55 mL and the illuminated area per flask was 28.3 cm^2. The culture was continuously mixed by a magnetic stirrer and was sampled and counted every 12 hours. As expected, the cells could not grow under either very low light intensity or extremely high light intensity. The results are summarized in Table 1. Assuming all the cells are spherical with identical size and a perfect mixing inside the flask, the compensation irradiance can be calculated. The average cell size under the red light from the LEDs were reported as 30 μm^3/cell [7], the average projection area per cell would be 12 μm^2/cell. The compensation irradiance was calculated to be 17 pW/cell from the values marked with '*a*' in Table 1 (pW stands for picowatt). The approximate saturation irradiance was calculated from the values marked with '*b*' and was about 160 pW/cell. Since the flask with the lowest inoculation density and the highest light intensity didn't show any growth (the point labeled with '*c*' in Table 1), per cell light intensity for photoinhibition was calculated from this point and was about 7200 pW/cell. The values obtained here are only rough estimations. However, they provide some figures on the relationship between light intensity and growth of *C. kessleri*. The light saturation irradiance calculated here can be validated by the theoretical light requirement.

The maximum photosynthesis rate (in other words, the photosynthesis rate when there is no mutual shading) of *C. kessleri* was found to be 200 fmol/cell/hr [5]. According to Z-scheme in photosynthesis, this amount of oxygen is equivalent to 1.6 pmol photons/cell/hr (pmol stands for picomol). Since the light used here was almost monochromatic with narrow spectrum centered at 680 nm, the energy of photon can be easily calculated. The energy of 1 einstein of 680 nm photon equals $Nh\nu$, where N is the Avogadro's number, h is Planck's constant, and ν is the frequency, or 1.76×10^5 J. Thus the theoretical power to produce 200 fmol/cell/hr or 1.6 pmol 680 nm photons/cell/hr is about 78 pW/cell. Considering that 11 quanta are needed to reduce one molecule of CO_2 in *Chlorella* [8] and the reported photosynthetic efficiency of *Chlorella* is in the range of 32 to 54% [9-11], the saturation irradiance calculated from the above experimental data is in accordance with the theoretical value. The reported value of saturation light intensity of microalgae is between 1.4 and 4.2 mW/cm^2 [12], whose range includes the value calculated above.

Table 1. Growth of *C. kessleri* under different light intensities

Cell concentration (cells/mL)	Net light energy supplied per flask (mW)			
	0.073	1.482	5.802	39.65
1×10^5	O[a]	O[b]	O	X[c]
1×10^6	X	O[a]	O[b]	O
1×10^7	X	X	O[a]	O[b]

1. The conditions supporting the culture within 12 hours of inoculation are marked with 'O'.
2. Compensation irradiance, saturation irradiance, and photoinhibiting irradiance were calculated from the numbers marked by *a*, *b*, and *c*, respectively.

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 in addition to the light absorption by the cells. Beer-Lambert's law is usually used to estimate light intensity within a culture medium. Some sophisticated models on the angular dependence of the scattered light for homogeneous spheres and a simpler anomalous diffraction can be found in the literature [13]. These models don't count the light absorption by the particles. Recently, numerous attempts have been made to formulate a numerical model for predicting light intensity within the culture by considering both scattering and absorption [14-19]. Scattering by unicellular algae tends to conform to these predictions [19], although there is some evidence for a disproportionally high percentage of wide-angle scattering in some algal cells [20]. However, these models are too complicated and sophisticated to be used in designing and analyzing photobioreactors.

In fact, PBRs has been conventionally designed based on this law to estimate the attenuation of light energy along the depth of liquid column [14]. However, light intensity distribution in a photosystem can be assessed by Beer-Lambert's law without considering the light scattering by the cells. Thus, it only holds for pigment solutions and is not strictly applicable to the cell suspension that scatters light. 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, referred to as the 'package effect' (also known as the sieve effect), explains the reduction of absorption at the absorption maxima of the pigments, while there is little reduction in absorption at those wavelengths that are scarcely absorbed by the pigments [21]. For unicellular algae, the magnitude of the package effect depends on cell size and intracellular pigment content [19]. Cells with large volumes and cells with a high pigment content have greater package effect than small cells or those with a lower pigment content. Since the average diameter of *C. kessleri* grown under LED light is only between 3 to 4 μm, Beer-Lambert's law can be a good approximation in calculating the light penetration depth of the *C. kessleri* culture. Beer-Lambert's law is

$$\text{Log}\,(I_o/I_x) = a \cdot X \cdot x$$

where, I_o and I_x are the light intensities at the surface and depth of x (distance from the illuminating surface), respectively, and X is the particle (or cell) concentration. a is the specific absorption coefficient. The validity of the assumption that the package effect of

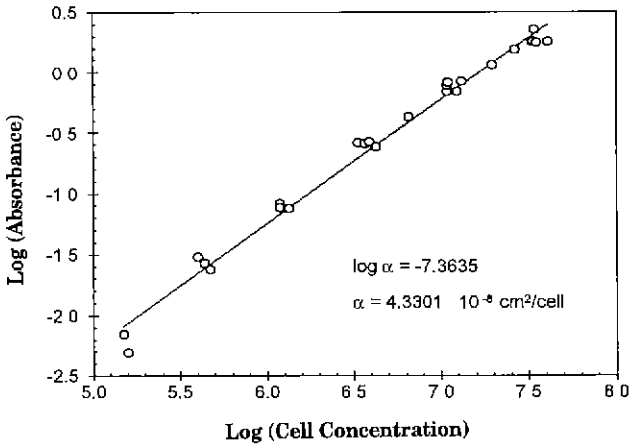

Fig. 1. Regression of measured absorbance (optical density) of various cell concentrations. Points are from actual measurement of culture at various cell concentrations. The cells used here had typical value of average cell volume and the average per cell chlorophyll contents (30 μm^3/cell and 0.5 fmol chlorophyll/cell, respectively).

small cells is negligible can be obtained from the linearity of measured absorbance (optical density) versus cell concentrations. The cell specific absorption coefficient, a, was obtained from Fig. 1. In order to make a valid in a wide range of cell concentrations, a was calculated from the linear regression of logarithmic values of the cell concentrations and their corresponding absorbance. From the best-fitted line shown in Fig. 1, an a value of 4.33×10^{-8} cm^2/cell was obtained.

Penetration depth must be defined first, to be calculated theoretically. It could be reasonably defined as the depth of photic zone, where the net oxygen production rate is positive. In other words, the penetration depth, x, is defined as the distance from the illumination surface to the point where the light intensity per cell reaches the compensation irradiance (17 pW/cell). The light intensity at the penetration depth, I_x, will be defined as the ratio of the saturation light intensity per cell to the average projection area per cell (about 12 μm^2/cell) or I_x is 1.4 pW/μm^2. Assuming a perfect light distribution and a uniform light attenuation within the culture (negligible package effect), the depth of photic zone can be calculated as a function of incident light intensity I_o and cell concentration (X) by Beer-Lambert's law using the I_x value. The penetration depth calculated based on this value will give the portion of culture which can be exposed to enough light in order to undergo photosynthesis at its maximum rate.

Fig. 2 shows the calculation results of penetration depth as a function of light intensity (mW/cm^2) and cell concentration (cell/mL). As can be seen in this figure, the algae are highly efficient in absorbing light. No matter what the maximum intensity of light source used is or what the geometry of a photobioreactor is, supplying enough photons to each cell in a 1 cm thick culture of 2×10^9 cell/mL is virtually impossible even with a light intensity of 1,000 W/cm^2 (Fig. 2). Besides, high intensity will also kill the algal cells. As a result, cells in algal culture over a concentration of 1×10^9 cell/mL cannot perform photosynthesis even though the incident light intensity is sufficient for the entire cells in the photobioreactor. This explains why the oxygen production rate showed a peak and started to decrease as the cell concentration increased (Fig. 3).

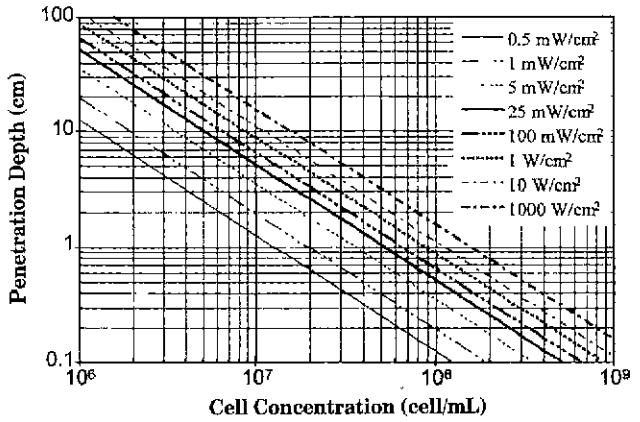

Fig. 2. Light penetration depth of *Chlorella kessleri* as a function of light intensity (W/cm^2) and cell concentration (cell/mL). The incident light intensities used are 1000, 10, and 1 W/cm^2, and 100, 25, 1, and 0.5 mW/cm^2, from top to bottom line.

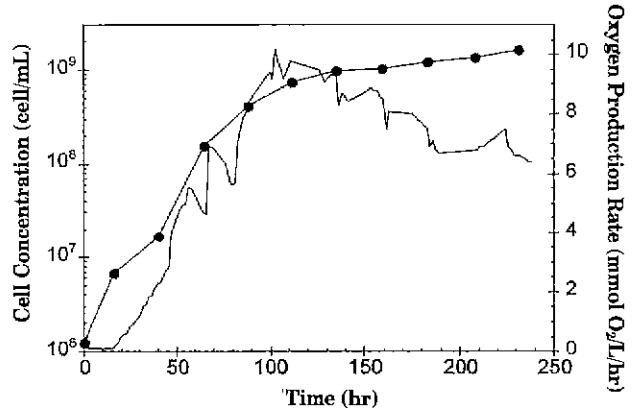

Fig. 3. The cell concentration [—●—, cell/mL] and the oxygen production rates [——, mmol O_2/L/hr] from a high-density *Chlorella* culture in a PBR with 1.0 cm thickness illumination chamber. Redrawn from [5].

The oxygen production rate showed a maximum at a concentration near 4×10^8 cell/mL, where the light intensity of the incidents light from both sides of illumination chamber decreases to the compensation irradiance at the center of the chamber. In other words, the light intensity at the darkest point in the chamber is the compensation point.

However, this theoretical penetration depth was calculated under the assumption that the culture is in laminar flow regime (that is, no mixing in the tangential direction to the flow). If the culture in the photobioreactor is in turbulent regime or at least there is some mixing between streamlines, the relative position of the cells with respect to photic zone will be shifted continuously. An individual cell will experience an alternating light and dark period as it travels with turbulent liquid. Thus, mixing will significantly increase the actual portion of the culture that can be exposed to the light. In a deliberately designed mixing system on the optimal time scale, each cell will absorb the light energy while it is exposed to the light and uses the absorbed photons up while it's in the dark layer. From a practical point of view, the major objective of mixing in a photobioreactor should be introducing some degree of turbulence to minimize the detrimental effect of mutual shading.

Light is undoubtedly the major limiting factor in high-

density algal cultures. As calculated in this paper, algae are so efficient in absorbing light energy and few photon can penetrate deep into the culture. High-density algal culture has vast potentials in producing pharmaceuticals, pigments, and other fine chemicals and in treating wastewater and carbon dioxide. However, light limitation or mutual shading must be eliminated or minimized to exploit this potential successfully. The calculation in this work suggests that simple increase in light intensity will not be a solution. Simply providing more light energy will not ease the situation and it will simply drop the overall light utilization efficiency. As a result, the absorbed but unused light energy will be exerted as heat, which should be removed from the culture. Other parameters such as the degree of turbulence should be considered in the optimal strategies. Theoretical calculation similar to this work will help the decision process in optimizing photobioreactor designs.

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REFERENCES

[1] Rabinowitch, E. (1961) Spectral efficiency of photosynthesis, p. 274-298. In: Campbell, P. A. (ed.), *Medical and Biological Aspects of the Energies of Space*. Columbia University Press. New York, NY.

[2] Krauss, R. W. and A. Osretkar (1961) *Minimum and maximum tolerances of algae to temperature and light intensity*. Columbia University Press. New York, NY.

[3] Thacker, D. R. and H. Babcock (1957) The mass culture of algae. *J. Sol. Energy Science. Eng.* 1: 37-49.

[4] Kyle, D. J., C. B. Osmond, and C. J. Arntzen (1987) *Photoinhibition*. Elsevier. Amsterdam, Netherlands.

[5] Lee, C.-G. and B. O. Palsson (1994) High-density algal photobioreactors using light-emitting diodes. *Biotechnol. Bioeng.* 44: 1161-1167.

[6] Vonshak, A. (1986) Laboratory techniques for the cultivation of microalgae, p. 117-145. In: Richmond, A. (ed.), *Handbook of Microalgal Mass Culture*. CRC Press. Boca Raton, FL, USA.

[7] 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. *Bioechnol. Prog.* 12: 249-256.

[8] 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.

[9] Kok, B. (1960) Efficiency of photosynthesis., p. 566-633. In: Ruhland, W. (ed.), *Encyclopedia of Plant Physiology*. Springer. Berlin, Germany.

[10] Myers, J. (1980) On the algae: thoughts about physiology and measurements of efficiency., p. 1-16. In: Falkowski, P. G. (ed.), *Primary Productivity in the Sea*. Plenum Press. New York, NY.

[11] 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.

[12] Richmond, A. (1986) Outdoor mass cultures of microalgae, p. 285-330. In: Richmond, A. (ed.), *Handbook of Microalgal Mass Culture*. CRC Press, Boca Raton, FL.

[13] Van de Hulst, H. C. (1957) *Light scattering by small particles*. John Wiley. New York, NY.

[14] Aiba, S. (1982) Growth kinetics of photosynthetic microorganisms, p. 85-156. In: Fiechter, A. (ed.) *Adv. Biochem. Eng.*, Vol. 23, Microbial Reactions. Springer-Verlag. Berlin, Germany.

[15] 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.

[16] 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.

[17] 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.

[18] Geider, R. J. and B. A. Osborne (1992) Light utilization and optical properties of algae, p. 122-155. In: Geider, R. J. and M. Melkonian (eds.) Current Physiology, *Algal Photosynthesis*. Chapman and Hal. New York, NY.

[19] 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.

[20] 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.

[21] 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.