

# Contrasting effects of blue and red LED irradiations on the growth of *Sargassum horneri* during the germling and immature stages

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1 **Contrasting effects of blue and red LED irradiations on the growth of *Sargassum horneri* during**  
2 **the germling and immature stages**

3

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16

1 **Abstract**

2 The brown seaweed *Sargassum horneri* is a member of the Sargassaceae family and is important for  
3 marine environment conservation. It could be used for a food material, medical applications, and future  
4 biofuel production. With the application of single wavelength blue and red light, we compared for the  
5 growth of *S. horneri* cultures during the germling and immature stages. The growth rate based on the  
6 thallus area of *S. horneri* during the germling stage was faster under blue LED irradiation than under red  
7 LED irradiation. Furthermore, based on the wet weight of *S. horneri*, during the immature stage, blue  
8 LED irradiation resulted in a faster growth rate than red LED irradiation. Moreover, during the immature  
9 stage, compared with red LED irradiation, blue LED irradiation tended to increase the content of  
10 photosynthetic pigments. We conclude that blue LED irradiation in indoor tanks during the germling and  
11 immature stages will improve the efficiency of *S. horneri* culture.

12

13 **Keywords** *Sargassum horneri*, Brown macroalgae, Blue LED, Red LED, Growth, Photosynthetic  
14 pigments

15

16

## 1 **Introduction**

2 *Sargassum horneri*, which belongs to the Sargassaceae family, is distributed widely in coastal areas of  
3 Japan. It is treated as edible brown macroalgae only in some limited areas of Japan (Tokuda et al. 1994;  
4 Murakami 2011). Sargassum is very important for the marine forest with a high potential to fix CO<sub>2</sub> (Ito  
5 et al. 2009; Pereira et al. 2015). The brown seaweed could be used for a food material, medical  
6 applications, and future biofuel production. (Ale et al. 2011; Murakami 2011; Ale et al. 2012; Borines et  
7 al. 2013).

8 Many methods for cultivating Sargassaceae species have been reported (Hwang et al. 2006; Choi et al.  
9 2008; Pang et al. 2009; Akimoto et al. 2010). Usually, *S. horneri* is cultivated in the immature stage in  
10 indoor tanks before culturing in large-scale open-water systems. Immature *S. horneri* is attached to ropes  
11 or concrete blocks and cultured in coastal areas (Akimoto et al. 2010). During the artificial cultivation of  
12 *S. horneri* at the early stage in indoor tanks, precisely controlling the environmental factors, such as  
13 temperature, nutrients, and light, is required (Hurd et al. 2014; Nagai et al. 2014; Miki et al. 2016).  
14 Moreover, the light source is crucial to the algal light utilization and the growth during the immature  
15 stages of *S. horneri*. The rate of photosynthesis depends on the absorbed irradiance (Hurd et al. 2014).  
16 Many studies on the indoor culture of *S. horneri* have addressed the effects of light quality and intensities  
17 of fluorescent lamps on their growth and proved that the growth of *S. horneri* is the best under white and  
18 blue light (Matsui et al. 1994).

19 The growth of germlings of *Sargassum thunbergii* cultured under blue LED was shown to be lower  
20 than that under white fluorescent lamp (Zhao et al. 2008), whereas blue LED irradiance has been shown  
21 to achieve superior growth compared with white LED irradiance for indoor seedling culture of  
22 *Saccharina japonica* (Wang et al. 2010). The growth and photosynthesis of *Ulva prolifera* were shown to  
23 be promoted using blue and/or red LEDs (Takada et al. 2011). Irradiance with white and blue LEDs

1 promote the growth and maturation of gametophytes of *Eisenia bicyclis*, whereas these are inhibited using  
2 red LEDs (Murase et al. 2014). These studies suggest that the effect of LED wavelength on the growth of  
3 seaweed greatly varies according to the seaweed species and the growth stage. Nevertheless, the effects of  
4 irradiation with blue LEDs on the growth of *S. horneri* during the germling and immature stages in indoor  
5 tanks remains unclear.

6 The present study aims to determine the effects of blue LED (peak wavelength: 445 nm) irradiation  
7 on the growth of *S. horneri* during the germling and immature stages, particularly compared with those of  
8 red LED (peak wavelength: 660 nm) irradiation on the growth of *S. horneri* during both stages.  
9 Furthermore, we investigated the effects of blue LED irradiation on the content of photosynthetic  
10 pigments of *S. horneri* compared with those of red LED irradiation.

11

## 12 **Materials and methods**

13

### 14 ***S. horneri***

15 Fertilized eggs of female *S. horneri* were collected in the nursery house of Sakaiovex Co. of the Fukui  
16 Prefecture, Japan. Fertilized eggs of *Myagropsis myagroides* and *S. patens* were collected in the same  
17 nursery house for comparison. Fertilized eggs of each Sargassaceae species were rinsed with filtered  
18 seawater, poured into amber glass bottles (500 mL) filled with filtered seawater, and stored at 4°C (Nagai  
19 et al. 2014; Miki et al. 2016).

20

### 21 **Culture medium**

22 The seawater used for the culture medium of Sargassaceae species was sampled using a suction pump at a  
23 depth of 320 m from the ocean off the Noto Peninsula, north of the Ishikawa Prefecture (Nagai et al.

1 2014; Miki et al. 2016). The seawater was filtered through a membrane filter (0.45  $\mu\text{m}$  pore size) before  
2 culture. Quality and analysis methods for assessing water quality of filtered seawater are described by  
3 Miki et al. (2016). Filtered seawater was sterilized by autoclaving at 121°C for 20 min (SN-200, Yamato,  
4 Japan) and then added to culture dishes or bottles. Provasoli Enriched Seawater (PES) media was added  
5 to the seawater (0.02 v/v) (Provasoli 1968), and Sargassaceae species were cultured using this enriched  
6 seawater. The nutrient quality of the enriched seawater is shown in **Table 1**.

## 7 8 **LEDs**

9 The effects of LED wavelength on the growth of Sargassaceae species were investigated using an  
10 irradiator (3LH-128, Nippon Medical & Chemical Instruments Co., Ltd, Japan), which can be adjusted to  
11 select specific wavelengths. The peak wavelengths and ranges of blue and red LEDs are 445 nm  
12 (420–480 nm) and 660 nm (620–680 nm), respectively. **Figure 1** shows the emission spectra of red and  
13 blue LEDs and the absorption spectra of the photosynthetic pigments in the thalli of *S. horneri*. The  
14 emission spectra of red and blue LEDs were compared using an LED meter (MK350, URPtek, Taiwan).  
15 LED irradiation intensities were measured using a quantum meter (MQ-200, Apogee Instruments, USA).

## 16 17 **Culture during the germling stage**

18  
19 *Comparison of the effect of blue and red LED irradiations on the growth of three Sargassaceae species*

20  
21 The growth rates of *S. horneri*, *M. myagroides*, and *S. patens* during the germling stage were compared in  
22 this experiment to determine the effects of blue and red LEDs. Three culture dishes were prepared for  
23 each Sargassaceae species. Fifteen fertilized eggs per culture dish (diameter = 8.7 cm) were cultured for

1 21 days in photoincubators (EYELA LTI-700, Tokyo Rikakikai Co., Ltd., Japan) (**Fig. 2a**). LED  
2 irradiation intensities were maintained at a constant  $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ . Culture dishes were  
3 separately irradiated using blue or red LEDs under a 12 h: 12 h light: dark cycle at  $20^\circ\text{C}$ .

4 Growth rates of Sargassaceae species during the germling stage were observed using a stereoscopic  
5 microscope (SMZ745T, Nikon, Japan) equipped with a digital camera (DS-Fi2-L3, Nikon, Japan). The  
6 areas of the algae thalli were analyzed using image analysis software (ImageJ, United States National  
7 Institutes of Health).

8 Growth of Sargassaceae species during the germling stage was observed using a stereoscopic  
9 microscope as described. The specific growth rate ( $\mu$ ) based on the area of the thallus was calculated as  
10 follows:

$$11 \quad \mu = \frac{\ln A_{t_2} - \ln A_{t_1}}{t_2 - t_1}, \quad (1)$$

12 where  $t_1$  is the initial time (day) of the logarithmic growth phase,  $t_2$  is the end (day) of the logarithmic  
13 growth phase,  $A_{t_1}$  is the thallus area ( $\text{mm}^2$ ) on the initial day of the logarithmic growth, and  $A_{t_2}$  is the area  
14 of the thallus ( $\text{mm}^2$ ) on the final day of logarithmic growth.

15 Differences between the specific growth rates ( $\mu$ ) under blue and red LED irradiations were evaluated  
16 using a one-sided Student's t-test. Results where  $p < 0.05$  were considered significant.

17

#### 18 *Comparison of the effects of blue and red LED irradiation intensities on the growth of S. horneri*

19

20 The effects of blue and red LED irradiation intensities on the growth of *S. horneri* during the germling  
21 stage were evaluated. Five culture dishes were prepared for each condition (10, 20, 40, 60, and  
22  $80 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ). Twelve fertilized *S. horneri* eggs were added to a culture dish and cultured for  
23 21 days in a photoincubator (**Fig. 2a**). Five culture dishes were irradiated with each LED under different

1 intensities under a 12 h: 12 h light: dark cycle at 20°C. The culture media in the culture dishes was  
2 renewed every 6–7 days.

3 The specific growth rate ( $\mu$ ) of *S. horneri* during the germling stage was calculated using the described  
4 method and equation (1). Moreover, the maximum specific growth rates ( $\mu_{\max}$ ) and saturation constants  
5 ( $K_s$ ) were estimated using Lineweaver–Burk plots (Nagai et al. 2014). Detailed methods used to study the  
6 germling stage are described by Miki et al. (2016).

7 Differences between the specific growth rates ( $\mu$ ) under blue and red LED irradiation intensities were  
8 evaluated using a one-sided Student’s t-test. Results where  $p < 0.05$  were considered significant.

## 10 **Culture during the immature stage**

11

12 *Comparison of the effects of blue and red LED irradiations on the growth of S. horneri*

13

14 **Figure 2b** shows the photoincubator used to culture *S. horneri* during the immature stage. Samples of  
15 healthy *S. horneri* that were cultured under white LED irradiation during the germling stage were  
16 prepared before the present experiment. Six samples for *S. horneri* per culture bottle (200 mL) were then  
17 cultured for 36 days with aeration in the photoincubator. Two culture bottles were separately irradiated  
18 using blue or red LED under a 12 h: 12 h light: dark cycle. The irradiation intensity of each LED was  
19  $100 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . The culture media in the two bottles was renewed every 6–7 days.

20 The growth of *S. horneri* at the immature stage was evaluated by measuring the wet weight of each  
21 sample every 3–4 days. During this period, a logarithmic increase in wet weight was confirmed.  
22 Therefore, the average specific growth rate ( $\mu^*$ ) based on wet weight was calculated as follows:

23 
$$\mu^* = \frac{\ln G_{t_2} - \ln G_{t_1}}{t} \quad (2)$$

1 where  $t$  is the culture time (day),  $G_{t1}$  is the wet weight (mg) on the initial day of the culture, and  $G_{t2}$  is the  
2 wet weight (mg) on the final day of the culture.

3 Differences between the average wet weight of the algae thalli after 21 culture days and the specific  
4 growth rates ( $\mu^*$ ) at the logarithmic growth phase were evaluated using a one-sided Student's t-test.  
5 Results where  $p < 0.05$  were considered significant.

6 After 36 days, the photosynthetic pigments in each culture bottle were analyzed. Six samples in each  
7 culture bottle were freeze-dried, sonicated for 10 min in 30 mL 80% acetone, and centrifuged at  
8 3,000 rpm for 10 min. After storage for 1 day at 4°C, the absorbance of the supernatant at 630 or 664 nm  
9 was measured. The concentrations of photosynthetic pigments were estimated using the methods  
10 described by Jeffrey and Humphrey (1975). Chl.  $a$  and Chl.  $c_1 + c_2$  contents on a dry weight basis were  
11 calculated using equations (3) and (4), respectively.

$$12 \quad \text{Chl. } a = 11.47 \times A_{664} - 0.40 \times A_{630} \quad (3)$$

$$13 \quad \text{Chl. } c_1 + c_2 = 24.36 \times A_{630} - 3.73 \times A_{664} \quad (4)$$

14

## 15 **Results**

16

### 17 **Comparison of the effects of blue and red LED irradiations on the growth of three Sargassaceae** 18 **species during the germling stage**

19

20 **Figure 3** shows the growth curves of the mean thallus areas of three Sargassaceae species cultured using  
21 blue or red LEDs. **Figure 4** shows the comparison of the thalli of the three Sargassaceae species after 21  
22 days of culture. The three Sargassaceae species grew under blue or red LED irradiations; however, blue

1 LED irradiation clearly promoted the growth of the three Sargassaceae species more efficiently than red  
2 LED irradiation.

3 **Table 2** shows the comparison of specific growth rates ( $\mu$ ). The average  $\mu$  values for *S. horneri*,  
4 *M. myagropisis*, and *S. patens* cultured under blue LED irradiation were  $0.40 \pm 0.01$ ,  $0.26 \pm 0.02$ , and  
5  $0.21 \pm 0.01 \text{ day}^{-1}$ , respectively, at  $100 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ . The  $\mu$  values for *S. horneri*, *M. myagropisis*,  
6 and *S. patens* cultured under red LED irradiation were  $0.20 \pm 0.02$ ,  $0.11 \pm 0.01$ , and  $0.16 \pm 0.01 \text{ day}^{-1}$ ,  
7 respectively, at  $100 \mu \text{ mol photons m}^{-2} \text{ s}^{-1}$ . Compared with  $\mu$  values under red LED irradiation,  $\mu$  values  
8 under blue LED irradiation increased significantly in three Sargassaceae species ( $p < 0.05$ ). Thus,  $\mu$   
9 values during germling growth using the blue LED were clearly higher than those using the red LED.

10 These data show that blue LED irradiation is more effective than red LED irradiation for the culture  
11 of the three Sargassaceae species during the germling stage. Furthermore, the results showed that  
12 *S. horneri* grew at the fastest rate among the three Sargassaceae species.

13

#### 14 **Comparison of the effects of blue and red LED irradiation intensities on the growth of *S. horneri*** 15 **during the germling stage**

16

17 **Figure 5** shows the growth curves of the mean thallus area of *S. horneri* cultured under different  
18 intensities of blue or red LED irradiation. **Figure 6** shows the comparison of the thalli of *S. horneri*  
19 cultured for 21 days under different intensities of blue or red LED irradiation. **Figure 7** shows the  
20 relationship between the average  $\mu$  and blue or red LED irradiation intensities.

21 The average  $\mu$  values under blue LED irradiation were  $0.35 \pm 0.10$ ,  $0.35 \pm 0.16$ ,  $0.50 \pm 0.14$ ,  
22  $0.53 \pm 0.09$ , and  $0.53 \pm 0.08 \text{ day}^{-1}$  for 10, 20, 40, 60, and  $80 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , respectively.

1 Compared with  $\mu$  values under blue LED irradiation of 10  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ ,  $\mu$  values increased  
2 significantly under blue LED irradiation of 40, 60, and 80  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  ( $p < 0.05$ ).

3 In contrast, the average  $\mu$  values under red LED irradiation were  $0.16 \pm 0.08$ ,  $0.25 \pm 0.05$ ,  $0.16 \pm 0.10$ ,  
4  $0.19 \pm 0.06$ , and  $0.19 \pm 0.06 \text{ day}^{-1}$  for 10, 20, 40, 60, and 80  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , respectively.

5 Compared with  $\mu$  values under red LED irradiation of 10  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , there were no significant  
6 differences between  $\mu$  values under red LED irradianations of 40, 60, and 80  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$   
7 ( $p > 0.05$ ).

8 In addition, the values of  $\mu_{\text{max}}$  and  $K_s$  under blue or red LED irradianations are summarized in **Table 3**.

9 For blue LEDs,  $\mu_{\text{max}}$  and  $K_s$  were estimated to be  $0.56 \text{ day}^{-1}$  and  $6.99 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ , respectively.

10 For red LEDs,  $\mu_{\text{max}}$  and  $K_s$  were estimated to be  $0.19 \text{ day}^{-1}$  and  $1.21 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ , respectively.

11 Therefore, there was a three-fold increase in  $\mu$  under blue LED irradiation compared with that under red  
12 LED irradiation.

13 These data show that blue LED irradiation  $> 40 \mu\text{mol photons m}^{-2} \text{s}^{-1}$  was effective for promoting the  
14 growth of *S. horneri* during the germling stage, whereas red LED irradiation was ineffective for  
15 promoting the growth of *S. horneri* during the germling stage.

16

### 17 **Effects of blue and red LED irradiation on the growth of *S. horneri* during the immature stage**

18

19 **Figure 8** shows the growth curves according to the wet weights of *S. horneri* cultured under blue or red  
20 LED irradianations during the immature stage. **Figure 9** shows the comparison of thalli between *S. horneri*  
21 algae cultured under blue or red LED irradiation during the immature stage.

22 The wet weight of *S. horneri* increased under blue or red LED irradianations; however, there was a clear  
23 difference between the effects of the LEDs on the growth during the immature stage. The average wet

1 weight of *S. horneri* under blue LED irradiation increased from  $2.7 \pm 0.4$  mg to  $113.6 \pm 37.8$  mg for  
2 36 days, whereas the average wet weight of *S. horneri* under red LED irradiation increased from  
3  $2.6 \pm 0.6$  mg to  $46.0 \pm 15.1$  mg for 36 days. The average wet weight of *S. horneri* under blue LED  
4 irradiation after 36 days was clearly larger than that under red LED irradiation ( $p < 0.05$ ).

5 In addition, the  $\mu^*$  value under blue LED irradiation was estimated to be  $0.10 \pm 0.01$  day<sup>-1</sup>, whereas  
6 the  $\mu^*$  value under red LED irradiation was estimated to be  $0.08 \pm 0.00$  day<sup>-1</sup>. Therefore, there was an  
7 approximately 1.25-fold increase in  $\mu^*$  using blue LED irradiation. Compared with  $\mu^*$  values under red  
8 LED irradiation, there was a significant difference between the samples under blue LED irradiation  
9 ( $p < 0.05$ ).

10 **Figure 10** shows the optical absorption spectra of thalli of *S. horneri* cultured under blue or red LED  
11 irradiations. The absorbance around 430 and 660 nm indicates the presence of Chl. *a*. And, the  
12 absorbance around 630 nm indicates the presence of Chl.  $c_1 + c_2$ . The optical absorption spectrum  
13 indicated that blue LED irradiation increased the contents of photosynthetic pigments compared with red  
14 LED irradiation. Chl. *a* contents in each thalli under blue or red LED irradiations were estimated to be  
15 6.64 and 4.31 mg/g dry weight, respectively. Thus, Chl. *a* content values of the thalli of *S. horneri*  
16 cultured using blue LED were greater than those cultured using red LED. Chl.  $c_1 + c_2$  contents in each  
17 thalli under blue and red LED irradiations were estimated to be 0.53 and 0.54 mg/g dry weight,  
18 respectively. In contrast, there was not clear difference as for Chl.  $c_1 + c_2$  contents.

19

## 20 **Discussion**

21

22 It is generally accepted that the absorption peaks of Chl. *a* of *S. horneri* are approximately 430 and  
23 660 nm (**Fig. 1**). The peak wavelength of a blue LED is 445 nm, and the range of wavelengths emitted by

1 a blue LED is 420–480 nm, whereas the peak wavelength of a red LED is 660 nm, and the range of  
2 wavelengths emitted by a red LED is 610–680 nm. The wavelengths emitted by blue and red LEDs in the  
3 present study completely include the absorption peak of Chl. *a* of *S. horneri*. However, during the  
4 germling stage, the growth of *S. horneri* was faster under blue LED irradiation than under red LED  
5 irradiation. The longer wavelength of the red LED did not promote the growth of *S. horneri* during the  
6 germling stage. A previous study using fluorescent light with a sharp cut-off filter showed a similar result  
7 of optimal growth of *S. horneri* under white and blue fluorescent light and very poor growth under red  
8 fluorescent lamp (Matsui et al. 1994). Therefore, it was concluded that the growth of *S. horneri* during the  
9 germling stage does not require red LED irradiation with a peak wavelength of 660 nm. However, the  
10 reason for this ineffectiveness of red LED irradiation with a peak wavelength of 660 nm remains unclear.  
11 The absorption peaks of Chl. *a* of *S. horneri* are located at the peak wavelengths of both blue and red  
12 LEDs. It is generally accepted that red LED irradiation is effective for the cultivation of land plants like  
13 vegetable. Further studies that include genetic analysis of the photosynthesis mechanism of *S. horneri*  
14 during the germling stage are required to solve this subject.

15 The values of  $\mu_{\max}$  of *S. horneri* during the germling stage under blue LED irradiation was estimated to  
16 be  $0.56 \text{ day}^{-1}$ , which was an approximate 1.3-fold increase compared with the values of  $\mu_{\max}$  ( $0.44 \text{ day}^{-1}$ )  
17 under white LED irradiation obtained in our previous study (Miki et al. 2016). Commercially available  
18 white LED consists of a mixture of blue and yellow light and has two peak wavelengths of  
19 approximately 470 and 560 nm (Takada et al. 2011, Murase et al. 2014). Yellow light of such white LED  
20 seems to be unnecessary for the culture of *S. horneri*.

21 Moreover, our present study clearly shows positive growth of *S. horneri* during the germling stage,  
22 even under the weak intensities of approximately  $40 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$  using the blue LED. A  
23 previous study shows an optimum irradiance for *S. horneri* using a white fluorescent lamp of

1 100  $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$  (Baba 2007). The difference appears to be caused by the difference in light  
2 quality between LEDs and fluorescent lamp, which comprises a broad spectrum. From these results, the  
3 culture of *S. horneri* under blue LED irradiation during the germling stage was more efficient than that  
4 under white fluorescent lamp or white LED irradiation. Moreover, strong light irradiation intensities often  
5 promote the growth of microalgae in indoor tanks (Ishikawa et al. 2012). Therefore, the use of weak blue  
6 LED irradiation for the culture of *S. horneri* at the germling stage in indoor tanks appears to be  
7 appropriate for protecting against growth inhibition caused by microalgae growth.

8 Blue LED irradiation promoted the growth of *S. horneri* during the immature stage more efficiently  
9 than red LED irradiation. Moreover, it was confirmed that blue LED irradiation tended to promote an  
10 increase in the content of photosynthetic pigments, such as Chl. *a*, in the thallus. Based on these results,  
11 the use of blue LED for the culture of *S. horneri* during the immature stage in indoor tanks will increase  
12 the efficiency of culture and improve the quality of *S. horneri*. However, compared with the difference in  
13 specific growth rates during the germling stage between blue and red LED irradiations, the difference in  
14 specific growth rates during the immature stage between blue and red LED irradiations was relatively  
15 small. The photosynthesis mechanism of *S. horneri* may change during the growth stages, which remains  
16 unclear and should be investigated further.

17 In conclusion, it is clear that blue LED irradiation is appropriate for increasing the growth of *S. horneri*  
18 during the germling and immature stages. The values of  $\mu_{\text{max}}$  based on the thallus area and  $K_s$  of  
19 *S. horneri* during the germling stage cultured under blue LED irradiation were estimated to be  $0.56 \text{ day}^{-1}$   
20 and  $6.99 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ , respectively. The average growth rate based on wet weight during the  
21 immature stage of *S. horneri* cultured under blue LED irradiation was estimated to be  $0.11 \text{ day}^{-1}$ . Blue  
22 LED could be applied for the light source in indoor tanks during the culture of *S. horneri*.

23

1

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4

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Table 1 Nutrient quality of enriched seawater

NH <sub>4</sub> -N	NO <sub>2</sub> -N	NO <sub>3</sub> -N	D-P	Fe
(mg L <sup>-1</sup> )	(µg L <sup>-1</sup> )			
<0.01	<0.01	11.6	1.04	550

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1 Table 2 Specific growth rates of three Sargassaceae species during the germling stage cultured  
 2 using blue or red light-emitting diodes (LEDs)

	$\mu$ under blue LED*	$\mu$ under red LED*	Logarithmic growth phase
	(day <sup>-1</sup> )	(day <sup>-1</sup> )	(day)
<i>S. horneri</i>	0.40 ± 0.01	0.20 ± 0.02	3–12
<i>M. myagroides</i>	0.26 ± 0.02	0.11 ± 0.01	3–12
<i>S. patens</i>	0.21 ± 0.01	0.16 ± 0.01	4–15

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 4 \*Values are mean ± standard deviation (SD). The number of replicates was n = 15 for blue or red  
 5 LEDs

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Table 3 Comparison of maximum specific growth rates and half-saturating constant for *Sargassum*

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*horneri* during the germling stage cultured using blue or red light-emitting diodes (LEDs)

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	$\mu_{\max}$ (day <sup>-1</sup> )	Ks ( $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ )	Logarithmic growth phase (day)
Blue LED	0.56	6.99	6–10
Red LED	0.19	1.21	12–18

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6

1 **Figure captions:**

2  
3 **Fig. 1** Relative emission spectra of red and blue light-emitting diodes (LEDs) and the optical absorption  
4 spectrum of *Sargassum horneri* thalli

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6 **Fig. 2** Photoincubators for culturing Sargassaceae species during the germling (a) and immature (b) stages

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8 **Fig. 3** Growth curves of the mean thallus area of three Sargassaceae species during the germling stage  
9 cultured under blue or red light-emitting diode (LED) irradiations. Values represent the mean of 15  
10 replicates  $\pm$  standard deviation (SD) (a: *Sargassum horneri*, b: *Myagropsis myagroides*, and c: *S. patens*)

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12 **Fig. 4** Comparison of the thalli of three Sargassaceae species cultured for 21 days under blue or red  
13 light-emitting (LED) irradiations (a: *Sargassum horneri*, b: *M. myagroides*, and c: *Sargassum patens*).  
14 Scale bar = 1 mm

15  
16 **Fig. 5** Growth curves of the mean thallus area for *Sargassum horneri* during the germling stage cultured  
17 under blue or red light-emitting (LED) irradiations (a: blue LED and b: red LED). Values represent the  
18 mean of 12 replicates  $\pm$  standard deviation (SD)

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20 **Fig. 6** Comparison of the thalli from *Sargassum horneri* cultured for 21 days under blue or red  
21 light-emitting diode (LED) irradiations (a: blue LED and b: red LED). Scale bar = 1 mm

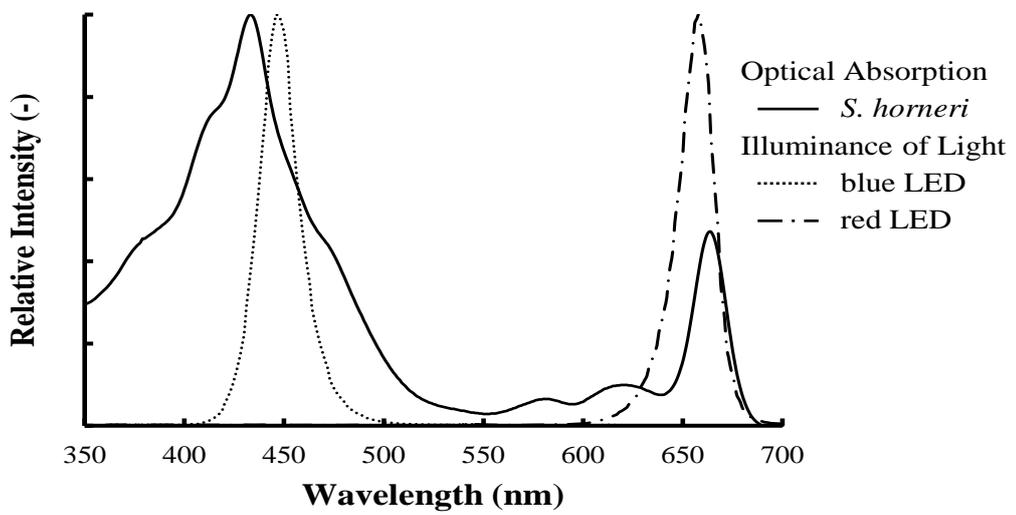
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23 **Fig. 7** Relationship between light-emitting diode (LED) irradiation intensities and  $\mu$  of *Sargassum*  
24 *horneri* during the germling stage. Values represent the mean of 12 replicates  $\pm$  standard deviation (SD)

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26 **Fig. 8** Growth curves according to the wet weights of *Sargassum horneri* cultured under blue or red  
27 light-emitting diode (LED) irradiations during the immature stage. Values represent the mean of six  
28 replicates  $\pm$  standard deviation (SD)

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30 **Fig. 9** Comparison of the thalli from *Sargassum horneri* cultured under blue or red light-emitting diode  
31 (LED) irradiations during the immature stage (a: blue LED and b: red LED). Scale bar = 10 mm

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33 **Fig. 10** Optical absorption spectrum of thalli from *Sargassum horneri* cultured under blue or red light  
34 emitting diode (LED) irradiations during the immature stage

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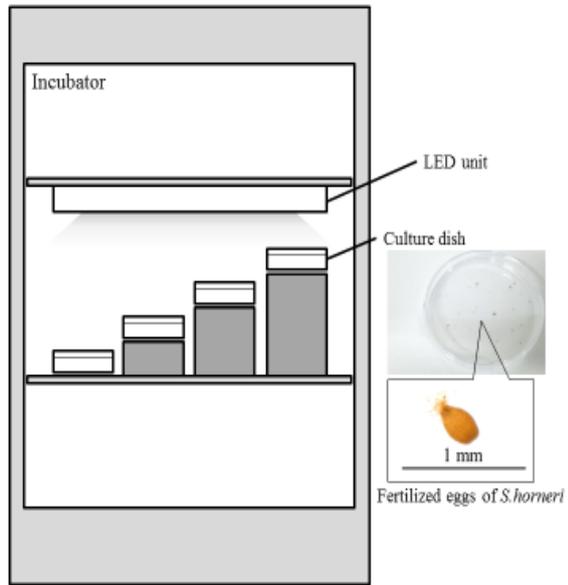


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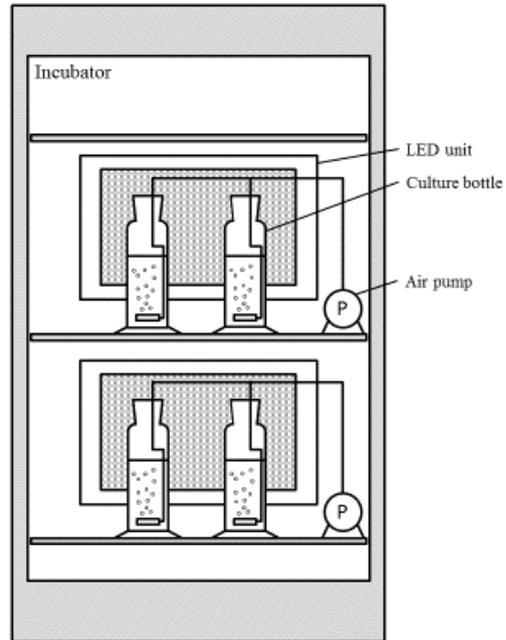
**Fig. 1**

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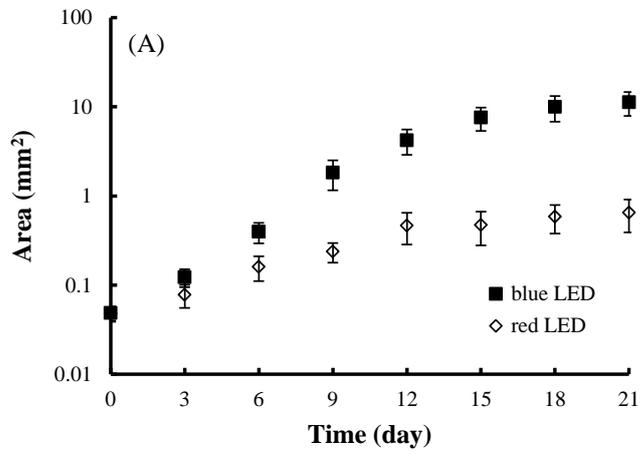
(A)



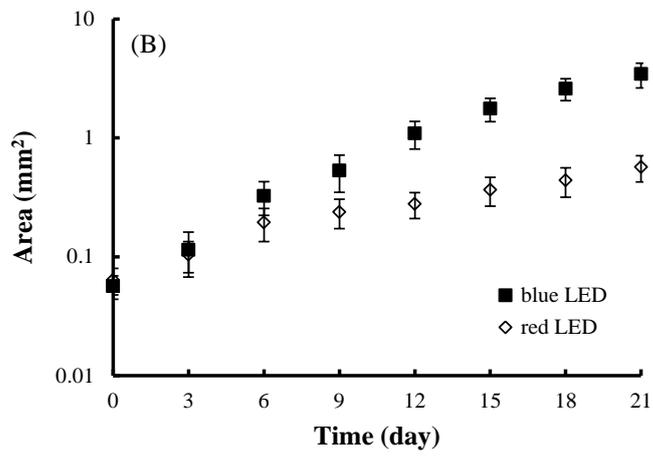
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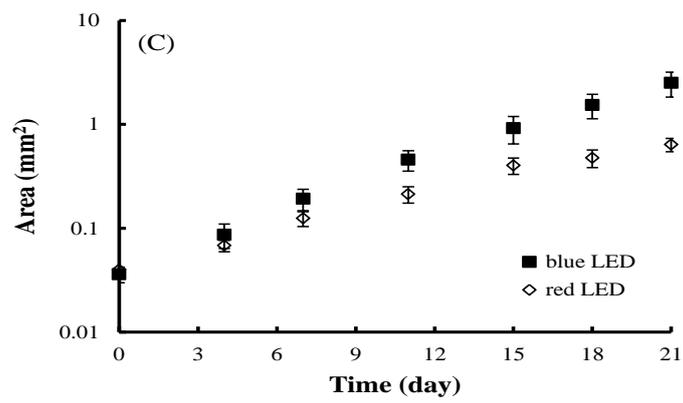
**Fig. 2**



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4 **Fig. 3**

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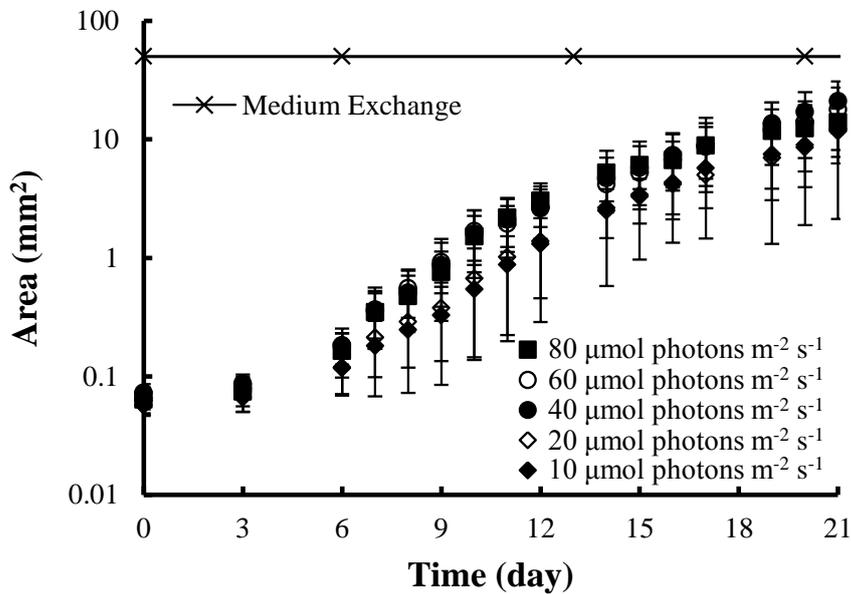


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**Fig. 4**

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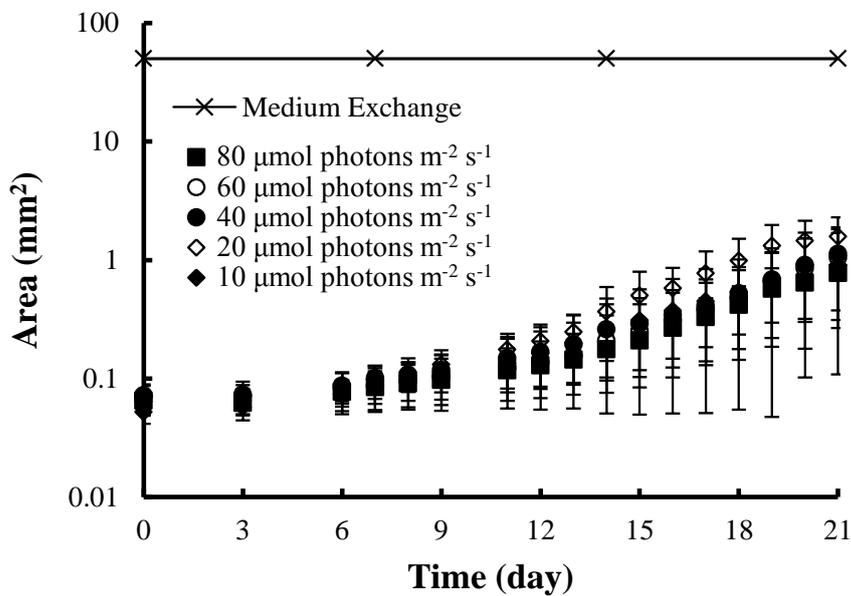
(A)



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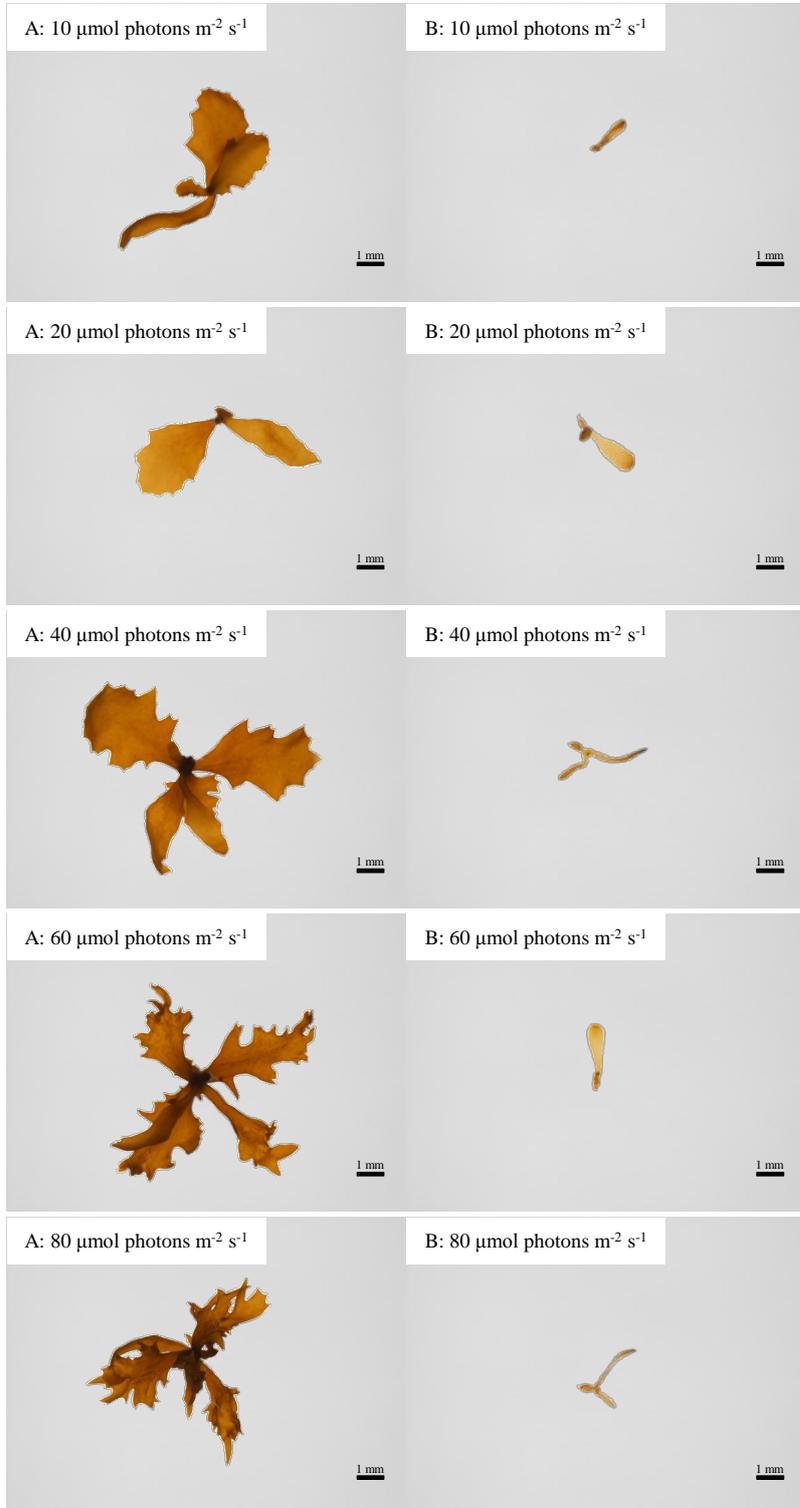


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5 Fig. 5

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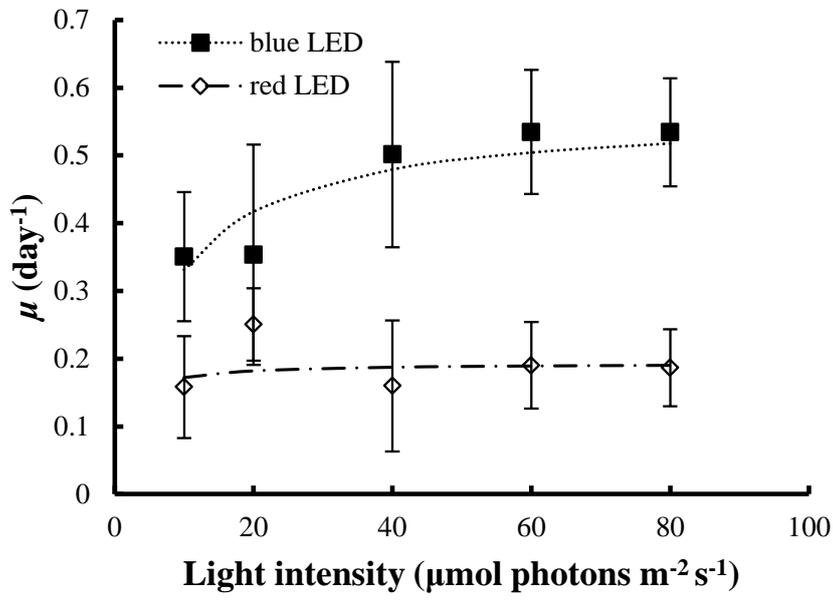
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4 **Fig. 6**

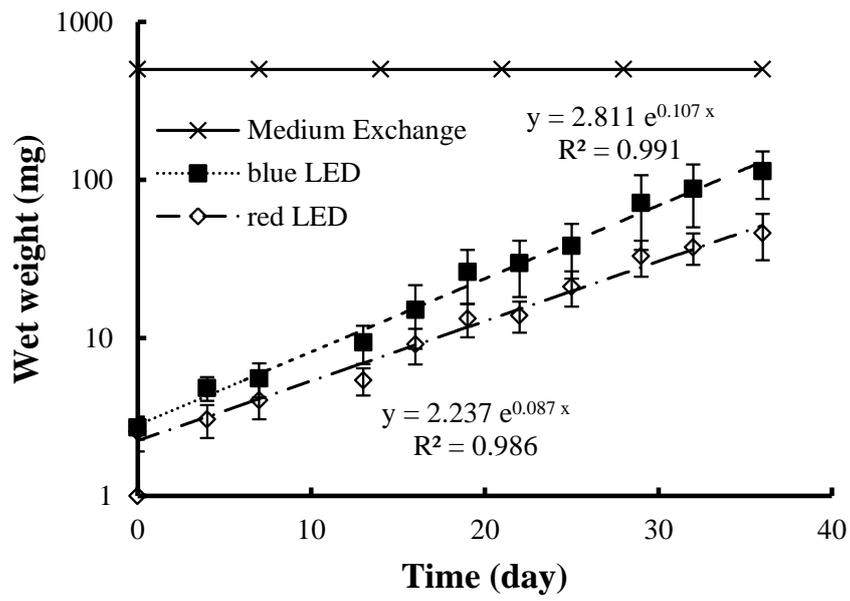
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**Fig. 7**

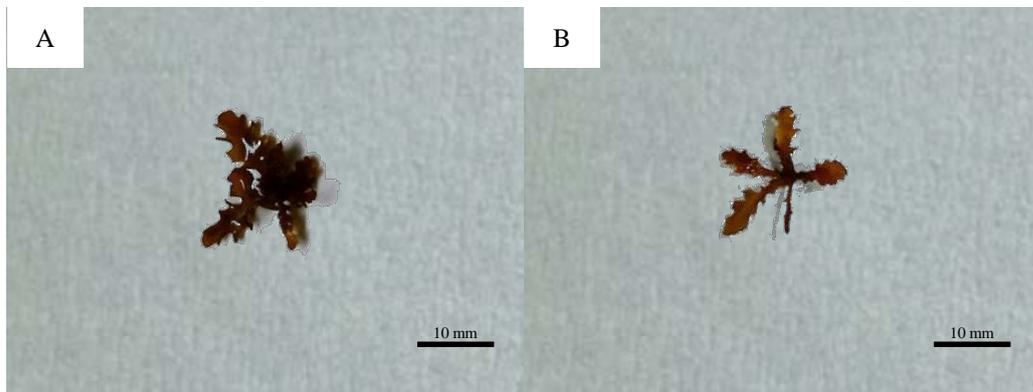
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**Fig. 8**

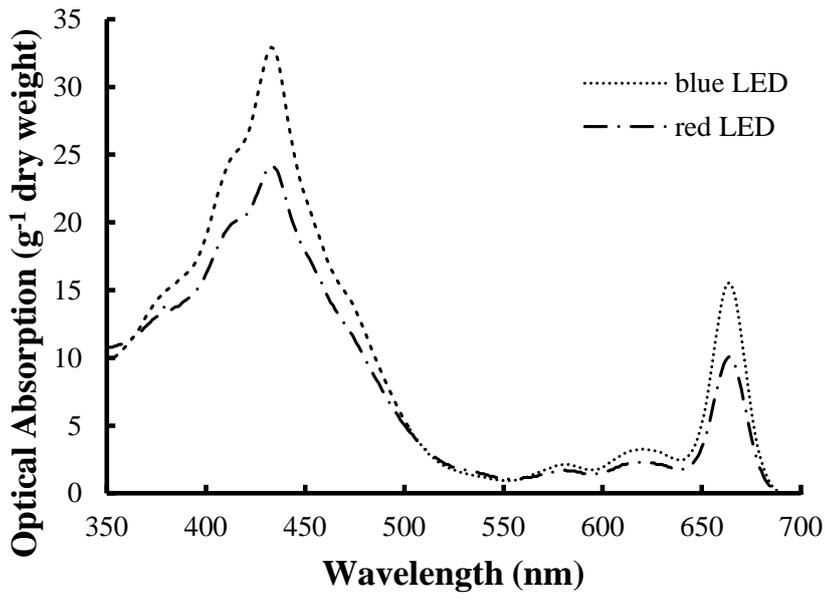
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**Fig. 9**

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**Fig. 10**