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Effectiveness of Flashing Light for Increasing Photosynthetic Efficiency of Microalgal Cultures over a Critical Cell Density

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Abstract Critical cell density (CCD), the maximum cell concentration without mutual shading in algal cultures, can be used as a new operating parameter for high-density algal cultures and for the application of the flashing light effect on illuminated algal cultures. CCD is a function of average cell volume and light illumination area. The CCD is thus proposed as an index of estimation of mutual shading in algal cultures. Where cell densities are below the CCD, all the cells in photobioreactors can undergo photosysnthesis at their maximum rate. At cell densities over the CCD, mutual shading will occur and some cells in the illumination chamber cannot grow photoautotrophically. When the cell concentration is higher than the CCD, specific oxygen production rates under flashing light were higher than those under continuous light. The CCD was found to be a useful engineering parameter for the application of flashing light, particularly in high-density algal cultures.

Keywords: critical cell density (CCD), flashing light, microalgae, mutual shading

INTRODUCTION

Microalgal biotechnology is one of the emerging fields in biotechnology era. In recent years, there has been a great interest using microalgae as sources of a wide range of fine chemicals, oils and polysaccharides [1-4]. The microalgae are particularly attractive as natural sources of bioactive molecules because algae have the potential to produce structurally complex compounds [5,6]. Many laboratory scale photobioreactors have been reported but most of them are extremely difficult to scale up due to the phenomenon of mutual shading at high cell densities.

Light, which is an essential substrate for the phototrophic growth of microalgae, cannot be stored in photobioreactors, so it must be supplied continuously. Due to the high light-harvesting efficiency of chlorophyll in microalgae, algae absorb all the light that reaches them even though they cannot use all the photons. This phenomenon cause a dramatic decrease in light utilization efficiency since the photon cannot penetrate deeply into the culture broth, even when enough photon are supplied at illumination surface. Many photobioreactors were developed to overcome this problem [7-13].

Both the spectral quality and the intensity of light are important for algal growth and metabolism. In high-density algal cultures, the light delivery becomes restricted as the cell concentration increases. This mutual shading, or self-shading will shield the cells that are apart from the illumination surface from receiving light. As a result, the light penetration depth should be calculated in order to achieve a successful photobioreactor [11]. Longer light penetration depth will increase the overall light utilization efficiency and thus algal productivity.

The effect of flashing lights can be theoretically explained using light and dark reactions of photosynthesis. Photosynthesis can be hypothesized as a discontinuous, linear, four-step process [14]. Emerson and Arnold [15] performed the first experiment on the application of flashing light in algal cultures in 1932. When algal cells were illuminated by a succession of very short flashes, it was found that the maximum rates of oxygen production and carbon dioxide uptake under this flashing light could be the same as those under continuous light. This means that photosynthetic cells do not need continuous illumination. For example, the cells will only receive the light for 1/1000 of the time when grown under a 100 Hz flashing light with a flash duration of 10 μs, and the cells can undergo photosynthesis as if they had received photons continuously [16]. Other studies were also reported on the effect of various frequencies and duty cycles on the oxygen production rate and the biomass production in algal cultures [17-20]. The existence of dark periods between the short flashes of the light can increase the efficiency of the photosynthesis, especially for a high-intensity light.

The aim of this study was to optimize high-density algal cultures by supplying a suitable flashing light, which has higher instantaneous photosynthetic photon flux (PPF) than continuous light with the same average intensity [20]. This study describes a method to estimate the critical cell density and its application to high-density algal cultures.

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MATERIALS AND METHODS

Cell Line and Culture Medium

Chlorella kessleri (UTEX 398) was obtained from The Culture Collection of Algae at UTEX (Austin, TX, USA) on proteose agar. N-8 medium was used throughout the study [17].

Seed cultures were usually prepared by suspending a single colony from a master plate in a 250-mL Erlenmyer flask containing 100 mL of N-8 medium. The seed culture flasks were cultured in illuminated shaking incubators (model HB-201S, HanBaek Scientific, Bucheon, Korea) at a constant light intensity of 50 $\mu E\ m^{-2}\ s^{-1}$).

LEDs, Light Measurement, and Power Supplies

Red DDH GaAlAs LEDs were obtained from Quantum Devices Inc. (Barneveld, WI, USA). The LEDs have narrow spectral outputs, whose central wavelength is approximately 680 nm. These red LEDs were powered by DC power supplies (Model GP-233, LG Precision, Seoul, Korea) at the constant voltages between 1.70 and 4.98 V depending on flashing frequencies and duty cycles.

Twenty-two watt cool-white circline fluorescent light (General Electric Co., Cleveland, OH, USA) was used as the light source for seed cultures.

The light intensities of LED units were measured by a silicon photo cell (model 0560.0500, Testoterm GmbH & Co., Germany) and by a quantum sensor (model LI-190SA, LI-COR, Lincoln, NE, USA). By monitoring the output of a photo cell located at the bottom of the culture flasks, the intensity (about 78 $\mu E\ m^{-2}\ s^{-1}$) of each LED unit in the same experimental set could be matched at desired flashing frequencies and duty cycles.

Flashing Light

A frequency modulator, based on a LM555C timing chip and IRF640 MOSFET was constructed in order to generate pulse power for the flashing light as described earlier [16]. The device provided the frequency range in 1-100 kHz. At the selected frequency, the device could provide the duty cycle of 10-50%. The duty cycle was achieved by a combination of a timing chip and a MOSFET. The average light intensity of the flashing light was equal to that of the control continuous light in order to deliver the same number of photons into the cultures in the same experimental set.

The frequency and duty cycle of the flashing light were measured by a digital oscilloscope (model HP54512B, Hewlett Packard, Colorado Springs, CO, USA).

Measurement of Photosynthesis

Chlorella cells in the exponential phase were centrifuged for 15 min at 1,000 rpm and resuspended in fresh media. These cells were transferred to specially designed illumination couvettes having a magnetic spin bar. The couvette was kept in the dark covered with aluminum foil until the dissolved oxygen tension reached an exhausted level. When the dissolved oxygen was exhausted, the specific oxygen production rate (SOPR) was measured using a dissolved oxygen (DO) electrode (model 023IP15-010BGV12, Phoenix, Houston, TX, USA). The DO level was recorded by a recorder (model 4156, Yokogawa, Tokyo, Japan) after LED units were turned on. The amount of photosynthetically produced oxygen under flashing light could be calculated from the slope of DO profiles as a function of the cell concentration, flashing frequency, and duty cycles.

Cell Analysis

The cell concentration was measured by a computer-controlled Coulter Counter (model Z2, Coulter Electronics Inc., Miami, FL, USA). The principle of sizing and counting particles using a Coulter Counter is based on measurable changes in electrical resistance produced by nonconductive particles that are suspended in an electrolyte. A small opening (aperture) between electrodes is the sensing zone through which suspended particles pass. In the sensing zone each particle displaces its own volume of electrolyte. Volume displaced results in a voltage pulse; the height of each pulse is being proportional to the volume of the particle. The quantity of suspension drawn through the aperture is precisely controlled to allow the system to count particles with an exactly reproducible volume. This method is independent of particle shape, color and density.

The computer acquires various data from Coulter Counter, such as the counted particle number, sample statistics, and sample information, and the cell volume distribution is displayed as a histogram. It also calculates the cell volume and surface area. All the data can be analyzed by AccuComp Software. This program can show all the statistical parameters, such as mean, median, skewness, and kurtosis from both volume and diameter statistics, as well as differential and cumulative displays for the histogram.

Estimation of Critical Cell Density in Algal Cultures

In order to estimate the critical cell density (CCD), an assumption was made: the cells in the illumination chamber were arranged on monolayer that was perpendicular to the light path without mixing. If all the illumination area was covered by cells, no more light could penetrate into the culture media. The CCD is therefore defined as the cell concentration where the sum of projection area of the cells in the illumination chamber is equal to the total illumination surface.

$$CCD = \frac{A_i}{\frac{\pi D_c^2}{4} V}$$

where D_c is diameter of cell, A_i is illumination area, and

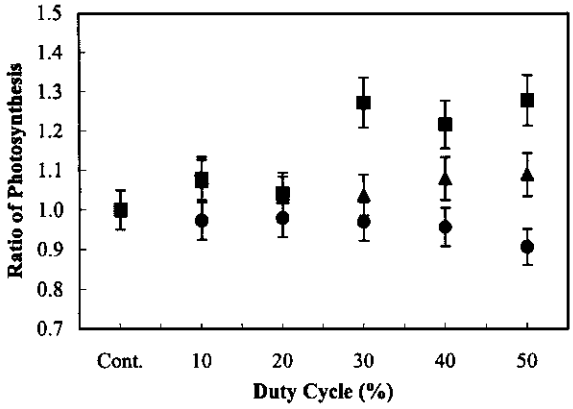

Fig. 1. The normalized SOPR under low frequency flashing light (10 kHz) with various duty cycles (*x*-axis) and cell concentrations (●: 1.2×10^6 cells/mL, ■: 1.4×10^7 cells/mL, ▲: 1.1×10^8 cells/mL)

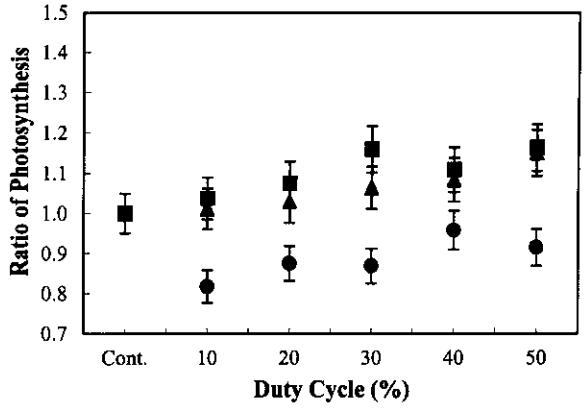

Fig. 2. The normalized SOPR under mid frequency flashing light (20 kHz) with various duty cycles (*x*-axis) and cell concentrations (●: 1.7×10^6 cells/mL, ■: 2.0×10^7 cells/mL, ▲: 1.1×10^8 cells/mL)

V is volume of culture. For example, the CCD in flask culture under fluorescence lamp is about 1×10^6-4×10^6 cells/mL, according to the average cell volume that varied from 30-90 μm^3/cell. Theoretically, the CCD is the maximum possible cell concentration without mutual shading in the culture media.

RESULTS AND DISCUSSION

SOPR under Flashing Light

A frequency modulator provided a switching power to LEDs at variable frequencies. The device successfully provided a wide range of frequencies (10-50 kHz) with various duty cycles (10-50%). At these frequencies, flashing times were very short (t_f = 2-50 μs), which was corresponding to the time constant of light adsorption in photosynthesis

In order to avoid the effect of ambient light, the entire experiments were performed in the dark. The flashing light effect was clearly observed. Figs. 1-3 showes the effect of flashing light on the SOPR at constant frequencies of 10, 20, and 50 kHz, respectively, with duty cycles of 10-50%. Cell concentrations were varied from 1×10^6 to 1×10^8 cells/mL. This range goes from a final cell density of flask cultures (1×10^6 cells/mL) to high-density cultures in PBRs ($> 1 \times 10^8$ cells/mL). The measured SOPR were in the range of 30-120 fmol cell^{-1} h^{-1}). The SOPR found to be functions of the cell growth stage, light condition and cell density.

For high-density cultures over 1×10^7 cells/mL, enhancements of 5 to 25% were observed in SOPR under flashing light conditions compared to that under continuous light. However, at a relatively low density (1×10^6 cells/mL), no enhancement of SOPR by flashing light was observed (Figs. 1-3). This result suggested that the photosynthesis of microalgae was photoinhibited by instantaneous high light intensity at lower cell concentrations. At higher cell densities, however, increased instantaneous photosynthetic photon flux helped the photons to penetrate deeper in to the cultures and thus reduced the mutual shading, resulting in an increased SOPR (Figs. 1-3). The increased light penetration depth and the decreased mutual shading would increase the volume of photic zone and thus the ratio of algal cells that could see enough light to perform photosynthesis. This is the flashing light effect. Interestingly, duty cycles of flashing lights were also found to be critical.

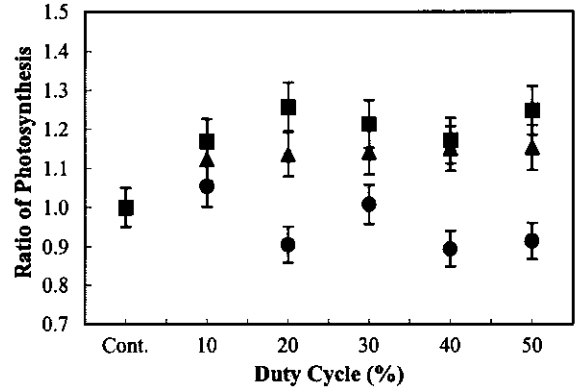

Fig. 3. The normalized SOPR under high frequency flashing light (50 kHz) with various duty cycles (*x*-axis) and cell concentrations (●: 1.2×10^6 cells/mL, ■: 2.1×10^7 cells/mL, ▲: 9.8×10^7 cells/mL).

In conclusion, SOPR under flashing light was higher than that of the continuous light in high-density algal cultures. From an economic point of view, the application of flashing light to the production of algal biomass as well as to algal-driven natural products and to CO_2 fixation would be beneficial, because of the efficient power usage in light illumination.

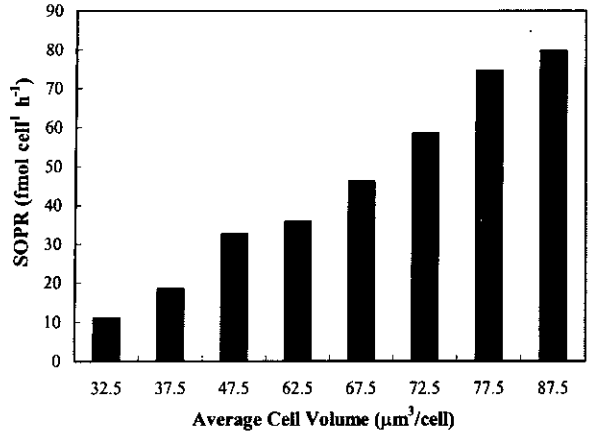

Fig. 4. The SOPR at various cell volumes (30-90 μm^3/cell).

Critical Cell Density

The SOPRs of microalgae under flashing light were compared with those under equivalent continuous light as a function of average cell volume. The SOPRs were increased as average cell volume increased (30-90 μm^3/cell) (Fig. 4). With the same average cell volume, the SOPRs in flashing light were higher than those under continuous light. The SOPRs under flashing light were enhanced by increased instantaneous PPF. The photosynthetic efficiency per unit cell volume was relatively constant (~0.8 fmol O_2 μm^{-3} h^{-1}) regardless of the flashing frequencies and duty cycles. The effects of the flashing frequencies and the duty cycles didn't seem to have a profound effect on SOPRs, unlike the effect of the average cell volume (Table 1).

The oxygen production rates per projected cell size in algal cultures under flashing and continuous lights are shown in Table 1. The result suggested that the larger the cell, the higher the oxygen production rate. Also, the oxygen production rate under flashing light was higher than that under continuous light.

The major limiting factor in high-density algal cultures is light delivery. The highly efficient chlorophyll antenna systems of microalgae cause mutual shading as cell concentration increases. The chlorophylls absorb excess light even though they cannot process all the photons absorbed. As a result, the light becomes a limiting factor especially in high cell densities. This affects the light penetration depth and thus the depth of photic zone. The degree of mutual shading was affected by cell concentration and size, light intensity, projection area, and mixing. If all the illumination area was covered by cells, no more light could penetrate into the culture media. At cell densities below CCD, light can penetrate to the other side of the illumination chamber. For the cell density above CCD, however, mutual shading occurred and dark zones were formed in the illumination chamber. CCD must be a function of cell concentration, average cell volume, and S/V ratio. Fig. 5 shows the CCD profile in specially-designed couvette as a function of cell condition when there was no mixing. The value of CCD will be increased as the degree of mixing increases, because mixing can mimic the flashing light effect. In other words, as the cell concentration exceeds CCD as the culture grows, photosynthetic efficiency will be decreased by mutual shading. In this situation, more effective light delivery to the culture media is required to increase light penetration depth, photosynthetic efficiency and algal productivity. The flashing light effect can improve this situation and the mutual shading can be decreased by supplying instantaneous high light intensity during the flashing times. The flashing light effect was observable only when the cell concentration was higher than the CCD (Figs. 1-3). Under fishing light conditions with the same average light intensity, the enhancement in SOPR by flashing light was clearly observed in the cell concentration above CCD. The SOPRs under flashing light were similar to those under continuous light in cell concentration below the CCD (Figs. 1-3). Clearly, flashing light was

Table 1. Comparison of photosynthesis efficiency as a function of cell volume under flashing and continuous lights (fmol μm^{-3} h^{-1})

Cell volume (μm^3/cell)	Cont.	Duty cycle (%)														
		10			20			30			40			50		
		10 kHz	20 kHz	50 kHz	10 kHz	20 kHz	50 kHz	10 kHz	20 kHz	50 kHz	10 kHz	20 kHz	50 kHz	10 kHz	20 kHz	50 kHz
31 - 35	0.341					0.320	0.476			0.573	0.326	0.375				
36 - 40	0.495			0.620											0.486	0.498
41 - 45								0.554	0.559	1.205		1.106	1.164	0.562	1.104	
46 - 50	0.691	0.480	0.464	1.039			1.117									1.109
51 - 55									1.437			1.120				
56 - 60			1.234			1.115							1.031		1.381	
61 - 65	0.575	0.570					0.869	0.567		0.776	0.928	0.970	0.685	1.116		1.011
66 - 70	0.685	0.883	0.938	0.676	1.191		1.002	1.219	0.838	1.053	1.349			0.997		
71 - 75	0.807	1.211	0.903	1.104	0.976	0.800	0.720		1.129		1.081		0.960	0.832	0.842	1.002
76 - 80	0.962		0.750	0.409					0.635	1.093				0.738		0.418
81 - 85		1.022				0.905				0.836			0.894			
86 - 90	0.912				0.878						0.871	0.877				

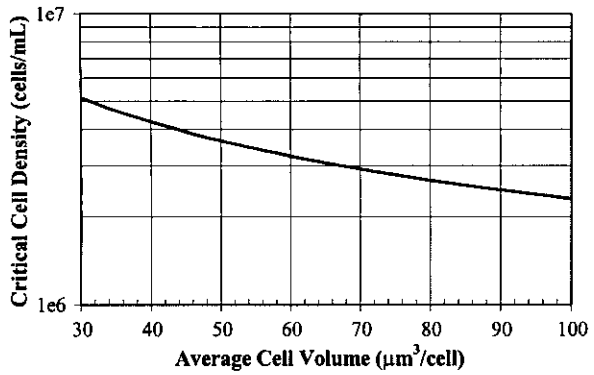

Fig. 5. The estimation of critical cell density in algal cultures as a function of the average cell volume.

effective to support high-density algal cultures above CCD. CCD was found to be a useful engineering parameter for application of flashing light in high-density algal cultures.

CONCLUSION

The SOPRs under flashing light were enhanced by increased instantaneous PPF only when the culture density was higher than CCD. However, the flashing frequency had little effect on the SOPR (10-50 kHz). The SOPRs were increased as the average microalgal cell volume increased (30-90 μm^3/cell). The photosynthetic efficiency per unit volume was relatively constant (~0.8 fmol O_2 μm^{-3} h^{-1}) under various frequencies and duty cycles. The effects of the flashing frequency and the duty cycle didn't seem to have a profound effect on SOPRs unlike the effect of the average cell volume.

The CCD could be calculated using cell concentration, average cell volume, and the S/V ratio of a culture chamber. Under flashing light, SOPR of the cell densities above CCD was higher than that under continuous light due to an increased PPF. CCD found to be a useful engineering parameter for application of flashing light in algal cultures.

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