Evaluation of the Effects of the Blinking Cycle and Duty Ratio of Red and Blue Light Emitting Diodes on the Photosynthetic Rate of Euglena

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ABSTRACT

This study is designed to clarify the optimum optical irradiation conditions for efficient cultivation and increased crop yields of Euglena gracilis. Specifically, we studied the light conditions (light blinking cycle, blinking light duty ratio, light quantity, etc.) to determine the optimum values for promoting efficient light usage for photosynthesis (energy efficiency) by measuring the photosynthesis rate while irradiating Euglena with light emitting diode (LED) blinking light.

We determined that the light conditions listed below produce the highest light energy usage efficiency.

1. Set the blinking cycle of the LED blinking light to the Optimum cycle area.
2. If the blinking cycle is set to a short period (e.g.: below $1.0 \times 10^{-3}$ s), the recommended range for the blinking duty ratio is 10-100%.

Key words: Blinking light, Euglena, Light emitting diode (LED), Photosynthetic rate, Red and blue LEDs

1. Introduction

In an effort to develop a highly efficient optical source device to increase the photosynthesis rate in Euglena, we have conducted various fundamental investigations, examining, among other things, the relationship between LED blinking lights and light cycles on photosynthesis rates.

Furthermore, we reported on the blinking cycle and light intensity conditions (as measured by the photosynthetic photon flux density (PPFD) value) that are suitable for Euglena (Ohashi et al., 2011).

However, in the previous report (Ohashi et al., 2011), we fixed the light quantity at PPFD = 35 $\mu$mol·m$^{-2}$·s$^{-1}$ and then measured the effect of varying the light blinking cycle values on the photosynthesis rate. Because only a single PPFD value was used, the effect of the light quantity on the photosynthesis rate was not elucidated. Evaluation of the importance of light quantity on the photosynthesis rate would allow us to assess whether there is a need to control the blinking cycle and the light quantity independently when designing an irradiation device for Euglena.

First of all, with the objective of elucidating the influence of light quantity on the "light blinking cycle-photosynthesis curve," we measured the photosynthesis rate of Euglena...
illuminated with an LED blinking light while systematically varying the light blinking cycle under several different light quantity conditions.

Furthermore, in the previous report (Ohashi et al., 2011), the blinking duty ratio was fixed to 50%. Thus, the influence of varying the blinking duty ratio on the photosynthesis rate was not assessed. In this study, we measured the effect of varying the light blinking duty ratio on the photosynthesis rate in order to determine the conditions for optimum energy efficiency.

Based on the condition of red-only monochromatic light irradiation (hereinafter, “red monochromatic irradiation”), we were able to clarify, from the measured results, the effect of varying the light quality conditions - changes in the light blinking cycle, the blinking duty ratio, and the light quantity - on the photosynthesis rate.

We feel that we have investigated nearly all the relevant conditions related to light quality that impact the photosynthesis rate.

Therefore, in search of methods to further enhance the light energy usage efficiency for photosynthesis, we shifted our focus to include conditions other than “red monochromatic irradiation” and consulted the literature.

The previous reports enumerated below elucidate the conditions for light quality that improve the photosynthesis rate for Euglena and other organisms that use photosynthesis.

(1) The photosynthesis rate improves on the basis of the Emerson effect (Emerson and Rabinowitch, 1960) when Euglena is exposed to continuous light from blue and red LEDs, where the R/B ratio (ratio of the photon numbers of red and blue light) = 9 (Matsumoto et al., 2006, 2009).

(2) The quantum yield of photosynthesis is maximized when Euglena is exposed to light with a PPFD of 30 μmol·m⁻²·s⁻¹ (Matsumoto et al., 2006).

(3) The growth ratio improves by 25% when lettuce is exposed to blinking light with a duty ratio (lighting span / blinking cycle) of 33% (Mori et al., 2002).

(4) It is reported that when lettuce is exposed to a blinking light with an LED blinking cycle of 4.0 × 10⁻⁴ s, the photosynthesis rate increases by 23% when compared to the result obtained with the use of continuous light (Mori et al., 2002).

Seeking a synergistic effect, we expect to further improve the photosynthesis rate by exposing Euglena to light conditions that incorporate all of the parameters mentioned above. There is no case study available in which Euglena has been exposed to such light conditions.

The objective of this study is to determine the light conditions that achieve a high crop yield specifically focusing on the effect of blinking cycle, blinking duty ratio, and wavelength of light on the photosynthesis rate.

2. Materials and Methods

2.1. Organisms and Culture

Studies were conducted using Euglena gracilis Z. For culturing Euglena, we used the method previously described by Ohashi et al. (2011).

2.2. Specimen preparation

Specimen preparation and measurement procedures were conducted as previously described by Ohashi et al. (2011).

Briefly, the solution was placed inside the measurement chamber with a liquid phase oxygen density indicator (OXY-1, Hansatech) and a Clark oxygen electrode.

2.3. LED light source specifications

The specifications of the LED lighting for experiments performed under monochrome red LED light, for the LED elements and the control methods for the LED optical sources have been described previously by Ohashi et al. (2011). Methods for establishing the timing wave of the blinking light and preparing the PPFD setup value have been described previously by Ohashi et al. (2011).

Moreover, for experiments performed under a mixture of blue and red LED lights, we prepared an LED optical source unit having the following specifications:

(1) The central wavelengths of the irradiated light (λ) used were 625 nm (red2) and 470 nm (blue) (Spectroscopic feature, Fig. 1).

Because there is a slight variation between the wavelengths of the two red LEDs used in this report (λ1 = 625 nm) and in the previous report (λ2 = 660 nm, Ohashi et al., 2011), these LEDs will henceforth be denoted as “Red1” and “Red2,” respectively.

(2) LED devices used: A total of 540 LED lights, namely OSHR5161 (red2) and OSUB5161 (blue) manufactured by OptoSupply Ltd., were installed on a printed circuit board for illumination. To uniformly blend the light, blue and “red2” LEDs were arranged alternately as shown in Fig. 2(a).
Furthermore, to uniformly diffuse the light, a diffuser plate (LSD80PC10-F12, Optical solutions Ltd.) was set on the front of the LED.

(3) The method proposed by Ohashi et al. (2011) was adopted as a control method for PPFD, blinking cycle, blinking duty ratio, and blinking operation characterization.

2.4 Photosynthesis rate measuring procedure

The system used to measure the rate of photosynthesis consisted of a Clark-type oxygen electrode (OXY-1; Hansatech Co.) combined with the LED light source unit (Fig. 2(b)). To correct for errors resulting from external light, a light interception cover encircling the chamber was used. The blinking light control technique for the LED optical source unit, measurement method for liquid oxygen density, calculation method for photosynthesis rate, measurement of PPFD value, and conditions of the surrounding environment have been described previously by Ohashi et al. (2011).

The calculation method we employed converted the photosynthetic rate into a value per number of cells.

Because the length of the light path of the measurement chamber was short (~1.0 cm) and the density of *Euglena* was low (~1.0 × 10^5 cell·ml^1^) our method for calculating the attenuation of light penetrating through the sample, carried out according to a previous report (Masuda et al., 2006), produced a very small value of 0.76%. Therefore, we ignored this parameter in this study.

2.5 Experimental methodology

2.5.1 Measuring the effect of the blinking cycle on the photosynthesis rate under red1 LED light

The blinking duty ratio was maintained at 50%. The lighting was turned on at the blinking cycle start time and when the PPFD value was twice (inverse multiplication of the blinking duty ratio) the set PPFD value (average time value). The light was turned off every time the transit time reached 50% of the cycle. In this way, the hourly average value of the PPFD was established as the set value. For convenience, the set value of the PPFD hourly average value is hereafter referred to as the PPFD set value.

The photosynthesis rate of the sample was measured upon exposure to blinking light with each of the five PPFD set values: 35, 64, 118, 218, and 400 μmol·m^−2·s^−1^. The blinking cycle was varied from 1.0 × 10^−6^ s to 10 s and the blinking duty ratio was maintained at 50%.

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*Fig. 1* Spectral distribution of blue and red2 LED lights. The dashed and solid lines represent the red2 and blue LED lights, respectively.

*Fig. 2(a)* Blue and red2 LED lights.

*Fig. 2(b)* The equipment used to measure the photosynthesis rate.
2.5.2. Measuring the effect of the blinking duty ratio under red1 LED light

We measured the effect of varying the blinking duty ratio of the LED light on the photosynthesis rate. Motivated by the previous report that showed that the photosynthesis and growth rates of lettuce increased when the blinking cycle was set at $4.0 \times 10^{-4}$ s (Mori et al., 2002), we used the same blinking cycle to test whether this is also true for *Euglena*.

The PPFD set value of each blinking duty ratio was maintained at $35 \mu$mol·m$^{-2}$·s$^{-1}$, and the blinking duty ratio was varied from 100% to 10%. For example, when the blinking duty ratio was 10%, the lighting was set to a PPFD value 10 times (inverse multiplication of the blinking duty ratio) of the PPFD set value at the blinking cycle start time. Then, the light was turned when the transit time reached 10% of the cycle (setup value of the blinking duty ratio).

This method was repeated continuously. In this manner, the specimen was exposed to blinking light by maintaining a constant PPFD set value (time average value).

In addition, we set the blinking cycle at $1.0 \times 10^{-6}$ s and $1.0 \times 10^{-2}$ s, then changed the blinking duty ratio, and measured the subsequent photosynthesis rate.

2.5.3. Measuring the effect of the blinking cycle on the photosynthesis rate under blue and red2 LED lights

The following conditions were kept constant: PPFD value $= 30 \mu$mol·m$^{-2}$·s$^{-1}$, R/B ratio $= 9$ (red2 $= 27 \mu$mol·m$^{-2}$·s$^{-1}$ and blue $= 3 \mu$mol·m$^{-2}$·s$^{-1}$). Hereafter, this irradiation condition is denoted as "blue-red2 blended irradiation"), and blinking duty ratio $= 33\%$. The photosynthesis rate was measured when *Euglena* was exposed simultaneously to blue-red2 blended irradiating blinking light under varying blinking light cycles.

In addition, as a control experiment, we performed the same measurement under the "red2 monochromatic irradiation" condition as well.

2.5.4. Measuring the effect on photosynthesis rate of the blinking duty ratio under “Blue-red2 blended irradiation”

The following conditions were kept constant: "Blue-red2 blended irradiation," and blinking cycle $= 4.0 \times 10^{-4}$ s. *Euglena* was exposed simultaneously to "Blue-red2 blended irradiation" blinking light under varying blinking duty ratios.

### 3. Results

#### 3.1. Effect of the blinking cycle on the photosynthesis rate under red1 LED light

The results showing the effect of varying blinking cycles on the rate of photosynthesis are plotted for PPFD set values of 35, 64, 118, 218, and $400 \mu$mol·m$^{-2}$·s$^{-1}$ (Fig. 3). For example, the curve for the PPFD set value of $400 \mu$mol·m$^{-2}$·s$^{-1}$ shows that the photosynthesis rate is remarkably stable in the blinking cycle range of $1.0 \times 10^{-6}$ s to $1.0 \times 10^{-2}$ s (Point B). However, the photosynthesis rate decreases if the blinking cycle time exceeds $1.0 \times 10^{-2}$ s, which we call a changing point.

On the other hand, the changing point cycle (Point A) at PPFD set value of $35 \mu$mol·m$^{-2}$·s$^{-1}$ occurs at $5.0 \times 10^{-2}$ s (Fig. 3), which is a longer time period than for Point B. This trend is similar for the curves for every other PPFD set value conditions. With an increase in PPFD set value, the changing point (Points C, D, and E) occurs at shorter times (refer to the dotted line in Fig. 3).

#### 3.2. Effect of the blinking duty ratio on the rate of photosynthesis under red1 LED light

In a comparison of the photosynthesis rates for all the blinking cycles, the curves showing the photosynthesis rates for cycles at $1.0 \times 10^{-3}$ s and $4.0 \times 10^{-4}$ s are nearly overlapping. Furthermore, the photosynthesis rates remain nearly constant even though the blinking duty ratio is varied from 100% to 10% (Fig. 4).

Fig. 3 Effects of the cycle time of blinking red1 LED lights on oxygen emission rates at several PPFD values and a duty ratio of 50%
- $400 \mu$mol·m$^{-2}$·s$^{-1}$
- $218 \mu$mol·m$^{-2}$·s$^{-1}$
- $118 \mu$mol·m$^{-2}$·s$^{-1}$
- $64 \mu$mol·m$^{-2}$·s$^{-1}$
- $35 \mu$mol·m$^{-2}$·s$^{-1}$
On the other hand, when the blinking cycle is set to $1.0 \times 10^{-1} \text{s}$, the photosynthesis rate tends to decline with a decreasing duty ratio. When the blinking duty ratio is 10% (Point M), the decrease in the photosynthesis rate is extreme (Fig. 4).

An additional point, the "Duty ratio = 100%" label on the left side of the figure signifies continuous exposure to light.

3.3. Effect of the blinking cycle on the photosynthesis rate under "Blue-red2 blended irradiation"

When the blinking cycle range is between $1.0 \times 10^{-6} \text{s}$ and $5.0 \times 10^{-3} \text{s}$, the photosynthesis rate as measured via oxygen evolution reaches $12 \text{nmol} \text{O}_2 \cdot 10^4 \text{cell}^{-1} \cdot \text{ml}^{-1} \cdot \text{min}^{-1}$ and remains nearly constant across the range (Fig. 5). The photosynthesis rate declines as the blinking cycle exceeds $5.0 \times 10^{-3} \text{s}$ (Point F). This point is the changing point. The photosynthesis rate declines sharply when the blinking cycle is within the range $1.0 \times 10^{-1}$ to $1.0 \text{s}$, as indicated by the arrow in Fig. 5.

Two curves were overlapped significantly in the region where the blinking cycle was $1.0 \times 10^{-6} \text{s} - 3.0 \times 10^{-1} \text{s}$ (Point H). The curve for "red2 monochromatic irradiation" was the control experiment. The photosynthesis rate was essentially the same. However, when the blinking cycle period was set to values longer than that at Point H, the photosynthesis rate under the "red2 monochromatic irradiation" declined sharply as compared to the rate obtained under "blue-red2 blended irradiation."

In addition, it was reported previously that the photosynthesis rate is optimal when the blinking cycle is $4.0 \times 10^{-4} \text{s}$, as shown in Fig. 5, Point G (Mori et al., 2002). However, our results indicate that the photosynthesis rate at this blinking cycle value is nearly identical to that observed under the other cycle conditions and does not represent an optimum condition (Fig. 5).

3.4. Effect of the blinking duty ratio on the photosynthesis rate under "Blue-red2 blended irradiation"

When the blinking duty ratio was changed from 10% to 100%, the photosynthesis rate reaches $12 \text{nmol} \text{O}_2 \cdot 10^4 \text{cell}^{-1} \cdot \text{ml}^{-1} \cdot \text{min}^{-1}$ and remains nearly constant (Fig. 6). It has been reported that the growth rate of lettuce is optimal when the blinking duty ratio is 33%, as shown in Fig. 6, Point J (Mori et al., 2002).

However, our results shown that this photosynthesis rate...
is almost identical to that observed for the other values of the blinking duty ratio and is not an optimum value (Fig. 6).

4. Discussion

While a previous study reported that a blinking cycle of 4.0 × 10^4 s increased the photosynthesis rate of lettuce by 23% as compared to the rate obtained under continuous light (Mori et al., 2002) and that the growth rate of lettuce increased by 25% when the blinking duty ratio was 33%, we did not observe an increase in the photosynthesis rate of *Euglena* when we used those parameters in this study (Fig. 5 and 6).

This disparity is most likely due to the difference in the types of organisms and the potential dissimilarities in their photosynthetic mechanisms. When duty ratio is set to 10% the photoperiod of lighting becomes very short as 10%. Owing to this short photoperiod, the PPFD value of the photoperiod can be as high as 500 μmol·m⁻²·s⁻¹. Since a PPFD value of 500 μmol·m⁻²·s⁻¹ exceeds the saturation point of lettuce, there is a possibility that the lettuce is stressed by this excess light (Mori et al., 2002). The photosynthetic process of *Euglena* differs from that of higher plants in several ways. (For a full discussion, see Ohashi et al., 2011.)

These fundamental differences in the biochemical mechanisms between higher plants such as lettuce and the organism used in the present work, *Euglena*, are the likely cause for the conflict in results observed in this study and in the previous work (Mori et al., 2002).

Moreover, when the results of “red2 monochromatic irradiation” and “blue-red2 blended irradiation” were compared, it was observed that the photosynthesis rate for “red2 monochromatic irradiation” declined when the blinking cycle was longer than approximately 3.0 × 10^4 s (Point H, Fig. 5). In particular, when we compared the photosynthesis rate under the two types of irradiation when the blinking cycle was 10 s, we observed that the photosynthesis rate for “red2 monochromatic irradiation” declined to nearly half of the value obtained for “blue-red2 blended irradiation.” Below are some possible reasons for the decline in photosynthesis rate.

The majority of photons used for photosynthesis are supplemented at the light-harvesting complex (LHC) of thylakoid membrane (Yamazaki, 2011). At the LHC, photons are supplemented with pigments such as chlorophyll a, chlorophyll b, carotenoid, etc. (Lincoln and Eduardo, 2004; Yamazaki, 2011).

Blue light is easily supplemented with any pigment described above. However, red light is not easily supplemented with carotenoid, due to its different emission wavelength (Yamazaki, 2011). Since blue light is more easily supplemented with carotenoid for “Blue-red2 blended irradiation” than is “Red2 monochromatic irradiation,” it is believed that more photons can be used for photosynthesis. The above reason explains the occurrence of the “decline phenomenon” where the photosynthesis rate for “red2 monochromatic irradiation” declined in comparison to “blue-red2 blended irradiation.”

It seems that the above-mentioned “decline phenomena” may have occurred because the blinking cycle period was longer than that at Point H and thus, the rate controlling step of photosynthesis was altered toward areas which were deeply related to LHC. However, it is difficult to confirm the aforementioned inferences based only on these experimental findings.

Next, we will discuss the “light energy usage efficiency” pertaining to photosynthesis. The “photosynthesis PPFD efficiency” (photosynthesis-derived oxygen generating rate for each PPFD value of the irradiated light (Ohashi et al., 2011)) appears to be optimum when the “light energy usage efficiency” of photosynthesis is also at its peak under “red2 monochromatic irradiation” (single-wavelength). The reason for this is that photon energy is the same for every photon if the irradiated light consists of a single wavelength. Thus, there is a proportional relationship between the irradiated light energy and photon number. As a result, for “red2 monochromatic irradiation,” the “light energy usage efficiency” of photosynthesis will also be at its highest value, which also causes the “photosynthesis PPFD efficiency” to be at its peak.

However, in the case of “blue-red2 blended irradiation,” the wavelength of light, and hence the photon energy, is different for each color. Therefore, some cases arise where there is no proportional relationship between the irradiated light energy and photon number. Thus, it is not necessarily true that “light energy usage efficiency” will be at its peak under conditions where the “Photosynthesis PPFD efficiency” attains its peak. For “blue-red2 blended irradiation,” based on the aforementioned result, the “Photosynthesis PPFD efficiency” cannot determine “light energy usage efficiency.”

In Fig. 5, based on the above results, we found that in a cycle area for time periods shorter than that at Point H, the “red2 monochromatic irradiation” curve overlapped with the
"blue-red2 blended irradiation." The photosynthesis rate is essentially the same. In particular, the photosynthesis rate near Point G at the flat cycle area is remarkably high. Therefore, the photosynthesis rate is also at its highest in the flat cycle area. Hereafter, this cycle area will be referred to as the "optimum cycle area."

To conclude, regarding LED blinking irradiation conditions, it is best to select the following light quality conditions in order to have optimum "light energy usage efficiency." Choose the blinking cycle within the "optimum cycle area."

However, in the present study, we fixed the PPFD value of irradiated light at 30 μmol·m⁻²·s⁻¹ to perform the measurements. Therefore, different results may be obtained using different PPFD values.

5. Conclusions

We consider these results useful for industrial applications, such as the design of energy-efficient LED-based light sources for Euglena. For example, if limited energy (lighting power) input is available, maximizing the yield of Euglena with cultivation equipment can be achieved if the light quality conditions are controlled in the following manner.

(1) Set the blinking cycle of the LED blinking light to the "optimum cycle area."

(2) If the blinking cycle is set to a short period (e.g.: below 1.0 × 10⁻³ s), the recommended setting range for the blinking duty ratio is 10–100%.

These results are very useful for developing highly efficient cultivation equipment for Euglena in the future. Future experiments should explore the optimum wavelengths of irradiated light to contribute to the set of conditions established here for high energy efficiency.

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