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RESEARCH ARTICLE

Production of Photosynthetic Pigments from *Spirulina platensis* Under Different Light Intensities

(Produksi Pigmen Fotosintesis dari *Spirulina platensis* pada Intensitas Cahaya Berbeda)

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ABSTRACT

Photosynthetic pigments from microalgae have great potential biotechnological applications, as healthy food colorants and cosmetics. The production of photosynthetic pigments depends on many environmental conditions, mainly light intensity during the cultivation period. The present study aimed to determine the productivity of photosynthetic pigments in the biomass of *S. platensis*, including total chlorophylls, carotenoids, and phycocyanin, under different light intensities: 40 µmol m^{-2} s^{-1} (lower light intensity) and 70 µmol m^{-2} s^{-1} (higher light intensity). The results showed that *S. platensis* culture responds to changes in light intensity by changing the composition of photosynthetic pigments as an adaptive mechanism. The higher carotenoids content (69.69 ± 7.47 µg/g dw) was found under high light intensity, meanwhile, the higher chlorophyll (1495.47 ± 279.00 µg/g dw) and phycocyanin (4995.49 ± 576.52 µg/g dw) contents were observed under low light intensity. The highest productivity of photosynthetic pigments in *S. platensis* was shown by phycocyanin (318.86 ± 44.22 µg/L/day) and cholophylls (95.38 ± 19.35 µg/L/day) which were produced under low light intensity. Our results show that changes in light intensity can contribute to a stronger effect on the productivity of algal pigments for human health benefits and food colorants.

Keywords: biomass, chlorophylls, carotenoids, phycocyanin, *Spirulina platensis*.

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INTRODUCTION

Spirulina platensis is one of the most promising microalgae for the production of dried biomass and/or pigment. Dried microalgal biomass has been commercially produced and used as food supplement and animal feed, because it is rich in protein, lipids, carbohydrates, and other important elements [1], [2]. *S. platensis* is rich in photosynthetic pigments, including chlorophylls, carotenoids, and phycocyanin. Chlorophyll and carotenoids are natural pigments that are present in all photosynthetic organisms, while C-phycocyanin is only found in blue-green algae. Chlorophyll absorbs energy from the sun for photosynthesis. *S. platensis* has two forms of chlorophylls, namely: chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*). Chl *a* is the main photosynthetic pigment present in all photosynthetic organisms that play a fundamental role in light harvesting, while Chl b participates as an accessory pigment in photosynthesis which plays a role to transfer light to the photosynthetic reaction center (photosystem I). In addition to chlorophyll b, there are several other accessory pigments in *S. platensis*, namely carotenoids, and phycocyanin. Carotenoids are responsible for the quenching of light and protect cells against photo-oxidative damage (light stress adaptation) [3]. *S. platensis* is also an excellent source of phycocyanin (PC) which is an accessory photosynthetic blue-colored and water-soluble pigment.

Photosynthetic pigments are bioactive compounds of great importance for the food, cosmetic, and pharmaceutical industries. They are not only responsible for capturing solar energy to carry out photosynthesis but also play a role in photo-protective processes and display antioxidant activity. The content of chlorophyll, carotenoids, and phycocyanin in *S. platensis* provides distinctive natural green, orange, and blue pigments, respectively. These photosynthetic pigments are regarding their use as natural color

agents for nutritional, pharmacological, and health-related benefits. Recently, the global market demand for natural food additives has increased along with increasing consumer awareness regarding the importance of natural agents. This included coloring agents that can successfully replace synthetic ones. As a result, the number of applications of natural pigments and *S. platensis* as a source of these pigments is increasing, especially in the food and cosmetic industries. Therefore, optimization studies are required to increase the natural pigments from *S. platensis* to achieve its industrialization.

The production and composition of the photosynthetic pigments in algal cultures are influenced by a variety of environmental factors, such as light, temperature, and nutrients. Light is absolutely necessary as an energy source for autotrophic organisms to conduct photosynthesis. Light conditions are the key factors affecting micro-algal physiology including the growth and biochemical compositions. The light intensity range for optimal growth of microalgae is 26 µmol m^{-2} s^{-1} to 400 μmol m^{-2} s^{-1} [4], [5]. Light intensity exceeding the optimum can cause photo-oxidative damage (photo-inhibition) on some microalgae, and inhibit their growth. Light intensity also has a regulating effect on microalgal pigments. Some previous studies have reported the effect of light intensity on the growth and pigment production of *S. platensis* [6], [7]. In this present study, we determined the productivity of photosynthetic pigments in *S. platensis* biomass, including total chlorophylls, carotenoids, and phycocyanin, under different light intensities: 40 μmol m^{-2} s^{-1} (lower light intensity) and 70 μmol m^{-2} s^{-1} (higher light intensity).

METHODS

Microorganisms and culture condition

Spirulina platensis was obtained from the Brackish Water Aquaculture Institute of Jepara, Central Java, Indonesia. This strain was grown axenically in batch culture of Walne media using simple air-lift photobioreactors (PBR) with a working volume of 900 mL. The stock cultures were periodically regenerated under a light intensity of 14 μmol photon.m^{-2}.s^{-1} at room temperature with a photoperiod of 24:0 (L:D), salinity 25 ppt, pH 8.0 – 9.0, and free air bubbling.

Determination of biomass productivity

The experiments were performed by using different light intensities of 40 μmol photon.m^{-2}.s^{-1} (low light) and 70 μmol photon.m^{-2}.s^{-1} (high light). Light intensity was measured in the middle of the PBR and was adjusting the distance of the PBR toward the light source. The growth of *S. platensis* in low light and high light intensity were evaluated separately. After 7 days of cultivation, biomass was harvested by centrifugation at 3,500 × g for 20 min to obtain an algal paste. The microalgae paste was washed two times with distilled water to remove residual salt from seawater, and then freeze-dried. The dried biomass was weighed (biomass yield, mg/L), and then used to calculate biomass productivity (P_B) using formulae (1) as follows:

$$P_B(mg/L/day) = \frac{Yield(mg/L)}{t(day)} \quad \ldots\ldots\ldots(1)$$

Chlorophylls and carotenoids content

All extraction procedures were performed under subdued light to avoid pigment degradation. Two hundred milligrams of dried samples were homogenized using a pestle in a pre-chilled mortar with 5 mL of 99,8% methanol. The suspension was kept at 4°C under a dark refrigerator for 24 h. Then, it was centrifuged at 4,000 × g for 10 min before the measurement of spectrophotometric. The concentration of chlorophyll a ($C_{Chl\text{-}a}$), chlorophyll b ($C_{Chl\text{-}b}$), and total carotenoids concentrations (C_{car}) were estimated according to equations (2), (3), and (4), as follows:

$$C_{Chl\text{-}a}\ (\mu g/g\ dw) = \frac{16.72\,A_{665} - 9.16\,A_{652}}{g\ biomass} \quad \ldots..(2)$$

$$C_{Chl\text{-}b}\ (\mu g/g\ dw) = \frac{27.05\,A_{652} - 11.21\,A_{665}}{g\ biomass} \quad \ldots\ldots(3)$$

$$C_{Car}\ (\mu g/g\ dw) = \frac{(1000 * A_{470} - 1.63 * C_{chl\text{-}a})/221}{g\ biomass} \quad .. \ (4)$$

The productivity of total cholophylls and carotenoids was calculated according to equations (5) and (6), as follows:

$$P_{chl}\ (\mu g.L^{-1}.day^{-1}) = \frac{(C_{chl-a} + C_{chl-b})}{1000} \times P_B \ldots\ldots \ (5)$$

$$P_{car}\ (\mu g.L^{-1}.day^{-1}) = \frac{C_{car}}{1000} \times P_B \ldots\ldots\ldots\ldots\ldots\ldots \ (6)$$

Phycocyanin content

Two hundred milligrams of dried samples were homogenized using a pestle in a pre-chilled mortar with 3 mL of cold buffer phosphate (pH = 6.8). The mixture was centrifuged at 17500 rpm with a temperature of 4°C for 30 min. The concentration of phycocyanin (C_{PC}) was determined using a spectrophotometer UV-Vis by measuring the optical density at 620 nm and 652 nm. The concentration of phycocyanin was determined as follows:

$$C_{PC}\ (\text{mg/g dw}) = \frac{OD_{620} - 0.474(OD_{652})}{5.43} \quad \ldots\ldots (7)$$

The productivity of phycocyanin was calculated according to equation (8) as follows:

$$P_{PC}\ (\mu g.L^{-1}.day^{-1}) = C_{PC} \times P_B \quad \ldots\ldots\ldots\ldots (8)$$

Statistical analysis

Experiments were analyzed in triplicate. The data were reported as mean ± SD. The means of all parameters were examined for significance using *a t*-independent test at $p < 0.05$.

RESULTS AND DISCUSSION

Effect of light intensity on biomass yield

Light is the basic source of energy and an important factor of photosynthesis for the cellular multiplication of microalgae. In a photoautotropic regime, light is essential for microalgae growth. Under low light intensity, the biomass productivity of *S. platensis* was significantly lower than that of high light intensity ($p<0.05$) (Figure 1). This is in line with previous works where a large amount of *Spirulina* biomass was produced with higher intensities of 2000 to 3500 lux [8]. It can be seen that the increase of light intensity up to 70 μmol photon.m^{-2}.s^{-1} can improve the biomass yield of *S. platensis*. This observation suggested that dynamically increasing light intensity as biomass yield increases may be a promising option for biomass production.

Change in pigments content

To understand the effect of different light intensities on *S. platensis* biomass production, changes in the composition of photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, carotenoids, and phycocyanins) were evaluated. The pigment content in the cells is regulated by light conditions [9].

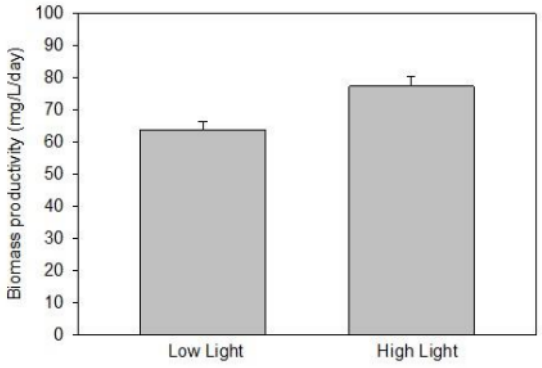

Figure 1. Biomass productivity of *S. platensis* cultured in different light intensity

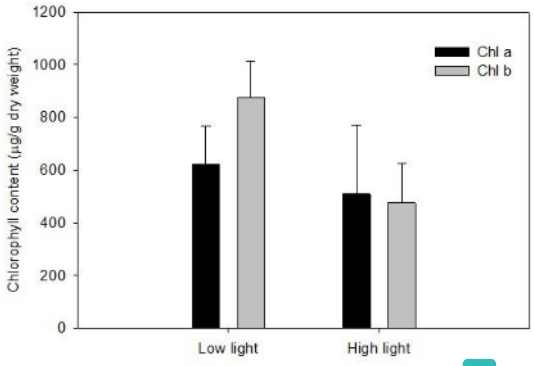

Figure 2. Composition of Chl *a* and Chl *b* in low light and high light intensity

The effect of two different light intensities (40 μmol photon.m^{-2}.s^{-1} and 70 μmol photon.m^{-2}.s^{-1}) on chlorophylls content of *S. platensis* for seven days of cultivation period is presented in Figure 2. The Chl *a* content of *S. platensis* grown at low light intensity (40 μmol photon.m^{-2}.s^{-1}) is about 1,2 times higher than when cultivated at high light intensity (70 μmol photon.m^{-2}.s^{-1}), whereas the Chl *b* content was about 1,8 higher (Figure 2). The increase in chlorophyll content during acclimation to low light is to increase light harvesting for algae growth. It is consistent with the results of Nicolo et al. [10] that chlorophyll content was higher in low-light microalgal cells. Indeed, the increase in light intensity induces a decrease in the photosystem concentration of the thylakoid membrane and a decrease in the size of the photosystem II (PSII). However, despite a decrease in the chlorophylls content, we can observe an increase

in photosynthetic activity because the capacity of absorption and conversion of photons into chemical energy caused the increase in biomass yield, and this has an impact on lower *S. platensis* biomass yields compared to that cultured at high light due to the need for nitrogen in the cell is more used for the production of chlorophyll than for cell division.

The reduced total chlorophyll content in the biomass cultured at high light intensity indicates chlorophyll degradation due to photo-inhibition (Table 1). Chlorophyll is a compound that is very susceptible to photo-oxidation by reactive oxygen species (ROS) and peroxide radicals generated in algal cells during photo-inhibition [9]. Changes in algal pigmentation usually indicate the occurrence of photo-acclimation in algal cells [11], resulting in lower chlorophyll content and higher carotenoid to higher irradiance. This is consistent with our results that the carotenoid content increased by 1.7 times at high light intensity (Table 1).

Table 1. Pigment content in *S. plantesis* biomass under different light intensity

Pigments	Content (µg per g dry weight)	
	Low light	High Light
Total chlorophylls	1,495.47 ± 279.00	1,023.87 ± 404.21
Total carotenoids	41.57 ± 6.65	69.69 ± 7.47
Phycocyanin	4,495.49 ± 576.52	1,216.76 ± 44.22

The increasing of the carotenoid content in the biomass cultured at high light intensity enables the microalgae to dissipate energy from excited chlorophyll and eliminate radical oxygen species (ROS) to maintain the photosystem structure [11],[12]. In addition, carotenoids scavenge triplet chlorophyll and quench singlet oxygen [14]. Carotenoids can be involved in the chemical or physical suppression of singlet oxygen which is formed under conditions of high light intensity [5]. At very high light irradiation, however, the protective mechanisms cannot sufficiently deal with the surplus of electrons, and formation of singlet oxygen, and the accumulation of reactive oxygen species (ROS) leading to cell damage. The increase in biomass productivity was observed at light intensity of 70 µmol photon.$m^{-2}.s^{-1}$ proves that this light intensity is still moderate and does not cause cell damage.

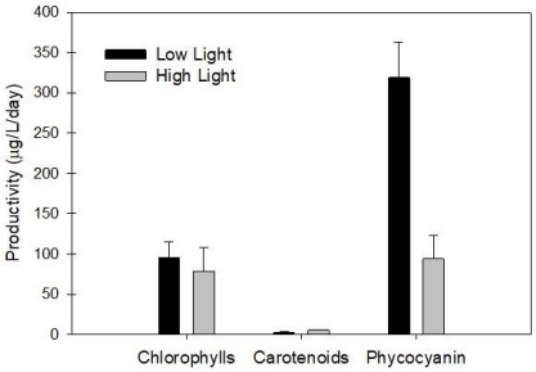

Figure 3. The productivity of total chlorophylls, carotenoids, and phycocyanin in *S. platensis* biomass

Unlike the chlorophylls and carotenoid pigments, the effect of light intensity on phycocyanin concentration is more significant. A constant light intensity of 40 μmol photon.$m^{-2}.s^{-1}$ promotes a higher production of phycocyanin (318.86 ± 44.22 µg.L^{-1}. day^{-1}). With regard to while greater light intensity (70 μmol photon.$m^{-2}.s^{-1}$), the lesser phycocyanin production of 93.78 ± 29.19 µg.L^{-1}. day^{-1} (Figure 3). This result is consistent with the reported by Kareem and Alghanmi [15] that moderate lighting increases phycocyanin production, but high light intensity inhibits it. As stated by Spangler et al. [16] that phycocyanin help microalgae absorb light in wavelenghts where chlorophyll poorly absorbs (500–650 nm), and light energy absorbed by phycocyanin is transferred to chlorophyll a in photosystem II to enhance the photosynthetic capacity of *S. platensis*. The increase in phycocyanin content when the cultures were cultivated under low light intensity reflects the process of chromatic acclimation. Chromatic acclimation is widely recognized as one of the photoacclimation types, whereby microalgae alter the absorbing light colors of a supermolecular antenna complex called phycobilisome [17]. These results revealed that a reduction in the light intensity can induce an increase in the phycocyanin content in *S. platensis* biomass.

CONCLUSION

Photosynthetic pigments production of cyanobacteria, in particular *Spirulina platensis*, depends on light intensity. Hence, the light manipulation could

be a feasible strategy for improving photosynthetic pigment production in *S. platensis* biomass. Overall, low light intensity is a recommendable light intensity for production of phycocyanin and chlorophylls from *S. platensis* biomass, while high light intensity is suitable for production of carotenoids. This study informed that the light intensity used to cultivate the *S. platensis* culture is depending on the type of pigments that want to produce in large amount in the biomass for industrial demand.

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