



Effects of light-emitting diode (LED) with a mixture of wavelengths on the growth and lipid content of microalgae

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Abstract

Integrations of two-phase culture for cell growth and lipid accumulation using mixed LED and green LED wavelengths were evaluated with the microalgae, *Phaeodactylum tricornutum*, *Isochrysis galbana*, *Nannochloropsis salina*, and *Nannochloropsis oceanica*. Among the single and mixed LED wavelengths, mixed LED produced higher biomass of the four microalgae, reaching 1.03 g DCW/L *I. galbana*, followed by 0.95 g DCW/L *P. tricornutum*, 0.85 g DCW/L *N. salina*, and 0.62 g DCW/L *N. oceanica* than single LED or fluorescent lights at day 10. Binary combination of blue and red LEDs could produce the high biomass and photosynthetic pigments in the four microalgae. The highest lipid accumulation during second phase with the exposure to green LED wavelengths was 56.0% for *P. tricornutum*, 55.2% for *I. galbana*, 53.0% for *N. salina*, and 51.0% for *N. oceanica*. The major fatty acid in the four microalgae was palmitic acid (C16:0) accounting for 38.3–47.3% (w/w) of the total fatty acid content.

Keywords Microalgae · Fatty acid · Mixed LED · Green LED · Two-phase culture

Introduction

Microalgal biomass is used as a feedstock for food or feed supplements, nutraceuticals, and cosmetics, and has been considered as a promising feedstock for aquaculture [1]. Microalgae play a vital role in aquatic food chain and are popularly used in rearing aquatic animals like mollusks, shrimps, and fishes at different growth stages [2]. In addition, microalgal photosynthesis has been recognized as one of the most cost-effective ways to solve global warming [3]. The four microalgae *Phaeodactylum tricornutum*, *Isochrysis galbana*, *Nannochloropsis salina*, and *Nannochloropsis oceanica* have desirable merits to contribute to environmental and economic issues such as their carbon fixation, high biomass productivity, and lipid synthesis [4–6].

Photoautotrophic microalgae absorb light energy (photons) and convert it into chemical energy, such as in the form of ATP and NADPH. These reactions occur in the microalgal photosystems and the absorption of light energy occurs via chlorophyll pigments and carotenoids [7]. Thus, the wavelength and intensity of light play key roles in the process of photosynthesis for photoautotrophic microalgal growth and also affect lipid production by microalgae [8]. The wavelengths absorbed by microalgae differ depending on the species.

Chlorophylls and carotenoids are the two major classes of photosynthetic pigments found in plants and algae. Chlorophyll *a* (Chl *a*) is the primary molecule responsible for photosynthesis, while chlorophyll *b* (Chl *b*) is an accessory pigment, the level of which increases upon exposure to a broad spectrum of light that transfers the energy to Chl *a*. Carotenoids as photosynthetic pigments play a role of excess energy disposal. The absorbance maxima of chlorophylls and carotenoids are in the red and blue wavelength regions of the light spectrum [7].

The use of specific narrow bands of light using light-emitting diode (LED) is more economical than using ordinary light sources with cost-effective low-wattage irradiance. Indeed, the light absorption ability of photosystem II was improved by red light, while that of photosystem I was

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improved by blue light [9]. Thus, mixed LED lights with red (660 nm) and blue (465 nm) wavelengths are more suitable for microalgal growth than other wavelengths and promote microalgal photosynthesis [10].

One of the most effective methods of improving microalgal lipid accumulation is nutrition stress [11]. However, higher lipid contents by nutrition stress conditions were accompanied by lower growth rates, which lead to decreased biomass and lipid productivity [12]. Gao et al. [13] studied the effect of nitrogen limitation on lipid accumulation of *Chaetoceros muelleri*. Under nitrogen-limited conditions, the lipid accumulation was increased to 23–46% and decreased in biomass productivity from 19.0 to 12 mg/L. On the other hand, little data of green LED wavelength stresses are available in the literature on lipid accumulation influence on microalgae. Thus, a two-phase culture was carried out to enhance the biomass and lipid productivities in the one photobioreactor. In the first phase, the microalgae are grown under mixed LED lights with red (660 nm) and blue (465 nm) wavelengths to obtain maximum biomass production. Then, lipid accumulation was performed with green (520 nm) wavelength at 10 days of culture as second phase.

The aim of this study was to use LED lights with a mixture of red and blue wavelengths, and green wavelength as two-phase culture process to enhance the production of components such as biomass, carotenoids, and lipids in four marine microalgae: *P. tricornutum*, *I. galbana*, *N. salina*, and *N. oceanica*.

Materials and methods

Microalgal strains and culture conditions

Four microalgae, *Phaeodactylum tricornutum*, *Isochrysis galbana*, *Nannochloropsis salina*, and *Nannochloropsis oceanica*, were obtained from the Korea Marine Microalgae Culture Center (Busan, Korea). They were cultured in sterilized seawater with modified *f/2* medium [14]. Four microalgae species were cultured in 2 L flasks with a 1.5 L working volume at 20 ± 1 °C, an aeration rate of 2.5 L/min, nitrate concentration of 16 mg/L, and a 12/12-h light/dark cycle under a light intensity of $100 \mu\text{mol}/\text{m}^2/\text{s}$ for 10 days. After the culture reached to stationary phase at 10 days, the second phase culture was carried out to accumulate lipid in microalgae until 13 days.

The two-phase culture process was employed to elucidate the link between mixed blue and red LED wavelengths for the biomass production and green LED wavelength stress condition for lipid accumulation as an energy storage product. This strategy included first phase allowing high biomass production without stress using blue LED (465 nm), red LED (660 nm), or mixed LED (blue:red = 1:1) in 10 days.

Then, green LED (520 nm) wavelength stress in the second phase culture was carried out for 3 days to find the optimum culture time to accumulate high lipid contents.

A control experiment was carried out using fluorescent light at the same light intensity and operating conditions. The initial cell density was determined using the standard curve of OD_{680} versus dry cell weight (DCW) using a UV spectrophotometer (Ultrospec 6300 Pro; Biochrom Ltd., Cambridge, UK). Aeration with filtered air was provided through an air stone at a rate of 2.5 L/min. Each of the flasks was then exposed to light with the mixture of red (660 nm) and blue (465 nm) wavelengths under a 12-h:12-h light–dark cycle for 10 days and green (520 nm) wavelength for day 10 to day 13.

Light source for microalgal culture

LED square panel lights ($28.5 \times 38.6 \times 4.4 \text{ cm}^3$; LUXPIA Co., Ltd., Suwon, Korea) were used as the light source for the photoautotrophic growth of microalgae, as shown in Fig. 1. Thirty blue (465 nm), green (520 nm), and red (660 nm) diodes were each spaced alternately in six strips at 1-cm intervals in both rows and columns. LED light at $100 \mu\text{mol}/\text{m}^2/\text{s}$ with a mixture of blue (465 nm) and red (660 nm) wavelengths at a 1:1 ratio and green (520 nm) were used for cell biomass and lipid production, respectively. The LED square panel lights were placed in parallel to illuminate the 2-L flask culture at a distance of 15 and 7 cm for cell mass (first phase) and lipid (second phase) production, respectively. The light intensity was measured with a light sensor (TES-1339; UINS Inc., Busan, Korea) as the photon flux at the center line of the flask filled with culture medium. A control experiment was conducted under the same intensity of fluorescent light and operating conditions for 13 days.

Lipid extraction and transesterification

The numbers of inoculated microalgal cells were determined by counting using a hemocytometer and a microscope (CK40-SLP; Olympus, Tokyo, Japan). The cultures were sampled at 24-h intervals. Based on the standard curve of OD_{680} and DCW, one unit of OD_{680} corresponded to 0.42-, 0.42-, 0.27-, and 0.22-g DCW/L dry biomass weights of *P. tricornutum*, *I. galbana*, *N. salina*, and *N. oceanica*, respectively. The specific growth rate (μ) of microalgae was calculated using the following equation:

$$\mu = \frac{(\text{DCW}_2 - \text{DCW}_1)}{(\text{DCW}_1 \times (T_2 - T_1))} \quad (1)$$

where μ is the specific growth rate (h^{-1}), and DCW_1 and DCW_2 are the dry cell weights at times T_1 and T_2 ,

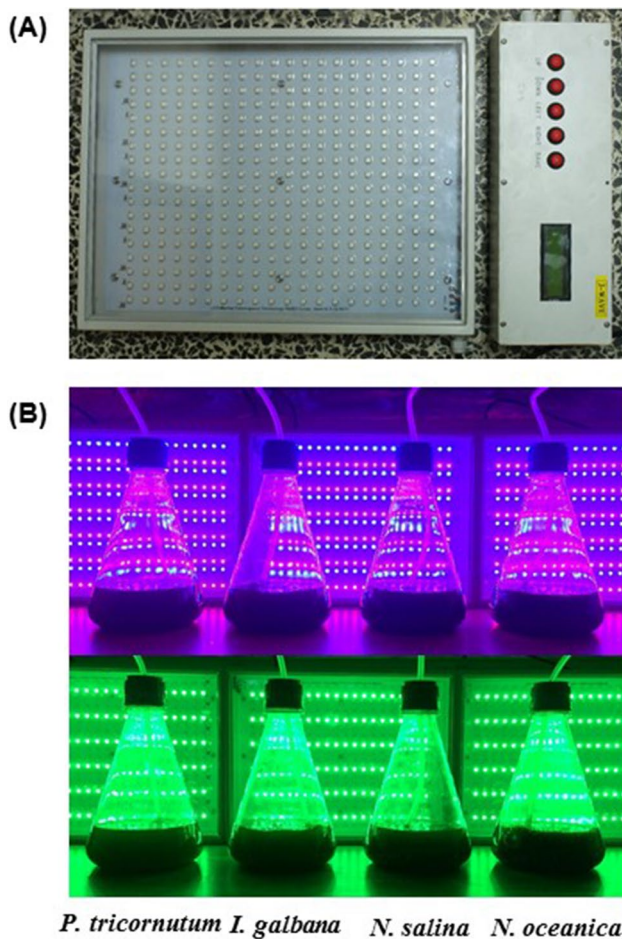


Fig. 1 Light-emitting diodes (LEDs) cultivation system. **a** Setup of LED lights and controller; **b** LED algal cultivation with a mixture of wavelengths and green LED as stress wavelength

respectively. Nitrate concentration was determined according to the standard methods using an ultraviolet–visible (UV–Vis) spectrophotometer (Ultrospec™ 6300 Pro; Biochrom Ltd.) [15]. Determination of chlorophyll *a*, chlorophyll *b*, and total carotenoid contents has been well established [16]. The determination of levels in whole pigment extract of microalgae using a UV–Vis spectrophotometer was performed using the following equations:

$$\text{Chl } a \text{ } (\mu\text{g/mL}) = 11.24 \times \text{OD}_{662} - 2.04 \times \text{OD}_{645} \quad (2)$$

$$\text{Chl } b \text{ } (\mu\text{g/mL}) = 20.13 \times \text{OD}_{645} - 4.19 \times \text{OD}_{662} \quad (3)$$

$$\text{TC } (\mu\text{g/mL}) = (1000 \times \text{OD}_{470} - 1.90 \times \text{Chl } a - 6.31 \times \text{Chl } b) / 214. \quad (4)$$

Lipid extraction and transesterification

After cell growth and lipid accumulation, suspended cells were harvested by centrifugation (994×*g*, 10 min) and lyophilized using freeze drier (SFDSM-24L; SamWon Industry,

Seoul, Korea). Total lipids were extracted with chloroform/methanol (2:1, v/v) and quantified gravimetrically [17]. The lipid content in dry weight was calculated using the following equation:

$$\text{Lipid content (\% of DCW)} = \frac{(W_2 - W_1)}{\text{DCW}} \times 100, \quad (5)$$

where lipid content is the cellular lipid content of the microalgae (% of DCW), W_1 (g) is the weight of the empty 20 mL glass tube, W_2 (g) is the weight of the lipid extracting 20 mL glass tube, and DCW (g) is the dry cell weight of the microalgae.

The lipid composition was determined as fatty acid methyl esters (FAMES) via the direct transesterification method [18]. FAME profiling was performed by gas chromatography (GC; YL 6100; YoungLin Inc., Anyang, Korea) with a flame ionization detector (FID) and a silica capillary column (HP-INNOWAX; 30 m × 0.32 mm × 0.5 μm; Agilent Technologies, Santa Clara, CA, USA). The column temperatures and holding times were set as follows: 140 °C for 5 min, followed by a temperature increase to 240 °C at a rate of 5 °C/min; this temperature was maintained for 5 min. Both injector and FID detector temperatures were set to 250 °C. The FAMES were identified by comparing their retention times to those of authentic standards.

Statistical analysis

Each experiment was carried out in triplicate. The statistical significance of differences in biomass and lipid contents was evaluated by one-way analysis of variance and Duncan's multiple range test ($P < 0.05$) using SPSS Software (ver. 23; SPSS Inc., Chicago, IL, USA)

Results and discussion

Effects of LED light with a mixture of wavelengths on the growth of microalgae

P. tricornutum, *I. galbana*, *N. salina*, and *N. oceanica* cultures under mixed LED lights of blue:red wavelengths at a ratio of 1:1 were carried out for four microalgae. The cell growth with mixed LED lights was compared to those with blue LED (465 nm), red LED (660 nm), or conventional fluorescent lights for 13 days, as shown in Fig. 2.

Among those wavelengths, mixed LED produced the highest biomass among the four microalgae, achieving 1.03 g DCW/L *I. galbana*, followed by 0.95 g DCW/L *P. tricornutum*, 0.85 g DCW/L *N. salina*, and 0.62 g DCW/L *N. oceanica* at day 10. This suggests that mixture of blue and red LEDs can be suitable light source for microalgae culture, because microalgal plastids are related to those of

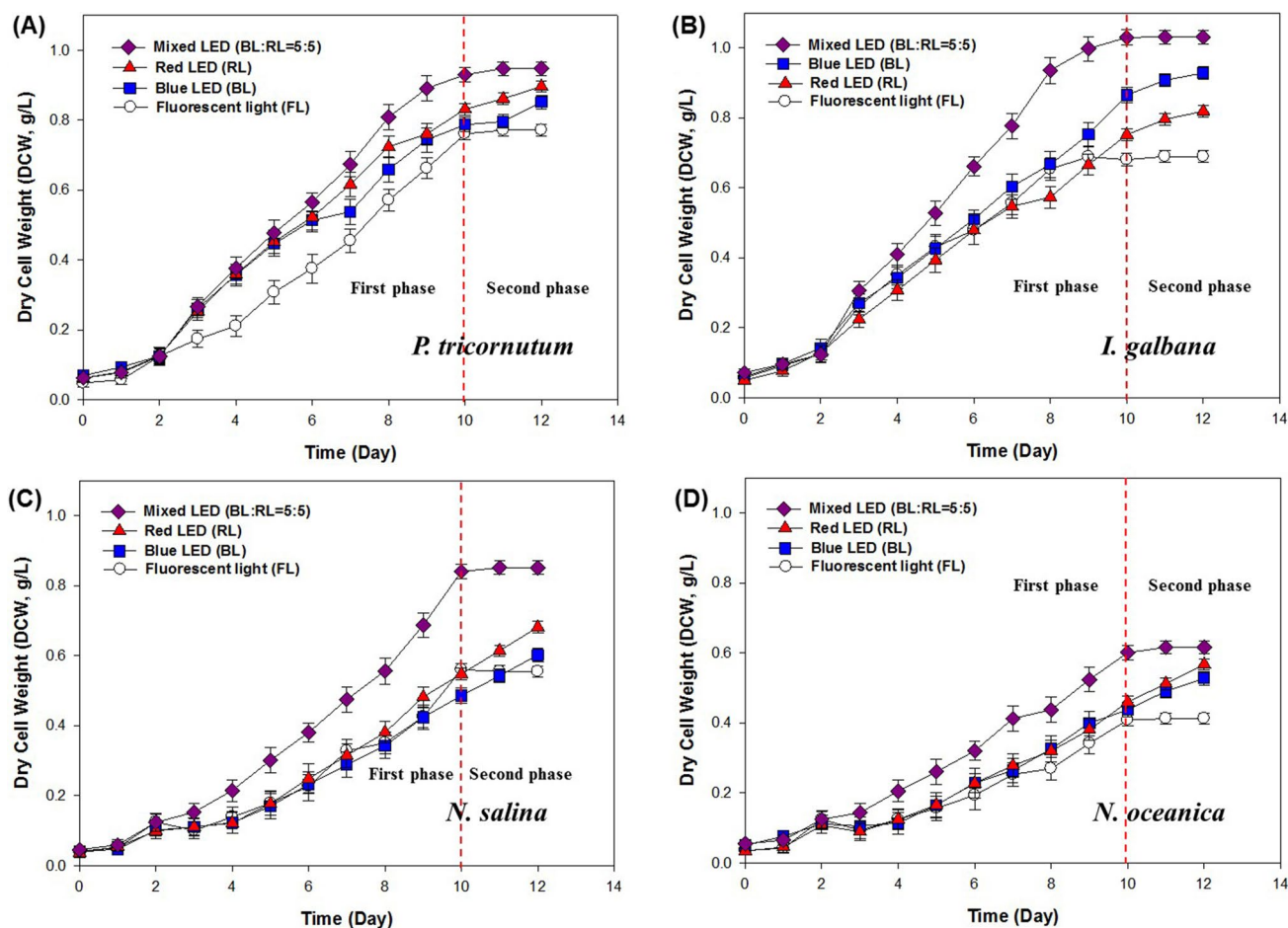


Fig. 2 Effects of LED with single and mixture of red and blue wavelengths and fluorescent light on the growth of four microalgae. **a** *P. tricornutum*, **b** *I. galbana*, **c** *N. salina*, and **d** *N. oceanica*. The vertical line in **a** indicates the start of second phase in microalgae cultures

terrestrial plants in terms of structure, metabolism, and biochemical composition [19]. The specific growth rates were calculated from the growth curve in Fig. 2 a–d. Specific growth rates (μ) of *P. tricornutum*, *I. galbana*, *N. salina*, and *N. oceanica* with mixed LED were 0.037, 0.042, 0.029, and 0.025 h^{-1} , respectively. However, four microalgae with the blue, red LEDs, and fluorescent light resulted in decrease of the specific growth rates such as 0.029, 0.030, and 0.025 h^{-1} for *P. tricornutum*; 0.025, 0.025 and 0.022 h^{-1} for *I. galbana*; 0.021, 0.024 and 0.021 h^{-1} for *N. salina*; and 0.019, 0.025 and 0.018 h^{-1} for *N. oceanica*, respectively. These results were consistent with those from Chen et al. [20]. The illumination with mixed blue (465 nm) and red LED (660 nm) wavelengths at 1:1 ratio led to higher microalgae biomass production than those of single LED wavelengths and fluorescent light. Similar results were obtained previously for the culture of *Chlorella* sp., which showed maximum biomass production of 0.49 g DCW/L using a mixture of red (660 nm) and blue (460 nm) LEDs at 1:1 ratio [21]. As shown in Fig. 3, the four microalgae reached the

stationary phase of growth at 10 days and reached to the stage of depleted nitrate concentrations at 10–11 days. Overall, mixed blue (465 nm) and red LED (660 nm) wavelengths at 1:1 ratio consumed higher nitrate than those of single LED wavelengths and fluorescent light. Using mixed LED lights, four microalgae showed the high and fast cell growth with 16.0 mg/L nitrate concentration.

Furthermore, several studies have applied LED with a mixture of wavelengths to promote microalgal photosynthesis [10, 20, 22, 23]. The biomass upon the treatment with red:blue light at a ratio of 1:1 was higher than those of the other treatments, such as at red:blue light ratios of 1:9, 3:7, 7:3, and 9:1 [10]. In addition, the opposite effect was observed in *Nannochloropsis* sp., which grew better under blue LED comparing to multi-chromatic white LED exposure [5]. This preference showed a correlation with the evolutionary pigment composition of the light-harvesting complexes in the chloroplasts of each microalgae [19]. In this present study, mixed LED was selected for biomass production of four microalgae.

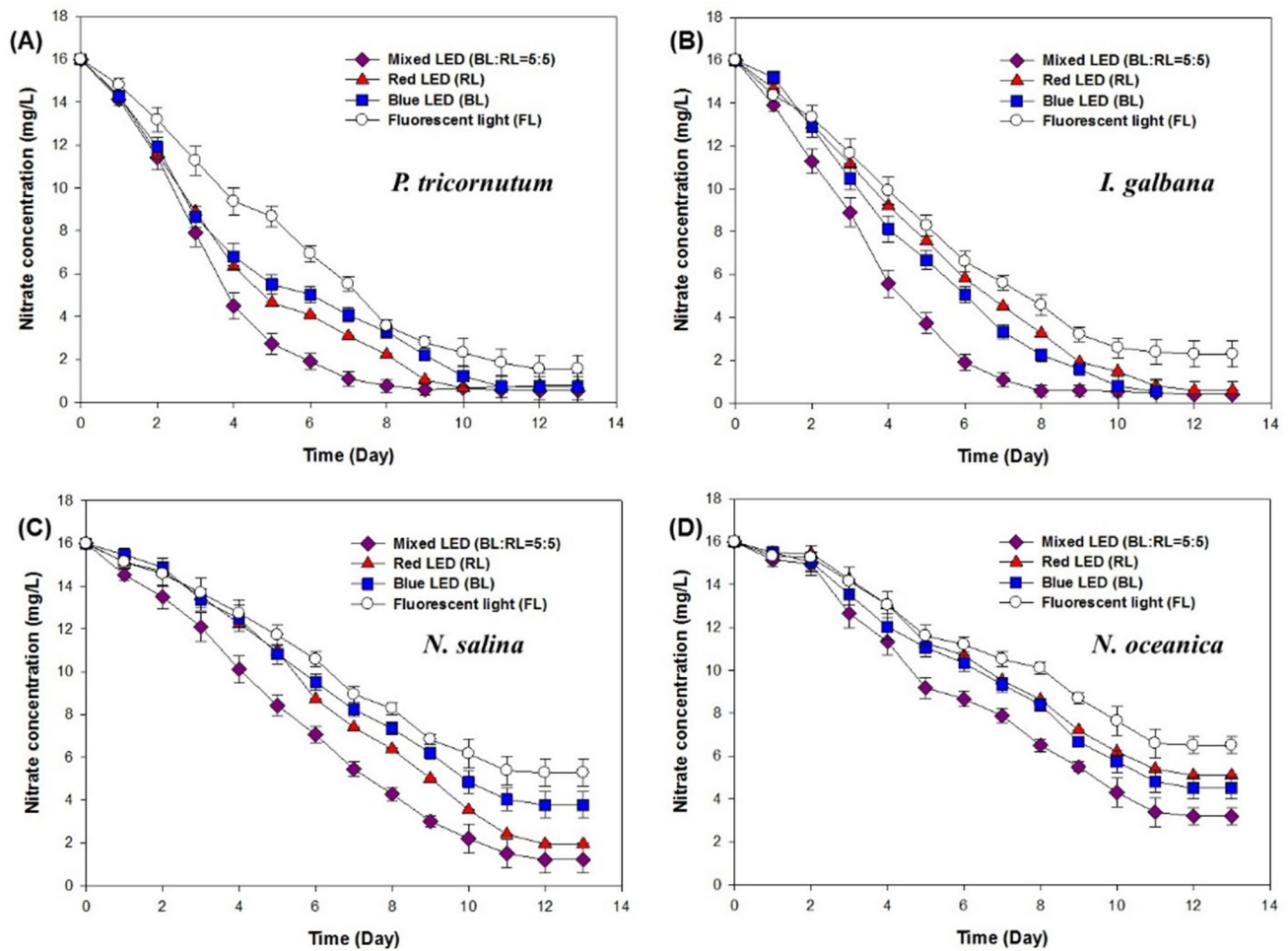


Fig. 3 Effects of LED with single and mixture of red and blue wavelengths and fluorescent light on the nitrate consumptions of four microalgae. **a** *P. tricornutum*, **b** *I. galbana*, **c** *N. salina*, and **d** *N. oceanica*

Effects of LED light with a mixture of wavelengths on Chl *a*, Chl *b*, and carotenoids

As shown in Fig. 4, higher concentrations of biomass and photosynthetic pigments (Chl *a*, Chl *b*, and carotenoids) were obtained with mixed red (660 nm) and blue (460 nm) LED at a 1:1 ratio cultivation than those with the conventional fluorescent light at 10 days in four microalgae. As shown in Fig. 4a, the growth of four microalgae was improved using a mixture of red and blue LEDs at a ratio of 1:1 comparing to those of single LED wavelengths and fluorescent light (Fig. 2).

Figure 4b, c shows that the pigment concentrations upon exposure to mixed LED wavelengths were as follows: *P. tricornutum* (Chl *a*: 9.91 mg/g, Chl *b*: 0.92 mg/g), *I. galbana* (Chl *a*: 11.38 mg/g, Chl *b*: 1.48 mg/g), *N. salina* (Chl *a*: 9.95 mg/g, Chl *b*: 2.06 mg/g), and *N. oceanica* (Chl *a*: 5.64 mg/g, Chl *b*: 0.66 mg/g). These results indicate that Chl *a* and Chl *b* contents of four microalgae cultured under

mixed LED were higher than those cultured under the conventional fluorescent light. Koller et al. [24] reported that the chlorophyll *a*, *b* and carotenoid contents in biomass for the photosynthesis reaction. Because chlorophyll *a* is the core of reaction pigment, while accessory pigments chlorophyll *b*, *c*, and *d* extend the range of wavelengths [7]. Thus, spectral quality is defined by the adsorption spectrum (light wavelengths) of the chlorophyll and other photosynthetically active pigments in microalgae such as carotenoids [25]. Figure 4d shows that the highest carotenoid content of 6.30 mg/g was obtained with *I. galbana* exposed to mixed LED wavelengths. *P. tricornutum*, *N. salina*, and *N. oceanica* showed carotenoid concentrations of 4.70, 3.12, and 2.54 mg/g, respectively. These results indicate that combination of blue and red LEDs is critical for the syntheses of photosynthetic pigments.

In addition, most photosynthetic pigments (Chl *a*, Chl *b*, and carotenoids) have two major wavelengths: blue wavelength range of 450–475 nm or the red wavelength range of

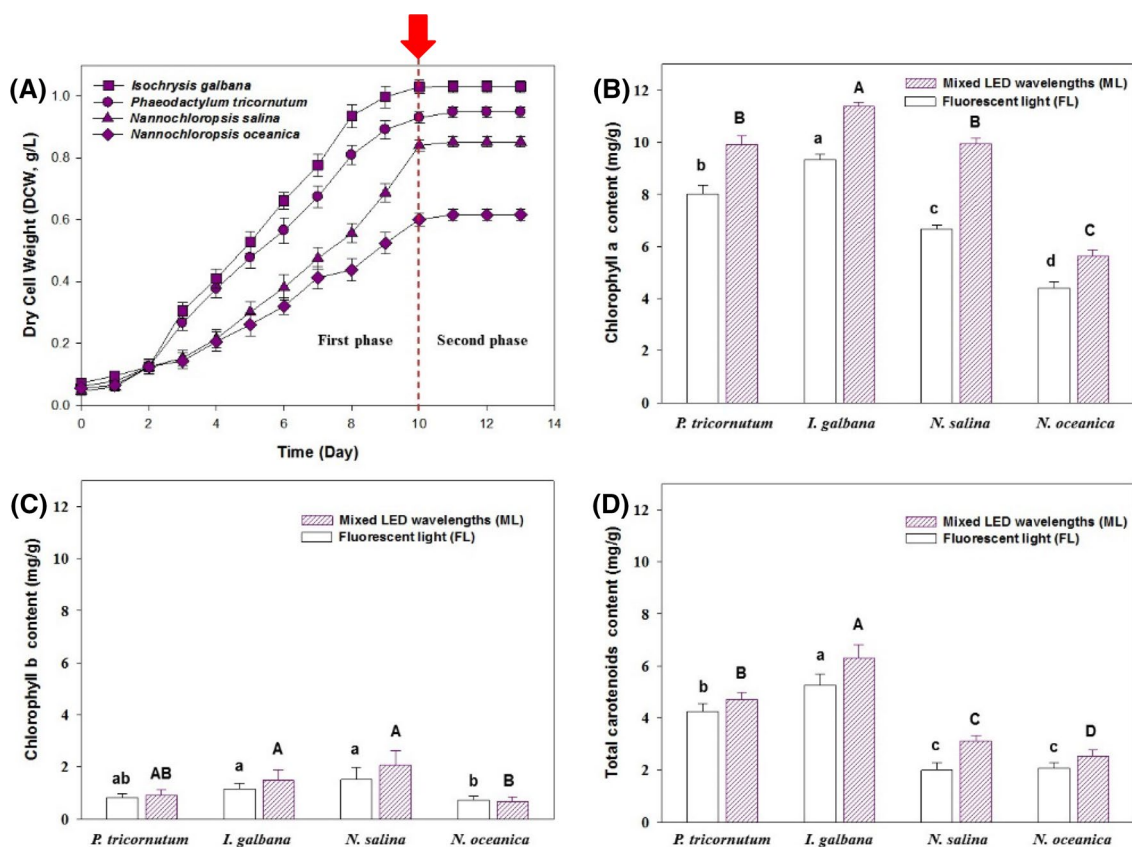


Fig. 4 Effects of mixed LED wavelengths on the growth and chlorophylls of microalgae during 10 days of culture. **a** Growth of four microalgae, **b** chlorophyll *a*, **c** chlorophyll *b*, and **d** carotenoids in

four microalgae. The vertical line in **a** indicates the start of second phase in microalgae cultures. Different capital and small letters indicate significant differences ($P < 0.05$, Duncan's test)

630–675 nm [23]. Due to the specific wavelength, microalgae require optimum light conditions within the narrow band of spectrum. However, the ordinary fluorescent light for microalgae culture contains a wide range of wavelengths and consists of a combination of growth-efficient and growth-in-efficient wavelengths [26]. Therefore, the production of two essential chlorophylls and carotenoids was sufficiently stimulated to achieve the highest growth rate of microalgae, especially upon exposure to mixed red (660 nm) and blue (465 nm) wavelengths.

Effect of lipid accumulation using two-phase culture process

Two-phase culture processes were carried out for high biomass production using mixed LED in 10 days, and then, stress condition with green (520 nm) LED wavelength in the second phase at day 10 was applied for following 3 days, as shown in Figs. 4a and 5. The effects of fluorescent light, mixed LED (blue:red = 1:1) and green LED wavelengths on lipid accumulation were evaluated with four species of microalgae, as shown in Fig. 5.

As shown in Fig. 5a–d, the high lipid accumulations at day 3 of second phase under fluorescent light were 45.0% for *P. tricornutum*, 41.4% for *I. galbana*, 32.0% for *N. salina*, and 32.0% for *N. oceanica*. The high lipid accumulations at day 2 of exposure to mixed LED wavelengths were 53.0% for *P. tricornutum*, 51.4% for *I. galbana*, 48.6% for *N. salina*, and 47.6% for *N. oceanica*. The highest lipid accumulation was achieved under green LED, reaching 56.0% of the dry cell weight by *P. tricornutum* in day 2, followed by *I. galbana* (55.2%) in day 3, *N. salina* (53.0%) in day 2, and *N. oceanica* (51.0%) in day 2. There were significant differences in lipid contents under fluorescent light, mixed LED, and green LED wavelength stresses.

In general, a number of researchers have reported on lipid content improvement using physical or chemical stresses, such as nitrogen starvation [27], high light intensity [28], high temperature [29], and salinity [30]. However, higher lipid contents by physical or chemical stress conditions were accompanied by lower growth rates, which lead to decreased biomass and lipid productivity [11, 12]. These reports indicated that it is mainly due to

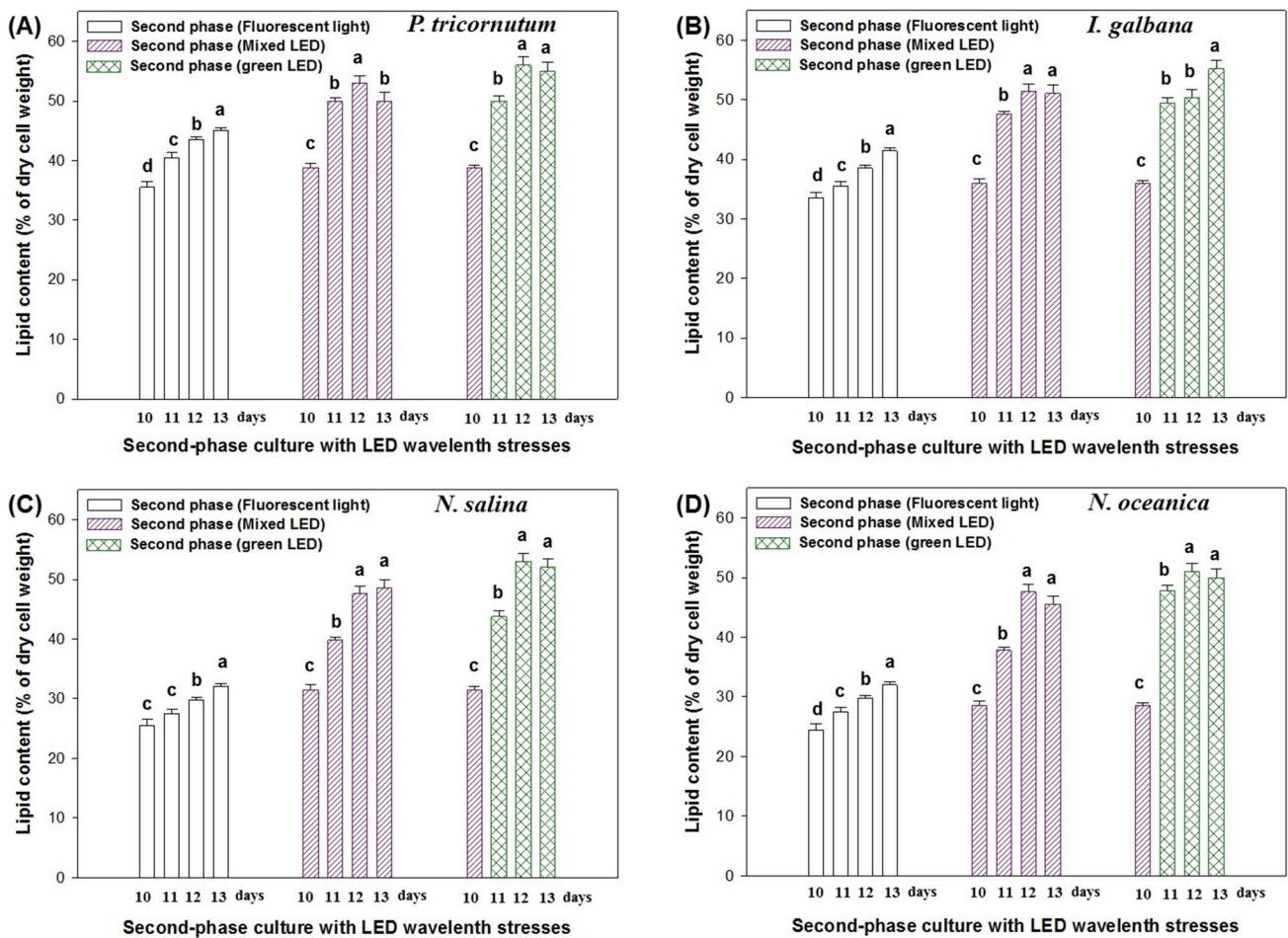


Fig. 5 Profiles of lipid production (% of dry cell weight) of four microalgae using mixed LED, green LED, and fluorescent light during 3 days of LED wavelength stresses. Different small letters indicate significant differences ($P < 0.05$, Duncan’s test)

reduced rate of metabolism under stress, the excess carbon generated is channeled towards as storage molecules predominantly triacylglycerol (TAG) or starch depending upon the microalgae species [26]. Therefore, the green LED as a light wavelength stress in the second phase could enhance the performance of lipid accumulation comparing to other stress factors. As observed in the previous report [6], the lipid accumulation by green LED wavelength was higher than those of different LED wavelengths. This may be due to the reason that green LED was not absorbed but reflected. Similar results were obtained for *Nannochloropsis* sp [23]. It was also observed that green microalgae species did not grow well under green LEDs. Whereas the culture was under dark period (100% dark phase) in the second phase, microalgae did not show cell growth or carbon dioxide consumption [31]. Thus, green LED wavelength stress could be used as the simple and convenient lipid accumulation method without changing culture medium from one photobioreactor. Figure 5.

Fatty acid composition

The lipids extracted from four microalgae, *P. tricornutum*, *I. galbana*, *N. salina*, and *N. oceanica*, were converted to FAMES and their compositions are shown in Table 1. Through the analysis of the fatty acid methyl esters (FAMES) composition, a useful comparison of four microalgae lipids with respect to the saturated (SFAs; C14:0, C16:0, C18:0, C20:0, and C22:0) and unsaturated (UFAs; C18:1, C18:2, C18:3, C20:5, and C22:6) fatty acids is provided in the present study.

Four species of microalgae were enriched in the common C14–C22 fatty acids such as myristic (C14:0), palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), arachidic (C20:0), eicosa-pentaenoic (C20:5, EPA), behenic (C22:0), and docosa-hexaenoic (C22:6, DHA) acids. Those indicated fatty acids of four microalgae varied significantly. Among these FAMES, 38.3–47.3% (w/w) palmitic acid (C16:0) was predominant form observed for four microalgae in

Table 1 Compositions of fatty acids, as fatty acid methyl esters (FAMES), in *P. tricornutum*, *I. galbana*, *N. salina*, and *N. oceanica* cultured in the second phase under green LED wavelength stress

Fatty acids	<i>P. tricornutum</i>		<i>I. galbana</i>		<i>N. salina</i>		<i>N. oceanica</i>	
	Control (10 day)	Green LED	Control (10 day)	Green LED	Control (10 day)	Green LED	Control (10 day)	Green LED
C14:0 (myristic)	17.08	14.84	25.94	19.01	7.24	5.65	13.56	7.24
C16:0 (palmitic)	51.01	38.34	43.90	39.63	65.08	47.33	55.39	46.52
C18:0 (stearic)	3.82	1.79	5.26	6.41	5.25	2.41	2.19	5.25
C18:1 (oleic)	17.01	25.79	9.37	13.89	18.54	32.99	20.13	33.53
C18:2 (linoleic)	0.51	4.38	2.03	5.28	0.36	3.28	0.80	3.57
C18:3 (lino- lenic)	0.74	0.98	0.20	0.49	0.43	1.12	0.26	0.43
C20:0 (ara- chidic)	7.17	10.21	8.82	10.49	2.50	6.10	7.09	2.50
C20:5 (EPA)	1.03	1.01	1.05	1.00	0.14	0.31	0.19	0.14
C22:0 (behenic)	1.43	1.63	1.74	1.08	0.36	0.65	0.15	0.36
C22:6 (DHA)	0.20	1.03	1.69	2.72	0.11	0.16	0.24	0.47
SFA	80.51	66.81	85.66	76.62	80.43	62.14	73.38	61.87
UFA	19.49	33.19	14.34*	23.38	19.57*	37.86	21.62	38.13

SFA saturated fatty acids (14:0, 16:0, 18:0, 20:0, and 22:0), UFA unsaturated fatty acids (18:1, 18:2, 18:3, 20:5, and 22:6)

the lipid accumulation. These results are similar to those reported by Converti et al. [32]. They reported that the palmitic acid (C16:0) found in *C. vulgaris* and *N. oculata* represented 47–66% (w/w) and 58–62% (w/w) of the total fatty acids, respectively. Green LED wavelength stress in the cultivation of the four microalgae induced a cascade of reactions leading to the formation of acetyl-CoA, which induced more synthesis of palmitic acid (C16:0). Thus, more syntheses of palmitic acid (C16:0) have been occurred due to palmitic acid (C16:0) acting as a precursor of fatty acids synthesis.

Furthermore, the palmitic acid (C16:0) level decreased from 51.01 to 38.34% (w/w) for *P. tricornutum*, 43.90–39.63% (w/w) for *I. galbana*, 65.08–47.33% (w/w) for *N. salina*, and 55.39–46.52% (w/w) for *N. oceanica* from control to green LED stress, respectively, as shown in Table 1. Overall, the synthesis pathway of fatty acids in microalgae starts from SFAs like palmitic acid (C16:0), which undergoes subsequent desaturation and elongation reactions leading to decrease in SFAs and increase in UFAs. The UFA component increased from 19.49 to 33.19% (w/w) for *P. tricornutum*, 14.34–23.38% (w/w) for *I. galbana*, 19.57–37.86% (w/w) for *N. salina*, and 21.62–38.13% (w/w) for *N. oceanica* (Table 1). Based on UFAs to SFAs fraction, the quality and usage of microalgae oil for aquaculture can be determined. High levels of UFAs and low levels of SFAs increase the quality of fish feed [1].

Conclusion

Two-phase culture processes of four microalgae species were carried out under mixed LED of red and blue wavelengths for biomass production, and green LED wavelength for lipid accumulation. Among the four strains with high growth and lipid production, the two species, *P. tricornutum* and *I. galbana*, can be used for aquaculture feedstock. The highest lipid contents of four microalgae in the two-phase culture increased from 38.8 to 56.0% (w/w) for *P. tricornutum*, 36.0–55.2% (w/w) for *I. galbana*, 31.5–53.0% (w/w) for *N. salina*, and 28.5–51.0% (w/w) for *N. oceanica* under green LED wavelength stresses. These results indicate that lipid accumulation under green LED wavelength had significant effects on lipid productivity in the second phase. Thus, two-phase culture process could enhance to accumulate lipid in the microalgae.

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