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Effect of Flashing Light on Oxygen Production Rates in High-Density Algal Cultures

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Abstract A proper flashing light is expected to enhance microalgal biomass productivity and photosynthetic efficiency. The effect of flashing light on high-density *Chlorella kessleri* (UTEX 398) cultures was studied using light-emitting diodes. A frequency modulator was designed to flash LEDs, and the device successfully provided wide range of frequencies and various duty cycles of flashing. A relatively high frequencies of 10, 20 and 50 kHz were used in this study. These frequencies have very short flashing time (2–50 μs), which corresponded to the time constant of the light reaction of photosynthesis. The specific oxygen production rates of photosynthesis under flashing light were compared with those under an equivalent continuous light in specially designed illumination cuvette. The specific oxygen production rates under flashing light were 5–25% higher than those under the continuous light. A range of cell concentration was discovered, where the benefit of flashing light was maximized. The photosynthetic efficiency was also higher under flashing light with frequencies of over 1 kHz, which was a clear indication of flashing light effect and the degree of mutual shading could be overcome by flashing lights, particularly at high-density algal cultures.

Key words: Flashing light effect, LED, microalgae, oxygen production rate, photosynthesis

Microalgae have vast potentials as a source for valuable pharmaceuticals, pigments, and other fine chemicals [9, 16]. The application of algae has been expanded in the areas of wastewater treatments [23], agriculture, and CO_2 fixation with atmosphere regeneration [25]. However, high-density algal cultures were hampered by light-limitation [14] and thus the widespread use of high-density algal cultures was limited as well.

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The key limiting factor in high-density algal cultures is the light penetration into the culture medium [1, 3, 14]. The highly efficient light harvesting systems in microalgae will collect all the photons that hit them, even if all of them cannot be used by the photosystems. Thus, the cells apart from the illuminating surface will be shaded by other cells near the surface, or mutually shaded particularly at high-density cultures. The degree of mutual shading will increase dramatically as the cell density increases. Consequently, incident photons cannot reach the cells apart from the illuminating surface [14]. Flashing light with proper frequencies and equal average intensities can be a possible solution to overcome this mutual shading because the flashing light will generate higher instantaneous photosynthetic photon flux (PPF) and thus deliver the photons deeper into the culture and lessen the degree of mutual shading [21]. For instance, flashing light with 10% of duty cycle can have 10 times higher instantaneous PPF than that of the continuous light with the same average intensity [4].

Microalgae experience intermittent light in nature. There is a seasonal change, periodic diurnal variations of solar light [5, 18] and intermittent lights by streams and waves. The key to an efficient photosynthesis lies in the adaptation of these changing-light conditions. It has been reported that flashing light enhances microalgal biomass productivity and photosynthetic efficiency in algal culture [2, 22], however, the previous studies used longer flashes with lower frequencies (0.5–1 kHz) and equal peak intensities, which did not show higher efficiency than the continuous light [10, 22, 29]. An existence of longer dark periods with shorter flashes (*i.e.* low duty cycle) and equal average intensities can increase the efficiency of photosynthesis, particularly for high-density algal cultures [19, 21]. The metabolic characteristics and time constants of photosynthesis enable the proper flashing light to outperform the steady light in high-density algal cultures. Photosynthesis consists of light and dark reactions. Microalgae can split water into oxygen and electrons and accumulate reducing power

(NADH) and energy (ATP) in the light reaction of photosynthesis. It fixes the carbon dioxide by using the energy sources (NADH and ATP), generated in the light reaction, and synthesizes organic compounds and biomass in the dark reaction of photosynthesis. Dark reaction does not need photons nor can use them despite how many photons are available. As a result, proper flashing lights will increase the overall light utilization efficiency by decreasing the photon losses during dark reactions and will enhance biomass productivity by delivering the photons deeper into the cultures.

Light emitting diodes (LEDs) have many desirable characteristics as a light source for high-density photosynthetic cultures, as described earlier [15, 21]. Furthermore, LEDs have extremely short rise and fall times of approximately 80 ns, so they can be flashed as rapidly as 6 MHz [24]. LEDs are undoubtedly the best light source for studying photosynthesis under the flashing light conditions.

In this paper, an increased photosynthetic efficiency by the enhanced instantaneous PPF of flashing light is studied using LEDs as light sources. The effect of flashing light with various frequencies and duty cycles on oxygen production rates was examined to enhance microalgal biomass productivity and photosynthetic efficiency.

MATERIALS AND METHODS

Cell Line and Culture Medium

Chlorella kessleri (UTEX 398) was obtained from The Culture Collection of Algae at UTEX, (Austin, U.S.A.) on proteose agar. N-8 Medium was used throughout the study [27]. The seed culture was prepared using 250 ml Erlenmeyer flasks with 100 ml working volume at a constant temperature of 25°C in an illuminated shaking incubator (Model HB-201S, HanBaek Scientific, Puchon, Korea).

LEDs, Light Measurement, and Power Supplies

Red DDH GaAlAs LEDs were obtained from Quantum Devices Inc. (Barneveld, WI, U.S.A.). 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 constant voltages between 1.70 and 4.98 V depending on flashing frequencies and duty cycles.

The light intensity of the LED unit was measured using a quantum sensor (model LI-190SA, LI-COR, Lincoln, NE, U.S.A.). The frequency and duty cycle of flashing light were measured by digital oscilloscope (model 54512B, Hewlett Packard, U.S.A.)

Measurement of Oxygen Production Rates

Chlorella cells in the exponential phase were centrifuged for 15 min at 1,000 rpm and resuspended in fresh medium.

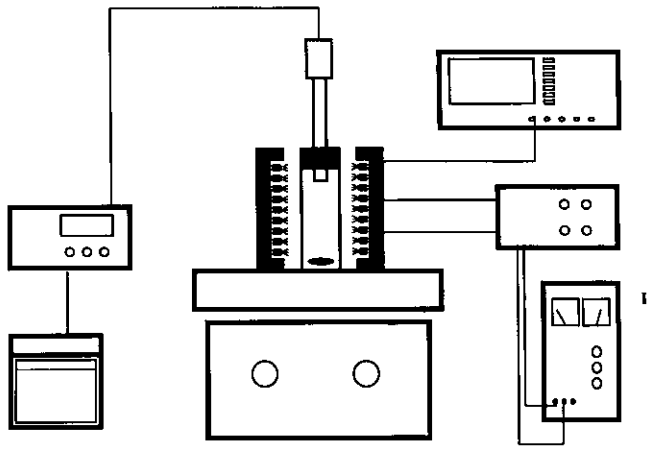

Fig. 1. Schematic representation of the SOPR measurement system.
A: the specially designed illumination couvettes, B: LED panel, C: DO probe, D: magnetic stirrer, E: DO transmitter, F: recorder, G: digital oscilloscope, H: frequency modulator. I: power supply.

These cells were transferred to a specially designed illumination cuvettes with a magnetic spin bar. The cuvette was kept in the dark covered with aluminum foil until the dissolved oxygen tension reached an exhausted level. When the dissolved oxygen was exhausted, specific oxygen production rates (SOPRs) were measured with a dissolved oxygen (DO) electrode (model 023IP15-010BGV12, Phoenix, Huston, U.S.A.). The schematic diagram of the apparatus was described in Fig. 1. The DO level was recorded by a recorder (model 4156, Yokogawa, Tokyo, Japan) after the LED unit was turned on. The amount of photosynthetically produced oxygen could be calculated from the slope of DO profiles as a function of cell concentration, frequency and duty cycle.

Cell concentration and average cell volume were measured by Coulter Counter (model Z2, Coulter Electronics, Inc., Hialeah, U.S.A.).

RESULTS AND DISCUSSION

Performance of the Frequency Modulator

Flashing light is a modulated light that consisted of light and dark cycles. Duty cycle is defined as $t_f/(t_f+t_d)\times 100$ (%), where t_f is the time of each light cycles (flashes) and t_d is the duration of a dark period. The flashing frequency is defined as the inverse of a cycle, $(t_f+t_d)^{-1}$ (Hz).

The frequency modulator was designed and made in order to provide switching powers to LEDs at variable frequencies and duty cycles. This device was based on an LM555C timing chip and an IRF640 MOSFET. The square wave for flashing LEDs is achieved by using the LM555C chip. A MOSFET acts like a switch that has a resistance level of 0.18 ohm. The device successfully

Table 1. Flashing light with various frequency and duty cycle.

Frequency (Hz)	Duty (%)	Period (μs)	Pulse (μs)	Off (μs)
10 k	10	100	10	90
10 k	20	100	20	80
10 k	30	100	30	70
10 k	40	100	40	60
10 k	50	100	50	50
20 k	10	50	5	45
20 k	20	50	10	40
20 k	30	50	15	35
20 k	40	50	20	30
20 k	50	50	25	25
50 k	10	20	2	18
50 k	20	20	4	16
50 k	30	20	6	14
50 k	40	20	8	12
50 k	50	20	10	10

provided a wide frequency range (10–50 kHz) with various duty cycles (10–50%), which are listed in Table 1. These frequencies have very short flashing times (t_f=2–50 μs), which corresponded to the time constant of light adsorption in photosynthesis. Figure 2 shows the actual frequency waveform by the frequency modulator, which is close enough to a square waveform that is suitable for the purpose of this experiment. The noise shown during the off time (dark time) was lower than the minimum forward voltage for lighting the LEDs and it had no effect on actual flashing.

Flashing Light Effect on Specific Oxygen Production Rates

There are two ways to compare flashing light effect with continuous light. The most common way is to chop the continuous light to obtain flashing light. The flashing light generated by this way has the same peak intensity as the continuous light (Fig. 3A). However, in this case, the rate of photosynthesis under flashing light cannot be greater than the rate under continuous light [10]. Since the peak intensity of flashing light is the same as that of the continuous light, the light penetration depth with flashing light will be the same as that with continuous light [14]. As a result, there will be no enhancement in biomass productivity with this type of flashing light, although the light utilization efficiency may be increased by this type of flashing light. This scheme is not suitable for high-density algal cultures, as the peak light intensity of flashing light is equal to the continuous light, and thus, the total light energy input (integral under the curve in Fig. 3A) of flashing light is lower than that of the continuous light, resulting in decreased total photosynthesis and biomass productivity.

The second method that was used in this study is to flash the light to have an equal average intensity as the continuous light (Fig. 3B). By supplying this type of

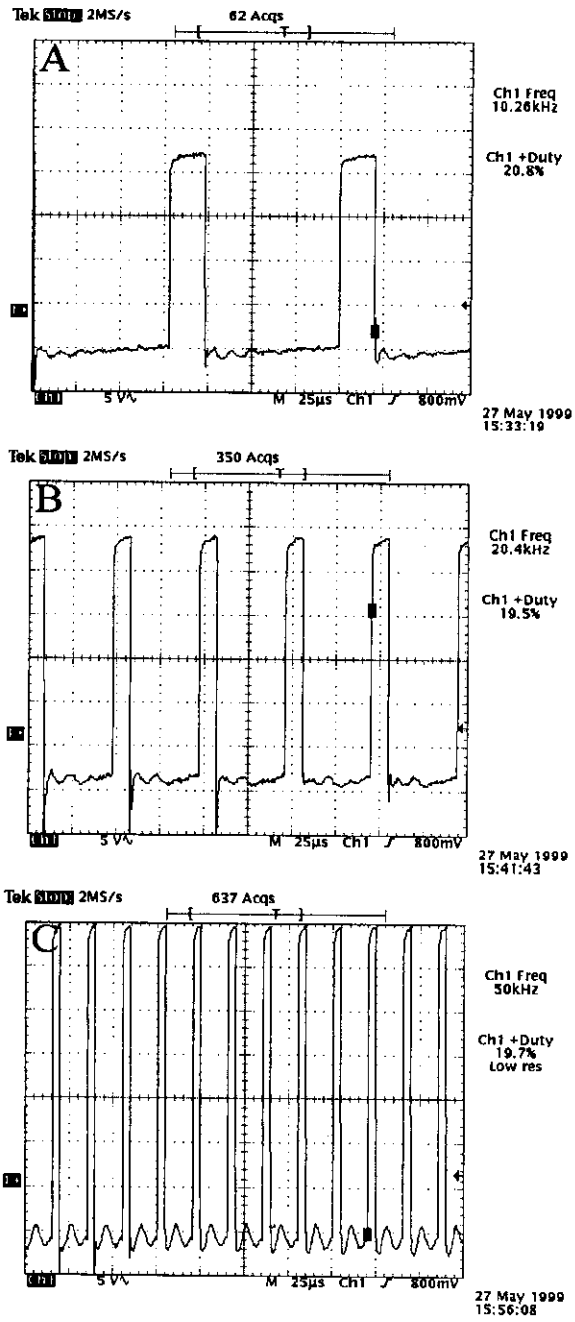

Fig. 2. Voltage output from the frequency modulator.
A: output at a constant flashing frequency of 10 kHz and a constant duty cycle of 20%, B: flashing with 20 kHz and 20%, C: flashing modulation at 50 kHz and 20%.

flashing light, 10 times greater instantaneous PPF can be delivered at 10% duty cycle. Higher PPF will help the photons penetrate deeper, and thus, increase photic zone by minimizing mutual shading [14]. Use of the second scheme of flashing light can increase the overall photosynthetic efficiency, particularly in high-density cultures. Conclusively, flashing light with an equal average intensity can serve as a

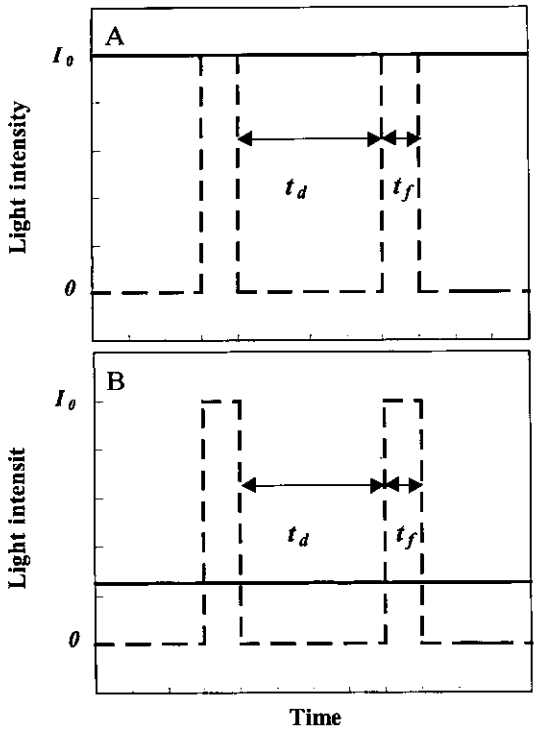

Fig. 3. Schematic diagram represents the two different ways to compare flashing lights (dotted line) and a continuous light (solid line).
t_d: dark time, t_f: flashing time. A: a flashing light and a continuous light with the same peak intensity; B: a flashing light and a continuous light with an equal average intensity.

solution to overcome the mutual shading in high-density algal cultures. The entire experiments were performed in the dark in order to elucidate the effect of flashing light by blocking ambient light.

Figure 4 shows the normalized effect of flashing frequencies on specific oxygen production rates. The cell concentrations used in this experiment were about 1.4×10^6, 1.9×10^7, and 1.1×10^8 cells/ml. All the data points were the average of 5 separate measurements with error bars of the standard deviation. The measured SOPR was in the range of 30–120 fmol/(cell · hr), which was also a function of the cell growth stage, light condition and cell density. The cell size distribution was shifted to a smaller average cell size after DO measurement (data not shown), however, the shift was small and the effect of the changes in the cell size was neglected. The flashing light effect was clearly observed especially at higher cell densities. For the reason discussed above, the effect of flashing light at lower cell concentrations (1.4×10^6 cells/ml, ● in Fig. 4) was not observed. In this lower concentration, the incident light can pass through well over 10 cm [14] and thus there is no mutual shading. In other words, all the cells in the cuvette can see the light in this concentration. On the other hand, an existence of the dark period will shorten the exposing time for each cell

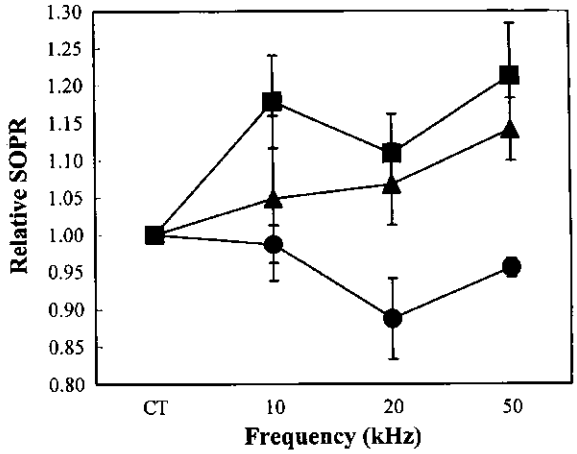

Fig. 4. The relative SOPRs under flashing lights with various frequencies (10, 20 and 50 kHz) and under a continuous light.
Cell concentrations: 1.4×10^6 cells/ml (-●-); 1.9×10^7 cells/ml (-■-); 1.1×10^8 cells/ml (-▲-).

to the light, resulting in a decrease in SOPRs. Also, the photosynthesis of microalgae may be photoinhibited by the enhanced instantaneous PPF [26]. At a higher cell concentration of 1.9×10^7 cells/ml (Fig. 4), where the light penetration depth has decreased down to a few centimeters, there are quite a number of cells which cannot see the light. As can be seen in Fig. 4, flashing lights with higher instantaneous PPF will help the light penetrate deeper into the cultures, and thus, reducing the number of cells in the dark zone. The increased light penetration depth and the decreased mutual shading would increase the volume of a photic zone, which would increase the portion of algal cells that could see enough light. This was a key of the flashing light effect. An increased photic zone helped more cells undergo photosynthesis and thus the measured SOPRs were increased about 10 to 25%. However, even higher cell concentration of 1.1×10^8 cells/ml (Fig. 4) gave a different picture. At this concentration, the light penetration depth would be shorter than 1 cm [14] and the increased instantaneous PPF would not help the photons much in penetrating deeper into the culture. As a result, the enhancement by the flashing light in higher cell density was smaller (5–10%) than that in a medium density (1.9×10^7 cells/ml). One interesting observation was that the flashing frequency (at least in the range of 10–50 kHz) was not a critical factor in obtaining the benefits from flashing light. However, previous works showed that the flashing frequency should be higher than 1 kHz [21].

Figure 5 shows the effect of the duty cycle of various frequencies of flashing light (10–50 kHz). Duty cycles of 10 to 50% were tested to find out their effect on the SOPR. Among tested duty cycles, the degree of enhancement by flashing light was relatively constant (5–10%). An interaction between duty cycle and flashing frequency was found. As the duty cycle increased, the difference between SOPRs

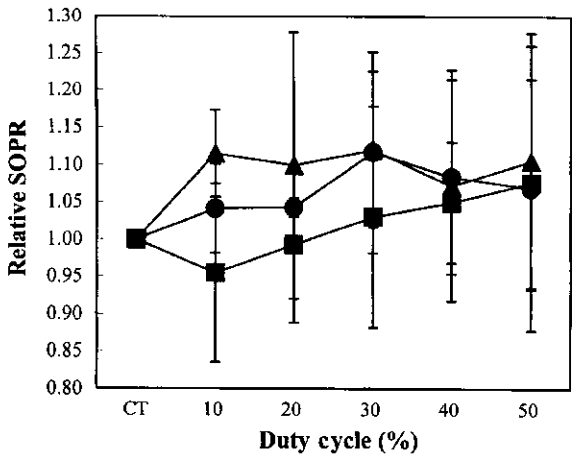

Fig. 5. The relative SOPRs under flashing lights with various duty cycles (10- 50%) and the continuous light.
Flashing frequencies: 10 kHz (-●-); 20 kHz (-■-); 50 kHz (-▲-).

under different frequencies decreased. This seems to be related to the time constant of photosynthesis and suggests that the flash duration (t_f) could be an important factor in optimizing the flashing light effect. An increased SOPR was observed under longer flash durations. As shown in Fig. 5, frequency was an important parameter in the low duty cycle of 10%. However, the flashing frequency had almost no effect in the high duty cycle of 50%. In general, the duration of flashes rather than the frequency of flashing mainly affected the measured SOPR. Also, the effect of duty cycle of flashing light on SOPR was clearer at high-density algal cultures, whose results implied the increase in photosynthesis efficiency (or overall light utilization efficiency) under the flashing light.

The Time Constants of Photosynthesis and Flashing Light Effect

The application of flashing light was based on the theoretical rate of light and dark reaction of photosynthesis. Photosynthesis is hypothesized as a discontinuous, linear, four-step process [11]. The existence of a longer dark period between short flashes of the light can increase the efficiency of photosynthesis, especially for the high intensity light. Emerson and Arnold [2] performed the first experiment in regards to the application of flashing light in an algal culture in 1932. In their work, 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 could be obtained with repetition rates of 100 per second, even though the cell only received light for 1/1000 of the time at a flash duration of 10 μs. Other studies were also reported on the effect of the various frequencies and duty cycles on the oxygen production rates and biomass production in algal cultures [13, 19, 22]. However, all these works used rather low frequencies (mostly 0.5- 1,000 Hz) and our previous work showed that the flashing light with the frequencies less than 1 kHz did not lessen the light-limiting problem in high-density cultures [21]. The effect of ultra-high frequency (up to 50 kHz frequency) with various duty cycles was not examined in previous reports. Besides, there was no report on the effect of duty cycles at a constant flashing frequency. According to the results of this study, the flashing light with 30- 50% duty cycle was effective for enhancing SOPR in high-density cultures. The time constants of photosynthesis are a supporting evidence of the results obtained in this study. The flashing time of 10- 90 μs corresponds to the light adsorption time in photosynthesis, which is in the range between 1 fs and 150 μs [4]. The difference in SOPR with different duty cycles could be interpreted as the difference of light intensity among different flashing times. In other words, if flashing time is long enough to be adsorbed by light reactions of photosynthesis, instantaneously higher light intensity could enhance the SOPR.

Mixing can also simulate the flashing light effect [12]. In this work, mixing was obtained by a magnetic stirrer in a specially designed illumination cuvette. Thus, the results reported here may be a synergistic combination of flashing light and mixing. Actually, there was a small difference between SOPRs obtained under different mixing speeds (data not shown). However, more experimental evidences are needed to clarify the synergistic effect.

In conclusion, flashing light does enhance SOPR, particularly at high-density algal cultures. An increased instantaneous PPF under flashing light makes incident photons penetrate deeper and decrease the degree of mutual shading. The results in this study will contribute to algal biotechnology, whose widespread use was hampered by light-limitation. For economical point of view, the application of flashing light to high-density algal cultures for production of biomass [7, 12, 17, 20], production of natural products [6, 30], and CO_2 fixation [8, 25, 28] would be beneficial because of the efficient usage of power for light supplement.

CONCLUSIONS

In order to test the effect of SOPR on flashing light, frequency modulator was designed and constructed. The device successfully provided wide range of frequencies and various duty cycles. Flashing light obtained by this modulator and LEDs enhanced photosynthetic oxygen production rates by increasing the light penetration depth, particularly at high-density algal cultures. The SOPRs under flashing lights were 5 to 25% higher than those under the continuous light with an equal average intensity (the same amount of photons). The increase in SOPR was higher at frequencies of 20 to 50 kHz flashing light with 30 to 50%

duty cycles, which was a clear indication of flashing light effect especially in high-density algal cultures.

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