

Growth, biomass, and chlorophyll-a and carotenoid content of *Nannochloropsis* sp. strain BJ17 under different light intensities

Pertumbuhan, biomassa, dan konsentrasi pigmen klorofil-a dan karotenoid *Nannochloropsis* sp. strain BJ17 pada intensitas cahaya berbeda

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ABSTRACT

Nannochloropsis sp. has been identified as sources of live feed and pigment in aquaculture. To increase the production, the optimal environmental conditions for microalgae are required. Light intensity is one of the important factors that significantly affects the biomass and pigment of microalgae. The study aimed to determine the effect of light intensity (1,500; 3,000; and 4,500 lux) on growth, biomass production, chlorophyll-a, and carotenoid content of *Nannochloropsis* sp. strain BJ17. The results showed that different light intensities significantly affected the growth, biomass, chlorophyll-a and carotenoid contents of *Nannochloropsis* sp. strain BJ17. Increasing light intensity resulted in the increase of the growth rate, biomass, chlorophyll-a, and carotenoid contents of *Nannochloropsis* sp. strain BJ17. The cell achieved the highest specific growth rate of 1.729 /day and the cell concentration of 43.333×10^6 cell/mL at a light intensity of 4,500 lux. The highest chlorophyll-a and carotenoid concentrations of algae were obtained at 4,500 lux (8.304 $\mu\text{g/mL}$ and 3.892 $\mu\text{g/mL}$, respectively). This study suggested that increasing light intensity led to the increase in the growth, biomass, chlorophyll-a, and carotenoid content of *Nannochloropsis* sp. strain BJ17.

Keywords: carotenoid, chlorophyll, biomass, growth rate, light intensity

ABSTRAK

Nannochloropsis sp. diketahui sebagai sumber pakan alami dan pigmen pada budidaya perikanan. Budidaya pada kondisi lingkungan yang optimal diperlukan untuk meningkatkan produksi mikroalga. Intensitas cahaya merupakan salah satu faktor esensial yang secara signifikan mempengaruhi biomassa dan pigmen mikroalga. Tujuan penelitian ini adalah untuk menentukan pengaruh intensitas cahaya yang berbeda (1.500, 3.000, and 4.500 lux) terhadap pertumbuhan, produksi biomassa, klorofil-a, dan karotenoid *Nannochloropsis* sp. strain BJ17. Hasil menunjukkan bahwa intensitas cahaya yang berbeda berpengaruh secara signifikan terhadap pertumbuhan, biomassa dan klorofil-a dan karotenoid *Nannochloropsis* sp. strain BJ17. Semakin tinggi intensitas cahaya maka laju pertumbuhan, biomassa, kandungan klorofil-a dan total karotenoid *Nannochloropsis* sp. strain BJ17 semakin tinggi. Laju pertumbuhan spesifik tertinggi 1,729 /hari dan konsentrasi sel maksimum tertinggi $43,333 \times 10^6$ sel/mL dihasilkan pada intensitas cahaya 4.500 lux. Konsentrasi klorofil-a (8,304 $\mu\text{g/mL}$) dan karotenoid (3,892 $\mu\text{g/mL}$) tertinggi juga diperoleh pada intensitas cahaya 4.500 lux. Studi ini menunjukkan bahwa peningkatan intensitas cahaya berperan dalam meningkatkan pertumbuhan, produksi biomassa, klorofil-a, dan karotenoid *Nannochloropsis* sp. strain BJ17.

Kata kunci: karotenoid, klorofil, biomassa, pertumbuhan, intensitas cahaya

INTRODUCTION

Microalgae is a photosynthetic organism with a rapid growth rate and capable of converting light energy, carbon dioxide, and nutrients into biomass and generating essential pigment (Mata *et al.*, 2010). Chlorophyll and carotenoids are essential, dominant pigment in microalgae (Koller *et al.*,

2014) that have antioxidant properties (Cardozo *et al.*, 2007) and pharmaceutical application (Koller *et al.*, 2014). Furthermore, the carotenoids are also used as micro-ingredients in aquaculture feed (Yaakob *et al.*, 2014).

Nannochloropsis sp. is microalgae that has been widely used as a live feed in aquaculture (Camacho-Rodríguez *et al.*, 2013; Freire *et al.*,

2016). It is also known as potential producers of pigments such as chlorophyll and carotenoids (Lubian *et al.*, 2000; Hosikian *et al.*, 2010).

Microalgae biomass and pigment production vary depending on culture conditions (Rao *et al.*, 2007), such as photoperiod, temperature and light intensity (Kitaya *et al.*, 2008; Fakhri *et al.*, 2015). Environmental adjustment can effectively optimize the growth rate of macroalgae (Mata *et al.*, 2010). Moreover, light intensity affects the physiology of microalgae (Khoeyi *et al.*, 2012). Photoautotrophic culture and the intensity of light are essential factors, which determine growth rate and biomass production of microalgae. Light intensity also affects the metabolism and pigment composition of microalgae (Sánchez-Saavedra & Voltolina, 1996). In response to the increase or reduction of light intensity, the pigments contents of microalgae may be altered (Sánchez-Saavedra & Voltolina, 2002).

The effect of light intensity on *Nannochloropsis* sp. growth has been previously studied (Wahidin *et al.*, 2013; Probir *et al.*, 2011). However, information related to the effect of light intensity on pigment contents (chlorophyll and carotenoid) of *Nannochloropsis* sp. is still limited. Furthermore, algae growth varies depending on the species (species-specific) (Banerjee *et al.*, 2011). This study aimed to determine the optimum light intensity for the growth, biomass and pigment production of *Nannochloropsis* sp. BJ17 strain. This study can also be applied to evaluate the potential *Nannochloropsis* sp. BJ17 strain to generate chlorophyll and carotenoids.

MATERIALS AND METHODS

Strain and growth medium of *Nannochloropsis* sp. strain BJ17

Nannochloropsis sp. BJ17 strains were obtained from Brackish Water Aquaculture Institute of Jepara, Central Java. These strains can rapidly growth in a wide range of salinity. Walne medium, which contained 100 g/L NH_4NO_3 ; 20 g NaH_2PO_4 ; 33.6 g H_3BO_3 ; 0.36 g MnCl_2 ; 1.3 g FeCl_2 ; 45.0 g EDTA/L and 12.01 g/L vitamin B12, was used as medium with seawater. Walne and vitamin dose of fertilizer were 1 mL/L and 0.5 mL/L, respectively.

Nannochloropsis sp. BJ17 stock culture conditions

Nannochloropsis sp. BJ17 culture in exponential phase was used as an inoculant.

The inoculant was then cultivated in 500 mL erlenmeyer. A total volume of media and inoculant was 350 mL. The cultures were incubated under continuous light 24:0 (light:dark cycle). The incubation conditions used were 29 ± 2 °C, the salinity of 15 g/L, the light intensity of 3,000 lux, and continuous aeration for four days (Fakhri *et al.*, 2015).

Culture treatments

The light intensity treatments (in lux) were 1,500; 3,000; and 4,500 with three replicates per light intensity treatment. Microalgae biomass was harvested after six days of culture, and used for biomass and pigment analyses.

Nannochloropsis sp. BJ17 was used as inoculants at the exponential phase or four-day-old cultures. The inoculant was cultured into a 2.5 L container with a total volume of 1.5 L seawater with 15 g/L salinity. Initial cell concentration for all treatments was 5.5×10^5 cells/mL. Microalgae cells were cultured at 28 °C, with photoperiod 24:0 and continuously aerated.

Analysis of growth

Microalgae growth was observed using cells concentration calculation method with 0.1 mm deep Neubauer haemocytometer (BOECO, Hamburg, Germany). Specific growth rate (μ) was calculated using the following formula (Fogg & Thake, 1987) :

$$\mu \text{ (/day)} = \frac{\ln(x_2) - \ln(x_1)}{t_2 - t_1}$$

Note:

μ = growth rate of per unit cell concentration

x_1 = cell concentration at time 1

x_2 = cell concentration at time 2

t_1 = time 1

t_2 = time 2

Cell doubling time (td) is the average generation time required for cell to double in concentration. Doubling time can be calculated using the formula (Ak *et al.*, 2008):

$$\text{td (day)} = \frac{\ln 2}{\mu} = \frac{0,693}{\mu}$$

Biomass analysis

The cell biomass of *Nannochloropsis* sp. BJ17 strains were analyzed following the method of Janssen (2002). Microalgae samples were taken

at the day-6 of microalgae growth. The filter paper GF/C (diameter 90 mm) were dried at 105 °C for 2 h until the weight is constant [A]. 25 mL of microalgae suspension was filtered through a filter paper GF/C, and washed with 25 mL of fresh water to avoid contamination with the insoluble salt in the media. The filter paper was placed in an oven at 105 °C for 2 h until the weight is constant. The filter paper was placed in a desiccator, and re-weighed [B]. The calculation used equation below:

$$\text{Dry weight/biomass (mg/L)} = \frac{[B] - [A]}{\text{sample volume}} \times 1,000$$

Note:

A = filter paper weight

B = filter paper weight + algae

Chlorophyll-a and carotenoids analysis

In this study, chlorophyll-a and carotenoids were analyzed using methanol extraction method (Ritchie, 2006). 10 mL of microalgae samples were centrifuged at 6,000 rpm for 10 min. 10 mL of absolute methanol was added to the centrifuged and vortexed pellet. Tube wrapped in aluminum foil and the mixture (pellet and solvent) was put in a water bath at a temperature of 70 °C for 10 min. The sample was vortexed and centrifuged at 6,000 rpm for 10 min. The clear supernatant was measured at the wavelengths of 480, 652, and 665 nm using a spectrophotometer (Spectroquant Pharo 300, Merck Millipore).

The content of chlorophyll-a was calculated using the method of Ritchie (2006), and total carotenoids were calculated following the method of Kim *et al.* (2014).

$$\text{Chlorophyll-a (}\mu\text{g/mL)} = 16,5169 \times A_{665} - 8,0962 \times A_{652}$$

$$\text{Total carotenoid (}\mu\text{g/mL)} = 4 \times A_{480}$$

Statistical analysis

Statistical analysis was performed using SPSS 16.0. Growth, biomass, chlorophyll-a and carotenoids data were analyzed by analysis of variance (ANOVA) test with 95% confidence. To determine the relationship between the light intensity and growth rate and biomass, regression analysis was carried out.

RESULTS AND DISCUSSION

Growth and biomass of *Nannochloropsis* sp. strain BJ17

The growth of *Nannochloropsis* sp. BJ17 strain cells under different light intensities (1,500; 3,000; and 4,500 lux) is illustrated in Figure 1. The results showed that similar growth pattern was found in cultured cells under different light intensities. The maximum specific growth rate occurred between day-2 and day-1. The highest cell concentration was achieved at day-6. In addition, cell growth curve did not show a lag phase on all treatments, and microalgae growth

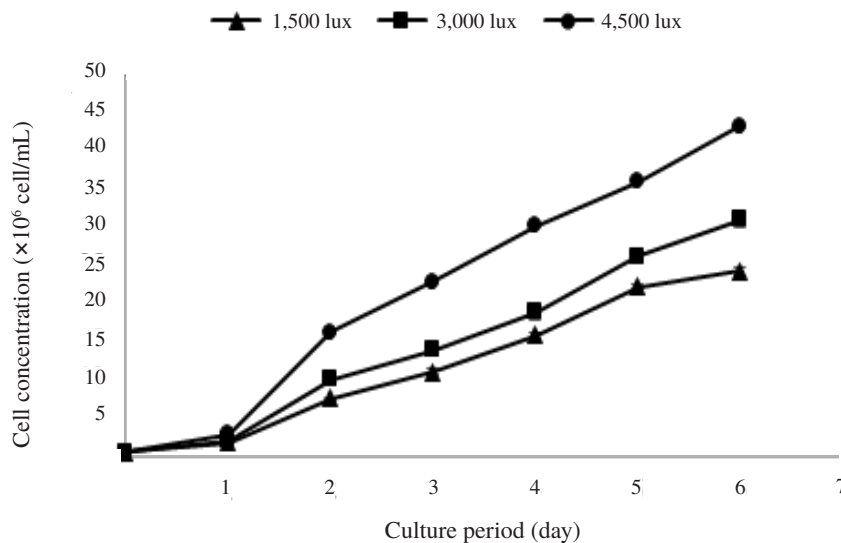


Figure 1. Growth of *Nannochloropsis* sp. BJ17 strain under different light intensities (1,500; 3,000; and 4,500 lux) for six days of culture period.

achieved rapid logarithmic phase. Similarly, Sforza *et al.* (2014) reported that the microalgae, *Scenedesmus obliquus* do not experience lag phase when they were treated with different light intensities. This indicates that some species of microalgae can grow in different conditions of light intensity without experiencing stress.

Table 1 shows that different light intensities have a significant ($P < 0.05$) effect on the specific growth rate, doubling time, maximum cell concentration and biomass of *Nannochloropsis* sp. BJ17 strain. The highest specific growth rate was 1.73/day ($P < 0.05$) with a doubling time about 0.401 days or 9.62 h. The highest cell concentration of 43.3×10^6 cells/mL ($P < 0.05$) was obtained at 4,500 lux of light intensity, while the highest biomass of 0.824 g/L ($P < 0.05$) was resulted from 4,500 lux of light intensity.

Figure 2 shows the relationship between the light intensity and the growth rate generates a linear pattern with the equation of $y = 0.0001x + 1.2761$ and $R^2 = 0.901$. Figure 3 shows the

relationship between the light intensity and biomass, which generate a linear pattern with the equation of $y = 0.0002x + 0.1893$ and $R^2 = 0.985$. The results indicate that the increase in light intensity can accelerate growth rate and biomass of *Nannochloropsis* sp. BJ17 strain.

Imaizumi *et al.* (2014) reported that the culture of *Chlorella zofingiensis* with light intensity between 75, 150, and 250 $\mu\text{mol photon/m}^2/\text{sec}$ produced specific growth rate between 0.399/day, 0.490/day, and 0.700/day, respectively. Furthermore, the increase in the light intensity from 75 $\mu\text{mol photons/m}^2/\text{s}$ to 250 $\mu\text{mol photon/m}^2/\text{sec}$ amplified the maximum cell concentration of *C. Zofingiensis* (Imaizumi *et al.*, 2014). The result showed that the growth rate and cell concentration increases with increasing light intensity. Nonetheless, the growth significantly decreased in lower light intensities. Additionally, Wahidin *et al.* (2013) reported that the increase in the light intensity from 50 $\mu\text{mol photon/m}^2/\text{s}$ to 100 $\mu\text{mol photons/m}^2/\text{s}$ boosted the

Table 1. Specific growth rate, doubling time, maximum cell concentration and biomass of *Nannochloropsis* sp. BJ17 strain under different light intensities

| Parameters | Light intensities (lux) | | |
|---|-------------------------|---------------------|---------------------|
| | 1,500 | 3,000 | 4,500 |
| Specific growth rate (/day) | 1.431 \pm 0.038a | 1.561 \pm 0.055b | 1.729 \pm 0.048c |
| Doubling time (day) | 0.484 \pm 0.013a | 0.444 \pm 0.015b | 0.400 \pm 0.011c |
| Maximum cell concentration ($\times 10^6$ cell/mL) | 24.333 \pm 0.577a | 31.133 \pm 0.981b | 43.333 \pm 1.433c |
| Biomass (g/L) | 0.434 \pm 0.022a | 0.620 \pm 0.004b | 0.824 \pm 0.008c |

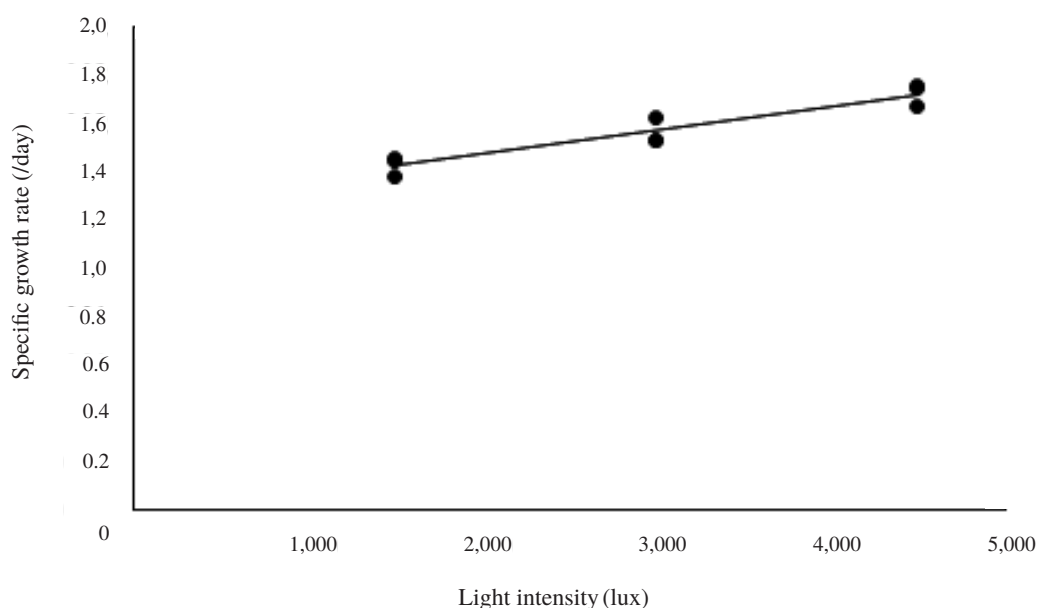


Figure 2. Regression analysis between the light intensity and the specific growth rate of *Nannochloropsis* sp. strain BJ17.

specific growth rate and the maximum cell concentration of *Nannochloropsis* sp. The growth rate of *Nannochloropsis* sp. BJ17 strain in the present study was higher than the growth rate of *Nannochloropsis* sp. in the previous study (Wahidin *et al.*, 2013). Solovchenko (2008) stated that the maximum biomass of 8 mg/mL resulted from light intensity of 400 $\mu\text{mol photon/m}^2/\text{s}$ and biomass production decreased at 4.2 mg/mL at 35 $\mu\text{mol photon/m}^2/\text{s}$ light intensity.

George *et al.* (2014) described that light is an essential parameter that becomes the limiting factor for *Ankistrodesmus falcatus* growth, and has been the major factor that determines the photosynthesis rate of microalgae. Similarly, Wahidin *et al.* (2013) reported that the light intensity controls the biomass production of photosynthetic organisms. Natural or artificial light provides the energy for the transfer of electrons from water to NADP + to form NADPH (nicotinamide adenine dinucleotide phosphate) and ATP (adenine tri-phosphate).

The content of chlorophyll-a and total carotenoids *Nannochloropsis* sp. strain BJ17

The chlorophyll-a and total carotenoid

contents of *Nannochloropsis* sp. BJ17 strain under different light intensities are presented in Table 2. Differences in light intensity significantly affected the chlorophyll-a and total carotenoid contents of *Nannochloropsis* sp. BJ17 strain ($P < 0.05$). Increasing the light intensity resulted in increased contents of chlorophyll-a and total carotenoid of *Nannochloropsis* sp. BJ17 strain. The highest chlorophyll-a and total carotenoid contents of *Nannochloropsis* sp. BJ17 strain were 8.304 mg/mL and 3.892 mg/mL, respectively, generated at 4,500 lux ($P < 0.05$) (Table 2). These results were consistent with George *et al.* (2014) who reported an increase in the chlorophyll content of *A. falcatus* from 10.29 mg/mL (light intensity of 30 $\mu\text{mol photon/m}^2/\text{s}$) to 11.73 $\mu\text{g/mL}$ when they were cultured in 150 $\mu\text{mol photon/m}^2/\text{s}$ of light intensity. A similar increase in chlorophyll was also found in *Chlorella* sp. (Cheirsilp & Torpee, 2012). Moreover, Junior *et al.* (2007) stated that the chlorophyll content was correlated with the concentration of microalgae cells. The higher concentration of chlorophyll cells, the greater values of chlorophyll produced.

George *et al.* (2014) illustrated that the carotenoid content of *A. falcatus* increased due

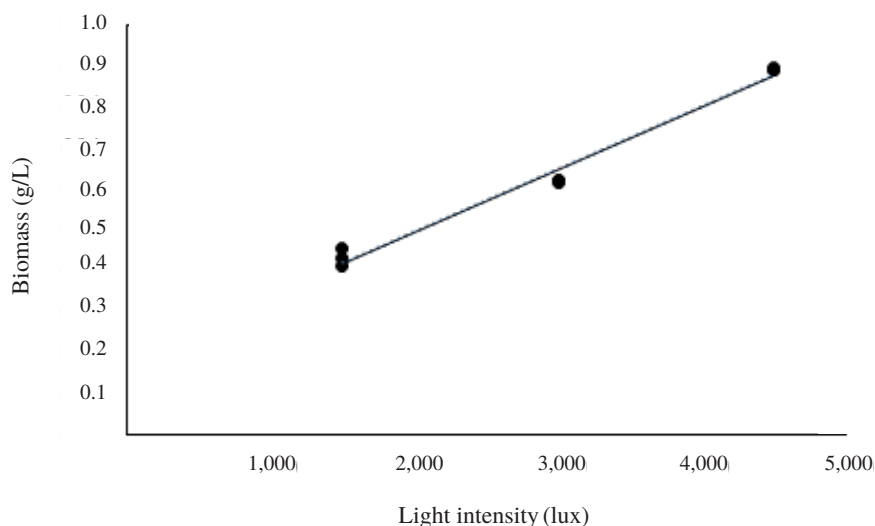


Figure 3. Regression analysis between the light intensity and biomass of *Nannochloropsis* sp. strain BJ17.

Table 2. The chlorophyll-a content and carotenoids of *Nannochloropsis* sp. BJ17 strain under different light intensities

| Parameters | Light intensities (lux) | | |
|------------------------------------|-------------------------|--------------------|--------------------|
| | 1,500 | 3,000 | 4,500 |
| Chlorophyll-a ($\mu\text{g/mL}$) | 4.977 \pm 0.095a | 6.520 \pm 0.049b | 8.304 \pm 0.248c |
| Carotenoid ($\mu\text{g/mL}$) | 2.830 \pm 0.014a | 3.518 \pm 0.018b | 3.892 \pm 0.016c |

to increased exposure to light. This is most likely as a mechanism of adaptation to high light intensity. Also, Richardson *et al.* (1983) reported pigment content and photosynthetic activity of microalgae cultured significantly changed due to differences in light intensities. In addition, Kumar *et al.* (2011) reported that *Spirulina platensis* carotenoid content increased with increasing light. This is a form of adaptation mechanisms of microalgae for photo-protection.

Chlorophyll-a and carotenoid contents of *Nannochloropsis* sp. BJ17 strain were respectively 36% and 11% higher than chlorophyll-a and carotenoid contents of *Chlorella* sp. (Fathi & Asem, 2013). In addition, the carotenoid content of *Nannochloropsis* sp. BJ17 strain was higher than the carotenoid content of *A. falcatus* cultured in bold basal medium, CHU-10 and Zarrouk Medium (George *et al.*, 2014). The results suggested that *Nannochloropsis* sp. BJ17 strain is a potential source of chlorophyll-a pigment and carotenoids.

CONCLUSION

The results showed that the different light intensities significantly affected the growth, biomass and chlorophyll-a and total carotenoids of *Nannochloropsis* sp. BJ17 strain. Furthermore, the higher of light intensity exposed to *Nannochloropsis* sp. BJ17 strain, the growth rate, biomass, chlorophyll-a, and carotenoids of *Nannochloropsis* sp. BJ17 strain were also increased.

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