Growth and Phycocyanin Productivity of *Spirulina fusiformis* under Various Light Regimes

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Abstract

A blue-green algae *Spirulina fusiformis* was grown under various light regimes in a laboratory-scale experiment. A 500 watt halogen lamp was employed as the light source, while the light variation of 2,000 lux, 4,000 lux, 6,000 lux, 8,000 lux, and 10,000 lux was obtained by placing a series of 2 L experimental bottles at various distances. The growth medium used was a modified-Zarrouk medium with initial pH of 8.72, and the room temperature was 28–30°C. After inoculation, the algae was let to grow for 30 days, and observation on the biomass, chlorophyll, crude protein, and phycocyanin content were carried out every 10 days. The algal biomass was determined gravimetrically, the chlorophyll, phycocyanin, and protein content were measured using spectrophotometer after extraction in 90% aceton, pH 7.0 buffered water, and folin-phenol dye binding, respectively. The result shows a remarkable effect of light intensity to the algal biomass as well as the biochemical content. The specific growth rate increased from 0.08 doubling/day at light intensity of 2,000 lux to 0.14 doubling/day at 10,000 lux, which was equivalent to an increase in the biomass productivity of more than 3 times. The highest algal chlorophyll content was observed at light intensity between 4,000–8,000 lux, indicating the optimum light condition at that irradiance range. The protein content was consistently lower with light intensity, from 42.96–52.91% DW at 2,000 lux to 33.71–41.08 % at 10,000 lux. A consistent drop in the protein content was also observed together with the culture growth phase, from 39.82–52.91% DW in the early growth stage down to 33.71–42.96% at day 30. Light intensity in concomitance with the growth phase remarkably increased the algal phycocyanin content. In the early growth stage the phycocyanin content ranged from 0.16% DW at 2,000 lux up to 1.229% DW at 10,000 lux, whereas at the end of the experiment the algal phycocyanin content were 1.04% DW and 2.436% DW, at 2,000 lux and 10,000 lux, respectively. It gave a consequence of more than 7 times higher phycocyanin productivity, which was from 0.09 mg/L/day at 2,000 lux to 0.62 mg/L/day at 10,000 lux. This result shows the importance of light factor in producing phycocyanin from the blue-green algae *Spirulina fusiformis*.

Keywords: blue-green algae, *Spirulina fusiformis*, light intensity, phycocyanin, productivity

Introduction

Spirulina is a potential blue-green algae for natural source of food and feed, particularly in terms of its high content of natural pigment phycocyanin, which has been reported as high as 20% of the total protein (Richmond, 1988). The high phycocyanin content could be of interest for economical scale production since the pigment has been reported to have various advantages, including antioxydant, anti-inflamatory, necrosis cancer inhibitor, and neural cell protection (Enriksen, 2008; Riss *et al.*, 2007; McCarthy, 2007; Romay *et al.*, 2003; Reddy, *et al.*, 2000) which is considered to have a prospective market in pharmaceutical industries. This high phycocyanin content is also supported by the ability of the algae to grow in extreemly basic media up to pH 11, so that it is easier to maintain it as monoalgal culture for a long period of time.

Light is one of the growth factors that greatly influence both the growth and phycocyanin content of spirulina (Pandey *et al.*, 2011; Tomaselli *et al.*, 1995). Pandey *et al.* (2011) for example, reported an enhancement in spirulina culture biomass with higher light intensity. On the contrary, a remarkable decrease in spirulina culture productivity in an outdoor high light intensity has been reported which was attributed to the photorespiration (Vonshak & Richmond, 1985). This is in an aggreement with Goldman (1979) which considered light as a resource

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factor that influences algal growth to form a model exhibiting a saturation point above which is harmful for the algal cells. At the same time, influence of light intensity on the biochemical composition of spirulina has also been reported. Chaiklahan *et al.* (2007) showed a decrease in protein content of *Spirulina platensis* with higher light intensity, whereas Walter *et al.* (2011) reported the influence of light on the phycocyanin content and purity in spirulina culture. Accordingly, light can be considered to have double important attribution in terms of phycocyanin production from algal culture, which is to control the biomass productivity as well as the phycocyanin content and quality. It is crucial, therefore, to find out the optimal light irradiance for phycocyanin production by spirulina culture.

Materials and Methods

The blue-green algae *Spirulina fusiformis* used in this experiment was obtained from microalgal collection of the Marine Culture Laboratory of Research Center for Oceanography LIPI in Jakarta.

A 500-watt halogen lamp was employed as the light source, while the light variation of 2,000 lux, 4,000 lux, 6,000 lux, 8,000 lux, and 10,000 lux was obtained by placing a series of 2 L experimental bottles at various distances. The growth medium used was a modified-Zarrouk medium with initial pH of 8.72, and the room temperature was 28–30°C. Aeration was provided for mixing the culture. After inoculation the algae was let to grow for 30 days, and observation on the biomass, chlorophyll, crude protein, and phycocyanin content were carried out every 10 days. The algal biomass was determined gravimetrically; the chlorophyll, phycocyanin, and protein content were measured using spectrophotometer after extraction in 90% aceton, pH 7.0 buffered water, and folin-phenol dye binding, respectively.

The culture medium was a modified-Zarouk medium (Borowitzka, 1988). The inoculum was from a 500-mL monoculture which was poured into the experimental bottles with dilution rate of 30 x. The culture was then grown in a batch mode for 30 days. The sampling for the biomass content and the measurement over the biochemical content were carried out every 10 days. The algal biomass is expressed by means of the dry weight, which was determined by filtering 20–100 mL culture aliquot through a tared Whatman GF/A filter paper. The filter paper was then dried at 60°C overnight and weighted. The algal dry weight was obtained by subtracting the weight of algal biomass containing filter paper by the initial weight. Ten ml culture aliquot was also filtered through Whatman GF/A filter paper for analysis of chlorophyll content, total protein and phycocyanin content. The chlorophyll content was determined by extraction in 90% acetone according to Jeffrey & Humprey (1975), while the total protein determination employed folin-phenol method of Lowrey *et al.* (1951). The phycocyanin measurement was carried out by extraction in pH 7.0 phosphate buffer (10.64 g K_2HPO_4 and 5.29 g KH_2PO_4 in a liter of aquadest) according to Boussiba & Richmond (1979).

Results and Discussion

Light intensity significantly affects the algal biomass as well as the biochemical content. The specific growth rate increased from 0.08 doubling/day at light intensity of 2,000 lux to 0.14 doubling/day at 10,000 lux, which was equivalent to an increase in the biomass productivity of more than 3 times (Figure 1). It is in consistence with Pandey *et al.* (2011) who reported 25% higher biomass concentration in spirulina culture with increase of irradiance from 3,000 to 5,000 lux. Indeed, this experiment shows that the highest irradiance employed of 10,000 lux was below the saturation point, as the algae still showed a trend to increase the growth. The productivity level obtained at 10,000 lux almost twice higher than what was reported by Wakte *et al.* (2011), which was 37.2 mg/L/day.

The highest algal chlorophyll content was observed at light intensity between 4,000–8,000 lux (Figure 2), indicating the optimum light condition in terms of the algal physiological processes is at that irradiance range. It supports the observation of a consistent decrease in the algal protein content with higher light intensity (Figure 3). The range of chlorophyll content in this experiment, which was 1.64–3.26 % DW, is in consistence with Wakte *et al.* (2011) observation on spirulina culture under low light intensity which contained chlorophyll 26.9 mg/g dried weight under low light condition. Pandey *et al.* (2011), however, reported a decrease in chlorophyll content of *S. platensis* culture when the light intensity increased from 3,000 lux to 5,000

lux, which might be attributed to the different growth optimum light intensity required between the algae. Chrismadha *et al.* (2010b) have also reported lower chlorophyll content when *S. fusiformis* was grown outdoor under sun light illumination. Beside the influence of light intensity, the chlorophyll content was also influenced by the culture growth phase, which was particularly obvious at the late stage of the experiment. A trend of chlorophyll content reduction with the time or culture stage has been widely reported (Chrismadha & Borowitzka, 1994; Chrismadha *et al.*, 2010b). This is particularly attributed to the nutrient depletion along with the culture development. N and P deficiency has been reported to inhibit chlorophyll synthesis in *S. fusiformis* culture (Chrismadha *et al.*, 2006).

Protein content of the *S. fusiformis* culture in this trial ranged from 33.71–52.91% DW which is comparable with which reported by Ogbonda *et al.* (2007) for *Spirulina* sp. to contain 39.71–57.31% AFDW. A decrease in protein content in *S.*

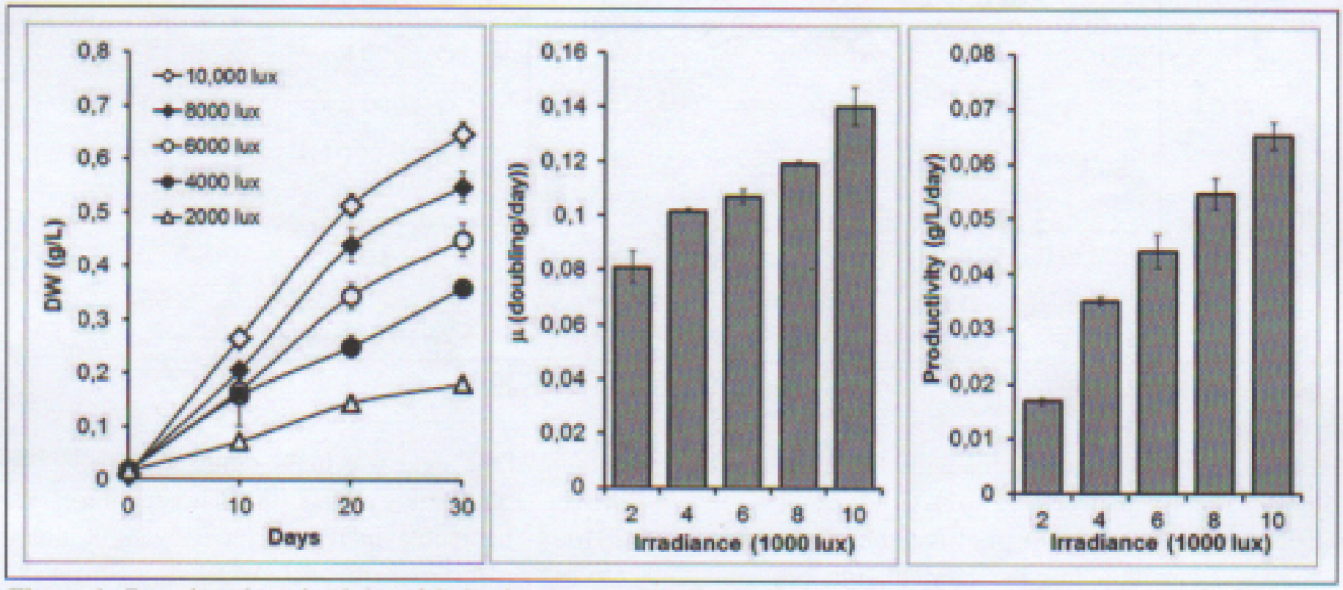

Figure 1. Growth and productivity of *S. fusiformis* at various light intensities.

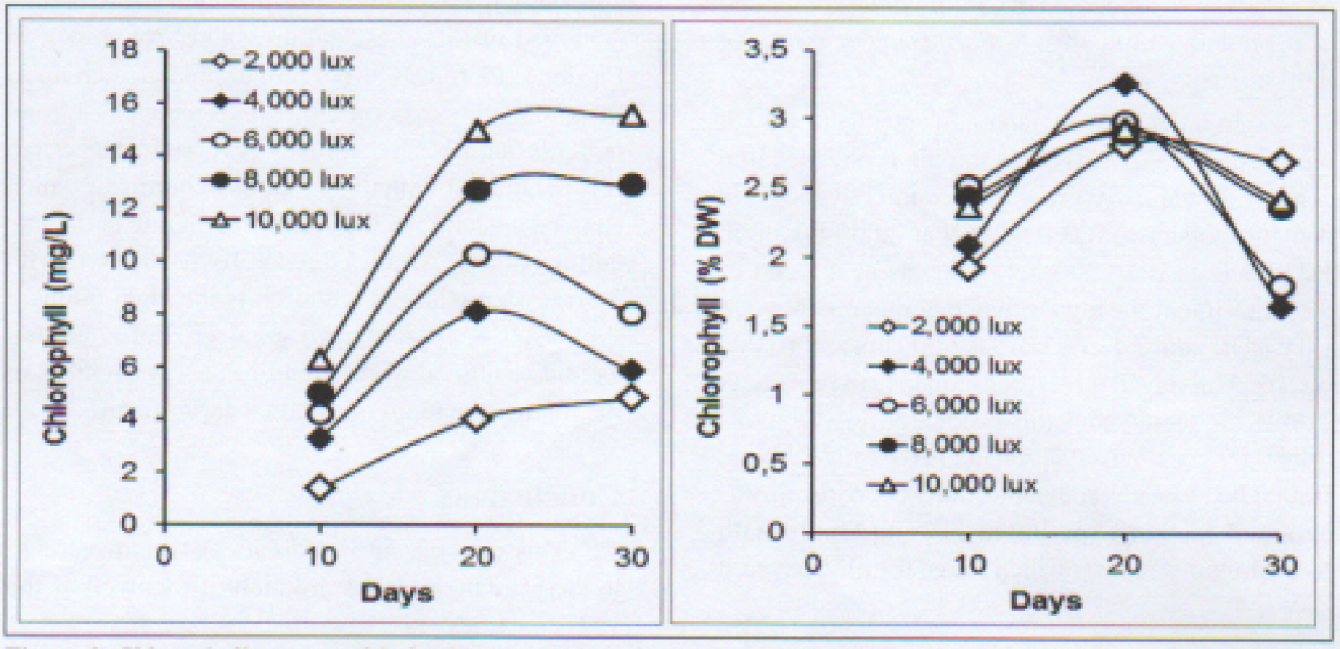

Figure 2. Chlorophyll content of *S. fusiformis* culture under various light intensities.

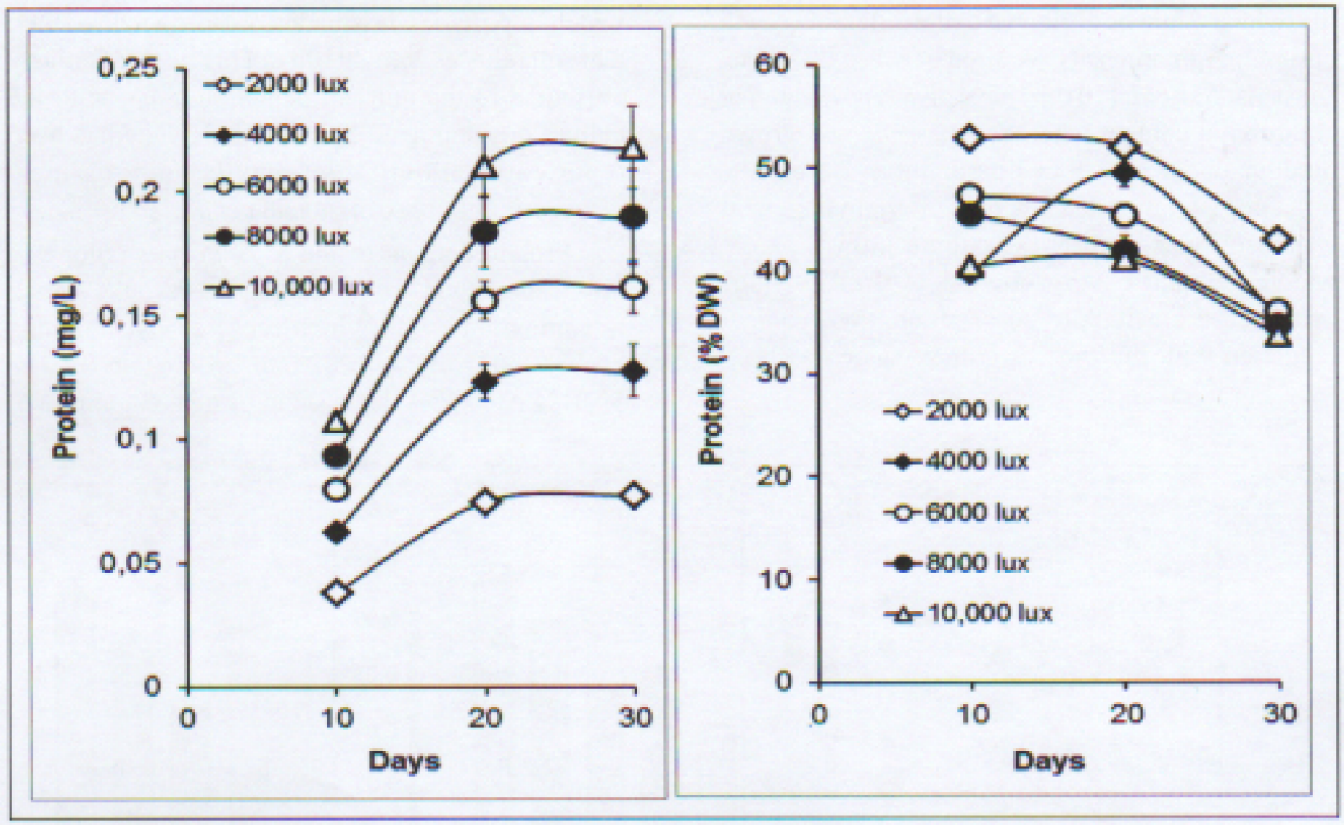

Figure 3. Protein content of *S. fusiformis* culture under various light intensities.

platensis culture with light intensity has also been reported (Chaiklahan *et al.*, 2007). Goldman (1979) attributed it to fast light photosynthetic process which was uncoupled by the dark catalytic process as the photosynthetic product is accumulated as carbohydrate. Chrismadha *et al.* (2010b) have also reported an accumulation of carbohydrate compound in an outdoor culture of *S. fusiformis* under excessive light intensity.

Light intensity in concomitance with the growth phase remarkably increases the algal phycocyanin content. In the early growth stage the phycocyanin content ranged from 0.16% DW at 2,000 lux up to 1.229% DW at 10,000 lux, whereas at the end of the experiment the algal phycocyanin content were 1.04% DW and 2.436% DW, at 2,000 lux and 10,000 lux, respectively. It gave a consequence of more than 7 times higher phycocyanin productivity, which was from 0.09 mg/L/day at 2,000 lux to 0.62 mg/L/day at 10,000 lux. The phycocyanin content of *S. fusiformis* obtained in this experiment is comparable with some previous reports which stated the phycocyanin content of the algae was in the range of 1.11–3.18% dried weight (Wakte *et al.*, 2011; Chrismadha *et al.*, 2010a). The trend of increasing phycocyanin content observed in this experiment, however, is inconsistent with the previous work which observed a decrease in the phycocyanin content along with the culture age. This is possible due to different growth state achieved at the latest culture stage. As shown in Figure 1, there was still a remarkable growth up to the end of this experiment, which indicates a good culture condition all the way the experimental period. It is in contrast with the previous experiment which showed a decline in the algae growth rate in the late culture phase, which is generally associated with nutrient depletion which inhibits the algal pigment synthesis. The maintained good growth condition can be attributed to the trend of steady increase in the pigment content during this experiment.

Conclusions

This experiment reveals a consistent increase in the growth, biomass productivity, as well as the

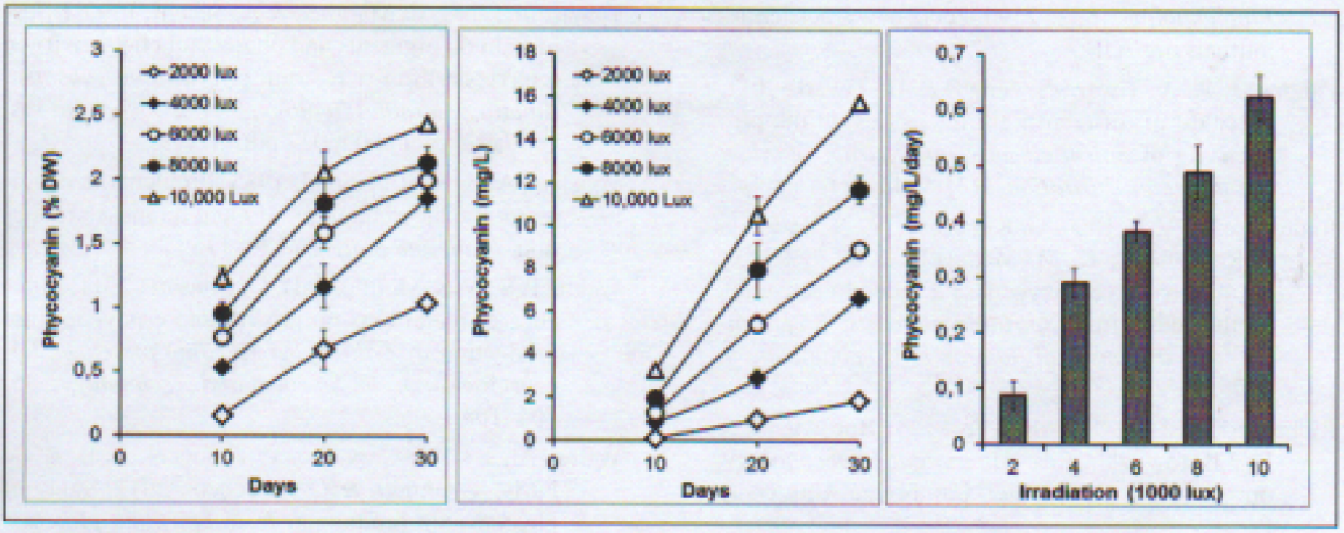

Figure 4. Phycocyanin content and productivity of *S. fusiformis* grown under various light intensities.

phycocyanin productivity in *S. fusiformis* culture with light intensity up to 10,000 lux, which indicates a potential to enhance a higher productivity by providing more light. This shows a remarkable influence of light on the phycocyanin productivity, so that it has to be considered carefully when producing phycocyanin from the algae.

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