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Optimization of Algal Photobioreactors Using Flashing Lights

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Abstract It has been reported that flashing light enhances microalgal biomass productivity and overall photosynthetic efficiency The algal growth kinetics and oxygen production rates under flashing light with various flashing frequencies (5 Hz-37 kHz) were compared with those under equivalent continuous light in photobioreactors. A positive flashing light effect was observed with flashing frequencies over 1 kHz. The oxygen production rate under conditions of flashing light was slightly higher than that under continuous light. The cells under the high frequency flashing light were also observed to be healthier than those under continuous light, particularly at higher cell concentrations. When 37 kHz flashing light was applied to an LED-based photobioreactor, the cell concentration was higher than that obtained under continuous light by about 20%. Flashing light may be a reasonable solution to overcome mutual shading, particularly in high-density algal cultures.

Keywords: photobioreactor, flashing light effect, mutual shading, *Chlorella*, high-density algal cultures

INTRODUCTION

The history of the commercial use of algal cultures spans over 50 years with various applications Microalgae have vast potentials as a source of valuable pharmaceuticals, pigments, and other fine chemicals. The applications of algae have been expanded to the area of wastewater treatment, agriculture, and CO_2 fixation with atmosphere regeneration. However, high-density algal cultures were hampered by light-limitation and thus the widespread use of high-density algal cultures.

The most important factor in designing a successful high-density photobioreactor (PBR) is undoubtedly the light [1] When selecting a light source, both spectral quality and intensity must be considered. However, the delivery and the distribution of the light into the culture, as well as the light scattering and attenuation in the culture, must be considered in order to support high-density algal cultures. The antenna structures of microalgal light harvesting complexes are so efficient that they can absorb most of the photons that hit them, although the cells cannot use all the absorbed photons in photosynthesis. This will cause mutual shading, where the cells apart from the illuminating surface will be shielded by the cells near the surface. As a result, delivering photons deep into the culture offers many challenges. The photosynthetic photon flux (PPF) cannot be increased to infinity in order to overcome this mutual shading because supplying so much light (i) may cause other problems such as heat and spatial efficiency; (ii) may not be economically favorable; and (iii) can damage algal metabolism by photoinhibition.

One possible solution to this problem is to supply light in short flashes. Photosynthesis is hypothesized to be as a discontinuous, linear, four-step process [2]. As a result, it cannot use the photons that are captured between steps. The existence of a longer dark period between the short flashes of light can increase the efficiency of photosynthesis, particularly for high intensity light [3-6]. In these previous works, flashing lights were obtained by a sectored, rotating disk to imitate the square wave. By assuming that the light obtained this way is a true square wave, with flashes of intensity I_0 and the duration t_f followed by dark periods of duration t_d, the average (or integrated) intensity, I, is the intensity that would prevail if the energy of the flashes was uniformly distributed in time; *viz.*, $I = I_0 \cdot t_f/(t_f + t_d)$.

By varying the values of t_f, t_d and I_0, Kok [3] obtained the ratios of the average rate of photosynthesis in flashing light, R_f, to the rate of photosynthesis in the same integrated intensity of steady light, R_s. From his work, the following results can be deduced: (i) The rate of photosynthesis in flashing light is never greater than the rate in steady light of the same intensity; (ii) The rate of photosynthesis in intermittent illumination is never greater than the rate in continuous illumination of equal average intensity; (iii) The efficiency with which light received by the algae is utilized tends to be greater in intermittent light than it is in steady light of an intensity equal to that of the light flashes; and (iv) A precise flash time is not necessary to achieve a considerable increase of efficiency when the incident intensity is high. Thus, by using a proper flashing light, algae can

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assimilate the same amount of light when using the same average intensity of steady light. Flashing the light will have the advantages over a steady light of the same net intensity, since the total amount of product from photosynthesis will be the same in either case.

Light-emitting diodes (LEDs) have several advantages over conventional light sources: (i) a narrow spectral output with a half-power bandwidth of 20-30 nm, which eliminated the need of a filter; (ii) a high-electrical conversion efficiency, so that the LED unit does not need a complex cooling system, (iii) a long life expectancy; and (iv) an extremely short rise and fall time, which enables flashing as fast as 5-6 MHz. Using a pulsed power to the LEDs (i) will reduce power consumption due to the existence of an off-cycle; (ii) will require a shorter cooling period, which will in turn, increase the energy conversion efficiency by reducing the electrical energy loss as heat; (iii) can enhance the instantaneous photosynthetic photon flux as much as 10 times the PPF maintained during the con-tinuous operation at a 10% duty cycle [7] (10% duty cycle means that the light is on only for 10% of on/off cycle); and (iv) will minimize the photon loss resulting from the difference in time constants between the light and dark reaction of photosynthesis [8]

Consequently, light can penetrate deeper using low duty cycle flashing light with much higher instantaneous PPF. In this study, the effect of flashing light on a eukaryotic green alga, *Chlorella kessleri* (UTEX 398), was investigated in high-density cultures.

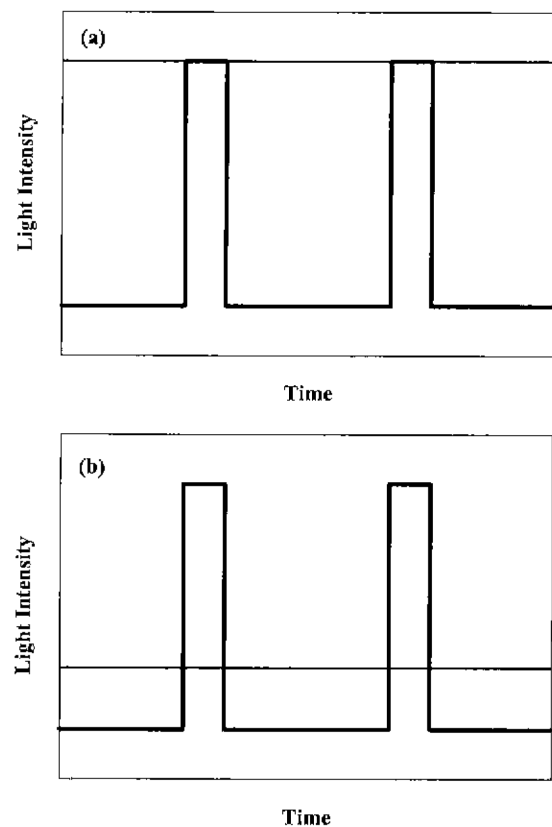

Fig. 1. Schematic diagram of flashing light (thick line) and continuous light (thin line). (a) flashing and continuous lights of the same intensity; (b) flashing and continuous lights of equal average intensity.

MATERIALS AND METHODS

Strains and Culture Condition

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 [9] This medium has no carbon source. The seed culture was prepared using 250 mL Erlenmeyer flasks at the constant temperature of 25°C in an illuminated shaking incubator (Model HB-201S, Han-Baek Scientific, Puchon, Korea). A subculture procedure was performed every week.

Light Source and Supply

Red DDH GaAlAs LEDs were purchased from Quantum Devices Inc. (Barneveld, WI, USA). The LEDs have narrow spectral output peaks at a wavelength of approximately 680 nm. These red LEDs were powered by DC power supplies (model GP-233, LG Precisions, Seoul, Korea) at a constant voltage between 1.70 and 4.98 V depending on frequency and duty cycle.

The light intensity of the LED unit was measured using a silicon photo cell (model 0560.0500, Testoterm GmbH & Co., Germany) and a quantum sensor (model LI-190SA, LI-COR, Lincoln, NE, USA). The experiment was performed with continuous and flashing light with an equal average intensity of 280 $\mu E/m^2/s$.

Flashing Light Modulator

A frequency modulator was designed and constructed to obtain flashing light. The frequency modulator based on the LM555C timing chip and IRF640 MOSFET formed flashing light at a constant frequency between 5 and 100 kHz with various duty cycles (5-50%) by changing the resistance. Flashing light from this frequency modulator was approximately square waveform. The flashing light used in this experiment is schematically presented in Fig. 1. The flashing light and continuous light were based on equal average light intensity.

The frequency and duty cycle of the flashing light was measured by digital oscilloscope (Model 54512B, Hewlett Packard, CA, USA)

Data Analysis

The specific oxygen production rate (SOPR) in the flask cultures was measured with a DO electrode and that in the PBRs was measured by Micro-Oxymax (Columbus Instrument, Columbus, OH, USA).

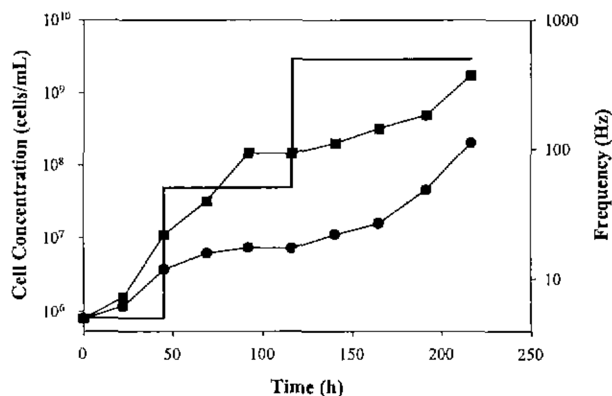

Fig. 2. Effect of low frequency flashing light on algal cultures (5-500 Hz). Cell concentrations under continuous light (-■-) and flashing light (-●-) were compared. The flashing frequency and the timing of frequency adjustment were also plotted (thick line).

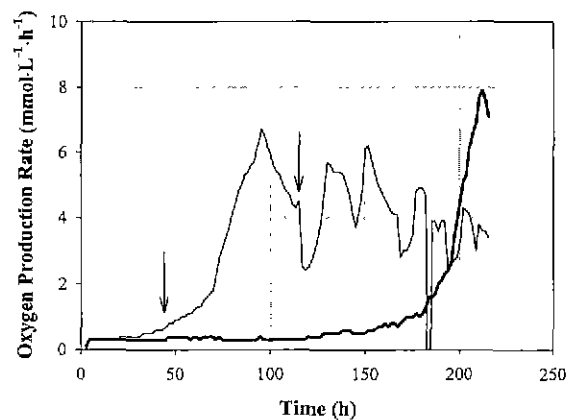

Fig. 3. Comparison of oxygen production rates under continuous light (thin line) and low frequency (5-500 Hz) flashing light (thick line). The arrows indicate the time when the flashing frequency was adjusted.

The samples for measurement were prepared by diluting the cell culture with the appropriate amount of isotonic diluent (Fisher Scientific, Pittsburgh, PA, USA). A microprocessor-controlled electric particle counter, Coulter Counter (Model Z2, Coulter Electronics, Inc., Hialeah, FL, USA) counted the cell concentrations. The Coulter Counter counts the number of particles and measures the size of the particles at the same time.

LED-Based Photobioreactor

The same PBR system, described earlier [10], was used after a minor modification to perform the application of flashing light. The total working volume of the PBR was about 70 mL and the total illumination area of PBR was about 100 cm^2. Each illumination chamber had two LED units consisting of 90 LED's each.

RESULTS AND DISCUSSION

The Effect of Low Frequency Flashing Light

The flashing light effect under low frequency has been reported earlier [5,6,11]. The frequencies tested by the previous studies were extremely low, ranging from 0.5 to 144 Hz. Based on these reports, relatively low frequency flashing light of 5-500 Hz was tested first (Fig. 2). Two identical PBR sets were prepared for this experiment and both PBRs were inoculated around 10^6 cells/mL. One PBR was illuminated with continuous light and the other PBR was illuminated at 5 Hz flashing with 10% duty cycles. However, the growth under 5 Hz flashing light was much slower than that under continuous light (control). Moreover, the oxygen production rate under 5 Hz flashing light was practically zero (Fig. 3), suggesting that the slight increase in cell concentration during this period (0-45 h) was due to the simple division of larger cells into smaller cells rather than actual growth. The average cell volume of *Chlorella* would be decreased by releasing daughter cells under higher light intensity, while maintaining the same total cell volume [12]. In this experiment, flashing light of 10% duty cycle with the same average intensity as control was used, resulting in much higher instantaneous PPF. An instantaneously higher PPF under flashing light could boost cell concentration as shown in the early period of the growth curve (Fig. 2) by the release of daughter cells.

After 45 h of cultivation, the flashing frequency was increased to 50 Hz in order to discern if the increase in flashing frequency helped the growth. However, practically no growth and no photosynthetic activity could be observed in the PBR with flashing light (45-116 h in Figs. 2 and 3). The flashing frequency was raised again to 500 Hz after 116 h, and a reasonable growth and high oxygen production rate were observed in the PBR with flashing light (116-220 h in Figs. 2 and 3). The final cell concentrations under flashing light were relatively comparable to those observed under the control conditions after culturing for 4 days under 500 Hz. This result clearly showed that the flashing frequency should be much higher than the frequency ranges tested by previous works (< 200 Hz).

The Effect of Medium Frequency Flashing Light

As the previous results showed that 500 Hz flashing could support algal cells as much as continuous light, another experiment was set up. This time, the flashing frequency was fixed at 500 Hz for the entire culture period. The cell growth profiles in both flashing and continuous lights were almost identical (Fig. 4). However, the cell concentration under flashing light was always slightly lower than that under continuous light. The difference in the oxygen production rate between flashing and continuous lights was greater than that in growth kinetics (Fig. 5). Again, the expected positive

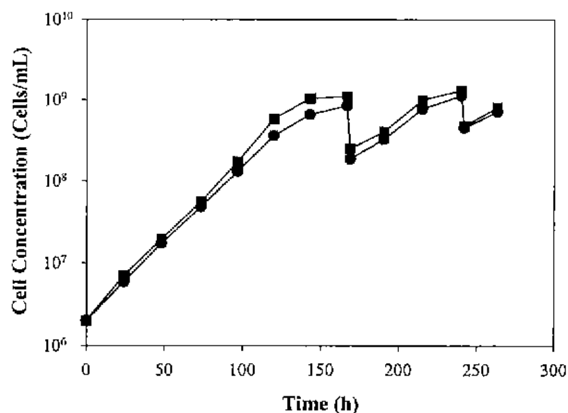

Fig. 4. Cell concentrations profiles under continuous light (-■-) and 500 Hz flashing light (-●-).

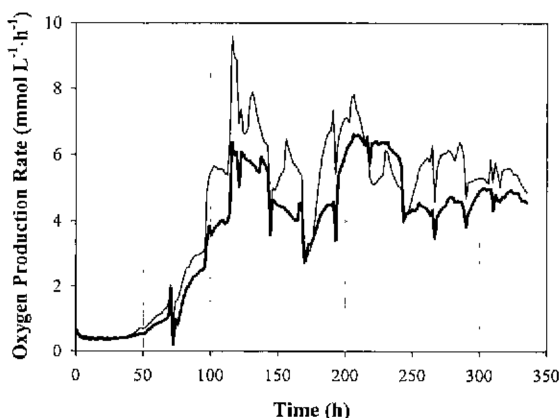

Fig. 5. Comparison of oxygen production rates under continuous light (thin line) and 500 Hz flashing light (thick line).

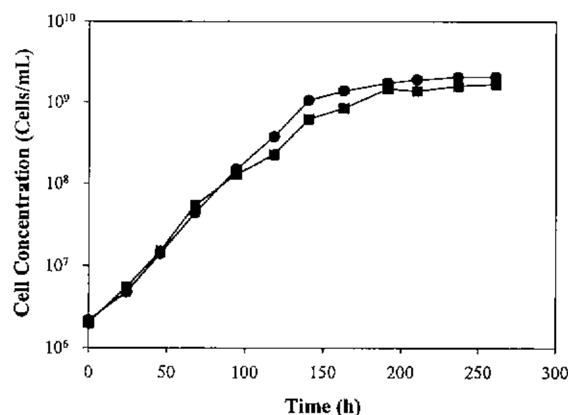

Fig. 6. Effect of high frequency flashing light on algal cultures (37 kHz). Cell concentration profile under flashing light (-●-) was compared with continuous light control (-■-).

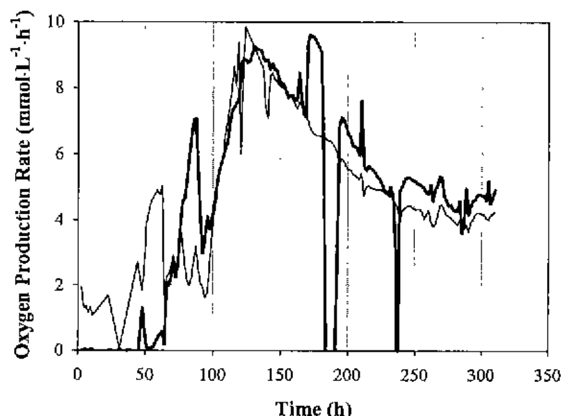

Fig. 7. Comparison of oxygen production rates under continuous light (thin line) and 37 kHz flashing light (thick line).

effect of flashing light on cell mass and oxygen production rate could not be observed. In order to verify the result, two dilutions were made at 169 h and 241 h (Figs. 4 and 5). The cell growth kinetics were reproducible in both light conditions, since both curves quickly recovered the same maximum concentration in an identical pattern after each dilution

According to these results, higher frequencies than 500 Hz may be needed to obtain the positive flashing light effect. Therefore, a new light frequency modulator was designed and constructed to generate higher flashing frequencies ranging 20-100 kHz.

The Effect of High Frequency Flashing Light

The cell growth curve under higher frequency flashing light (37 kHz) is shown in Fig. 6. When the flashing frequency was 37 kHz, the cell concentration was about 20% higher than that under continuous light. This positive flashing light effect was able to be confirmed under other frequency ranges over 1 kHz (data not shown). The cells under high frequency flashing light were also healthier than those under continuous light particularly at higher cell concentrations over 10^8 cells/mL. The per cell chlorophyll concentrations, which can be used as viability parameter for algal cultures [13], were also higher under flashing light (data not shown).

The oxygen production rate also demonstrated the same confirming positive flashing light effect. The oxygen production rate under flashing light was slightly higher than that under continuous light (Fig. 7). The fluctuations in the oxygen production rate reading (Figs. 3, 5, and 7) were introduced due to the way the gas analyzer was configured. The instrument required a hermetically sealed loop to measure the gas composition. Sampling and on-line ultrafiltration will disturb the gas pressure in the gas loop and thus the fluctuation in oxygen production reading was inevitable.

The flashing light effect can generally observed when light is supplied discontinuously. Flashing light has been used as a tool for improving the efficiency of photosynthesis or for understanding the photosynthetic mechanism. It has been reported that flashing light is a more efficient way to culture microalgae, because a faster photosynthetic rate, higher growth rate and productiv-

ity were observed under flashing light [5,6,14,15]. However, there are two significant differences in this report. First, this study was performed with a much higher frequency flashing light (37 kHz) than previous works. Most of the reported works have been done in order to study photosynthesis or in order to increase photosynthetic efficiency by (1) reducing power consumption (due to the existence of off-duty cycle), and (2) minimizing photon losses during the dark reaction. Naturally, those works did not need higher frequencies. Second, this study used flashing light for a different purpose. The reported works used a flashing light with the same peak intensity of continuous light (Fig. 1(a)) while this study used a flashing light with equal average intensity (Fig. 1(b)) to prove mutual shading can be minimized by using high intensity flashing light with a low duty cycle The instantaneous PPF can be 10 times greater than the level maintained during continuous operation at a 10% duty cycle. A positive flashing light effect reported here showed that increased instantaneous PPF could lessen the degree of mutual shading.

Consequently, flashing light may increase the performance of a photobioreactor by increasing the instantaneous PPF and thus decreasing mutual shading. This is a very promising result since the performance of PBR can be optimized without genetic modification or improvement of photosynthesis mechanisms. Only the delivery of light in the PBR system was the significant parameter in the improvement of the performance of PBR.

CONCLUSION

The algal cultures under flashing light with a relatively low frequency showed lower growth kinetics and oxygen production rates than those performed under continuous light. For the flashing frequency below 1 kHz, the cell growth curve and photosynthetic rate under the flashing light were less than or nearly identical to those under continuous light. The final cell concentration under flashing light was increased as the flashing frequency increased The final cell concentration under 37 kHz flashing light was higher than that under a steady light by about 20% The results demonstrate that the application of flashing light to a PBR system can improve the cell concentration, photosynthesis rate and chlorophyll concentration.

In conclusion, flashing light can lessen the degree of mutual shading by penetrating deeper into the cultures owing to the increased instantaneous PPF. The flashing light effect was more evident in high-density algal cultures. These results will contribute to the production of high-valued metabolites and natural products from microalgae since the lack of proper PBR has to date prevented the commercial use of microalgae.

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