

Research Article

Light Emitting Diode (LED) Illumination for Enhanced Growth and Cellular Composition in Three Microalgae

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Abstract

Light wavelength is an essential parameter of microalgal growth and light spectrum quality affects the algal metabolism. Light-emitting diodes (LEDs) emit a very narrow wavelength which addresses fundamental as well as applied aspects of light color on biomass and value-added compound formation in microalgae. In this study, *Arthrospira platensis*, *Chlorella vulgaris* and *Scenedesmus obliquus* were cultivated in Bold's basal medium under five different LED illuminations (white, blue, green, yellow and red) to augment growth, pigment, protein, carbohydrates and lipid production. Dry weight of microalgae at the end of 10 days cultivation period revealed that higher content under the LED illumination was in the order of red>white>blue. Highest Chlorophyll a content of 8.4 mg g⁻¹ was recorded in *C. vulgaris* under red LED. Whereas green LED illumination has resulted higher content of Chlorophyll b with 2.0, 1.98 and 1.68 mg g⁻¹ in *A. platensis*, *C. vulgaris* and *S. obliquus* respectively. Similarly highest carotenoid content of 6.1 mg g⁻¹ in *A. platensis* was recorded in green LED. Total carbohydrates of microalgae were increased under blue light and the maximum content of 52.1 mg g⁻¹ was found in *C. vulgaris*. Protein content in the range of 102.7-97.5 mg g⁻¹, 77.9-76.5 mg g⁻¹ and 51.9-49.4 mg g⁻¹ was observed in *A. platensis*, *C. vulgaris* and *S. obliquus* under blue and green LED. Lipid content of 5.68 % and 4.96 % was obtained in *C. vulgaris* and *S. obliquus* under blue LED. This was followed by 4.37% and 3.86% under red LED illumination for the same species. Based on the results, selection of blue and red LED illumination to enhance microalgal growth and cellular composition is possible in the selected microalgae.

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Keywords: *Arthrospira*; *Chlorella*; Illumination; LEDs; Microalgae; *Scenedesmus*

Introduction

Microalgae are phototrophic organisms and promising resources for the mass production of lipids, biomass and nutraceuticals. Microalgae use sunlight to derive energy and expanded their applications were expanded and diversified in recent years. Light quality and quantity are the most important parameters for microalgae and other phototrophic organisms, as it is required for photosynthesis and the regulation of several cellular processes [1]. As a photoautotroph, microalgae cannot utilize all spectra of light but only some fractions, in particular between 400 and 700 nm, which are called as photosynthetic active radiation [2]. In most laboratories and industries, artificial light is used to control the microalgal growth and physiology. However, the competitiveness of any artificial light-driven microalgal production hinges on energy consumption. Better light energy usage by phototrophs can be achieved by tailoring species-specific emission spectra of artificial light sources [3]. Although artificial light costs more than sunlight, it allows control of microalgal biochemistry and growth, increasing the reliability of industrial processes for the production of high-value biomolecules from microalgae. Artificial light also provides better regulation of the photosynthetic photon flux density, photoperiod, and light spectra in microalgal production [4]. Among the artificial light sources, Light-Emitting Diodes (LEDs) are being used as an alternative light source in microalgae cultivation [5].

Light wavelength is an essential parameter of microalgal growth [6]. Algal metabolism and growth can be also affected by spectrum and spectrum quality is known to influence biochemical composition, pigment content and photosynthesis rate of various species [7-9]. It is important to study the effects of different light qualities on microalgae and screen out the most efficient one as there is a possibility that high production efficiency can be achieved by optimizing the artificial light quality [10].

The main objective of the present work is to investigate the effect of LED as a light source to augment growth, pigment, protein, carbohydrates and lipid production of *Arthrospira platensis*, *Chlorella vulgaris* and *Scenedesmus obliquus*.

Material and Methods

Microalgae strains and culture conditions

Three microalgae, *Arthrospira platensis*, *Chlorella vulgaris*, *Scenedesmus obliquus* were used in this study. Initial concentration of microalgal cells was adjusted to 0.05 g/L and the microalgae were cultured in sterilized Bold's basal medium in 2 L flasks with a 1.5 L working volume at 20 ± 1°C and a 12/12-h light/dark cycle. Light emitting diodes (LEDs) strips were used as light source for the cultivation of microalgae for a period of 10 days. The LED lights emitting blue (465 nm), green (520 nm), yellow (640 nm), red (660 nm) and white light were used.

Dry weight determination

Microalgae grown under different LED wavelengths were harvested by centrifugation at 5000 rpm for 10 mins. The algal pellets were dried at 95°C in a hot air oven until a constant weight was obtained to determine the dry weight.

Cellular pigments assay

Determination of chlorophyll a, chlorophyll b, and total carotenoid contents was done according to Lichtenthaler [11]. The determination of levels in whole pigment extract of microalgae using a UV-Vis spectrophotometer was performed using the following equations:

$$\text{Chl a } (\mu\text{g ml}^{-1}) = 11.24 \times \text{OD662} - 2.04 \times \text{OD645}$$

$$\text{Chl b } (\mu\text{g ml}^{-1}) = 20.13 \times \text{OD645} - 4.19 \times \text{OD662}$$

$$\text{Ct } (\mu\text{g ml}^{-1}) = (1000 \times \text{OD470} - 1.90 \times \text{Chl a} - 6.31 \times \text{Chl b}) / 214$$

where Chl a is the chlorophyll a, Chl b is the chlorophyll b, and Ct is the total carotenoids ($\mu\text{g mL}^{-1}$).

Protein assay

The extraction of proteins from microalgae was performed using alkali method. Aliquots of algal sample were centrifuged and 0.5 N NaOH was added to the pellet followed by extraction at 80°C for 10 mins. The mixture was centrifuged and protein content of the supernatant was estimated using Bovine Serum Albumin (BSA) as standard [12].

Carbohydrate assay

Cellular carbohydrates were estimated using the anthrone method after hot alkaline extraction [13, 14]. Briefly, microalgal pellets were resuspended in distilled water and then heated in 40% (w/v) KOH at 90°C for 1 h. After cooling down, ice cold ethanol was added and stored at -20°C overnight followed by centrifugation. The pellet was resuspended in distilled water and then reacted with anthrone reagent. D-glucose was used as standard and the colour development was read at 578 nm in a spectrophotometer.

Total lipid estimation

Lipid extraction from dried algal cells were carried out by chloroform: methanol extraction method [15]. Dried algal cells added with distilled water were ultrasonicated and mixed with chloroform: methanol (2:1). The mixture was left for 30 mins in a water bath (30°C) and filtered through a Whatman No.1 filter paper. The filtrate was transferred to another screw cap tube containing NaCl solution (0.9%) and the purified chloroform layer was evaporated to a constant weight in a fuming hood under vacuum at 60°C. The total lipid content of dry weight was calculated using the following equation.

$$\text{Lipid content \%} = (m_2 - m_0) / m_1 \times 100 \dots\dots\dots \text{Eq. (3)}$$

where m_1 is the weight of the dried algal cells, m_0 is the weight of the empty new screw cap tube and m_2 is the weight of the new screw cap tube with the dried lipids.

Results and Discussion

The quality and regulation of the illumination is crucial in microalgae cultivation processes. Light-emitting diodes (LEDs) emit a very narrow wavelength address fundamental as well as applied

aspects of light color on algae biomass and value-added compound formation. In this respect, effect of various LEDs on microalgal growth and biochemical characteristics were examined in this study. Three microalgae namely *Arthrospira platensis*, *Chlorella vulgaris* and *Scenedesmus obliquus* were cultivated in Bold's basal medium under five different LED illuminations (white, blue, green, yellow and red) for a period of 10 days.

(Figure 1) shows the dry cell weight of microalgae under the different LED wavelengths ranging from white to red. Dry weight of microalgae at the end of 10 days cultivation period revealed that higher content under the LED illumination was in the order of red>white>blue. Highest dry weight of 1.43 g⁻¹ was recorded in *S. obliquus* under red light illumination followed by 1.41 g⁻¹ by *A. platensis*. Whereas, white LED illumination resulted in highest dry weight content in *C. vulgaris*. Both green and yellow LEDs produced lower biomass in the form of dry weight. In a study by Ajayan, highest biomass (5.2 g L⁻¹) was observed under red illumination with reflector in *Chlorella* species [16]. At the same time, combination of Blue-Red LED combination gave maximum biomass in terms of cell density in *Chlorella* sp found that the combination of red and blue light is more conducive to the growth of *Arthrospira platensis* [17,18]. In another study, red LED has produced higher biomass in *Picochlorumatomus* [19]. It was also reported that binary combination of blue and red LEDs could produce the high biomass and photosynthetic pigments in the four microalgae [20].

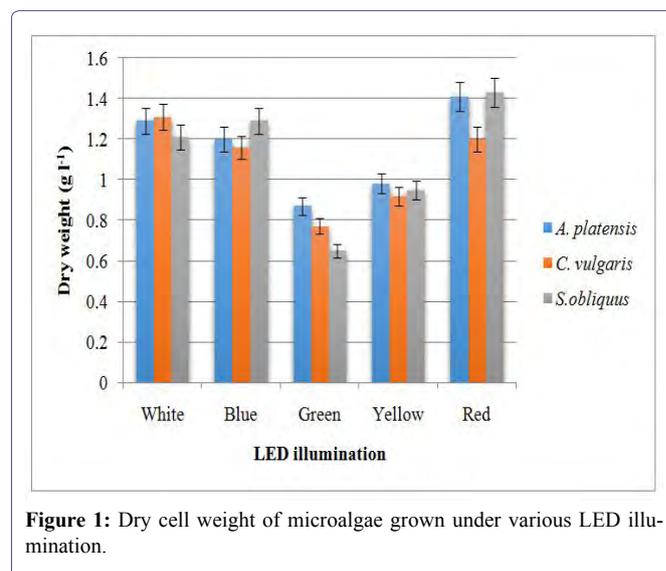


Figure 1: Dry cell weight of microalgae grown under various LED illumination.

To understand the relations between pigment concentrations under various light sources, chlorophyll and carotenoid contents were measured and recorded (Figure 2,3 and 4). The Chlorophyll content of the green, yellow and blue LEDs was significantly lower than that produced under the red and white light. Among the LED lights tested on pigment production of three microalgae studied, red LED has resulted in higher chlorophyll a followed by white LED. Highest Chl a content of 8.4 mg g⁻¹ was recorded in *C. vulgaris*. Both red and white LED illumination produced 7.8 mg g⁻¹ Chla in *A. platensis*. In the case of Chl b, green LED illumination has resulted higher content in all the three microalgae with 2.0, 1.98 and 1.68 mg g⁻¹ in *A. platensis*, *C. vulgaris* and *S. obliquus* respectively. Similarly, carotenoids were

high in microalgae grown in the presence of green LED illumination with highest carotenoid content of 6.1 mg g^{-1} in *A. platensis*. The second highest was recorded with yellow LED illumination.

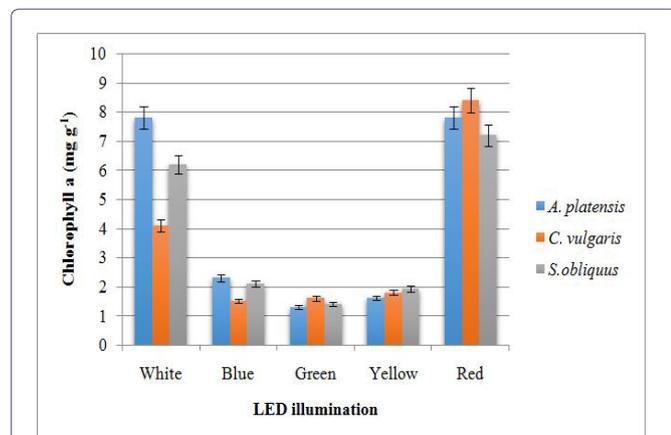


Figure 2: Chlorophyll a content of microalgae grown under various LED illumination.

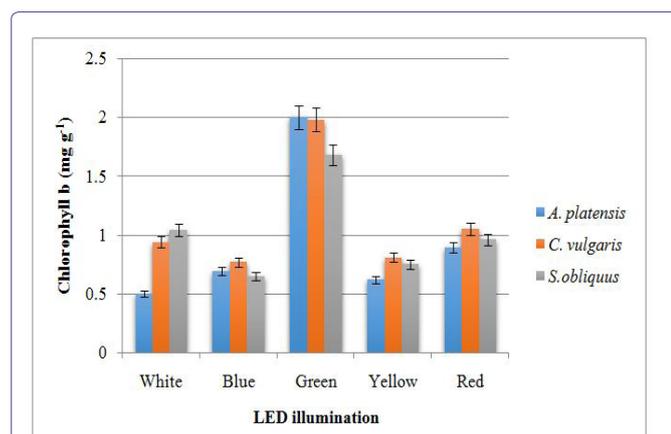


Figure 3: Chlorophyll b content of microalgae grown under various LED illumination.

Chlorophylls and carotenoids are the two major classes of photosynthetic pigments found in plants and algae. Chlorophyll a is the primary molecule responsible for photosynthesis, while chlorophyll 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 (Richmond, 2003). Microalgal photosystem II can be enhanced by red light wavelength, whereas the microalgal photosystem I can be induced by blue light wavelength [21,22]. Therefore, blue and red light wavelengths are more suitable for microalgal growth than other wavelengths and should be adequately and selectively provided for microalgal photosynthesis [23]. The ability of microalgae to use different spectra is possibly related to the photosynthetic pigment compositions [24]. Red light can be efficiently absorbed by chlorophyll and phytochrome, and then be transmitted for photosynthesis Hohm [25]. Blue light can be efficiently absorbed by chlorophyll and carotenoids, and green light can be used by phycocyanin [26].

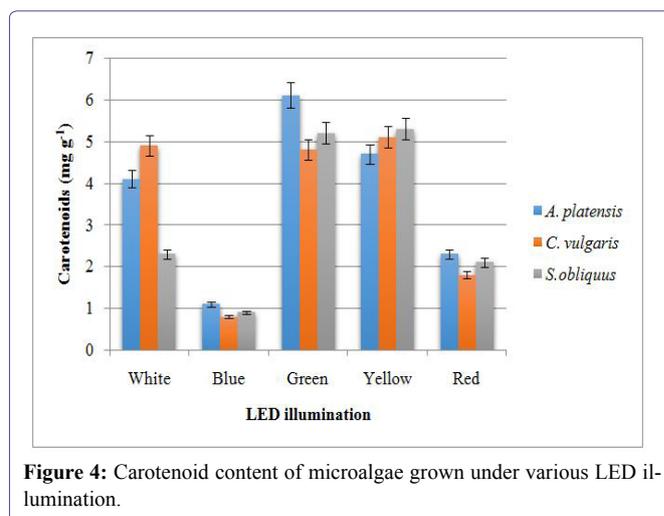


Figure 4: Carotenoid content of microalgae grown under various LED illumination.

Total protein content of microalgae grown under LED illumination revealed that blue and green LED produced higher amounts. Protein content in the range of $102.7\text{-}97.5 \text{ mg g}^{-1}$, $77.9\text{-}76.5 \text{ mg g}^{-1}$ and $51.9\text{-}49.4 \text{ mg g}^{-1}$ was observed in *A. platensis*, *C. vulgaris* and *S. obliquus* respectively (Figure 5). In general, blue LED has increased the protein content followed by green light. The results were in contradictory with who found the highest content of protein in microalgae under green light and the lowest under blue light. Total carbohydrates of microalgae were increased under blue light and the maximum content of 52.1 mg g^{-1} was found in *C. vulgaris* [21,27]. The second highest carbohydrate content was produced under red LED illumination in *A. platensis* and *S. obliquus* (Figure 6). The results were in accordance to findings in which blue light increased the carbohydrate content, and adding green light can improve protein content in *Arthrospira platensis* [18]. Blue light influences gene expression and several metabolic pathways in algae via photoreceptors and several other growth factors. But low carbohydrate content was observed in *Isochrysis* sp. when exposed to the blue light [28,29].

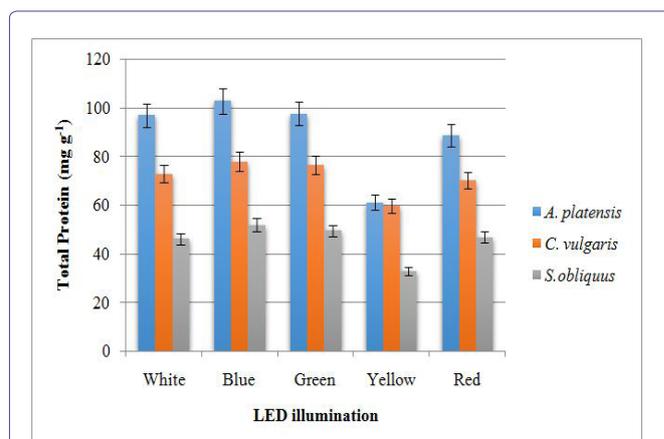


Figure 5: Total protein content of microalgae grown under various LED illumination.

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 [30]. Microalgae require optimum

irradiation conditions with specific narrow bands of light to achieve maximum photosynthetic rates with minimum energy consumption. LED could be the optimal light source for microalgae systems as they have the characteristics of narrow-band wavelength and cost-effective power consumption. In this study, the percentage of lipid content was determined from the microalgal samples grown under different LED illumination. Blue and red LED was proven to be the best sources for lipid accumulation in *A. platensis*, *C. vulgaris* and *S. obliquus*. Lipid content of 5.68 % and 4.96 % was obtained in *C. vulgaris* and *S. obliquus* under blue LED (Figure 7). This was followed by 4.37% and 3.86% under red LED illumination for the same species. Different kinds of algae may require different photon flux density, different light wavelengths for their growth reported that blue LED light was preferable for lipid accumulation as opposed to red LED light in *Chlorella* species [31-33]. In another study, *Skeletonema costatum* preferred blue and red light to green light and the saturated light intensity is significantly different under the three monochromatic lights [34]. Severes reported that LED of red light 220 lux wavelength doubled the lipid dry weight in *Chlorella* sp. Whereas, total lipid (53.3%), lipid productivity (27.7 mg L⁻¹ day⁻¹) and total fatty acid (79.4%) were observed under blue LED with reflector [16,17].

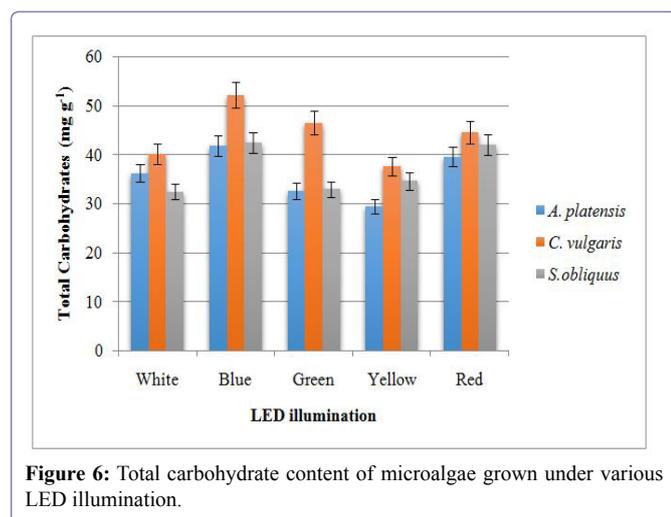


Figure 6: Total carbohydrate content of microalgae grown under various LED illumination.

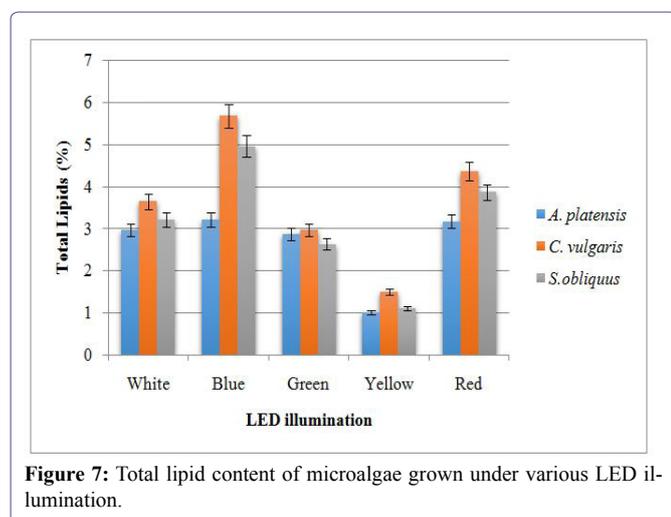


Figure 7: Total lipid content of microalgae grown under various LED illumination.

Conclusion

All the three microalgae produced better growth and had increased biochemical composition under LED light exposure. Blue LED has produced greater total protein, carbohydrate and lipid content. Red light has increased the dry cell weight, chlorophyll a content. Both chlorophyll b and carotenoids were increased with green light illumination. There were not many changes in term of microalgal growth and their cellular composition under yellow LED source. Based on the results, selection of blue and red LED illumination to enhance microalgal growth and cellular composition is possible in the selected microalgae.

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