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

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

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Abstract

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

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

Introduction

Sargassum horneri, which belongs to the Sargassaceae family, is distributed widely in coastal areas of Japan. It is treated as edible brown macroalgae only in some limited areas of Japan (Tokuda et al. 1994; Murakami 2011). Sargassum is very important for the marine forest with a high potential to fix CO_2 (Ito et al. 2009; Pereira et al. 2015). The brown seaweed could be used for a food material, medical applications, and future biofuel production. (Ale et al. 2011; Murakami 2011; Ale et al. 2012; Borines et al. 2013).

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

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

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

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

Materials and methods

S. horneri

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

Culture medium

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

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

LEDs

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

Culture during the germling stage

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

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

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

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

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

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

where t_1 is the initial time (day) of the logarithmic growth phase, t_2 is the end (day) of the logarithmic growth phase, A_{t1} is the thallus area (mm^2) on the initial day of the logarithmic growth, and A_{t2} is the area of the thallus (mm^2) on the final day of logarithmic growth.

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

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

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

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

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

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

Culture during the immature stage

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

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

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

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

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 wet weight (mg) on the final day of the culture.

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

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

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

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

Results

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

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

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

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

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

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

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

The average μ values under blue LED irradiation were 0.35 ± 0.10, 0.35 ± 0.16, 0.50 ± 0.14, 0.53 ± 0.09, and 0.53 ± 0.08 day^{-1} for 10, 20, 40, 60, and 80 µmol photons m^{-2} s^{-1}, respectively.

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

In contrast, the average μ values under red LED irradiation were 0.16 ± 0.08, 0.25 ± 0.05, 0.16 ± 0.10, 0.19 ± 0.06, and 0.19 ± 0.06 day^{-1} for 10, 20, 40, 60, and 80 µmol photons m^{-2} s^{-1}, respectively. Compared with μ values under red LED irradiation of 10 µmol photons m^{-2} s^{-1}, there were no significant differences between μ values under red LED irradiations of 40, 60, and 80 µmol photons m^{-2} s^{-1} ($p > 0.05$).

In addition, the values of μ_{max} and Ks under blue or red LED irradiations are summarized in **Table 3**. For blue LEDs, μ_{max} and Ks were estimated to be 0.56 day^{-1} and 6.99 µmol photons m^{-2} s^{-1}, respectively. For red LEDs, μ_{max} and Ks were estimated to be 0.19 day^{-1} and 1.21 µmol photons m^{-2} s^{-1}, respectively. Therefore, there was a three-fold increase in μ under blue LED irradiation compared with that under red LED irradiation.

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

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

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

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

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

In addition, the μ^* value under blue LED irradiation was estimated to be 0.10 ± 0.01 day^{-1}, whereas the μ^* value under red LED irradiation was estimated to be 0.08 ± 0.00 day^{-1}. Therefore, there was an approximately 1.25-fold increase in μ^* using blue LED irradiation. Compared with μ^* values under red LED irradiation, there was a significant difference between the samples under blue LED irradiation ($p < 0.05$).

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

Discussion

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

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

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

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

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

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

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

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Table 1 Nutrient quality of enriched seawater

NH_4–N (mg L^{-1})	NO_2–N (mg L^{-1})	NO_3–N (mg L^{-1})	D–P (mg L^{-1})	Fe (μg L^{-1})
<0.01	<0.01	11.6	1.04	550

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

	μ under blue LED* (day^{-1})	μ under red LED* (day^{-1})	Logarithmic growth phase (day)
S. horneri	0.40 ± 0.01	0.20 ± 0.02	3–12
M. myagroides	0.26 ± 0.02	0.11 ± 0.01	3–12
S. patens	0.21 ± 0.01	0.16 ± 0.01	4–15

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

Table 3 Comparison of maximum specific growth rates and half-saturating constant for *Sargassum horneri* during the germling stage cultured using blue or red light-emitting diodes (LEDs)

	μ_{max} (day^{-1})	Ks (μmol photons m^{-2} s^{-1})	Logarithmic growth phase (day)
Blue LED	0.56	6.99	6–10
Red LED	0.19	1.21	12–18

Figure captions:

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

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

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

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

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

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

Fig. 7 Relationship between light-emitting diode (LED) irradiation intensities and μ of *Sargassum horneri* during the germling stage. Values represent the mean of 12 replicates ± standard deviation (SD)

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

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

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

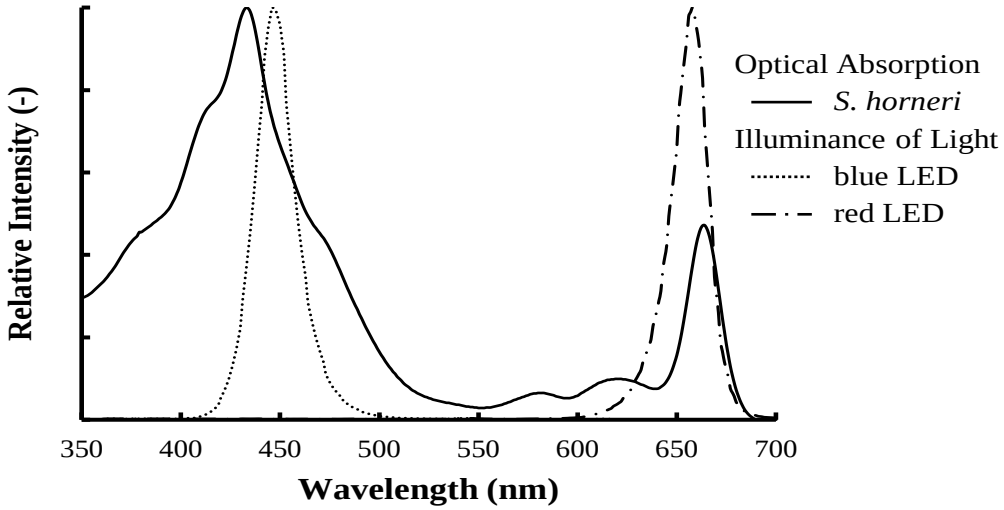
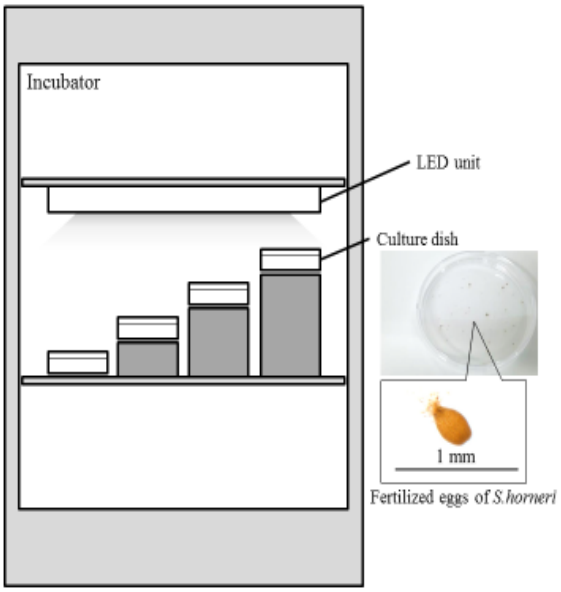

Relative Intensity (-)
350
400
450
500
550
600
650
700
Wavelength (nm)
Optical Absorption
S. horneri
Illuminance of Light
blue LED
red LED

Fig. 1

(A)


Incubator
LED unit
Culture dish
1 mm
Fertilized eggs of S.horneri

(B)

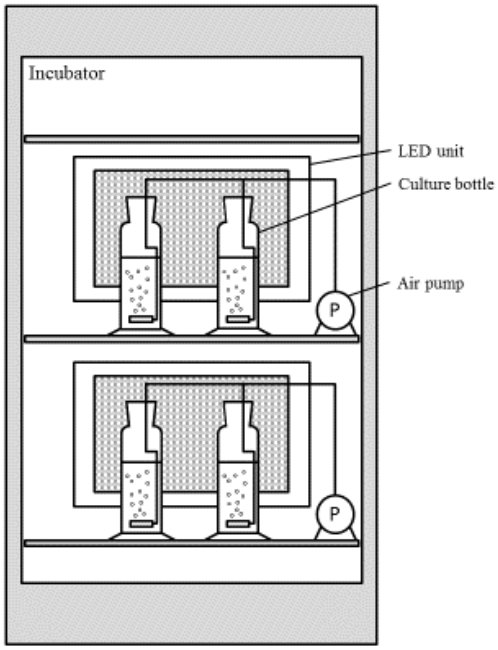
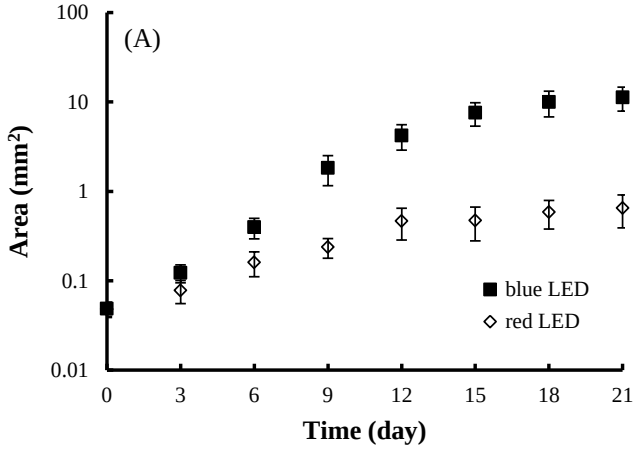
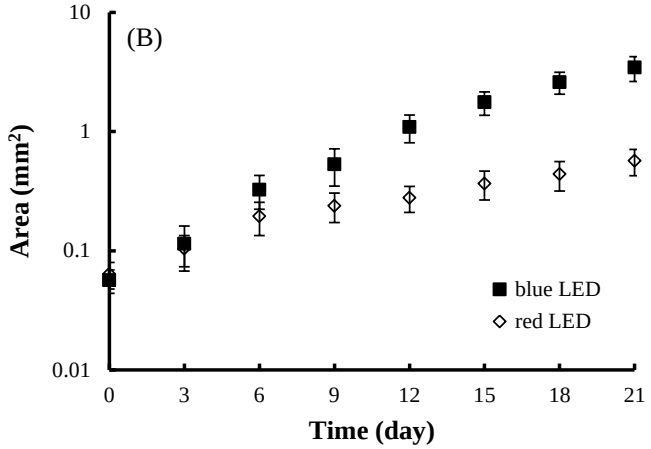

Incubator
LED unit
Culture bottle
Air pump
P
P

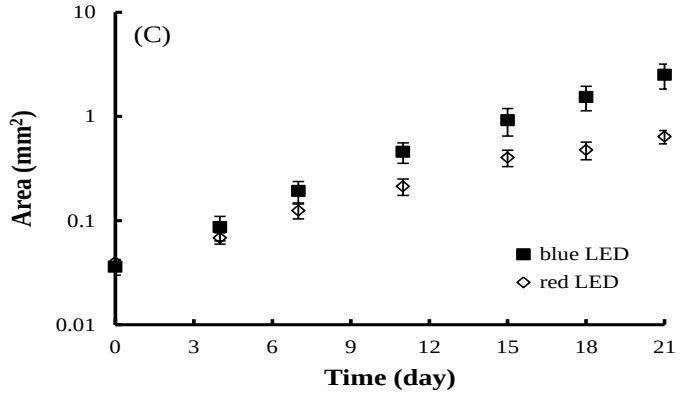
Fig. 2


(A)
100
10
1
0.1
0.01
Area (mm²)
blue LED
red LED
0
3
6
9
12
15
18
21
Time (day)

1


(B)
10
1
0.1
0.01
Area (mm²)
blue LED
red LED
0
3
6
9
12
15
18
21
Time (day)

2


(C)
10
1
0.1
0.01
Area (mm²)
blue LED
red LED
0
3
6
9
12
15
18
21
Time (day)

3

4 **Fig. 3**

5

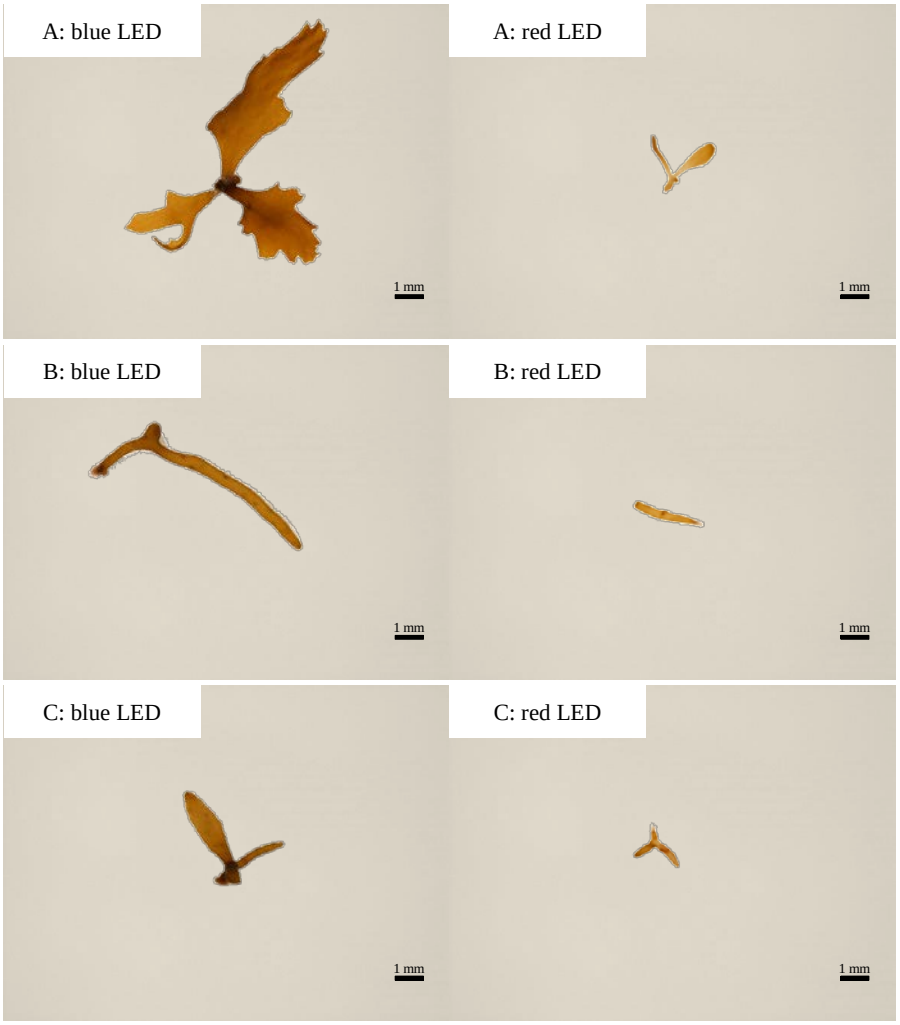
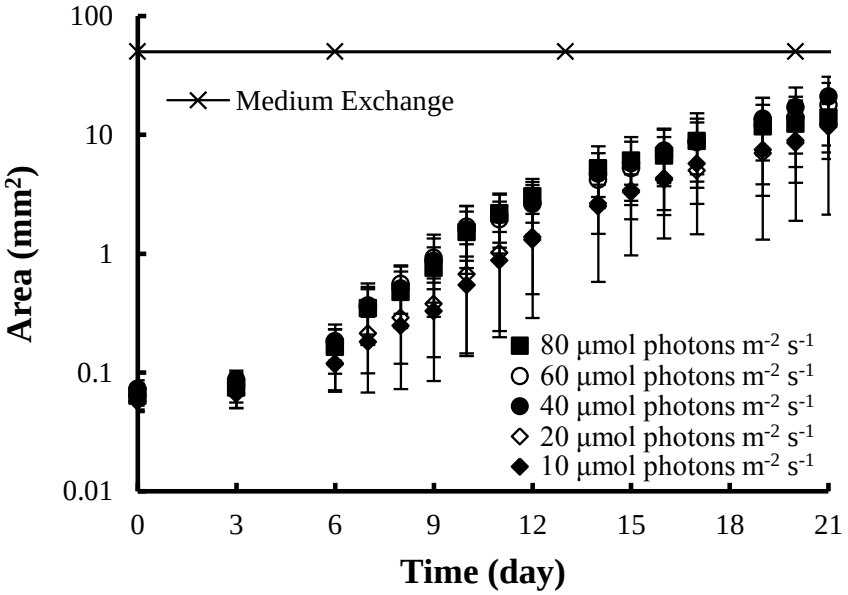


Fig. 4

(A)


100
10
1
0.1
0.01
Area (mm²)
Medium Exchange
80 µmol photons m-2 s-1
60 µmol photons m-2 s-1
40 µmol photons m-2 s-1
20 µmol photons m-2 s-1
10 µmol photons m-2 s-1
0
3
6
9
12
15
18
21
Time (day)

(B)

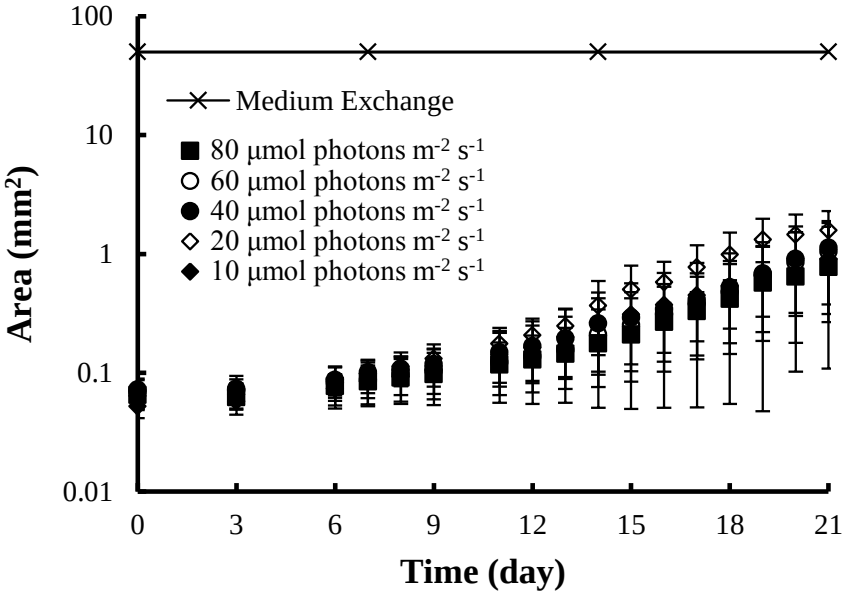

100
10
1
0.1
0.01
Area (mm²)
Medium Exchange
80 µmol photons m-2 s-1
60 µmol photons m-2 s-1
40 µmol photons m-2 s-1
20 µmol photons m-2 s-1
10 µmol photons m-2 s-1
0
3
6
9
12
15
18
21
Time (day)

Fig. 5

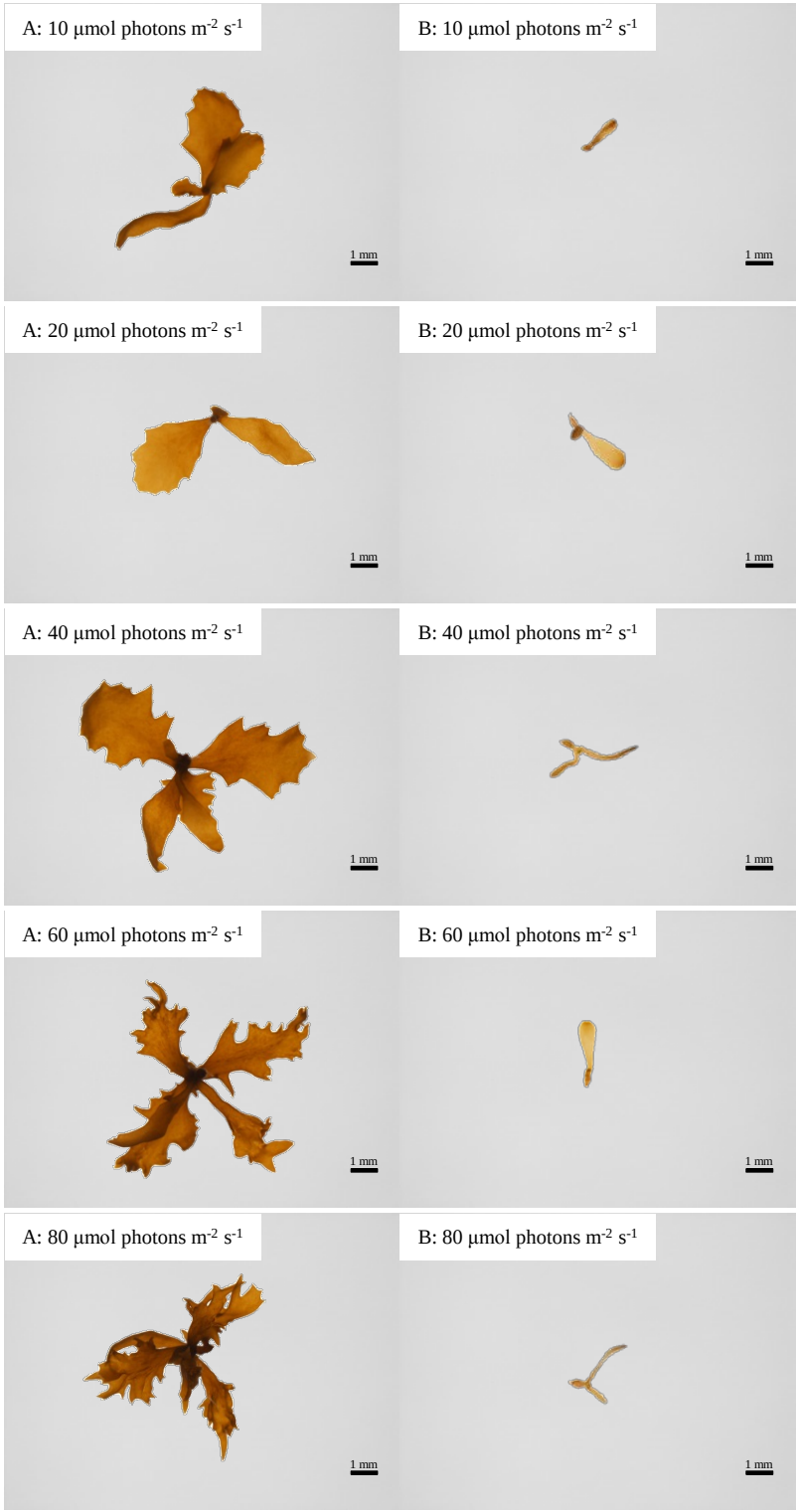

A: 10 µmol photons m-2 s-1
B: 10 µmol photons m-2 s-1
1 mm
1 mm
A: 20 µmol photons m-2 s-1
B: 20 µmol photons m-2 s-1
1 mm
1 mm
A: 40 µmol photons m-2 s-1
B: 40 µmol photons m-2 s-1
1 mm
1 mm
A: 60 µmol photons m-2 s-1
B: 60 µmol photons m-2 s-1
1 mm
1 mm
A: 80 µmol photons m-2 s-1
B: 80 µmol photons m-2 s-1
1 mm
1 mm

Fig. 6

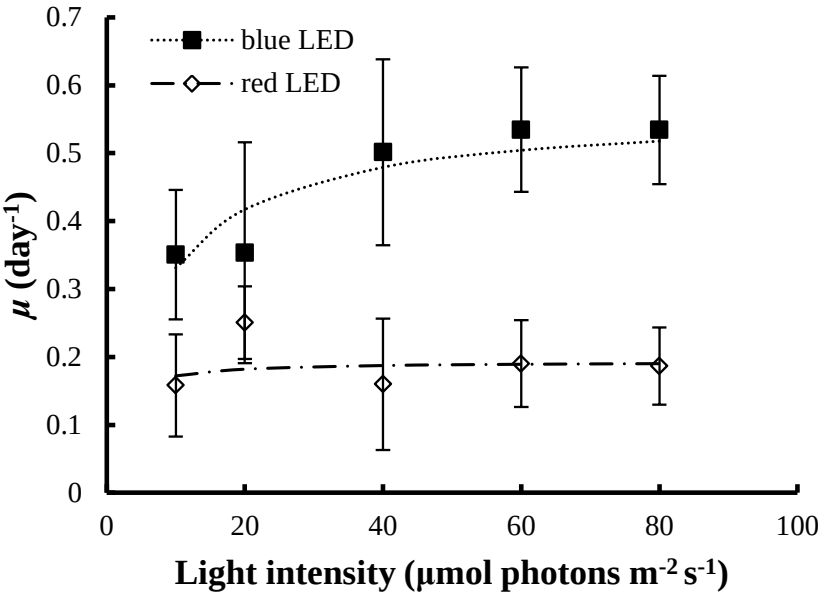

blue LED
red LED
μ (day-1)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
20
40
60
80
100
Light intensity (μmol photons m-2 s-1)

Fig. 7

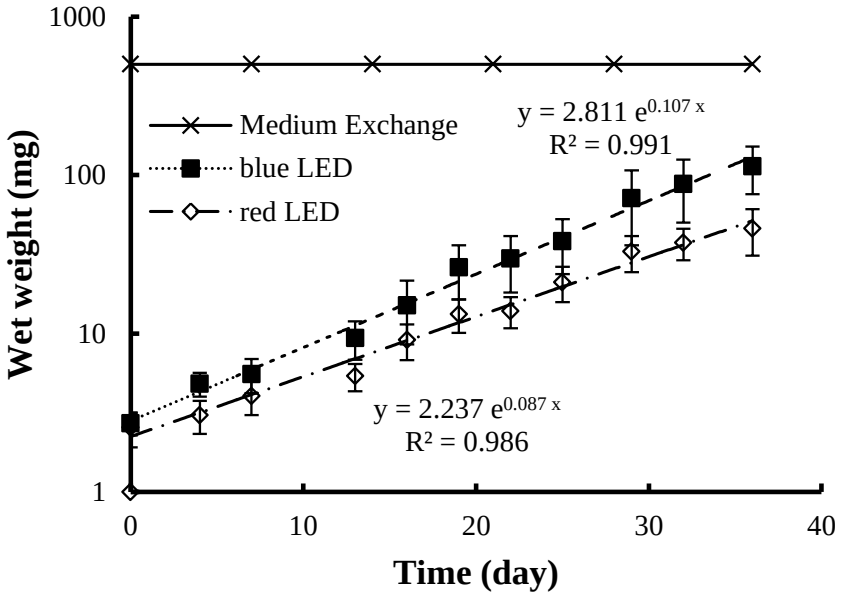

1000
100
10
1
Wet weight (mg)
Medium Exchange
blue LED
red LED
y = 2.811 e0.107 x
R² = 0.991
y = 2.237 e0.087 x
R² = 0.986
0
10
20
30
40
Time (day)

Fig. 8

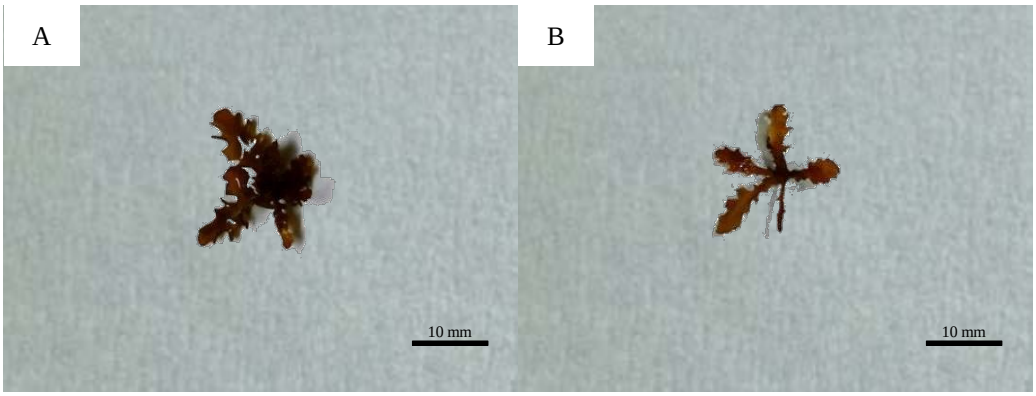


Fig. 9

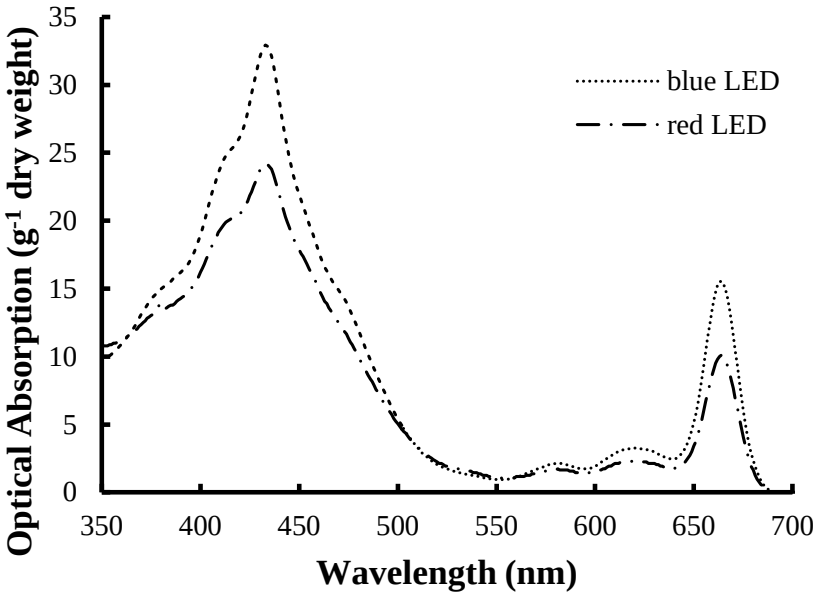

Optical Absorption (g-1 dry weight)
35
30
25
20
15
10
5
0
350
400
450
500
550
600
650
700
Wavelength (nm)
blue LED
red LED

Fig. 10