

Effect of light quality on the cultivation of *Chlorella pyrenoidosa*

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Abstract. Effect of light quality, including red light, blue light, white light, red and blue mixing light with 8:1, 8:2 and 8:3, on the growth characteristics and metabolite accumulation of *Chlorella pyrenoidosa* was conducted based on light emitting diode (LED). Results showed that *Chlorella pyrenoidosa* grew best under blue light, and the optical density, specific growth rate and biomass of *Chlorella pyrenoidosa* was about 2.4, 0.10 d⁻¹ and 6.4 g·L⁻¹, respectively, while the optical density of *Chlorella pyrenoidosa* was between 1.0 and 1.7, specific growth rate was between 0.06-0.10 d⁻¹ and biomass was between 2.7 and 3.8 g·L⁻¹ under other light quality after 30 days of cultivation. The optical density, specific growth rate and biomass of *Chlorella pyrenoidosa* was approximately 2.05 times, 1.33 times and 2.06 times under blue light than red light, respectively. Moreover, Red and blue mixing light was conducive to the synthesis of chlorophyll a and carotenoids of *Chlorella pyrenoidosa*, and blue light could promote the synthesis of chlorophyll b. Chlorophyll a and carotenoids content of *Chlorella pyrenoidosa* was 13.5 mg·g⁻¹ and 5.8 mg·g⁻¹ respectively under red and blue mixing light with 8:1, while it was 8.4 mg·g⁻¹ and 3.6 mg·g⁻¹ respectively under blue light. Red and blue mixing light was more conducive to protein and total lipid content per dry cell of *Chlorella pyrenoidosa*. Protein and total lipid content was 489.3 mg·g⁻¹ and 311.2 mg·g⁻¹ under red and blue mixing light with 8:3, while it was 400.9 mg·g⁻¹ and 231.9 mg·g⁻¹ respectively under blue light.

1 Introduction

Light is one of the most important environmental factors in the growth and biochemical composition of microalgae cell, and it also has significant impact on microalgae's growth, photosynthesis, algae color, cell morphogenesis and metabolites accumulation [1, 2]. The variation of illumination in nature has regularity and stability, and microalgae form the specific reaction to light in the long evolutionary. Therefore, it is one of the important ways to improve microalgae's yield and quality to apply appropriate light technology on microalgae cultivation to accelerate its growth reproduction and to adjust its nutrient composition [3-5]. Light is a complex ecological factor, and effects on the microalgae growth include light quality, light intensity and light photoperiod [6-8]. However, it is easy to realize the adjustment of light intensity and light photoperiod, and therefore studies of light environments on microalgae growth have stressed on light intensity and light photoperiod recently. Few study was carried out to focus on light quality on the growth and metabolism of microalgae.

The light sources used for microalgae cultivation are mainly fluorescent lamps, mercury lamps, halogen lamps and so on, in which fluorescent lamps are the major light sources [9, 10]. Fluorescent lamps are the ideal choices from both spectral structure and luminous efficiency as the tool for everyday lighting. However, the emission spectrum of fluorescent lamps and aquatic selective

absorption spectrum does not match, and fluorescent lamps have great limitations to be chosen as ecological illumination source to cultivate microalgae due to poor targeting, low luminous efficiency, energy consumption, and much heat affecting water temperature. Recently, Light Emitting Diode (LED) has been considered as a novel cold light source with its advantages of low voltage and power consumption, wide operating temperature range, high mechanical strength, energy efficiency, strong stability, multicolor, narrowband, excellent color rendering, safety, environment protection, high brightness, long life, small size, less heat, fast response, no flicker, no pollution and no radiation and so on [11]. LED has been demonstrated broad application prospects in the field of eco-lighting [2, 12, 13]. What's more, LED has a series of characteristics such as low cooling load, high electro-optical conversion efficiency and change in specific wavelength of light quality. LED has small heat source, pure quality and it is available to get pure monochromatic light and composite spectra, which is very suitable artificial light for microalgae cultivation [13-15]. Unfortunately, we most focused on the studies of LED light source on tissue culture, and also effects of monochromatic light on plant growth and development was highlighted in China. However, few studies of LED-based light quality on microalgae cultivation were reported. Consequently, effects of LED-based light quality on growth characteristics and metabolite accumulation of *Chlorella pyrenoidosa* was carried out to provide theoretical basis for the application

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of LED light source on microalgae cultivation in the present study.

2 Materials and methods

2.1 Experimental materials and culture medium

2.1.1 Tested microalgae

Chlorella pyrenoidosa (FACHB-9), which was bought from freshwater algae species pool in the institute of hydrobiology, Chinese academy of sciences, was selected as the tested microalgae.

1.1.2 Culture medium

Selenite Enrichment (SE) culture medium was used for the cultivation of *Chlorella pyrenoidosa*. The main ingredients of SE culture medium are as follows (1L): NaNO₃, 0.25 g; K₂HPO₄, 0.075 g; MgSO₄·7H₂O, 0.075 g; CaCl₂·2H₂O, 0.025 g; KH₂PO₄, 0.175 g; NaCl, 0.025 g; FeCl₃·6H₂O, 0.0005 g; EDTA-Fe, 1 mL; Trace element solution 1 mL; Soil extracts 40 mL. Trace elements (1L) include H₃BO₃, 2.86 mg; MnCl₂·4H₂O, 1.86 mg; ZnSO₄·7H₂O, 0.22 mg; Na₂MoO₄·2H₂O, 0.39 mg; CuSO₄·5H₂O, 0.08 mg; Co(NO₃)₂·6H₂O, 0.05 mg. Preparation methods of EDTA-Fe are as follows: a. HCl, Take 4.1 mL of concentrated hydrochloric acid to 50 mL diluted with distilled water; b. EDNA-Na₂, Take 0.9306 g dissolved in 50 mL of distilled water; c. Take 0.901 g of FeCl₃·6H₂O dissolved in HCl 10 mL that have been prepared, and mix it with the finished EDNA-Na₂, then use distilled water to dilute it to 1000 mL. Soil extract manufacturing method is as follows. Firstly, take not fertilized garden soil 200 g in a beaker and add 1000 mL of distilled water sealed with vent plug. Secondly, put it in boiling water for 3 hours and then cool it to precipitate for 24 hours. This process is repeated for 3 times. Lastly, filtration and the supernatant sterilized in an autoclave and stored in a refrigerator at 4°C.

2.2 Measurement method

For determination of microalgae cell growth, we take sample once every 24 hours and measure algae liquid with a spectrophotometer of 722N to test the optical density value at 680 nm. We use the change of optical density of 680 nm to represent the growth of microalgae cells.

The specific growth rate of *Chlorella pyrenoidosa* can be calculated as follows:

$$\mu = (\ln X_2 - \ln X_1) / t \quad (1)$$

In the equation, μ is the specific growth rate (d⁻¹), X₁, X₂ are the optical density value at 680 nm at the beginning and the end respectively, and t is the culture time (day).

For determination of chlorophyll content, harvested by centrifugation of 20 mL volume of algal cells and then add 90% acetone. At 4 °C darkness extracted 12 hours, we test the optical density value at 630 nm and 664 nm respectively, and then chlorophyll content is calculated by the formula.

For determination of aryttenoids content, harvested by centrifugation of 20 mL volume of algal cells and then add 90% acetone. At 4 °C darkness extracted 12 hours, we test the optical density value at 450 nm, and then aryttenoids content is calculated by the formula.

We take some algae fluid in certain volume after centrifugation, discard the supernatant and then dry it overnight at 50 °C and weight it. The biomass can be calculated in the following formula.

$$Biomass = \frac{\text{dry algal biomass weight}}{\text{algae solution volume}} \quad (2)$$

For determination of algal protein, we measure the total nitrogen content in the fluid and the supernatant after centrifugation (1000r/min, 5min) respectively. The difference is the nitrogen content of algae and algae nitrogen content of 6.27 times is the algal protein content.

2.3 Experimental design

In our experiments, *Chlorella pyrenoidosa* are cultivated with the SE culture medium. When the algae are in logarithmic phase, we transfer them to the flask (250 mL) containing 150 mL of sterile mixing medium with the initial optical density at 680nm of 0.1. LED light quality is set at red and blue light with 8:1, 8:2 and 8:3, respectively, and pure blue, red and white lights. The temperature is set at 25 Celsius degree with error range of 1 Celsius degree. Light cycle is 12 hours/ 12 hours. We study the different light quality's influence on the growth characteristics and metabolites accumulation of *Chlorella pyrenoidosa* in the same CO₂ condition in one-time culture. 30 days after inoculation culture, *Chlorella pyrenoidosa* for growth characteristics and indicators of metabolites are measured.

3 Results

3.1 Effects of light quality on growth characteristics of *Chlorella pyrenoidosa*

3.1.1 Growth curve of *Chlorella pyrenoidosa* under different light quality

The growth curves of *Chlorella pyrenoidosa* under different light quality were demonstrated in Fig. 1. Algal cells are in the lag phase and the growth rate is slow, and no significant difference in algal cell growth was found among different light quality on the first 15 days after inoculation. Algal cells turn into the logarithmic phase and significance difference in cell growth was detected among different light quality on 20th day after inoculation. *Chlorella pyrenoidosa* grows best under blue light, and the growth advantages present more significant

with the incubation time increasing. The optical density of chlorella pyrenoidosa at 680nm is 2.4 under blue light, 1.2 under red light and 1.0-1.7 under the other lights after 30 days' inoculation.

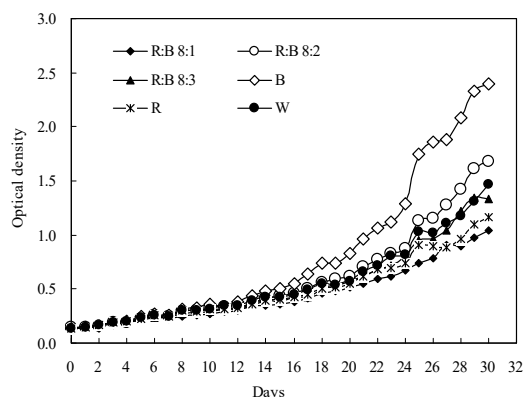


Fig. 1. Growth curve of chlorella pyrenoidosa under different light quality

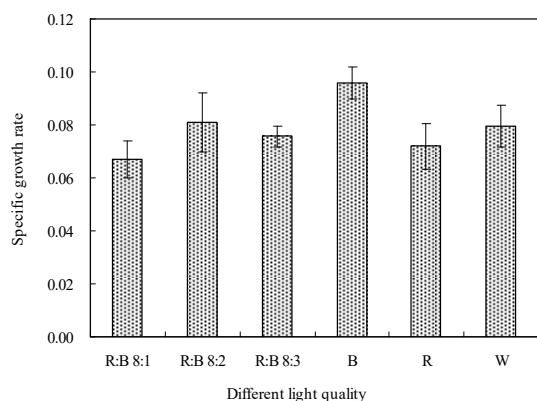


Fig. 2. Growth rate of chlorella pyrenoidosa under different light quality

3.1.2 Specific growth rate of chlorella pyrenoidosa under different light quality

The specific growth rate of chlorella pyrenoidosa are between 0.06 d^{-1} and 0.10 d^{-1} under different light quality (Fig. 2). The specific growth rate is highest under blue light, accounting for 0.1 d^{-1} , and no significant difference in specific growth rate was found under other different light quality.

3.1.3 Biomass of chlorella pyrenoidosa under different light quality

Significant differences in biomass accumulation of chlorella pyrenoidosa exist among different light quality (Fig. 3). The biomass of chlorella pyrenoidosa under red and blue mixing light with 8:1, red and blue mixing light with 8:2, red and blue mixing light with 8:3, red light, and white light is $2.7 \text{ g}\cdot\text{L}^{-1}$, $3.6 \text{ g}\cdot\text{L}^{-1}$, $3.2 \text{ g}\cdot\text{L}^{-1}$, $3.1 \text{ g}\cdot\text{L}^{-1}$ and $3.8 \text{ g}\cdot\text{L}^{-1}$, respectively. The biomass of chlorella pyrenoidosa reach a maximum of $6.4 \text{ g}\cdot\text{L}^{-1}$ under the blue light, which is 2.4 times of red and blue mixing light with 8:1, 1.8 times of red and blue mixing light

with 8:2, 2.0 times of red and blue mixing light with 8:3, 2.1 times of red light and 1.7 times of white light.

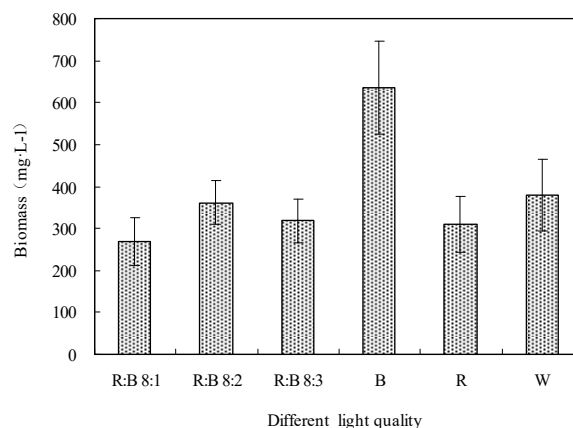


Fig. 3. Biomass of chlorella pyrenoidosa under different light quality

3.2 Effects of light quality on the pigment synthesis of chlorella pyrenoidosa

2.2.1 Photosynthetic pigment content of chlorella pyrenoidosa under different quality

The Photosynthetic pigment content of chlorella pyrenoidosa under different light quality were showed in Fig. 4. chlorophyll a content is lower under blue light, while it is much higher under red and blue mixing light. The chlorophyll a content of blue light, red light, red and blue mixing light with 8:1, red and blue mixing light with 8:2, red and blue mixing light with 8:3 is $8.4 \text{ mg}\cdot\text{g}^{-1}$, $11.5 \text{ mg}\cdot\text{g}^{-1}$, $13.5 \text{ mg}\cdot\text{g}^{-1}$, $12.8 \text{ mg}\cdot\text{g}^{-1}$, and $13.2 \text{ mg}\cdot\text{g}^{-1}$, respectively. Moreover, chlorophyll b content is much lower under red light, and no significant difference in chlorophyll b content was found among other light quality. The chlorophyll b content of blue light, white light, red light, red and blue mixing light with 8:1, red and blue mixing light with 8:2, red and blue mixing light with 8:3 is $6.4 \text{ mg}\cdot\text{g}^{-1}$, $6.4 \text{ mg}\cdot\text{g}^{-1}$, $4.8 \text{ mg}\cdot\text{g}^{-1}$, $6.3 \text{ mg}\cdot\text{g}^{-1}$, $6.1 \text{ mg}\cdot\text{g}^{-1}$, and $6.6 \text{ mg}\cdot\text{g}^{-1}$, respectively. The variation pattern of chlorophyll a+b content is similar to the chlorophyll a, and chlorophyll a+b content is much lower under blue light, while it is much higher under red and blue mixing light. The chlorophyll a+b content of blue light, red and blue mixing light with 8:1, red and blue mixing light with 8:2, red and blue mixing light with 8:3 is $14.8 \text{ mg}\cdot\text{g}^{-1}$, $19.8 \text{ mg}\cdot\text{g}^{-1}$, $18.8 \text{ mg}\cdot\text{g}^{-1}$, and $19.8 \text{ mg}\cdot\text{g}^{-1}$, respectively.

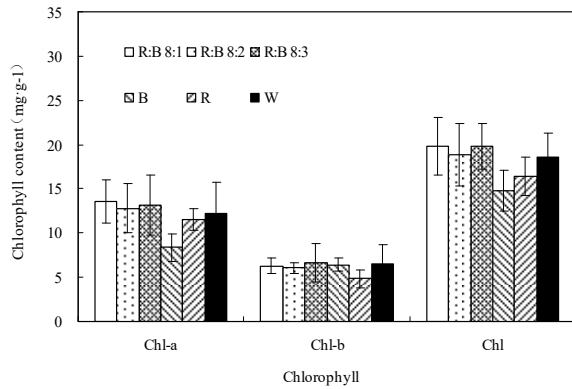


Fig. 4. Chlorophyll content of chlorella pyrenoidosa under different light quality

3.2.2 Carotene content of chlorella pyrenoidosa under different quality

Lower carotene content of chlorella pyrenoidosa was detected under blue light, while it is higher under red and blue mixing light (Fig. 5). The carotene content of blue light, red light, red and blue mixing light with 8:1, red and blue mixing light with 8:3 is 3.6 mg·g⁻¹, 4.9 mg·g⁻¹, 5.8 mg·g⁻¹, 5.7 mg·g⁻¹.

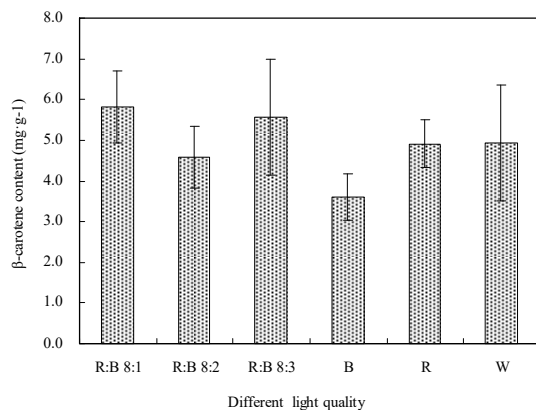


Fig. 5. Carotene content of chlorella pyrenoidosa under different light quality

3.3 Effects of light quality on metabolite accumulation of chlorella pyrenoidosa

3.3.1 Protein content of chlorella pyrenoidosa under different light quality

Lower protein content of chlorella pyrenoidosa was detected under blue light, while it is higher under white light and red and blue mixing light with 8:3 (Fig. 6). The protein content of blue light, red light, white light, red and blue mixing light with 8:3 is 400.9 mg·g⁻¹, 456.7 mg·g⁻¹, 488.5 mg·g⁻¹, 489.3 mg·g⁻¹.

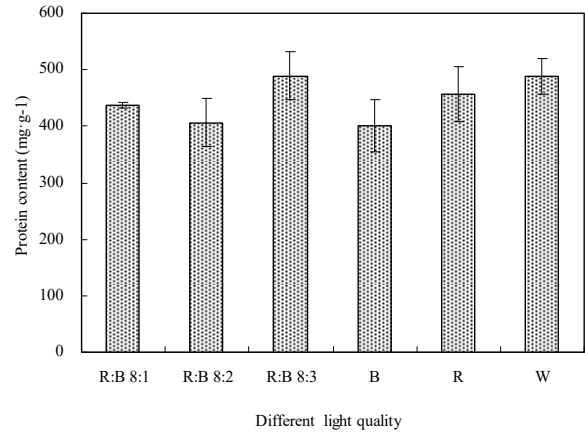


Fig. 6. Protein content of chlorella pyrenoidosa under different light quality

3.3.2 Total lipid content of chlorella pyrenoidosa under different light quality

Lower total lipid content of chlorella pyrenoidosa was detected under blue light, while it is higher under red and blue mixing light with 8:1 (Fig. 7). Total lipid content of blue light, red light, white light, red and blue mixing light 8:1 is 231.9 mg·g⁻¹, 251.6 mg·g⁻¹, 311.2 mg·g⁻¹.

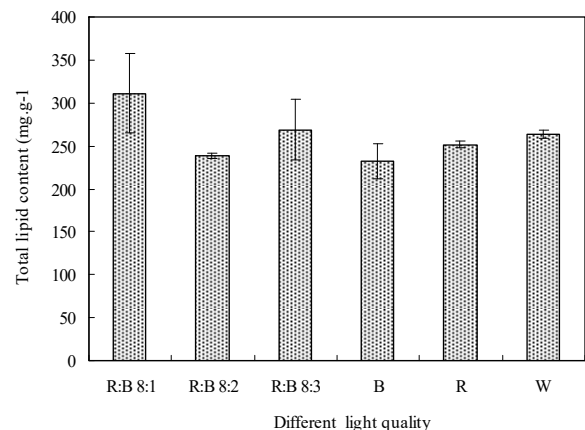


Fig. 7. Total lipid content of chlorella pyrenoidosa under different light quality

4 Discussion

Photosynthesis is a basic method for synthetic substances in the cell of microalgae. They convert light energy into their own needed material through absorption, transmission, and transformation of light energy [16]. Compared with high plants, chromatophore and pigment composition of microalgae are relatively complicated. For example, chromatophore of cyanophyta doesn't have plastid. What's more, its pigment disperses and contains chloroplasts a, β carotene, and phycocyanin, pyhcerythrin and xanthophyll pigments etc. Chromatophore and chloroplasts of chlorophyta are cups, links spiral ribbon, star, mesh etc, and different chromatophore and pigment composition of microalgae

don't have the identical requirements on light [17]. Aidar et al. [3] reported that *Cyclotella caspia* had the highest growth rate under the blue-green light while the *Tetraselmis gracilis* were under red light. Red light could improve the growth rate of *Spirulina platensis* while its conversion efficiency from light quantum to biomass was lowest under blue light [15]. Blue light could improve the growth rate of *Nannochloropsis* sp., while it had the lowest growth rate under red light [5]. Red light was conducive to biomass accumulation in the mixed culture system of *Chlorella* sp. and *Saccharomyces cerevisiae* [18]. *Richelia sinica* grew slowly under blue light [19]. Wang et al. [20] discovered that the cell proliferation rate of *Biddulphia sinensis* was highest under white light, and followed by blue light. Monochromatic LED light and fluorescent lamp were used to estimate the effect of light optical spectrum on the growth characteristics of *Chlorella vulgaris* and *Isochrysis galbana* Parke 8701 from efficiency and growth rate, and result showed that continuous spectrum could promote growth rate, and blue light had high efficiency on promoting growth rate [13]. All above indicate that different microalgae have different pigment composition, and require different light quality during photosynthesis. *Spirulina platensis* needs lower light intensity of 330 $\mu\text{mol}/\text{m}^2/\text{s}$, and blue light has higher quantum and photosynthetic efficiency. Therefore, red light can improve the growth of *Spirulina platensis* [4]. What's more, red light can active the chlorophyll molecules of benthic microalgae, and accelerate the rate of photosynthesis and cell division, and promote chlorophyll accumulation of benthic microalgae [21].

The light energy captured by photosynthesis in microalgae is mainly performed by photosynthetic pigment. As a result, the changes of light quality, light intensity and light photoperiod have important influence on the formation of microalgae photosynthetic pigment [22]. Our results showed that red and blue mixing light was conducive to the synthesis of chlorophyll a and carotene of *Chlorella pyrenoidosa*, and blue light could accelerate the synthesis of chlorophyll b. Other reports indicated that blue light was helpful to synthesize chlorophyll a and carotene of *Richelia sinica* [19]. Phycoerythrobilin of porphyridium was higher under green light, while it was lower under red light. Therefore, further studies should be carried out to estimate the effect of light quality on the changes of photosynthetic pigments.

Light energy captured by photosynthetic pigments is the main source of microalgae photosynthesis. Therefore, the levels of photosynthetic pigment have close relation with photosynthetic rate of microalgae. Red light can regulate the synthesis of pigment protein complexes (LHC), and the regulation of blue and near-ultraviolet light on photosynthesis is mainly concentrated in the differentiation and movement of chloroplast, and the adjustment of photosynthetic enzyme activity. Photosynthetic oxygen release and absorption rate of *Richelia sinica* is higher under yellow and white light, and lower under green, red and blue light [19]. Red light can promote photosynthesis rate of benthic algae, and blue light can inhibit the growth of benthic algae cells

[21]. Therefore, effects of light quality on microalgae's growth and metabolite are decided by different photosynthetic pigment composition in different types of microalgae. The changes of environmental factors such as light environment and nutritive salt will affect the flow and transformation of photosynthetic pigment and pigment precursor, which can result in the changes of photosynthetic pigment. The changes of synthetic pigments in the cell of *Chlorella pyrenoidosa* under different light quality is probably caused by the hypothesis that different light quality can affect the intracellular turnover and transformation of photosynthetic pigment inside the cell, and cause pigment synthesis and degradation of algal cells.

Substance accumulation in microalgae cell is affected by the selection of light quality, and significance difference in the synthesis of protein and total lipid of *Chlorella pyrenoidosa* is detected under different light quality. In the present study, blue light can promote the growth and biomass accumulation of *Chlorella pyrenoidosa*, while red and blue mixing light conducive to the accumulation of protein and total lipid. Wang et al. [20] reported that blue light could promote the protein synthesis of *Biddulphia sinensis*, while red light did benefit to the carbohydrate synthesis. Red and blue light could promote photosynthetic efficiency and the accumulation of extracellular polysaccharides of *Porphyridium cruentum* [6]. Korbee et al. [23] found that blue light was more beneficial to the accumulation of nitrogen metabolites of *Porphyra leucosticta* such as mycosporine amino porphyra-334, palythine and asterine-330, while red, yellow, green, blue and white light was conducive to shinorine accumulation. Blue light can promote lipid accumulation in the mixed culture system about the *Chlorella* sp. and *Saccharomyces cerevisiae* [18], and it also promote fatty acid methyl esters (FAME) accumulation of *Nannochloropsis* sp [5]. All of above suggested that significance difference in the metabolite accumulation of microalgae was found under different light quality. However, little is known about the mechanism how light quality affect the metabolite synthesis of microalgae, and it needs further exploration in future.

5 Summary

Based on LED light source, we study the influence of light quality on the growth characteristics and metabolites accumulation of *Chlorella pyrenoidosa*. The results of study are as follows. Firstly, blue light can clearly help the growth of *Chlorella pyrenoidosa* and promote their biomass accumulation. Secondly, red and blue light is conducive to chlorophyll a and carotene synthesis of *Chlorella pyrenoidosa* while blue light can promote the synthesis of chlorophyll b. Finally, mixed red and blue light is more conducive to *Chlorella pyrenoidosa* protein and total lipid accumulation. Unit cell dry weight of protein and total lipid content are lower under blue light conditions.

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