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Effect of high frequency of intermittent light on the growth and fatty acid profile of *Isochrysis galbana*

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ABSTRACT

The marine microalga *Isochrysis galbana* was cultured under different light regimes to examine the changes in growth and fatty acid profile. We have obtained preliminary results that *I. galbana* cultured under white intermittent light for 24 h day^{-1} shows better growth than continuous white light with light/dark (L/D) cycles of 12 h/12 h. In this study, we searched for an effective intermittent light color for the growth of *I. galbana*. Control cultures were grown under white continuous light, with a photon flux density at 104 μmol m^{-2} s^{-1} with L/D cycles of 12 h/12 h. The other cultures were grown under a photon flux density of 52 μmol m^{-2} s^{-1} of 24 h flashing per day, and white, red, and blue intermittent light at 10,000 Hz as L/D cycles of 50 μs/50 μs. After 6 days of cultivation, the cell density of the sample cultured under blue intermittent light was significantly higher than those of the others. The lipid contents in *I. galbana* were 98 mg L^{-1} from the culture under constant white light and 155 mg L^{-1} from the culture under blue intermittent light. Total lipids from *I. galbana* were separated into neutral lipids (29–35%), glycolipids (38–47%), and phospholipids (20–28%). The light condition did not affect the ratio of lipid classes or the fatty acid composition of total lipids, neutral lipids, glycolipids, or phospholipids from *I. galbana*. The amounts of neutral lipids, glycolipids, and phospholipids obtained from culture medium were the highest under blue intermittent light (3.27, 4.71, and 2.48 mg L^{-1}, respectively). The highest amounts of phospholipids and DHA were recovered from *I. galbana* cultured under blue intermittent light.

1. Introduction

Marine microalgae are widely used in aquaculture and play an important role in aquaculture development. The microalgal species *Isochrysis galbana* is known to have good nutritional qualities, particularly in its polyunsaturated fatty acid content. It is widely used in aquaculture as a food in the early larval stages of mollusks, fish, and crustaceans. Some microalgal species, including *I. galbana*, are known to synthesize and accumulate large amounts of polyunsaturated fatty acids, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). To obtain larger amounts of polyunsaturated fatty acids from *I. galbana* cultivation, different components of the culture medium (Guihéneuf et al., 2009; Lin et al., 2007) and changes in the nitrogen source (Fidalgo et al., 1998) have been studied.

Concerning the effect of different light regimes on phytoplankton culture, there have been many reports related to light strength, light/dark interval, and intermittent light effect. In the 1980s, Grobbelaar (1989, 1994) reported the effects of different light/dark cycles on phytoplankton or algal cultures, including intermittent light. He used intermittent light of photon flux density at a maximum 800 μmol m^{-2} s^{-1} at 0.05 to 50 Hz. He tested different flashing lights with 1 light/1 dark (1L/1D) or with 1 light/2 dark (1L/2D) periods. These studies found an increase of productivity by intermittent light stimulation with the use of frequencies over 10 Hz. Grobbelaar also found that light energy is generally used more efficiently at the 1L/2D ratio compared to the 1L/1D ratio. From these results, it was concluded that a flashing light was effective in increasing the light utilization efficiency of photosynthetic microorganisms due to light integration when subjected to alternating light and dark periods.

For T-ISO (*I. aff. galbana*, also known to be rich in essential fatty acids) biomass production, different photoperiods (24:0 to 12:12 h L/D) combined with photon flux densities of 120–460 μmol photons m^{-2} s^{-1} have been studied (Tzovenis et al., 2003a,b). These studies considered the relationship between photon flux density and L/D cycle, and obtained a maximum biomass of 350 mg L^{-1} at total photon flux of 40 mol photons m^{-2} day^{-1} (24:0 h L/D). These studies also obtained optimal n3/n6 and DHA/EPA ratios under 24 h/0 h or 12 h/12 h L/D cycles. Mouget et al. (2004) reported different growth rates of marine diatoms cultured under white, blue, green, yellow, and red lights of 20 μmol photons m^{-2} s^{-1}, and a photoperiod of 14 h/10 h, L/D. They observed significantly higher growth rates under blue light than under the other light conditions tested.

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However, marine diatom cultivation under blue light did not show a significant increase of growth rate under irradiance of 100 μmol photons $m^{-2} s^{-1}$. Sánchez-Saavedra and Voltolina (2006) reported differential light source effects on the growth of diatoms. They cultivated diatoms under an irradiance of 125 μmol photons $m^{-2} s^{-1}$ and three types of lights (including a fluorescent light). They obtained significantly higher cell numbers from cultivation under standard white light both in exponential and in stationary phases. Seyfabadi et al. (2010) reported the effects of irradiance and photoperiod on growth rates and fatty acid composition of microalgae. They obtained maximum growth rate at 100 μmol photons $m^{-2} s^{-1}$ and a photoperiod of 16 h/8 h, L/D. Additionally, they observed that the sum of saturated fatty acids increased while the monounsaturated and polyunsaturated fatty acids decreased, following the increase of irradiance and light duration. Taken together, these studies show that there is a variety of light conditions to be evaluated to identify the most optimal cultivation conditions for *I. galbana* with an optimal fatty acid profile.

Considering reports on the culture of *I. galbana*, T-ISO, and species of diatoms, as mentioned above, Morinaga and Fukui (2007) conducted preliminary research that was published in a patent (Japanese published patent application P2007-236277A; published on September 20, 2007). They attempted to find the optimal stock culture condition and tested several cell concentrations of *I. galbana* at the starting point of intermittent light cultivation. They also tested *I. galbana* at 100 μmol photons $m^{-2} s^{-1}$, 10^2–10^5 Hz, and a duty ratio of 40–80%. After several trials, they found that intermittent light with the duty ratio set to 50%, and light intensity to 52 μmol photons $m^{-2} s^{-1}$, was optimal for the culture of *I. galbana*. The recommended intermittent light condition reported, however, made no reference on the effect of the light color and resulting chemical compositions. Thus, the present study was carried out to evaluate the effect of intermittent light color on biomass production and biochemical composition, with a focus on the fatty acid profile, of *I. galbana.*

2. Materials and methods

All chemicals used in the experiments were of analytical grade and were purchased from Sigma-Aldrich Co. (St. Louis, MO)

2.1. Phytoplankton, apparatus, and methods

The species of microalga used for the experiment was *I. galbana* (Haptophyceae). The strain was obtained from the National Research Institute of Aquaculture in Japan. Conditions of *I. galbana* cultivation were fixed following the Japanese published patent application P2007-236277A. Sample of *I. galbana* were precultured with F/2 medium (Guillard and Ryther, 1962) in an Erlenmeyer flask, under an irradiance of 20 μmol photons $m^{-2} s^{-1}$, L:D = 12:12, and at a water temperature of 20 °C. The apparatus consisted of a light source and a plankton culture section. As a light source, an LED panel was used. The panel consisted of 1520 bulbs of the same color LED. The colors were white, blue, and red. The light intensity, the frequency, and the light/dark time ratio (hereafter, duty ratio) of intermittent light of the light source were controlled using the panel controller.

The phytoplankton culture section consisted of 500-mL, glass beakers for plankton culture, a water bath, and a magnetic stirrer. The outer surfaces of the beakers were painted black. Each beaker was placed just below the LED panel. The water temperature in the culture beaker was maintained at 20 ± 0.5 °C using the water bath. The culture equipment was covered with a blackout curtain to prevent effects from external light. The distance between the LED light panel and culture medium was 18 cm.

An inoculum with a high concentration of phytoplankton, used for cultivation, was diluted with F/2 culture medium. The cell concentration on the first day of the experiment was adjusted to 2.0×10^5 cells mL^{-1}. The experimental medium was poured into the culture beaker (depth: ca. 10 cm), and was agitated by a magnetic stirrer during the experimental period. Then, different optical conditions were assigned for each beaker and the phytoplankton was cultivated over a period of 6 days. The number of cells was determined on the first day, and 3 and 6 days after the start of cultivation. A 10 mL sample was collected from each cultivation beaker, and diluted with 190 mL of filtered seawater. The concentration and sizes of sample cells were measured using a Coulter counter. The growth rate was calculated using the following equation (Guillard, 1973):

$$\text{Growth rate}\left(\mu, \text{day}^{-1}\right) = \ln(\text{Nt}-\text{Ns})/(\text{t}-\text{s})$$

Nt : Cell concentration at day t
Ns : Cell concentration at day s(t > s).

2.2. Light conditions

To determine the effect on phytoplankton multiplication caused by intermittent light, we used continuous light and intermittent light under the following conditions. Both light sources were the white LED panel. The intensity of continuous light was set to 52 μmol photons $m^{-2} s^{-1}$ on the surface of the culture medium. For the intermittent light, the duty ratio was set to 50%, and the light intensity was 104 μmol photons $m^{-2} s^{-1}$. The frequency of intermittent light was 10,000 Hz in L/D cycles of 50 μs/50 μs. The waveform of a pulse of intermittent light was rectangular. To equalize the amount of radiation per day (hereafter, daily light exposure), the irradiation times were 12 h (12L:12D) of continuous light and 24 h of intermittent light. The daily light exposure in this experiment was 4.5 mol photons $m^{-2} day^{-1}$. Panels of white, blue, and red LEDs were used to test the effect on growth and fatty acid profile of phytoplankton caused by differences in the color of intermittent light.

2.3. Cell dry weight

I. galbana cells were recovered from culture medium on day 6 by filtration using glass fiber filters, and cells on the filter were washed with water to remove any salt. *I. galbana* cells were then freeze-dried and weighed to obtain the cell dry weight.

2.4. Extraction of total lipids

The extraction of total lipids (TL) was carried out in accordance with the method described by Bligh and Dyer (1959), using a chloroform–methanol (2:1, v/v) solvent system. The chloroform layer, containing the lipids, was collected and the solvents were removed by evaporation under a vacuum, and all the samples were dissolved in a known volume of chloroform. The lipid extracts were stored at −80 °C under nitrogen gas to avoid oxidization until analysis.

2.5. Lipid class separation

Total lipid extracts were fractionated on silica gel cartridge columns after an activation step with methanol followed by chloroform following the method of Devos et al. (2006). Neutral lipids (NL) were eluted using chloroform, glycolipids (GL) were eluted with chloroform/methanol (5/1, v/v), and the phospholipids (PL) were recovered in methanol. Separation of lipid classes was confirmed by thin-layer chromatography (TLC) with TLC-reference standard lipids (18-5A). The developing solvent of TLC was methyl acetate/propan-2-ol/chloroform/methanol/0.25% aqueous KCl (25:25:25:10:9, v/v/v/v/v). Stains were identified by comparison of their Rf (rate of flow) values.