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Vol. 8(47), pp. 3815-3820, 19 November, 2014
DOI: 10.5897/AJMR2014.6980
Article Number: B02F4E648992
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Effects of flashing light-emitting diode (LED) of several colors on the growth of the microalga *Isochrysis galbana*

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Received 23 June, 2014; Accepted 13 November, 2014

The growth rate of *Isochrysis galbana* was determined under flashing light conditions (104 µmol $m^{-2}s^{-1}$ light intensity, 10 kHz frequency, and 50% of the duty cycle) of several colors. The most suitable light for the growth of *I. galbana* was blue (dominant wavelength: 470 nm). After six days, the cell density under blue light was 34.0 × 10^5 cells mL^{-1}, and was 1.4, 1.6, 1.8,and 2.2 times higher than those under white, red, white non-flashing, and green light, respectively. The peak wavelengths of white and blue lights are nearly consistent with the absorbance maxima of major pigments, which are chlorophylls and xanthophylls, in *I. galbana*. White and blue light were considered to be most effective for *I. galbana* growth.

Key words: Algal growth, light conditions, light-emitting diode (LED), duty cycles, flash.

INTRODUCTION

Phytoplankton is valuable as a diet for cultured bivalve and shrimp larvae, and the haptophyte *Isochrysis galbana* is widely used in aquaculture (Kaplan et al., 1986; Sukenik and Wahnon, 1991; Saoudi-Helis et al., 1994; Phatarpekar et al., 2000; Sánchez et al., 2000). Mass quantities of this microalga are often cultured indoors to avoid the effects of external factors (Toba and Miyama, 1993a, b; Ueno, 2003). Stable algal cultures are difficult to achieve outdoors because of the influence of weather, low temperature, and contamination by other species. Optimal growth conditions such as nutrients, salinity and temperature have been investigated in previous studies (Kaplan et al., 1986; Toba and Fukayama, 1993a, b). Light conditions are also important factors for controlling indoor microalgae culture.

Although fluorescent lamps are still used as a common light source (Kaplan et al., 1986), light-emitting diodes (LEDs), which have become more affordable, have several advantages (Park and Lee, 2000). For example, LEDs use less electricity than fluorescent lamps and do

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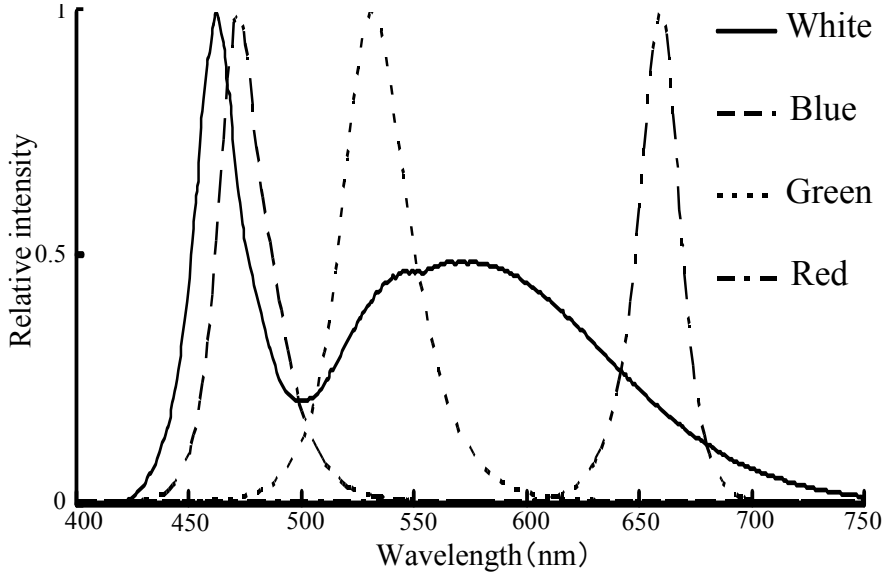

Figure 1. Spectral distributions of the four LED panels used in this study.

not cause the room temperature to rise (Ishikawa and Isowa, 2012). Since the rise in temperature caused by fluorescent lamps decreases the phytoplankton growth rate, LEDs may be a suitable light source for indoor algal culture. This study tested the growth of several phytoplankton species, including *Chaetocerosneo gracile*, *Chlorella pyrenoidosa*, *Heterocapsa circularisquama*, *Nannochloropsis* sp., *Pavlova lutheri*, *Skeletonema costatum* and *Spirulina platensis* under different-colored LEDs (Matthijs et al., 1996; Wang et al., 2007; Oh et al., 2008; Das et al., 2011; Ishikawa and Isowa, 2012). The use of LED lighting has also been reported to be difficult for the growth of the phytoplankton (Miguel et al., 2013). The goal of our study was to determine the optimal light conditions for the mass culture of *I.galbana* using different-colored LEDs as a light source. In our previous study, we investigated suitable flashing light conditions using white light (Yago et al., 2012).

Pigment profiles vary with different classes of phytoplankton (Jeffrey and Wright, 1997). The suitable light colors for phytoplankton growth are known to differ with species (Wang et al., 2007; Ravelonandro et al., 2008). To effectively produce commercially valuable substances in phytoplankton, growth tests using LEDs were conducted (Katsuda et al., 2004; Konishi et al., 2007). In this study, we determined the light colors that produce the highest growth rates of *I. galbana*.

MATERIALS AND METHODS

The basic experimental procedure is the same as in our previous report (Yago et al., 2012).

Experimental organisms and apparatus

I. galbana was obtained from the National Research Institute of Aquaculture, Fisheries Research Agency, Japan, and cultured in f/2 medium (Guillard and Ryther, 1962) at 20°C and 20 µmol photons m^{-2} s^{-1} on a 12:12 h light: dark cycle before experimental use. During experiments, the algae were grown in beakers, each containing 500 mL of f/2 medium. The experiments for each light color were repeated eight times with two beakers for each treatment. The beakers were held in a water bath at 20 ± 0.5°C. Magnetic stirrers kept cells in suspension for uniform illumination. Initial cell densities for each experiment were approximately 2.0 × 10^5 cells mL^{-1}. The experimental and control cultures were grown for 12 days, and cell densities were measured on days 2, 3, 4, 6, 8, 10 and 12.

Effect of the wavelength of flashing light on growth

Flashing or non-flashing light was provided by LED panels (INL-S305x302-WWWW; CCS Inc., Kyoto, Japan). Control cultures received non-flashing white light on a 12:12 h light : dark cycle at 104 µmol m^{-2} s^{-1} of photosynthetically available radiation, which provided a daily irradiance of 4.5 mol m^{-2} d^{-1} (104 µmol m^{-2} s^{-1} × (60×60 ×12) s). Flashing light conditions continued over 24 h, so instantaneous irradiances were reduced to half those of controls to equalize the daily irradiance. We prepared various LEDs with different light colors: white, blue, green and red (Figure 1). The peak wavelengths of each color are 460 and 560 (white), 470 (blue), 525 (green) and 660 nm (red). The flashing light irradiance (52µmol m^{-2} s^{-1}), frequency (10 kHz), and duty cycle (50% light and 50% dark; 52µmol m^{-2} s^{-1}× (60 × 60 ×24) sec) were regulated with a panel control device (ISC-101-4, CCS Inc., Kyoto, Japan). The flashing light pulse profile was rectangular. Irradiance was measured with a LI-COR LI-250 photometer (LI-COR Inc.,Lincoln, NE, USA).

Cell counts and pigment analysis

To compare the growth rates in different treatments and the control cultures, a 10-mL sample was collected from each culture vessel every t days and diluted 20 times with filtered seawater. Microalgae were counted using a Coulter Counter Multisizer II (Beckman Coulter Inc., Brea, CA, USA) with a 100-µm aperture tube.

Chlorophyll*a* (Chl. *a*) concentration was analyzed by the

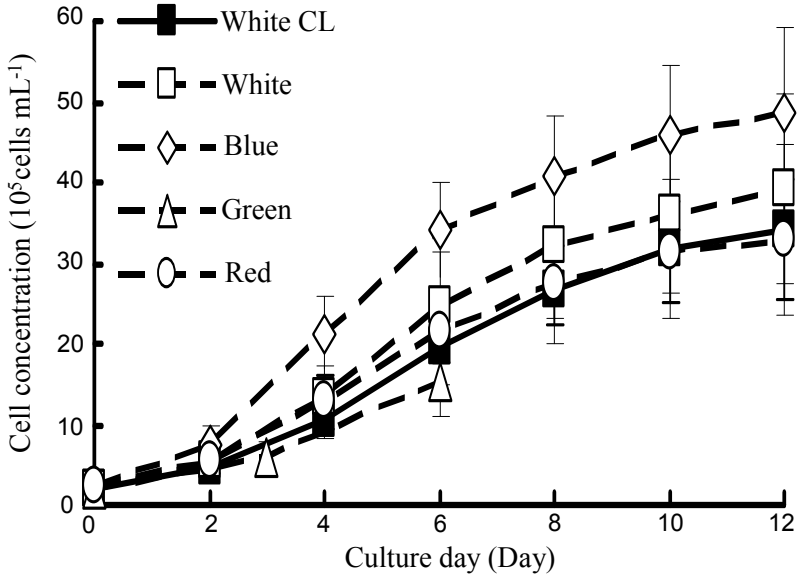

Figure 2. Relationships between culture day and cell concentration under five different light conditions. Points: means ± standard deviations (n = 16). CL: non-flashing light.

fluorometric method (Holm-Hansen et al., 1965). Every 2 days, the 1-mL sample was filtered through a glass fiber filter (47-mm Whatman GF/F filter; Whatman PLC, Springfield Mill, Maidstone, UK). Chl. *a* pigment were extracted from the filter for 24 h in 6 mL of N,N-dimethylformamide (Wako Chemicals, Kyoto, Japan). Chl. *a* concentrations were measured using a Turner Designs fluorometer (Model 10-005R; Sunnyvale, CA, USA).

The major xanthophyll pigments, fucoxanthin (Fuco) and diadinoxanthin (Diadino), were analyzed using high-performance liquid chromatography (HPLC). At the end of the experiment, the 10-mL sample was filtered through a glass fiber filter (25-mm Whatman GF/F filter, Whatman PLC, Springfield Mill). The pigments were extracted from the filter for 24 h in 1 mL methanol (Merck), and the extract was centrifuged at 10000 ×*g* for 10 min. After dilution of the supernatant to 80% with distilled water in an HPLC auto-sampler (Shimadzu, Kyoto, Japan), the pigments were analyzed using the methods of Zapata et al. (2000), with a slight modification whereby a guard column was attached between the injection valve and the analytical column.

Statistical methods

Significant tests of cell density and pigment concentrations were conducted by PSAW ver.17 software (IBM Corporation, Armonk, NY, USA). We used Mann-Whitney's U test of nonparametric measures for a comparison of the average of each group of cell densities, and the Tukey HSD test of parametric measures for a comparison of the average of each group of pigments.

RESULTS AND DISCUSSION

Effects of different colors on the growth of *I. galbana*

I. galbana grew in all experimental plots during the experimental period (Figure 2). On day 6, the cell density decreased in the order, blue light > white light > red light >white non-flashing light > green light (Figure 3). The cell density under blue light was 34.0 × 10^5 cells mL^{-1}, and was 1.4, 1.6, 1.8 and 2.2 times higher than those under white, red, white non-flashing and green light, respectively ($p<0.01$).

Although the suitable light colors for phytoplankton growth differ with species (Wang et al., 2007; Ravelonandro et al., 2008), blue light increased the growth of the diatoms *Biddulphia* sp. (Humphrey, 1983), *Cyclotella nana* (Wallen and Geen, 1971) and *Skeletonema costatum* (Oh et al., 2008), the dinoflagellate *Heterocapsa circularisquama* (Oh et al., 2008), and the green algae *Dunaliella tertiolecta* (Wallen and Geen, 1971) and *Nannochloropsis* sp. (Das et al., 2011). We observed a similar positive effect of blue light on the growth of *I. galbana*.

Major pigments in the haptophyte *I.galbana* include chlorophylls, such as Chl*a*, Chlc_1, Chlc_2 and xanthophylls, such as Fuco and Diadino (Figure 4). These pigments are also major pigments in diatoms (Lavaud et al., 2003).

Photosynthetic pigments are components of the light-harvesting complexes of phytoplankton. Light is trapped in pigments in the photosynthetic system (Matthijs et al., 1996), and it is important for photosynthesis and phytoplankton growth to match the wavelength of light and absorption bands of pigments. The absorbance maxima of Chl.*a* and Diadino are about 430 and 478 nm, respectively (Jeffrey et al., 1997) (Figure 4). In our study, the peak of blue and white light is 470 and 460 nm. The peaks of blue and white lights are nearly consistent with the absorbance maxima of major pigments in *I. galbana*

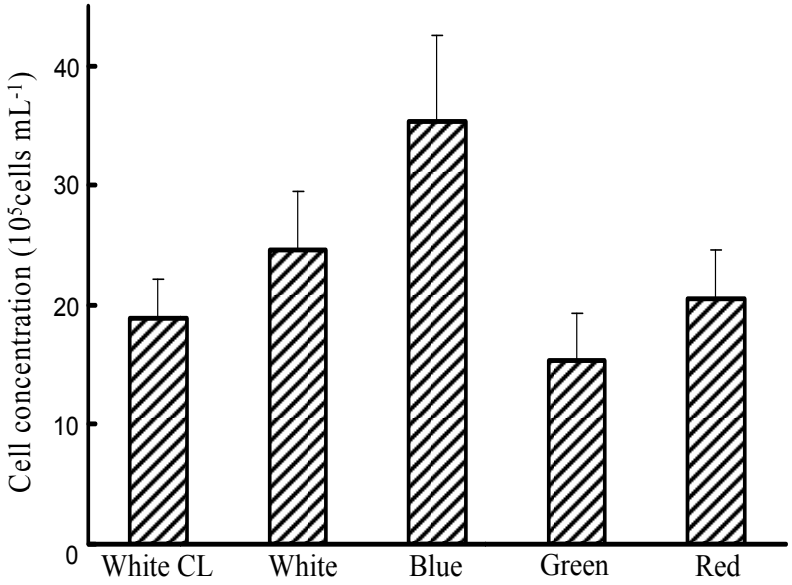

Figure 3. Cell concentrations of five different light conditions on the sixth day of testing. Values are means. Error bars indicate standard deviations (n = 16). CL: non-flashing light. Cell concentration under blue light was significantly higher than all other colors. Cell concentration under green light was significantly lower than blue, white and red light.

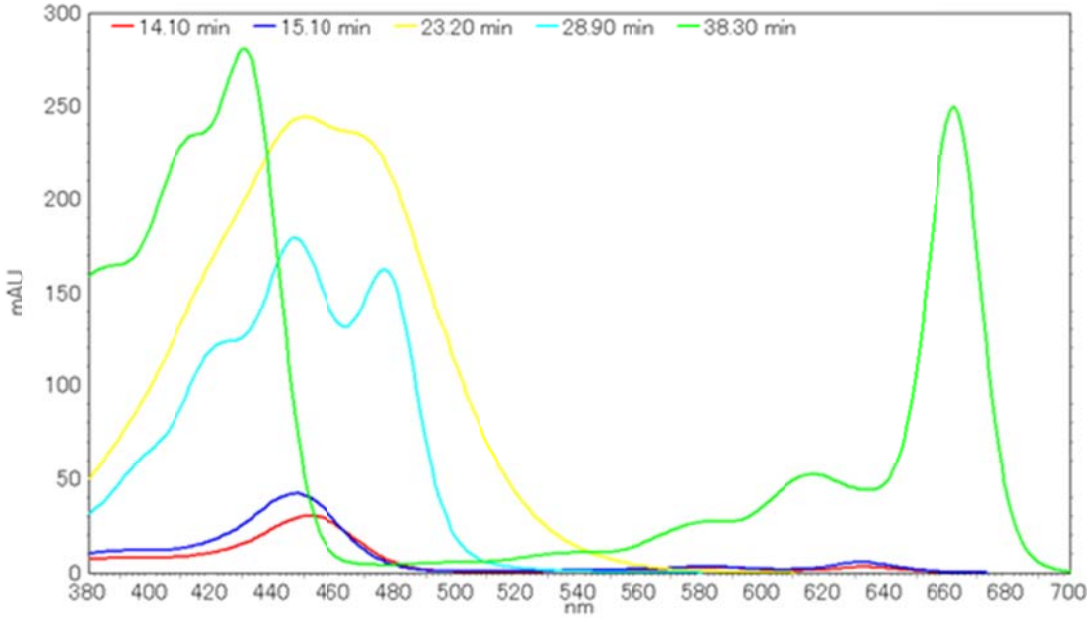

Figure 4. Spectrum of major pigments of *I. galbana*. Red line: chlorophyll c2; retention time for HPLC analysis was 14.1 min. Blue line: chlorophyll c1; retention time was 15.1 min. Yellow line: fucoxanthin; retention time was 23.2 min. Light blue line: diadinoxanthin; retention time was 28.9 min. Green line: chl *a*; retention time was 38.3 min.

and blue and white lights were effective for *I. galbana* photosynthesis. Although blue light has a single absorption peak, white light has a dual peak (Figure 1). In the white light spectrum, the peak area around 560 nm is approximately equal to the peak area around 460 nm. When light intensity is equivalent, the blue light intensity

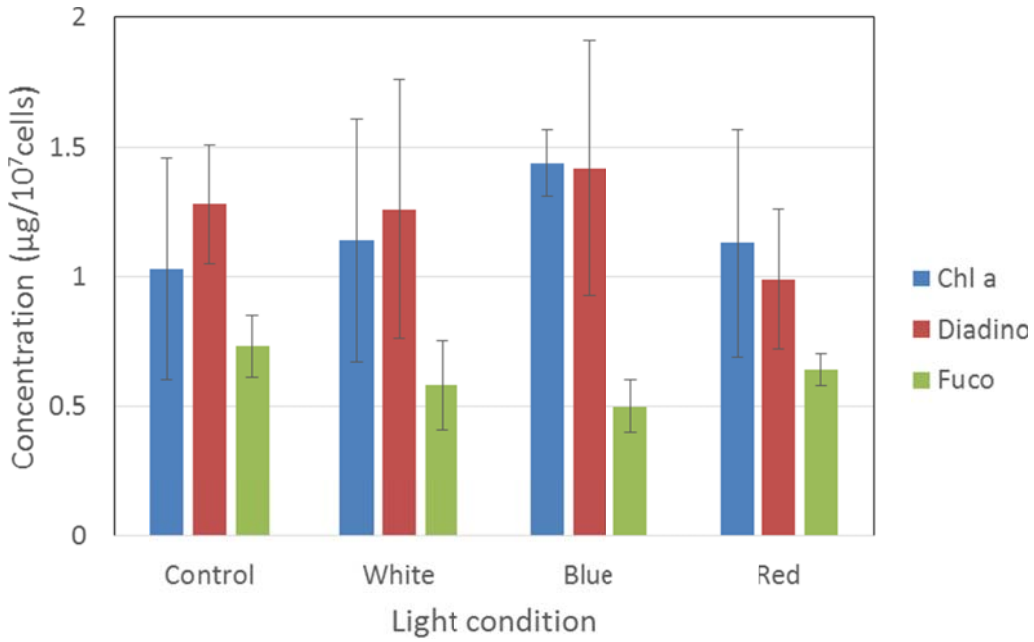

Figure 5. Cell pigment contents (10^{-7}µg $cell^{-1}$) of *I. galbana* under four different light conditions on the 12th day of testing. Chl.*a*: Chlorophyll *a*; Fuco: Fucoxanthin; Diadino: Diadinoxanthin. The values are means±standard deviation (n=4). Control: white continuous light, White: white flashing light, Blue: blue flashing light, Red: red flashing light. As the growth under green light was poor, pigment contents under green light were not measured. Chl*a* concentration under blue light was significantly higher than all other colors. Fuco and diadino were not detected with significant difference by light colors.

around 470 nm was equivalent to about twice that of white light around 460 nm. Therefore, we conjecture that growth under blue light would be higher than that under white light. Chl. *a* has two absorbance maxima (about 435 and 665 nm) (Jeffrey et al., 1997) (Figure 4). The peak of red light is 660 nm, and is nearly consistent with the secondary absorbance maxima of Chl. *a* (about 665 nm). Red light was also effective for photosynthesis of *I. galbana*. Except for cyanobacteria and red algae that possess phycobilin, the green region (500-600 nm) for all plants is of lower absorbance than the blue and red regions (Seki and Naganuma, 1996) (Figure 4). As the peak wavelength of green light (525 nm) does not coincide with the absorbance maxima of major pigments in *I. galbana* (about 430 to 478 and 665 nm), green light is not used for growth by the microalgae (Katsuda et al., 2004).

Effects of different colors on the pigment contents of *I. galbana*

Although Chl *a* concentration under blue light was higher than under other light conditions, there was not a clear difference between Fuco and Diadino concentrations under several light color emissions (Figure 5). In general, Chl. *a* and Fuco concentrations are thought to be higher under low light conditions to increase photosynthesis (Kebede and Ahlgren, 1996; Anning et al., 2000; Macintyre et al., 2002). However, variation in the fucoxanthin concentration was not consistent with Chl*a* concentrations in this study. The concentrations of Diadino and diatoxanthin are thought to be high under high light conditions to enhance a photoprotective effect, which relates to the diadinoxanthin cycle (Anning et al., 2000; Macintyre et al., 2002; Jeffrey and Mantotra, 1997). The variation of Chl. *a*, Fuco and Diadino concentrations under several light colors did not follow a clear trend. The light intensity of the midsummer sun, which can induce light injury is approximately 2,000 µmol m^{-2} s^{-1}, and is 20 times higher than the light intensity used in this study (104 µmol m^{-2} s^{-1}). It is probable that the light intensity in this study was not high enough to induce a photoprotective effect on the pigment profile of *I. galbana*. Therefore, the relationship between light color and each pigment concentration is still unclear.

In our previous study, we determined suitable light conditions for the growth of *I. galbana* under white flashing light (104 µmol $m^{-2}s^{-1}$ light intensity, 10-100 kHz frequencies and 40-80% of the duty cycle) (Yago et al., 2012). Under the same light regime, blue light was found to be particularly suitable for the growth of *I. galbana*. The elongation and leaf formation of the plant are influenced by light quality (Fukuda, 2008). In the future, we would

like to examine the influence of light quality on the growth of phytoplankton from a biochemical perspective.

Conflict of Interest

The author(s) have not declared any conflict of interests.

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