

Effects of Various Light-Emitting Diode (LED) Wavelengths on the Growth of *Scenedesmus Obliquus* Fachb-12 and Accumulation of Astaxanthin

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Abstract: Given the central role of light in the algal photosynthesis, respiration, cell division, growth and the accumulation of value products, the effects of light-emitting diodes (LEDs) light wavelengths (blue, white, red and green) were studied in *Scenedesmus obliquus*. Biomass, residual nutrient amount, soluble protein, astaxanthin and reactive oxygen species, superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) activity were analyzed to determine the effects of different monochromatic light wavelengths via biochemical methods. The results showed that blue light wavelength is the optimal light wavelength for phosphorus removal efficiency and the accumulation of biomass and astaxanthin in *S. obliquus*. Meanwhile, high reactive oxygen species content under the blue light might induce the accumulation of astaxanthin. The high activity of SOD, CAT and POD might participate in clearing the reactive oxygen species to facilitate the growth of microalgae. Furthermore, we found mixed blue/green lights treatment is the most appropriate mixture for the nitrogen removal. Under the blue light treatment, high light intensity and 18L:6D light cycle is the best condition for biomass and astaxanthin accumulation. Optimal nitrogen/phosphorus removal efficiency was observed under a 24L:0D light cycle. These results might provide a foundational data for the optimizing the productivity of high-value metabolites and treatment of wastewater.

Keywords: Light-emitting diodes; wavelength; cell density; nutrient removal efficiency; astaxanthin

1 Introduction

Algae, ideal candidates for producing renewable biofuels and high-value products (e.g., proteins, fatty acids, and carbohydrates), are becoming more and more important in ecosystem. The growth of algae is a function of many essential parameters, including abiotic factors (e.g., light, pH, temperature, salinity, as well as medium composition) and biotic factors (e.g., the influence of other organisms and the age of the cells) [1,2]. Among these factors, light is the most determinant factor. This is because light exerts a direct influence on photosynthesis. Photosynthetically active light radiation is absorbed, and the energy is used to synthesize nutrients. Additionally, light controls the circadian rhythm of the organism, influencing respiration, cell division and the growth rate, as well as the production of pigments, unsaturated fatty acids, carbohydrates and protein [3-5]. Therefore, the properties of light (e.g., wavelength, intensity, and photoperiod) are particularly critical for the growth of algae.

To optimize culture conditions for microalgae, an efficient light source is necessary. Previous studies

showed that the conventional lights (e.g., fluorescent lamps, incandescent lamps and cold cathode sources) are less economical and efficient for algal metabolism, since the wavelength of conventional lights may not include the absorption bands of the algal chlorophyll pigments or may contain only a combination of efficient and inefficient light spectra [6,7]. However, light-emitting diodes (LEDs), in contrast to conventional lights, is more economical, durable, reliable and highly efficient [8,9]. Studies have indicated that LEDs are considered to be the optimal light sources for microalgal growth because they emit narrow-band wavelength light, exhibit high conversion efficiencies (low heat emission and low power consumption) and are available in a variety of wavelengths ranging from red to violet [10-12]. A number of studies have been conducted to evaluate the effects of LEDs at different light conditions (photoperiod, intensity and wavelength) in microalgal production systems [13-15]. For example, Ifeanyi V. et al. (2011) demonstrated that the cell growth of *Aphanocapsa* is noticeably increased under the 2,000-5,000 light intensity [16]. Additionally, different algae require different wavelength light for their optimal growth. For example, the ratio of red light: blue light = 5:5 is the optimum light quality for *Chlorella sp.* reproduction and nutrient removal efficiency [17]. The highest productivity and concentration of *Spirulina platensis* were observed under green light at 1,200 lux [4]. Conversely, white LEDs resulted in highest biomass production and better lutein production efficiency when compared to red, blue and green monochromatic LEDs in *S. obliquus* FSP-3 [18]. Hongli Miao proposed that compared with red and green light, *Skeletonema costatum* grows best under blue light, and the saturated light intensity declines with an increasing spectrum absorption coefficient [19]. Nevertheless, very few studies have been undertaken to determine the optimal wavelength for *Scenedesmus obliquus* FACHB-12 growth, astaxanthin production or nutrient removal efficiency.

The green alga *S. obliquus* FACHB-12 is an important freshwater alga (Chlorophyta), often the dominant species in aquaculture ponds. Previous studies showed that *S. obliquus* can serve as a major source of protein, food and cosmetics production [20]. Recently, *S. obliquus* is being evaluated for biodiesel and edible oil extraction [21-24]. Consequently, in this study an assessment of using LEDs with four light spectra (blue, white, green and red) shall be experimented on the growth and chlorophyll, residual nutrients and soluble protein, as well as astaxanthin content of *S. obliquus* FACHB-12. The antioxidant response mechanisms of *S. obliquus* FACHB-12 under different spectra were also investigated. Furthermore, the comparison of the optimal blue and mixed light wavelength (blue-green and blue-red) on the effect of *S. obliquus* was analyzed. Finally, *S. obliquus* cells were treated by different light intensities and photoperiod cycles in order to select the optimal light schedules. The results of this study will provide a fundamental resource for the application of LEDs to microalgal production and wastewater treatments.

2 Materials and Methods

2.1 Materials

The *S. obliquus* strain (FACHB-12) was purchased from Institute of Hydrobiology, Chinese Academy of Sciences. The strain was maintained in 200 ml BG11 medium of 500 ml flasks. The culture conditions were as follows: cool-white fluorescent light with a surface light intensity of 3000 lux, temperature of $25 \pm 0.5^\circ\text{C}$, light-dark cycle of 12 h:12 h (light period from 6:00 am to 6:00 pm, dark period from 6:00 pm onward). The cells were cultured for 8 days, and were shaken three times per day (8:00 am, 2:00 pm and 8:00 pm).

2.2 Experimental Procedure

Cells were cultivated in 500 ml Erlenmeyer flasks containing 150 ml BG11 medium. The initial cell density was adjusted to be 10^6 cells/ml and temperature was maintained at $25 \pm 0.5^\circ\text{C}$ by air-condition. Three different monochromatic wavelength LEDs (blue wavelength ($\lambda_{\text{max}} = 460\text{-}470$ nm), red wavelength ($\lambda_{\text{max}} = 620\text{-}630$ nm), green wavelength ($\lambda_{\text{max}} = 525\text{-}550$ nm), and one white (full-spectrum) ($\lambda_{\text{max}} = 380\text{-}760$ nm)) (T8-36W LED, Opm lighting co., LTD, China) were chosen for microalgal cultivation. By adjusting the distance between the light source and the cultivation vessel, the designated

irradiances were achieved. The experiment was carried out in triplicate using $150 \mu\text{mol}/\text{m}^2\cdot\text{s}$ irradiance and 12:12 h (light/dark) cycles. The flasks were artificially and intermittently shaken thrice a day. The experiments were performed in a dark room to avoid interference by natural light and every flask was separated by a black board. Cell density, chlorophyll a (chl a), chlorophyll b (chl b) were measured at 48 h intervals during the 8 days, the other indexes were collected on the 8th day. The mixed LED wavelengths treatment was the same as the conditions used in monochrome wavelengths, except the irradiances were combined to $150 \mu\text{mol}/\text{m}^2\cdot\text{s}$.

S. obliquus FACHB-12 cells were treated at three different light intensities and photoperiod cycles, as shown in Tab. 1.

Table 1: All treatments of *S. obliquus* FACHB-12 under the three different light intensities and photoperiod cycles

Treatment	Light intensity	Light photoperiod (Light/Dark)
L12		12L:12D
L18	$50 \mu\text{mol}/\text{m}^2\cdot\text{s}$	18L:6D
L24		24L:0D
M12		12L:12D
M18	$150 \mu\text{mol}/\text{m}^2\cdot\text{s}$	18L:6D
M24		24L:0D
H12		12L:12D
H18	$250 \mu\text{mol}/\text{m}^2\cdot\text{s}$	18L:6D
H24		24L:0D

2.3 Measurement

Cell density was measured using an improved Neubauer hemocytometer at 48 h intervals with three replications per-ample during the experimental period. The specific growth rates (μ) were determined with the following exponential growth equation: $\mu = (\ln N_2 - \ln N_1) / (t_2 - t_1)$, where μ is the specific growth rate, t_1 is time at the start of the experiment (day), t_2 is time at the end of the experiment (day), N_2 and N_1 are the number of algal cells at time t_2 and t_1 , respectively.

At every 48 h interval, 5 ml of the culture was filtered through a glass microfiber filter (GF/F, 0.7 μm , Whatman, USA), the filter with attached microalgal cells was used to determinate the chlorophyll a (chl a) and chlorophyll b (chl b), which was extracted by acetone and determinate by spectrophotometry. The culture filtrates were analyzed to determine total nitrogen (TN) and total phosphorous (TP) according to the persulfate method and ascorbic acid method, respectively.

For determination of astaxanthin, 5 ml culture sample was centrifuged for 5 min at 5000 r/min, and the pellet was first saponified by using a solution of 5% KOH in 30% (v/v) methanol at 70 degree for 5 min to destroy the chlorophyll, and the supernatant was discarded. Three to five drops of acetic acid were added to reduce pH and the remaining pellet was extracted twice with 5-ml DMSO in 70 degree for 5 min to recover the astaxanthin. The absorbance of the combined extracts was measured at 490 nm. Astaxanthin concentration was calculated using pure astaxanthin (Aladdin, China) as a standard. 25 milliliters of harvested microalgae were centrifuged for 10 min at 4 degree at 4,000 rpm, washed, and centrifuged again. Pellets of the 25 ml samples were washed in 25 mM phosphate buffer, pH 7.4, and then resuspended in 10 ml phosphate buffer with 1% (w/v) SDS. Cells were disrupted by sonication on ice. After ultrasonic decomposition the cells were centrifuged at 5,000 rpm for 10 min at 4°C. The supernatant was used for soluble protein analyses. Superoxide dismutase (SOD), catalase (CAT) and Peroxidase (POD) activities were determined using commercial kits (Nanjing Jiancheng Bioengineering Institute, China).

ROS was measured according to the instructions supplied with the Reactive Oxygen Species Assay Kit (Beyotime Institute of Biotechnology, Nanjing, China). In this kit, the non-fluorescent probe 2', 7'-dichlorofluorescein diacetate (H₂DCF-DA) passively diffuses into cells and is deacetylated to form nonfluorescent 2', 7' dichlorofluorescein (DCFH). DCFH reacts with ROS to form the fluorescent product DCF, which is trapped inside the cells. Fluorescence is read at 485 nm for excitation and 530 nm for emission with a fluorescent microplate reader (Bio-TEK, Winooski, Vermont, USA).

2.4 Statistical Analyses

The results were analyzed by one-way ANOVA with a significance level of $p < 0.05$ in SPSS 18. One-way ANOVA was performed to evaluate the differences among the light wavelength treatments. Duncan's multiple range tests were conducted to assess the significant differences among the light wavelength treatments. B, R, W and G represent blue, red, white and green wavelength, respectively. Different superscript letters in columns indicate that means differ significantly from each other ($p < 0.05$).

3 Results

3.1 The Effect of Different Light Wavelengths on the Growth and Chlorophyll Content of *S. obliquus*

The cell density of *S. obliquus* FACHB-12 under four different spectra (blue, red, white, green) was investigated during the 8 days (Fig. 1(a)). The highest cell density was found under the blue wavelength, which is markedly higher than that of the other three wavelengths. Compared with the rest of the light spectra, the blue light wavelength achieved a significantly higher ($p < 0.05$) specific growth rate (0.4 ± 0.01 d⁻¹) at the 8th day, whereas the green wavelength achieved a significantly lower ($p < 0.05$) specific growth rate. No significant difference was observed between the results of the white and red light spectra ($p > 0.05$) (Fig. 1(b)). Generally, the descending order of specific growth rate is blue > white > red > green.

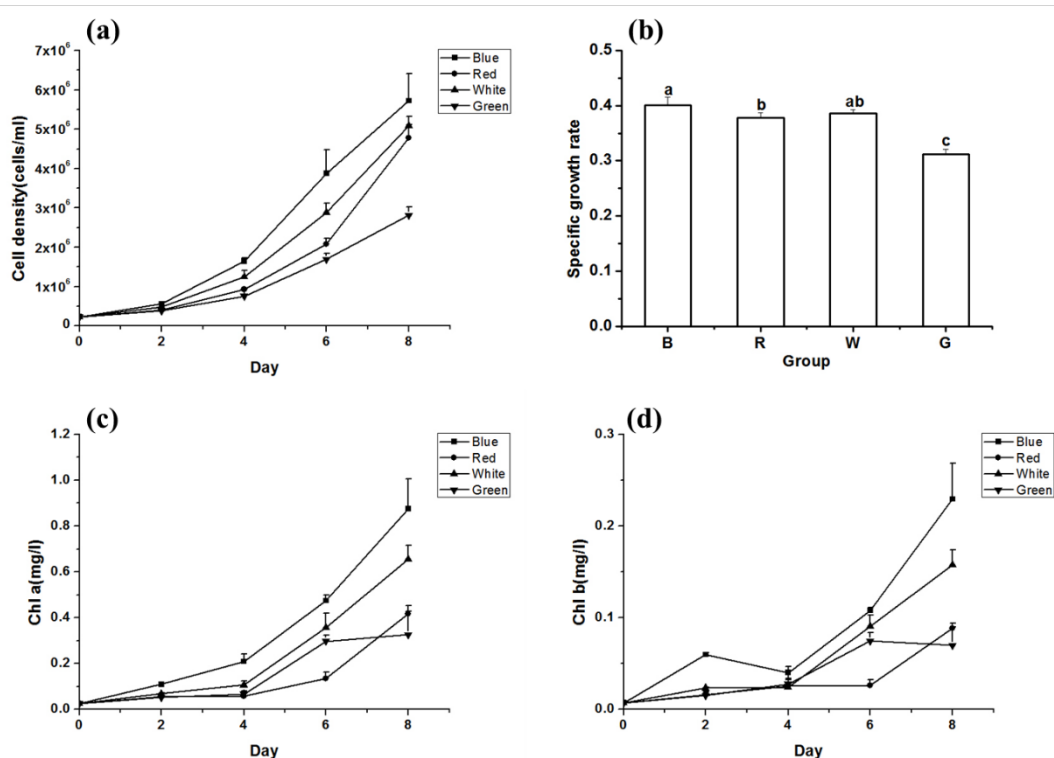


Figure 1: Cell density (a), Specific growth rate (b), Chlorophyll a (c) and Chlorophyll b (d) of *S. obliquus* FACHB-12 under different light wavelengths

Results of chlorophyll content of *S. obliquus* FACHB-12 influenced by different light wavelengths are shown in Fig. 1(c) and Fig. 1(d)) *S. obliquus* FACHB-12 revealed distinct chlorophyll content in response to different color treatments. The content of chl a and chl b in the culture reached the highest point under the blue light wavelength at the 8th day. The contents of chl a and chl b of *S. obliquus* FACHB-12 were 0.87 ± 0.13 mg/l and 0.23 ± 0.04 mg/l, respectively. Interestingly, the chlorophyll content did not increase under the red and green light wavelengths for the first four days, but gradually increased at the sixth day. Chlorophyll content under red light was increased dramatically to higher than that of green light wavelength at the eighth day.

3.2 The Effect of Different Light Wavelengths on Nutrient Removal Efficiency

Total residual nitrogen and phosphorus (TN and TP) in medium of various light colors at the 8th day are presented in Fig. 2. The residual nitrogen amount under green light was significantly higher ($p < 0.05$) than that of the other light colors. Furthermore, no significant difference ($p > 0.05$) was observed among the blue, white and red lights. However, the residual phosphorus amount under the blue light was significantly lower ($p < 0.05$) than that of the other light color treatments. Conversely, the residual phosphorus amount under the green light treatment was significant higher ($p < 0.05$) compared with the rest light colors. The residual nutrient amount was in the descending order of green > red > white > blue. In other words, the nutrient removal efficiency is in the order of blue, white, red and green.

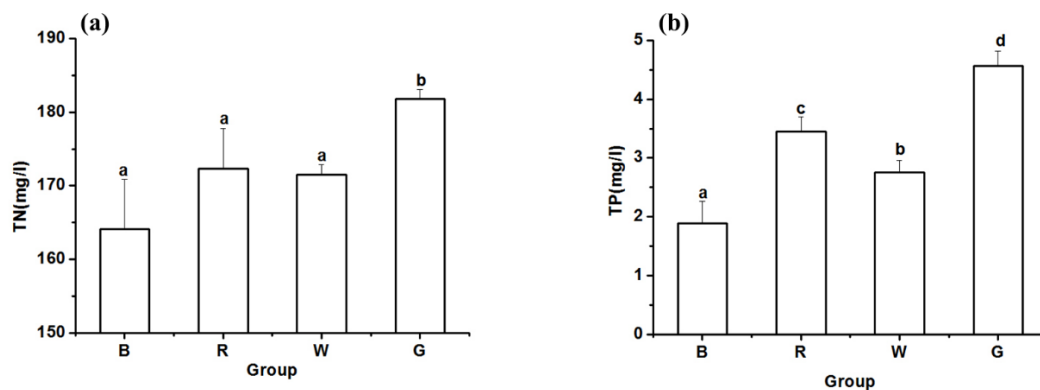


Figure 2: Residual Nitrogen (a) and Phosphorus (b) in the culture medium of *S. obliquus* FACHB-12 under different light wavelengths

3.3 The Effect of Different Light Wavelengths on Soluble Protein and Astaxanthin Content of *S. obliquus*

The effects of light wavelengths on the contents of soluble protein and astaxanthin at the 8th day are shown in Fig. 3. The content of soluble protein (13.2 ± 1.1 mg/l) under the blue light wavelength was considerable higher ($p < 0.05$) than that of the other light colors. However, no significant difference ($p > 0.05$) was observed among the results of blue, white and red wavelengths. The contents of astaxanthin under blue and white colors were significantly higher ($p < 0.05$) than that of red and green wavelengths. Nevertheless, there was no significant difference ($p > 0.05$) between blue and white colors. The descending order of soluble protein and astaxanthin contents were blue > white > red > green.

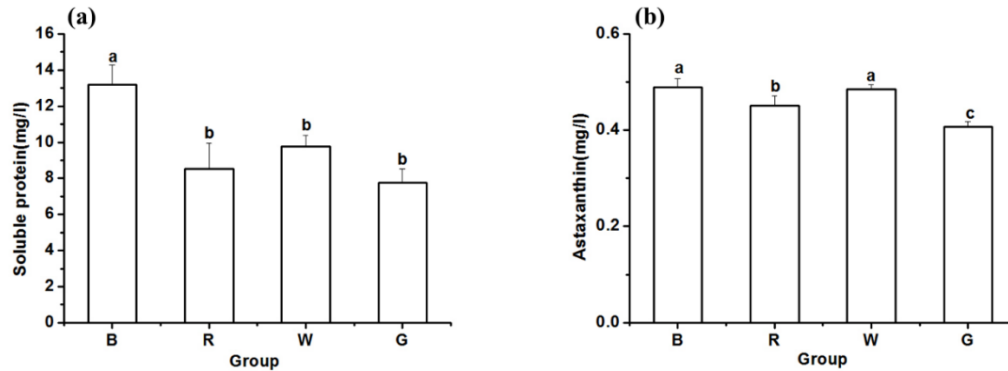


Figure 3: Soluble protein (a) and astaxanthin (b) of *S. obliquus* FACHB-12 under different light wavelengths

3.4 The Effect of Different Light Wavelengths on ROS and on SOD, CAT and POD Activity in *S. Obliquus*

The reactive oxygen species (ROS) levels of *S. obliquus* FACHB-12 under four different wavelengths is shown in Fig. 4(a). Quantification analyses demonstrated that cells cultivated under the blue light wavelength displayed higher ROS levels ($p < 0.05$) than those under the green or red light. There was no significant difference ($p > 0.05$) between the blue and white light colors (Fig. 4(a)). The ROS activity of *S. obliquus* FACHB-12 was in the order of blue > white > red > green.

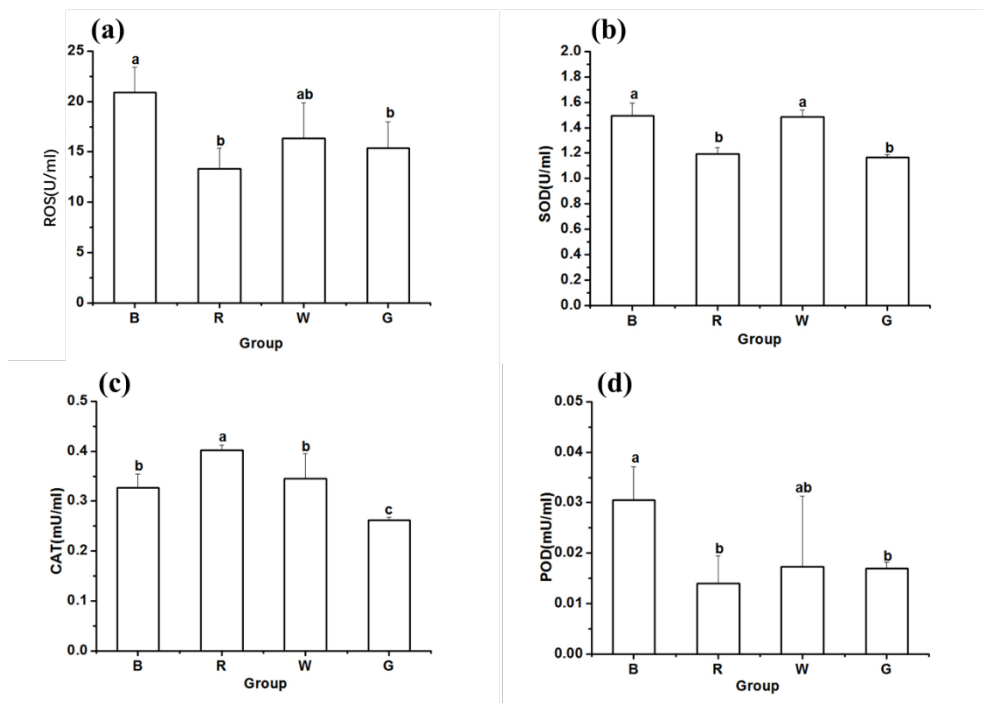


Figure 4: ROS (a) and the SOD (b), CAT (c) and POD (d) activity of *S. obliquus* FACHB-12 under different light wavelengths

The activity of SOD, CAT and POD influenced by the light colors are shown in Figs. 4(b)-4(d). The SOD and POD activity under the blue light were significantly higher ($p < 0.05$) than that of red and green

light wavelengths. However, no obvious difference ($p > 0.05$) was observed between the blue and white light colors. The activity of SOD and POD were in the order of blue > white > red > green. CAT activity induced by the red light wavelength was significant higher ($p < 0.05$) than the CAT activity when cultivated under the other light colors. CAT activity under green light was significantly lower ($p < 0.05$) than the other light colors.

3.5 The Effect of Mixed LED Light Wavelength Treatments on *S. obliquus*

Given that monochromatic blue light gave optimal results for dry weight, soluble protein and astaxanthin. We further tested whether a combination of blue plus other wavelengths could provide additional benefit. No obvious difference was observed in the amount of dry weight, chlorophyll a and b, astaxanthin and residual P amount between three treatments (Tab. 2).

Table 2: Dry weight, chlorophyll, the nitrogen & phosphorus residual amount and metabolite composition of *S. obliquus* under the mixed LED light wavelengths and monochromatic blue light

	Blue-Green (1:1)	Blue-Red (1:1)	Blue
Dry weight (mg/l)	0.2 ± 0.03	0.18 ± 0.02	0.2 ± 0.01
Chlorophyll a (mg/l)	0.53 ± 0.01	0.5 ± 0.04	0.51 ± 0.14
Chlorophyll b (mg/l)	0.11 ± 0.01	0.1 ± 0.01	0.09 ± 0.03
Nitrogen residual amount (mg/l)	143.64 ± 8.73 ^a	170.1 ± 1.68 ^b	160.91 ± 2.3 ^b
Phosphorus residual amount (mg/l)	2.58 ± 0.29	2.38 ± 0.04	2.22 ± 0.09
Astaxanthin (mg/l)	0.49 ± 0.03	0.51 ± 0.02	0.44 ± 0.04
Soluble protein (mg/l)	0.22 ± 0.01 ^a	0.2 ± 0.01 ^b	0.21 ± 0.01 ^b

Values with different superscript letters (a and b) in the same column indicate a significant difference at $p < 0.05$ according to Duncan's multiple range tests.

However, residual nitrogen amount under blue-green light was significantly lower ($p < 0.05$) than the blue-red and monochromic blue wavelengths. Conversely, the amount of soluble protein (0.22 ± 0.01 mg/l) under the blue-green LED light was significantly higher ($p < 0.05$) than that of blue-red and blue light wavelengths.

3.6 The Effect of Light Intensity and Photoperiod Cycle on the Dry Weight and Astaxanthin of *S. obliquus*

Given the results of the previous wavelength trials, blue light was selected as the optimal light wavelength for testing the effects of photoperiod length and light intensity. Generally, the dry weight increased with the photoperiod length, and dry weight increased with light intensity (Fig. 5(a)). However, under the most intense light and longest photoperiod (H24 group) dry weight was lower than either the H18 or M24 group. Thus, the group that exhibited the significant high dry weight (0.41 ± 0.05 mg/l) was the H18 group (250 $\mu\text{mol}/\text{m}^2\cdot\text{s}$ and the light/dark cycle of 18:6) ($p < 0.05$). Astaxanthin accumulation was also evaluated under varying photoperiods and light intensities (Fig. 5(b)). Here, optimal astaxanthin accumulation (0.61 ± 0.07 mg/l) occurred in the H18 group, which experienced light intensity of 250 $\mu\text{mol}/\text{m}^2\cdot\text{s}$ and the light/dark cycle of 18:6.

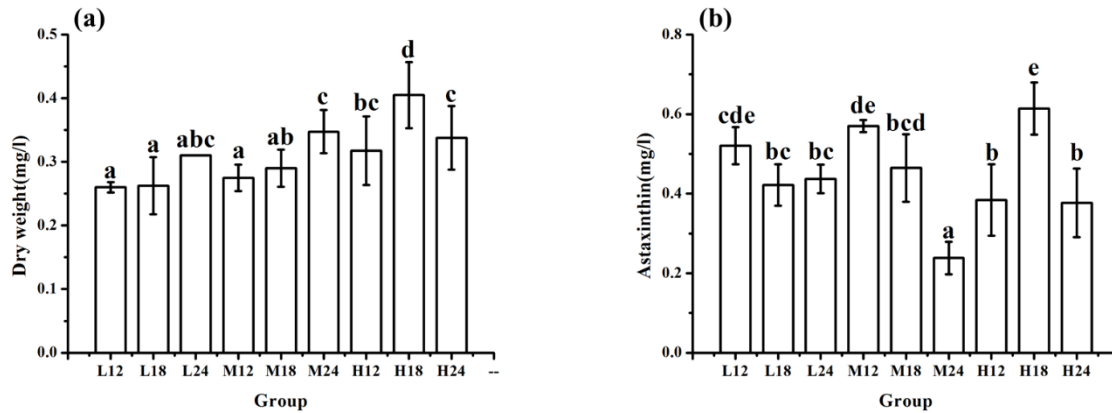


Figure 5: Dry weight (a) and astaxanthin (b) of *S. obliquus* FACHB-12 at 50, 150 and 250 $\mu\text{mol}/\text{m}^2\cdot\text{s}$ light intensities and different photoperiod cycles

3.7 The Effect of Light Intensity and Photoperiod on Chlorophyll Content of *S. obliquus*

The amount of chlorophyll present in *S. obliquus* under different light intensities and photoperiod cycles is shown in Fig. 6. Under the light/dark 12:12 cycle, chlorophyll increased with increasing light intensity. Under the light/dark 18:6 cycle, the middle light intensity (150 $\mu\text{mol}/\text{m}^2\cdot\text{s}$) was the optimal condition for the accumulation of chlorophyll a. Under the light/dark 24:0 cycle, chlorophyll a content decreased with the increasing light intensity. The optimal chlorophyll a content (1.47 ± 0.07 mg/l) was observed under a high light intensity (250 $\mu\text{mol}/\text{m}^2\cdot\text{s}$) and a 12:12 light/dark cycle. The optimal chlorophyll b content (0.43 ± 0.04 mg/l) was observed under a high light intensity (250 $\mu\text{mol}/\text{m}^2\cdot\text{s}$) and a 18:6 light/dark cycle.

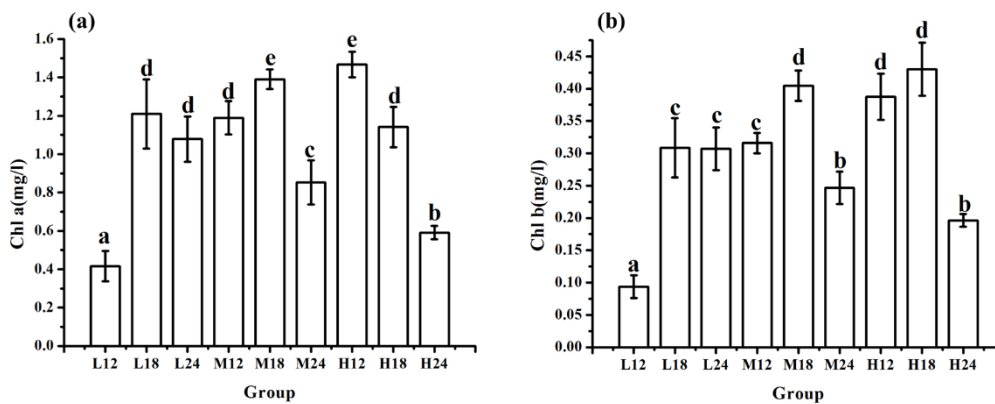


Figure 6: Chlorophyll a (a) and chlorophyll b (b) of *S. obliquus* FACHB-12 at 50, 150 and 250 $\mu\text{mol}/\text{m}^2\cdot\text{s}$ light intensities and different photoperiod cycles

3.8 The Effect of Light Intensity and Photoperiod Cycle on the Nitrogen and Phosphorus Residual Amount of *S. obliquus*

Residual nitrogen and phosphorus were also analyzed in response to differing light intensities and photoperiods (Fig. 7). It was found that nitrogen levels were not affected strongly by light intensity or photoperiod (Fig. 7(a)). However, the H24 group (250 $\mu\text{mol}/\text{m}^2\cdot\text{s}$ light intensity, 24:0 photoperiod cycle) exhibited the lowest residual nitrogen content of any group (Fig. 7(a)). Conversely, residual phosphorous was dramatically affected by photoperiod length, with 24:0 photoperiod groups showing the lowest

phosphorous amounts (Fig. 7(b)). Higher light intensities also reduced the amount of residual phosphorous (Fig. 7(b)). At the longest photoperiods (24:0, very little differences were seen in residual phosphorous between low, medium, and high intensities (Fig. 7(b)).

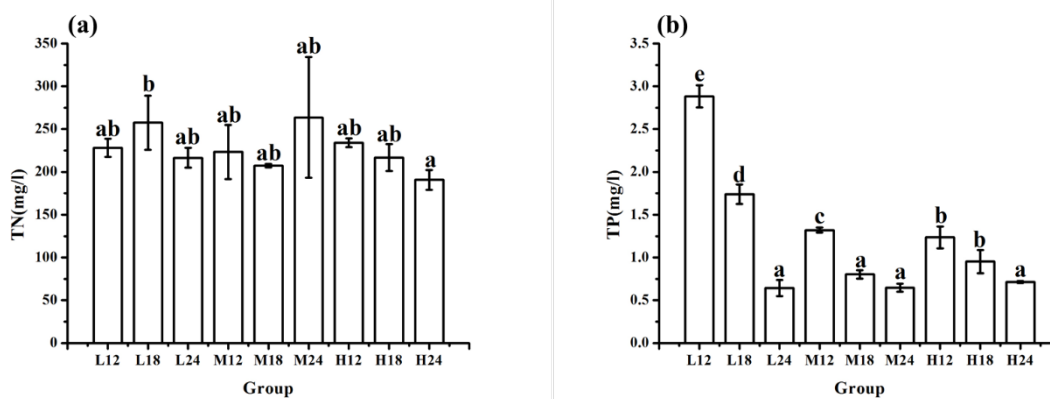


Figure 7: Residual Nitrogen (a) and Phosphorus (b) amount in the culture medium of *S. obliquus* FACHB-12 at 50, 150 and 250 $\mu\text{mol}/\text{m}^2\cdot\text{s}$ light intensities and different photoperiod cycles

4 Discussions

Previous studies indicated that light with a specific wavelength could not only facilitate the growth of microalgae, but also promote the productivity of high-value products (e.g., proteins, pigments) [10,25,26]. In this study, the cell density and specific growth rate of *S. obliquus* FACHB-12 decreased in the order of blue, white, red and green LEDs (Figs. 1(a) and 1(b)). Different results were obtained by Ho et al. wherein the biomass productivity decreased in the order of white, red, green and blue LEDs in *S. obliquus* CNW-N, and in the order of green, red, white and blue LEDs in *S. obliquus* FSP-3 [18]. Moreover, this discrepancy was also observed in different algal species, such as white light was the best color in marine green alga *Tetraselmis suecica* growth [27], green LED condition was suitable for the growth of rhodophyta microalga *Porphyridium purpureum* [28], and red light wavelength was optimal for *Haematococcus pluvialis* production [29]. These various results indicate that the influence of light wavelength on the growth and biomass productivity of algae is not only species-specific, but also strain-specific. Additionally, research showed that certain mixed LED light wavelength treatments (red: blue) were better for the growth of algae than other LED light color treatments (red, blue and white) [17,30]. However, no significant difference was observed between monochromatic blue light compared with mixed LED light colors (blue: red and blue: green, Tab. 2) in this study. One possible explanation for this result is that the appropriate intensity ratio of monochromatic light wavelengths may be important for optimal algal growth. This hypothesis will be investigated in future studies. In addition to light wavelengths, irradiance and photoperiod also influence the biomass production and the most suitable conditions for the growth of specific algae. In this study, *S. obliquus* showed the highest dry weight under the light intensity of 250 $\mu\text{mol}/\text{m}^2\cdot\text{s}$ and 18L:6D photoperiod cycle. Chen X. et al. (2011) found that the best growth performance of *Chlorella sp.* was conducted under the light intensity range of 82-590 $\mu\text{mol}/\text{m}^2\cdot\text{s}$ [31]. *Aphanocapsa* have a very high and noticeable increase in the biomass density under the effect of 5,000, 3,500 and 2,000 lux light intensity on the 9th day of cultivation [16]. Therefore, different algae have different optimal conditions for the best productivity.

The biomass productivity of algae mainly depends on the characteristic of the light wavelength [26]. Studies showed that light with shorter wavelengths could strike the light-harvesting complex for higher efficiency photosynthesis [13,32]. This might be the reason to explain the blue light with short wavelength (460 nm) has an efficiency influence on the growth and pigment content of algae. However, the white color displayed a medium level of biomass (Fig. 1(a)). This could be explained by the

observation that the white light is a combination of the red light wavelength and other growth inefficient light wavelengths [33]. As described previously, specific growth rate is the best parameter to explain the ecological or adaption success of species under the special environment condition [34]. Since the highest cell density and specific growth rate was observed under the blue light wavelength, we have determined that the blue light is the most suitable light wavelength for the productivity of *S. obliquus* FACHB-12.

Furthermore, studies have shown that the pigment content is a major factor on the ability of algae to photo-acclimate [35-37]. In the present results, the chlorophyll a/b contents of *S. obliquus* FACHB-12 show a similar trend as that of the growth pattern (Figs. 1(c) and 1(d)). This indicates the uniformity in the light utilizing ability of algal cells under different light wavelengths. Under the blue light wavelength, we found *S. obliquus* FACHB-12 had significantly higher chl a and chl b contents than those of white, red and green colors. Similar results were observed in *Nitzschia* sp. [38], blue LED resulted in highest chl content compared to red and yellow LEDs. Costa B.S. et al. 2013 who noted that higher chl a content was discovered under the blue light wavelength compared to red and white LEDs in *Phaeodactylum tricorutum* [39]. Conversely, blue light gave rise to lowest chl content in *Arthrospira platensis* [40]. Previous studies suggested that blue light could induce the accumulation of pigment via photoreceptors (e.g., phototropins) [41,42]. A possible explanation that green algae lost the phycobilin during the evolutionary process, mainly through chl a and chl b to absorb light energy [43]. Under the optimal blue light, chl a and chl b were largely accumulated under the high light intensity ($250 \mu\text{mol}/\text{m}^2\cdot\text{s}$), and 12L:12D and 18L:6D photoperiod cycles, respectively. chl a and chl b have a significant effect on photosynthetic capacity of *S. obliquus*. Therefore, high productivity of dry weight and astaxanthin were observed in this study.

Afterward, the residual nutrient amounts were also analyzed in this study. It was shown that the order of the residual nutrient amounts is green > red > white > blue LEDs in *S. obliquus* (Fig. 2). Hence, the nutrient removal efficiency of blue light wavelength was higher than that of the other treatments. This result is coincident with the variation of cell density and specific growth rate during the cultivation period. Studies showed that microalgal cells need abundant nitrogen and phosphorous sources during the synthesis of nucleic acids, phospholipids and proteins [44,45]. Furthermore, it was shown that red and blue light wavelengths are associated with algal photosystems I and II, the blue LED light could induce photosystem I [4,46]. Therefore, the biological N: P rate decreases with the increasing of the algal growth rate, higher photosynthesis in *S. obliquus* benefits for the degradation of organic matter in wastewater. A similar result was found in Kim et al. (2013) study where they found the phosphorus removal rate was very high (90%) with blue light wavelength compared to the white light, and N and P removal rates were about 15 mg/l/day at a wavelength mixing ratio of red and blue lights in wastewater treatment using *Scenedesmus* sp. [26]. While the white light color was approximately under the range of the blue and green LEDs, consequently, the nutrient removal rate of white light is intermediate of all treatments. In addition to the effects of monochromatic light wavelengths on the N/P removal, mixed light wavelength treatments (blue: green and blue: red) were also evaluated. The results indicated that the mixture of blue and green light wavelengths was the most appropriate mixture in the nitrogen removal efficiency, but not for phosphorus removal (Tab. 2). Therefore, the blue: green light is determined as the best light wavelengths for nitrogen removal in microalgae *S. obliquus*, and this might provide a promising route for the wastewater treatment. Finally, it was determined that minimal residual nutrient levels were obtained under long photoperiods (24L: 0D) and moderate to high intensities $150 \mu\text{mol}/\text{m}^2\cdot\text{s}$ or $250 \mu\text{mol}/\text{m}^2\cdot\text{s}$.

This work has shown that different light wavelengths have different effects on cell density and chlorophyll content. These results led us to examine the soluble protein and astaxanthin production of *S. obliquus* under different LED lights. Similarity, soluble protein and astaxanthin content can effectively be induced by the blue light at a light intensity of 3000 lux. It was observed that blue light could not only increase the high-value metabolites (e.g., chlorophyll, soluble protein and astaxanthin), but also has a positive effect in the wastewater treatment. However, it was shown that green light was ineffective for the accumulation of soluble protein and astaxanthin, because the absorption efficiency of chlorophyll in this range of green light wavelength is low. A similar result was obtained in *H. pluvialis*, LEDs with short

wavelengths (blue light) can induce the change of morphology and the increase of astaxanthin content [10]. Xi T et al. found that astaxanthin production under the wavelength shift from red to blue was dramatically increased in green microalga *Haematococcus pluvialis* [47]. In previous studies the highest astaxanthin content of *Haematococcus pluvialis* was obtained under the blue wavelength, while the growth of microalgae was inhibited at blue wavelength [10,48]. Conversely, the content of astaxanthin and the cell biomass of *S. obliquus* in this study were both increased under the blue wavelength. Additionally, high temperature, nutrient starvation, salt stress and oxidative stress (reactive oxygen species, free radicals and dissolved oxygen) and the other environmental conditions can also induce intracellular accumulation of astaxanthin [49]. It was known that astaxanthin is widely studied and used in industry, such as in nutraceutical, cosmetic, pharmaceutical and food industries [47]. Further research showed a mixture of blue and green light could induce the accumulation of astaxanthin and soluble protein better than monochromatic blue light. In the mixed LED light wavelength treatments, the photosynthetic efficiency of algae was enhanced by simultaneously providing both blue and green lights. Additionally, the light intensity and photoperiod cycle are also important. Insufficient light intensity and inappropriate light cycle resulted in the biomass loss and slower growth rates because the algae consumed oxygen and carbohydrates during the process of photorespiration [32]. This might be a reason to explain that the highest astaxanthin content was observed under the high light intensity and 18L: 6D light cycle treatments. These results here provide a new route for obtaining astaxanthin.

Previous studies showed that astaxanthin can not only be a light protection agent to prevent chloroplast from light damage, but also a strong antioxidant to clear a variety of reactive oxygen species (ROS) caused by the strong light and excessive oxidation [50]. ROS plays an important role in plant processes such as growth, development, and different resistant responses [51]. The steady-state level of ROS is dependent on the ROS gene network [52,53]. Although the ROS gene network has a key role in various biological processes, the detailed function of only few genes were determined until now in plants [52,54,55]. Here, we found that high amounts of ROS might induce the accumulation of astaxanthin under blue light. Logically, *S. obliquus* FACHB-12 produced more corresponding antioxidant enzymes (SOD, CAT and POD) activity under the blue wavelength, which could inhibit the ROS damage to algae and ensure the rapidly growth of microalgae.

In conclusion, our results suggested that *S. obliquus* FACHB-12 could produce more biomass and accumulate more astaxanthin under the blue wavelength, which provides a new route for the productivity of astaxanthin. The nutrient removal efficiency is high under the blue light wavelength, mixed LED light wavelengths (blue: green = 1:1) is the optimal nitrogen removal treatment, suggesting a new possibility of wastewater treatment. It was proposed that the amount of astaxanthin and antioxidant enzymes (SOD, CAT and POD) increase to protect algae from the ROS damage. However, the detailed defense mechanism needs further investigation. Under the blue light, appropriate light intensity and photoperiod cycle play an important role in the biomass, astaxanthin and nutrient removal.

5 Conclusion

This study indicated that blue light wavelength is the optimal light wavelength for phosphorus removal efficiency and the accumulation of biomass and astaxanthin in *S. obliquus*. Furthermore, mixed blue/green lights treatment is the most appropriate mixture for the nitrogen removal. By using the blue light treatment at high light intensity and 18L: 6D light cycle, the highest biomass and astaxanthin accumulation were observed. Optimal nitrogen/phosphorus removal efficiency was found under a 24L: 0D light cycle. These results might provide a scientific data for the optimizing the productivity of high-value metabolites and treatment of wastewater, *S. obliquus* FACHB-12 could thus use to be an ideal candidate to commercial produce astaxanthin from microalgae.

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