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The Growth and Lipid Accumulation of *Spirulina* sp. Under Different Light Conditions

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Abstract: *Spirulina* is a filamentous, spiral-shaped cyanobacterium (blue green alga), known as a great resource of natural and bioactive compounds. The colour of *Spirulina* sp. cell under the red and white light conditions rapidly transferred from green to yellow after 5 days of cultivation. High biomass and lipid accumulation of *Spirulina* sp. were achieved after 5 days of culture under the red light condition. The results showed that the red and white light conditions induced the growth and biosynthesis organic compounds such as carotenoid and lipid with high concentration compared to the blue condition in *Spirulina* sp.

Keywords: *Spirulina*, Lipid Accumulation, Sulfo-Phospho-Vanillin Assay

1. Introduction

Cyanobacteria are one of the oldest and morphologically most diverse prokaryotic phyla on our planet. The early development of an oxygen-containing atmosphere approximately 2.45 - 2.22 billion years ago is attributed to the photosynthetic activity of cyanobacteria [3]. The genus *Spirulina* of the Oscillatoriaceae family contains the group of flamentous cyanobacteria characterized by spiral-shaped chains of cells (trichomes) enclosed in a thin sheath [16]. They form massive populations in tropical and subtropical water bodies [14], [2].

Spirulina algae are an important source of nutrients in the traditional diet of some populations of Africa and Mexico [14]. *Spirulina* contains a high content of protein (up to 70%), along with high amounts of essential fatty acids, essential amino acids, minerals, vitamins (especially B12), antioxidant pigments (phycobiliproteins and carotenoids) and polysaccharides [1], [2]. *Spirulina plutensis* contains 13.6% carbohydrate, the sugar composition of which is comprised principally of glucose along with rhamnose, mannose, xylose, galactose and two unusual sugars [7]. Moreover, it contains other components such as ω-3 and ω-6 polyunsaturated fatty acid, provitamins and phenolic compounds [14]. Consequently, the commercial production of *Spirulina* has gained worldwide attention for use in human food supplements, animal feed and pharmaceuticals. In aquaculture, *Spirulina* is used as a feed additive to improve growth, feed efficiency, carcass quality, and physiological response to disease in several species of fish [10]. Biochemical composition of microalgae is known to be related culture condition. Many environmental factors such as temperature, nutrient concentration, light irradiance. Therefore, the light quality was used to induce growth and biosynthesis of lipid in *Spirulina* sp.

2. Material and Methods

2.1. Spirulina Strain and Cultural Conditions

Spirulina strain obtained from Department of Algal Biotechnology, International University, Viet Nam, provided by Dr. Tran. The alga was grown in Zarouk medium, pH 9.0 according to [6]. The experiment carried out on three different lights including the blue (455-492nm), red (622-780nm) and white lights by LED lights, at 30 µmol photon.m^{-2}.s^{-1} continuous light and 25 ± 2°C temperature.

2.2. Spirulina sp. Morphology

The morphology of *Spirulina* sp. cells observed by microscope (X40) every 5 days of culture.

2.3. Biomass Estimation and the Growth Rate of Spirulina sp

10 mL cultural suspensions were filtered through 47mm glass fiber filters with 0.7µm nominal pore size. The filter was washed with distilled water, dried at 103°C for 6 hours for dry weight. The dry weight was further burned in furnace at 550°C to obtain ash weight. Biomass is calculated as dry weight (103°C) - ash weight (550°C) [4], [19], [15].

2.4. Sulfo-Phospho-Vanillin Assay for Lipid Accumulation

Phosphovanillin reagent was prepared by initially dissolving 0.06 g vanillin in 2 ml absolute ethanol; 8 ml deionized water and stirred continuously. Subsequently 50 ml of concentrated phosphoric acid was added to the mixture, and the resulting reagent was stored in the dark until use. To ensure high activity, fresh phospho-vanillin reagent was prepared shortly before every experiment run [17].

For SPV reaction of the algal culture for lipid quantification, One mL of algal suspension was centrifuged at 13000 rpm for 15 min and the pellet was extracted with 2 mL of concentrated (98%) sulfuric acid was added to the sample and was heated for 10 min at 100°C, and was cooled for 5 min in ice bath. 5 mL of freshly prepared phospho-vanillin reagent was then added, and the sample was incubated for 15 min at 37°C incubator shaker at 200 rpm. Absorbance reading at 530 nm was taken in order to quantify the lipid within the sample [17].

3. Data Analysis

Data was processed in Excel 2013 and analyzed by one-way ANOVA using SPSS (Statistical Package for the Social Sciences) software. All significant levels were set at $p < 0.05$.

4. Results and Discussion

4.1. Morphology of Spirulina sp

Structure of *Spirulina* sp. cells was longer filamentous shape and transfer from green to yellow in color after 5 days of culture under the white and red light conditions. However, *Spirulina* sp. cell remained green in color after 10 days of culture under blue light condition. It was clear that *Spirulina* cells increased immediately carotenoid biosynthesis under white and red light conditions (figure 1). More studies demotrated that *Spirulina* can respond to changes in light spectra by changing its pigment composition and content such as chlorophyll a and carotenoid [12]. Therefore, color of *Spirulina* cells also changed when they exposured to different spectra (white, blue and red lights) (figure 1). Total carotenoid content of *Spirulina* was similar under white and red light conditions, especially β-carotene increased with time. For blue light condition total carotenoid content decreased initially, but after total carotenoid content increased, corresponding to an increase in β-carotene content [12].

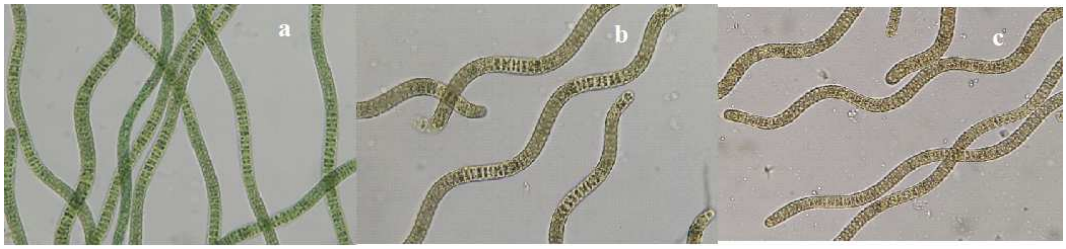

Figure 1. *Morphology of Spirulina sp. cells under different light conditions, (a) blue light, (b) red light and (c) white light at 5th day of culture.*

4.2. Growth of Spirulina sp

Spirulina sp. grew fast and reached highest biomass at 5th or 10th days of culture under the white and red light conditions. For blue light condition, the growth was slower and maximum biomass after 15 days of culture. This results showed that the white and red light conditions induced biosynthesis of high concentrated organic compounds such as carbohydrate, lipid and protein in *Spirulina* cells. The blue light condition with high energy can cause damage for cells at initial days of culture, so the growth of *Spirulina* and content of these organic compounds was lower. Biomass under blue light condition obtained high concentration with adaptation of cells, while the growth of *Spirulina* remarkably declined after 15 days of culture. This decrease in the growth of *Spirulina* can caused by starvation of nutrients in cultural medium. The growth of *Spirulina* wasn't significant difference under the white and red light conditions ($p= 0.969$) at initial 15 days, while under blue light condition the growth was significant difference compared with in the red and white light conditions at 5th and 10th days in culture ($p = 0.000$ and $p=0.001$, respectively) (figure 2).

According to [12], the growth rate of *Spirulina platensis* increased rapidly under white and red lights, while under blue light the growth rate obtained lower. Chlorophyll a content was lower during the first two generations in blue light, but increased by the end of the experiment (8 generations). This final value (1.3%) was equal to the chlorophyll a content of the cultures grown in red light.

The green microalga *Chlorella vulgaris* had significantly higher biomass in treatments with yellow, red and white light compared with blue, green and purple light. The growth of *Chlorella* as dry biomass under yellow, red and white light

treatments reached the exponential phase earlier than blue, green and purple light treatments [11]. Plant, especially microalga *Chlorella* absorbed efficiently and had high level in chlorophyll in the red wavelength area, so they obtained high biomass productivity [18].

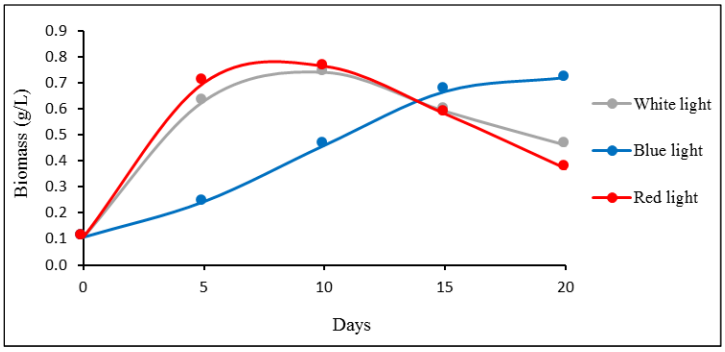

Figure 2. *The growth of Spirulina sp. under different light conditions.*

4.3. Lipid Accumulation of Spirulina sp

Figure 3 showed lipid accumulating capacity of *Spirulina* sp. under different light conditions. Lipid accumulation per volume and cell of *Spirulina* sp. increased significantly and reached maximal value at 10th day of culture under almost light conditions. Lipid accumulation under white and red light conditions obtainded higher and was significant difference (p= 0.025) under blue light condition. It was clear that the white and red light conditions caused lipid biosynthesis in high amount as well as other organic compounds immediately when exposure to light. However, for blue light condition lipid accumulation per volume increase slowly from 5th day to 10th day of culture (figure 3a). Lipid accumulation of *Spirulina* sp. dropped rapidly after 10 days according to decrease in biomass under all light conditions (figure 3).

The cyanobacteria *Spirulina* is rich in nutrients, such as proteins, lipids, carbohydrates, vitamins, minerals. The lipid composition is similar to vegetable oils and rich in essential fatty acids such as linoleic 18:2n6 and α-linolenic 18:3n3 acids and their C20 derivatives, eicosapentaenoic acids 20:5n3 and arachidonic acids 20:4n6 [8], [5], [13].

According to [11], the light quality significantly effected biomass productivity, total lipid concentration and fatty acid profile in the microalga C. *vulgaris*. Under green light condition, the content of of hexadecatrienoic acid (16:3) was significantly increased and concentration of a-linolenic acid (18:3) was the highest, while the contents of other fatty acids such as stearic acid (18:0), oleic acid (18:1) and linolenic acid (18:2) were significantly decreased compared with in the other treatments.

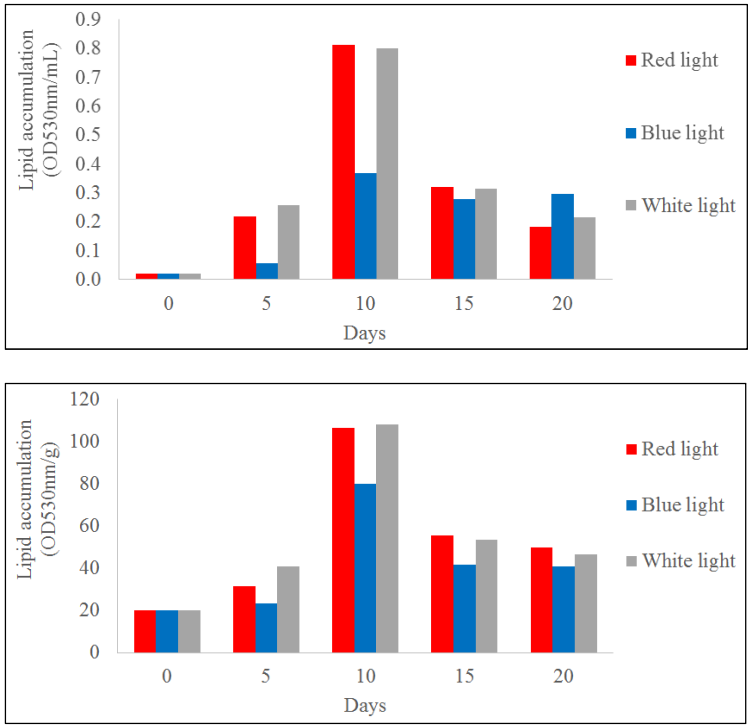

Figure 3. *Lipid accumulation in lipid per volume (a) and lipid per biomass (b) of Spirulina sp. under different light conditions.*

5. Conlusion

Spirulina sp. was used as functional food with concentration of higher nutrients such as carbohydrate, lipid and protein. *Spirulina* sp. was cultured under the red and white light conditions produced with the larger concentration of lipid and high growth rate compared with in the blue light condition.

References

[1] A. Belay, Y. Ota, K. Miyakawa, H. Shimamatsu, "Current knowledge on potential health benefits of *Spirulina*," Journal of Applied Phycology, Vol. 5, 1993, pp. 235–241.

[2] A. Vonshak, *Spirulina platensis* (Arthrospira): Physiology, Cell Biology and Biotechnology. Taylor and Francis, London, 1997.

[3] B. E. Schirrmeister, A. Antonelli, H. C. Bagheri, "The origin of multicellularity in cyanobacteria," BMC Evolutionary Biology, Vol. 11 (45), 2011, pp. 1-21.

[4] C. J. Zhu and Y. K. Lee, "Determination of biomass dry weight of marine microalgae," Journal of Applied Phycology, VOL. 9 (2), 1997, pp. 189-194.

[5] C. S. Singh, P. R. Sinha, P. D. Hader, "Role of lipids and fatty acids in stress tolerance in cyanobacteria," Acta Protozoologica, Vol. 41, 2002, pp. 297–308.

[6] J. P. Pandey, A. Tiwari and R. M. Mishra, "Evaluation of Biomass Production of *Spirulina* maxima on Different Reported Media," J. Algal Biomass Utln., Vol. 1 (3), 2010, pp. 70–81.

[7] K. M. Shekharam, L. V. Venkataraman and P. V. Salimath, "Carbohydrate composition and characterization of two unusual sugars from the blue green alga, *Spirulina platensis*," Phytochemistry, Vol. 26 (8), 1987, pp. 2267-2269

[8] K. P. Quoc, M. Pascaud, "Effects of dietary gammalinolenic acid on the tissue phospholipid fatty acid composition and the synthesis of eicosanoids in rats," Annals of Nutrition and Metabolism, Vol. 40, 1996, pp. 99–108.

[9] L. Tomasselli, Morphology, ultrastructure and taxonomy. In: *Spirulina platensis* (Arthrospira): Physology, cell biology and biotechnology (ed. Vonshak A.), Talor and Francis, London, UK, 1997, pp. 1-15.

[10] L. H. Pelizer, I. O. Moraes, "A method to estimate the biomass of *Spirulina platensis* cultivated on a solid medium," Brazilian Journal of Microbiology, Vol 45 (3), 2014, pp. 933-936.

[11] M. Hultberg, H. L. Jönsson, K. J. Bergstrand, A. S. Carlsson, "Impact of light quality on biomass production and fatty acid content in the microalga *Chlorella vulgaris*," Bioresource Technology, Vol. 159, 2014, pp. 465–467.

[12] M. Olaizola and E. O. Duerr, "Effects of light intensity and quality on the growth rate and photosynthetic pigment content of *Spirulina platensis*," Journal of Applied Phycology, Vol. 2, 1990, pp. 97-104.

[13] M. F. Ramadan, M. M. S. Asker and Z. K. Ibrahim, "Functional Bioactive compounds and Biological Activities of *Spirulina platensis* Lipids," Czech J. Food. Sci., Vol. 26, 2008, pp. 211–222.

[14] M. S. Miranda, R. G. Cintra, S. B. M. Barros and J. Mancini-Filho, "Antioxidant activity of the microalga *Spirulina maxima*," Brazilian Journal of Medical and Biological Research, Vol. 31, 1998, pp. 1075-1079.

[15] N. D. Tran, T. N. N. Doan, K. Q. M. Ho, T. M. L Nguyen, S. Portilla, T. Hoang, D. T. Duong, "A potential low cost medium for cultivation of *Dunaliella salina* DCCBC15 in Vietnam," Biological Journal, Vol. 35 (3), 2013, pp. 328-332.

[16] O. Ciferri and O. Tiboni, "The biochemistry and industrial tential of *Spirulina*," Ann. Rev. Microbiol., Vol. 39, 1985, pp. 503-526.

[17] S. K. Mishra, W. I. Suh, W. Farooq, M. Moon, A. Shrivastav, M. S. Park, J. W. Yang, "Rapid quantification of microalgal lipids in aqueous medium by a simple colorimetric method," Bioresource Technology, Vol. 155, 2014, pp. 330-333.

[18] W. Fu, O. Gudmundsson, A. M. Feist, G. Herjolfsson, G. Brynjolfsson, B. Ø. Palsson, "Maximizing biomass productivity and cell density of *Chlorella vulgaris* by using light-emitting diode-based photobioreactor," J. Biotechnol., Vol. 161, 2012, pp. 242–249.

[19] Y. K. Lee and H. Shen, Basic culturing techniques. In: A. Richmond, *Handbook of microalgal culture: Biotechnology and applied phycology*, UK: Blackwell Science Ltd, 2004, pp. 44-82.