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ORIGINAL ARTICLE

Aquaculture

Effects of light quality and photoperiod on growth and photosynthetic pigment content of a Rhodophyta, *Gloiopeltis furcata*

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Abstract
The objective of the present study was to determine the optimal incubation conditions for *Gloiopeltis furcata* culture. Three experiments, each lasting 30 days, were conducted to study the effects of photoperiod (6:18, 8:16, 12:12, 14:10, and 16:8 h light/dark), different wavelengths of LED light (blue, green, yellow, red, and white), and solar radiation filtered through different plastic films (blue, green, yellow, red, and white) on the growth and photosynthetic pigment content of *G. furcata*. The results of these experiments demonstrated that the growth rate of *G. furcata* was significantly higher under 14:10 and 16:8 light/dark than under 6:18 and 8:16 light/dark, while the pigment content of *G. furcata* was significant higher under 6:18 and 8:16 than under 14:10 and 16:8 light/dark. The growth rate of *G. furcata* was the lowest when the algae were exposed to blue LED and the highest under yellow LED illumination, while the phycobiliprotein content was the highest under blue LED and the lowest under yellow LED. Solar radiation filtered through different plastic films had no significant effect on the growth rate of *G. furcata*, but affected its pigment content. The results indicate that a photoperiod of 12 h or more of light and yellow LED are the optimal parameters for culturing of *G. furcata* thalli on land.

Keywords *Gloiopeltis furcata* · Light wavelength · Photoperiod · Photosynthetic pigments · Growth

Introduction

Gloiopeltis furcata is a red alga widely distributed along the shorelines of China, Korea, Japan, and the Pacific coast of Russia (Tseng 1983). The species has been used extensively in Japan, China, and Korea as food, medicine, and a source of funoran, which has been traditionally employed as a sizing agent for textiles and a thickener in mortar and plaster (Xia 2004; Yu et al. 2007, 2010; Tuvikene et al. 2014). Most of the *G. furcata* harvest originates from wild stocks, and its cost of collection is approximately US$200–300 per kilogram dry weight on the Chinese and Japanese markets. A growing demand for *G. furcata* and the decline in its natural populations has sparked an increased interest in *G. furcata* culture (Chen et al. 2014a). Different aspects of *G. furcata* have been studied to develop successful culture management protocols, such as the effects of temperature and light intensity (Chen et al. 2011a, 2014b), salinity (Chen et al. 2013), nutrients (Zhang et al. 2016), and triacontanol (Chen et al. 2011b) on the growth and development of this species, as well as its spore culture in tanks (Chen et al. 2014b) and filament formation and differentiation (Yin et al. 2007).

Harvested thalli of *G. furcata* are only 4–10 cm in length and 4 mm in diameter, and its vegetative fragments are too short to be attached to ropes for culturing in the sea. Therefore, a culture technique using a designed device was devised for culturing of vegetative fragments of *G. furcata* on land (Chen et al. 2012, 2016). Light conditions are an important factor influencing the incubation of *G. furcata* thalli in land cultures. Although the effect of light intensity has been investigated, the effect of light quality and photoperiod on the growth of *G. furcata* thalli remains unknown.

Light quality and photoperiod affect algal growth and synthesis of photosynthetic pigments (Dring 1988; Katz et al 2000; Talarico and Maranzana 2000; Godínez-Ortega et al.

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2008; Barufi et al. 2015; Green and Neefus 2015, 2016). The objective of the present study was to assess the effect of different light wavelengths and photoperiods on the growth and physiological status of *G. furcata* culture on land. To this end, we conducted a series of experiments designed to elucidate the responses to photoperiodic and light quality in thalli of *G. furcata* using different-colored LED lights and solar radiation filtered through plastic films of different colors.

Materials and methods

Young thalli of *G. furcata* (approximately 1 cm long) were collected from the seashore of Yangmeikeng, Shenzhen, China (22°32′52″ N; 114°34′28″ E) and transported to a laboratory, where they were cleaned and incubated under different photoperiod and light quality schemes.

Incubation of thalli under different photoperiods

The experiment was conducted in a laboratory with controlled temperature of 18 °C. Different photoperiods [6:18, 8:16, 12:12, 14:10, and 16:8 h light/dark (L/D)] were established using six separate incubation chambers (Chen et al. 2015, China, ZL201520216205.3). White LED (color temperature 6500 K) was used as the light source. In each incubation chamber, four Erlenmeyer flasks (each with 0.5 g of *G. furcata* thalli and 500 mL seawater at 18 °C) were placed under the same light intensity (105 µmol photons m^{-2} s^{-1}). The light level was measured using a quantum meter (MQ-500; Apogee Instruments, Logan, UT, USA).

Incubation of thalli under LEDs of different colors

The experiment was conducted in a laboratory with controlled temperature of 18 °C. Different LED colors (red, blue, green, yellow, and white) were used as light source. Four Erlenmeyer flasks (each with 0.5 g of *G. furcata* thalli and 500 mL seawater at 18 °C) were placed under the same light intensity (105 µmol photons m^{-2} s^{-1}) and a photoperiod of 12:12 L/D for each type of light. The wavelengths of the blue, green, yellow, and red LEDs were 450 nm, 520 nm, 585 nm, and 630 nm, respectively, and the color temperature of the white LED was 6500 K.

Incubation of thalli under solar radiation filtered through plastic films of different colors

Gloiopeltis furcata thalli (4 g) were placed in plastic tanks (size 24 cm × 17 cm × 16 cm), each filled with 4 L of seawater. The tanks were covered with plastic films (0.2 mm thick) of different colors (red, blue, green, yellow, or white). Eight replicates were prepared for each film color. The tanks were placed outdoors and exposed to the same solar radiation. During the experiment, the water temperature varied with air temperature (ranging from 16 to 23 °C), but the water temperature of all tanks was the same. The light transmittance of the plastic films and the percentage of different wavelengths of solar radiation passing through the films are shown in Table 1.

Incubation management

The three experiments described above were conducted for 30 days. During the experiments, water motion was provided by aeration. The incubation water was exchanged three times per week. A solution (1 mL L^{-1}) composed of 10 g L^{-1} $NaNO_3$ and 1 g L^{-1} KH_2PO_4 was added to the refreshed seawater. The seawater used in the experiments was filtered through fine sand and stored in the dark for 5 to 10 days. At the end of the experiment, the thalli from each tank were weighed. The relative growth rate (RGR) was calculated using the formula: $RGR = [\ln(N_t/N_0)/t]\ 100\%$, where N_0 is the initial biomass and N_t is the biomass at day t (Korbee et al. 2005).

Pigment analysis

Pigment analysis was performed at the end of the experiment. Two hundred milligrams of thalli from each sample was ground in liquid nitrogen and extracted in 50 mM phosphate buffer (pH 7.8). Crude extracts were centrifuged at 4000 rpm for 20 min to obtain phycobiliproteins. Chlorophyll *a* (Chl-*a*) was extracted after dissolving the pellet in 90% acetone and centrifugation at 4000 rpm for 15 min. The pigments were quantified in a spectrophotometer (UV-1800; Shimadzu, Kyoto, Japan), and concentrations of phycobiliproteins (allophycocyanin [APC], phycocyanin [PC], and phycoerythrin [PE]) and Chl-*a* were calculated as described by Kursar et al. (1983) and Moran and Porath (1980), respectively.

Table 1 Light transmittance and percentage of different wavelengths of solar radiation through different-colored film (%)

Film color	Blue	Green	Yellow	Red	White
Wavelength (nm)					
300–400	3.6	3.3	2.8	1.6	2.8
400–510	19.0	11.0	8.5	1.8	8.0
510–610	3.4	21.5	20.6	1.2	19.2
610–720	12.8	9.6	21.3	27.4	21.6
720–1100	61.2	54.6	46.8	67.8	48.4
Light transmittance	69.5	71.2	72.1	56.5	75.0

Data for different wavelengths of solar radiation through different-colored film was provided by the producer of the color film, and the light transmittance was measured by a quantum meter

Statistical analysis

Data were analyzed by one-way analysis of variance using SPSS 21 for Windows (IBM Corp., Armonk, NY, USA). The differences among the treatments were tested for significance using a least significant difference (LSD) multiple comparisons test ($P < 0.05$), and all the data were reported as mean ± SD.

Results

Thalli incubated under different photoperiods

After 30 days of incubation, the color of *G. furcata* under 16:8 L/D became yellow, while the color of *G. furcata* under 6:18 L/D was still reddish purple (Fig. 1).

The statistical analysis showed that the growth rate (% growth day^{-1}) of *G. furcata* was significantly affected by the photoperiod ($P < 0.05$), and it increased with increasing illumination time from 6 to 14 h per day (Fig. 2). The LSD analysis revealed a significantly higher growth rate under 14:10 L/D and 16:8 L/D than under 6:18 L/D and 8:16 L/D photoperiods. There was no significant difference in growth rate among the treatments with 12:12L/D, 14:10 L/D, and 16:8 L/D photoperiods.

Similarly, the pigment content in *G. furcata* thalli was significantly affected by the photoperiod ($P < 0.05$). Based on the LSD analysis, the PE content was significantly higher under 6:18 L/D and 8:16 L/D than under 14:10 L/D and 16:8 L/D photoperiods (Fig. 3). There was no significant difference in the PE levels among the treatments with 12:12 L/D, 14:10 L/D, and 16:8 L/D photoperiods. The PC content was significantly higher under a 6:18 L/D than a 16:8 L/D photoperiod, and there was no significant difference in the PC levels among the treatments with other photoperiods (Fig. 3). The APC and Chl-*a* content were significantly higher in thalli subjected to a 6:18 L/D photoperiod than in treatments with any other photoperiod (Figs. 3 and 4).

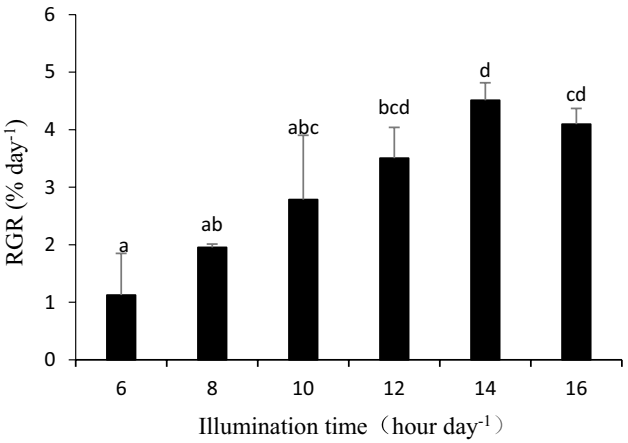

Fig. 2 Growth rate (% growth day^{-1}) of *Gloiopeltis furcata* under different photoperiods. Each data point is the mean ± SD ($n = 4$). Bars with different letters indicate significant differences according to one-way ANOVA and LSD multiple comparisons test ($P < 0.05$)

Thalli incubated under different-colored LED lights

The different-colored LED lights significantly affected the growth rate and pigment content of *G. furcata* thalli

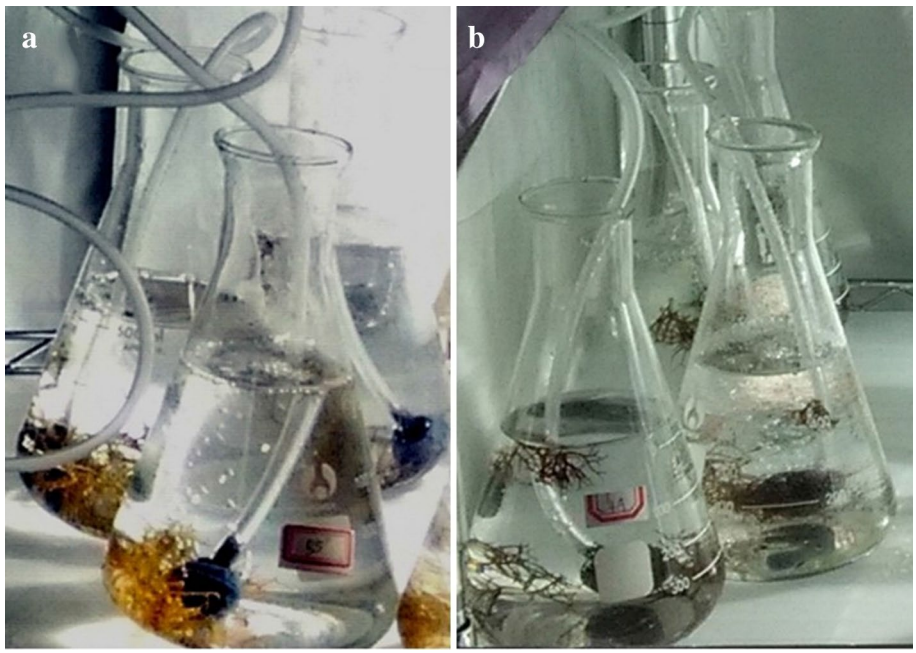

Fig. 1 Thalli of *Gloiopeltis furcata* incubated under photoperiods of 16:8 L/D (**a**) and 6:18 L/D (**b**)

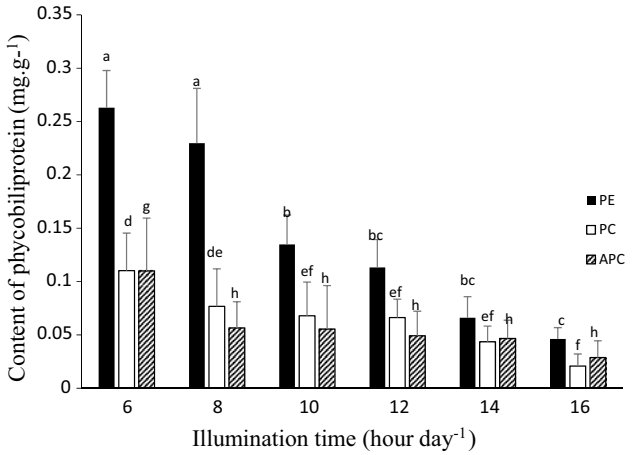

Fig. 3 Content of phycobiliprotein of *Gloiopeltis furcata* under different photoperiods. Data points are mean ± SD ($n = 4$). Bars with different letters indicate significant differences in the same kind of phycobiliprotein ($P < 0.05$)

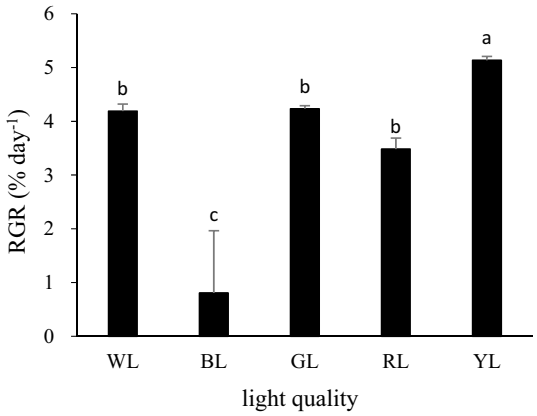

Fig. 5 Growth rate (% growth day^{-1}) of *Gloiopeltis furcata* under different-colored LEDs. Data points are mean ± SD ($n = 4$). Bars with different letters indicate significant differences according to one-way ANOVA and LSD multiple comparisons test ($P < 0.05$)

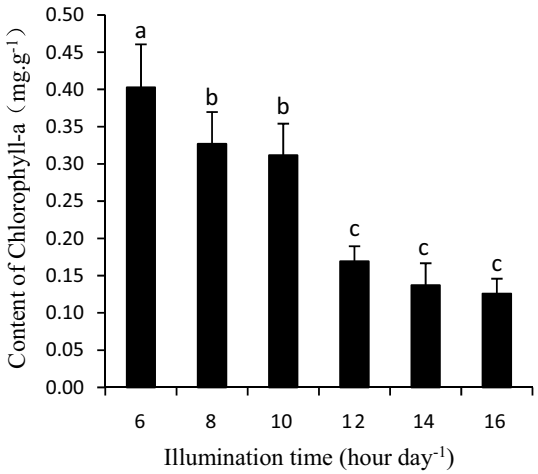

Fig. 4 Content of chlorophyll *a* of *Gloiopeltis furcata* under different photoperiods. Data points are mean ± SD ($n = 4$). Bars with different letters indicate significant differences according to one-way ANOVA and LSD multiple comparisons test ($P < 0.05$)

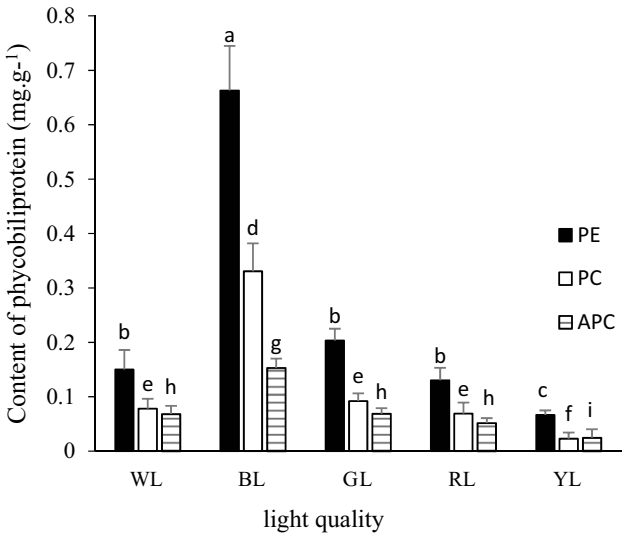

Fig. 6 Content of phycobiliprotein of *Gloiopeltis furcata* under different LED colors. Data points are mean ± SD ($n = 4$). Bars with different letters indicate significant differences in the same kind of phycobiliprotein ($P < 0.05$)

($P < 0.05$). The growth rate was the highest under yellow light and the lowest under blue light (Fig. 5). According to the LSD analysis, there was no significant difference in the growth rate of thalli among the treatments with white, green, and red light. The content of PE, PC, and APC was highest under blue light and lowest under yellow light (Fig. 6). The content of Chl-*a* was the highest under blue light, and there was no significant difference in Chl-*a* levels among the treatments with lights of other colors (Fig. 7).

Thalli incubated under solar radiation filtered through different-colored plastic films

Solar radiation through different plastic films had no significant effect on the growth rate of *G. furcata* (Fig. 8), but it significantly affected the pigment content in its thalli ($P < 0.05$). The PE level was higher in thalli cultured under red plastic film than under white, blue, and yellow film. The content of PC and APC was higher under blue film than under green film and yellow film (Fig. 9). The content of Chl-*a* was the highest in thalli exposed to light passing through green plastic film (Fig. 10).

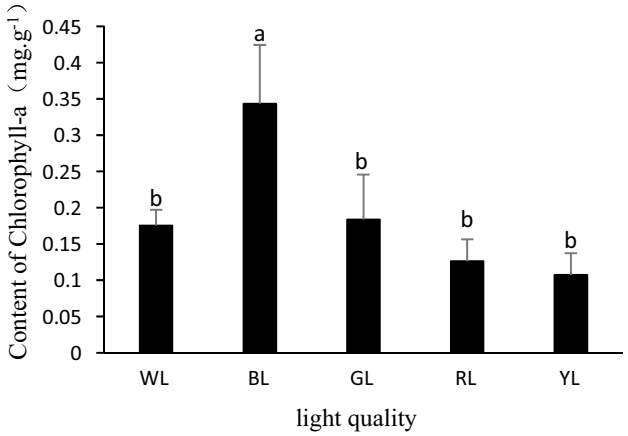

Fig. 7 Content of chlorophyll *a* of *Gloiopeltis furcata* under different LED colors. Data points are mean ± SD ($n = 4$). Bars with different letters indicate significant differences according to one-way ANOVA and LSD multiple comparisons test ($P < 0.05$)

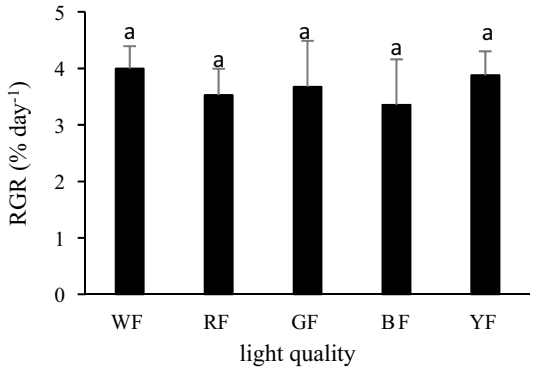

Fig. 8 Growth rate of *Gloiopeltis furcata* under solar radiation through film of different colors. Each data point is mean ± SD ($n = 8$). Bars with different letters indicate significant differences according to one-way ANOVA and LSD multiple comparisons test ($P < 0.05$)

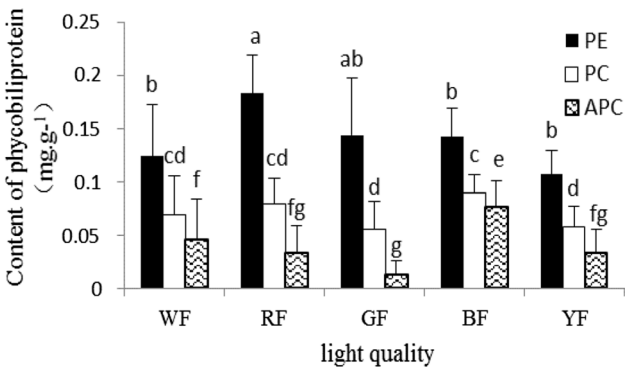

Fig. 9 Content of phycobiliproteins of *Gloiopeltis furcata* under different plastic film. Each data point is mean ± SD ($n = 8$). Bars with different letters indicate significant differences in the same kind of phycobiliprotein ($P < 0.05$)

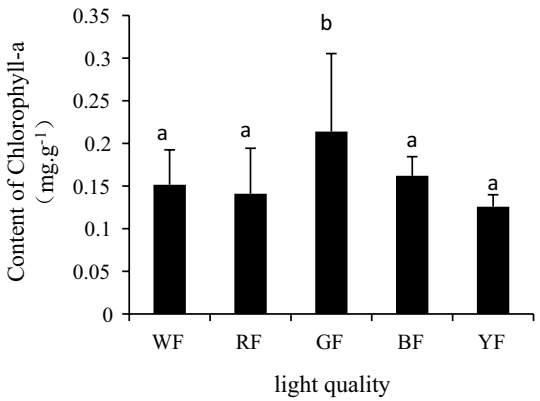

Fig. 10 Content of chlorophyll *a* of *Gloiopeltis furcata* under different-colored plastic film. Each data point is mean ± SD ($n = 8$). Bars with different letters indicate significant differences according to one-way ANOVA and LSD multiple comparisons test ($P < 0.05$)

Discussion

Effect of photoperiod

Based on a previous study that examined the effects of light intensity on the growth of *G. furcata*, the optimal photon flux density for growth in this species was 105 µmol photons m^{-2} s^{-1} (Chen et al. 2014b). Therefore, in the present study, this optimal light intensity was implemented to investigate the effect of different photoperiods. The results showed that the growth rate of *G. furcata* was significantly higher when thalli were exposed to at least 12 h of light daily. Similar effects of the photoperiod on the growth rate were reported for *Pyropia leucosticta* and *Porphyra umbilicalis* (Green and Neefus 2015, 2016), whereas the growth rate of *Chondrus ocellatus* was the highest at a 12:12 h L/D photoperiod (Kim et al. 2006). The pigment content in *G. furcata* decreased with increasing growth rate under different light photoperiods. This decrease in pigment content may be due to the rapid growth of thalli, which effectively diluted the concentration of pigments (Green and Neefus 2015).

Effect of light quality

Light quality has a strong influence on the vegetative development, reproductive induction, and growth rate of macroalgae (Figueroa et al. 1995). The effect of light quality on growth has been found to vary among different algae species. For example, *Gelidium sesquipedale* exhibited higher growth rates under blue and red light than under white light (Carmona et al. 1996), *Pyropia haitanensis* experienced slower growth under red light than under white, blue, and green light (Wu 2016), and the growth rate of *Kappaphycus alvarezii* was higher under red light than under blue light (Thien et al. 2016). The growth rate of *G. furcata* was

the lowest under blue light and the highest under yellow light. This result was in contrast to microalgae *Amphora* sp. (Romero-Romero and Sánchez-Saavedra 2017), which showed lower growth rates under yellow light, and it also differed from that of red alga *Porphyra leucosticta* incubated under blue, green, yellow, red, and white light. *P. leucosticta* did not exhibit a significantly higher growth rate under yellow light than under blue light (Korbee et al. 2005). There are few reports on the effect of yellow light on plant growth, which may be attributable to misclassification of light with 500–600 nm wavelength as green light by researchers (Dougher and Bugbee 2001). In the present experiment, wavelengths of 520 nm and 585 nm were considered to be green light and yellow light, respectively. There was a significant difference in the growth rate of *G. furcata* between the treatments with these two wavelengths. Therefore, it is necessary to separate yellow light from green light. Our experiments showed that the concentration of phycobiliproteins in *G. furcata* was the highest under blue LED and significantly different from those measured in other treatments. This was in accordance with the suggestion by Godínez-Ortega et al. (2008) that the accumulation of phycobiliproteins stimulated by blue light is a general feature in red algae. The lowest levels of phycobiliproteins in *G. furcata* thalli were measured under yellow LED, and they were significantly different from the values obtained under other LEDs. This may have resulted from diluted concentrations of these proteins as a consequence of the high growth rate of *G. furcata* observed under yellow light.

During the 30 days of the experiments, lower pigment content had not effect on the growth of *G. furcata*. However, the thalli with lower pigment content became white and decayed about 20 days after the end of experiments. This indicates that lower pigment content does affect the growth of *G. furcata* after a prolonged incubation period. Therefore, it is recommended that thalli be exposed to yellow light (wavelength of 585 nm) and a photoperiod of at least 12 h of light per day for no more than 30 days for optimal growth of *G. furcata* thalli in culture.

Effect of plastic films of different colors

Yellow LED can be used to provide the optimal light quality and photoperiod, although its use will add to production costs. A more economical alternative is the use of cheap colored plastic films to manipulate solar radiation and provide optimal light quality for *G. furcata* culture on land, especially under greenhouse conditions. Some studies showed that solar radiation through colored plastic films affected the growth and secondary metabolite accumulation in higher plants such as *Chrysanthemum morifolium* (Jin et al. 2011), *Panax notoginseng* seedlings (Kuang et al. 2014), *Perilla frutescens* (Grbic et al. 2016), and *Capsicum annuum* (Casierra-Posada et al. 2014). The use of plastic films of different colors to filter solar radiation before it reached *G. furcata* thalli had no significant effect on the growth rate of this alga, but it affected the content of the pigments significantly. The plastic films used in our experiment were not of the quality of optical filters, and solar radiation that passed through the monochrome plastic film was of multiple wavelengths (Table 1). This explains the difference between the effects of LED and plastic films of the same color on *G. furcata* growth. However, the use of optical filters in greenhouses is cost-prohibitive. Further studies should investigate other types of plastic films that will provide better control of filtered light and thus be suitable for the growth of *G. furcata* in cultures on land.

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