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The Influence of Salinity on the Growth and Chlorophyll Content of *Nannochloropsis* sp. BJ17

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ABSTRACT

In this study, the effect of salinity (5, 15, 25 and 35‰) on growth and total chlorophyll content of *Nannochloropsis* sp. BJ17 was evaluated. Moreover, chlorophyll content during logarithmic and stationary phases was investigated. The results showed that maximum cell growth and chlorophyll concentration of *Nannochloropsis* sp. BJ17 were achieved at the salinity of 15‰. The algae had the higher total chlorophyll concentration in stationary phase than in logarithmic phase. The authors conclude that, the optimum growth and chlorophyll content of *Nannochloropsis* sp. BJ17 were achieved in salinity of 15‰.

INTRODUCTION

Marine microalgae are potential producers of favourable special compounds such as pigments, vitamins, sugars, and lipids with valued fatty acids (Pal et al. 2011, Koller et al. 2014). Pigments are regarded as one of the most potential product from microalgae (Rao et al. 2007, Forján et al. 2007, Granado-Lorencio et al. 2009). The significant pigment groups are found in microalgae, that is chlorophylls, carotenoids, and phycobilins. All these pigments can be applied in food technology, pharmaceuticals and cosmetics industry (Dufosse et al. 2005, Koller et al. 2014). Chlorophyll is one of the useful bioactive compounds that can be extracted from biomass of microalgae. It has been used as a natural food colouring agent and has antioxidant property (Hosikian et al. 2010).

The production of biomass and pigments can vary significantly depending on the culture conditions of microalgae (Rao et al. 2007). Salinity is one of the most essential parameters affecting the growth and biochemical composition especially chlorophyll content of marine microalgae (Hu 2004, Ghezelbash et al. 2008, Zhila et al. 2011). The adaptability of microalgae to salinity is species-specific and is depending on the physiological characteristic of species (Richmond 1986) and species origin (Banerjee et al. 2011). Adenan et al. (2013) reported that *Chlorella* sp. showed optimum growth when cultured at low salinity. Ak et al. (2008) reported that chlorophyll *a* content of *Dunaliella viridis* increased with increasing salinity. *Nannochloropsis* sp. is highly potential microalgae which has been widely utilized as animal feed in aquaculture (Richmond & Zheng 2001, Rodolfi et al. 2003). Moreover, it has been recognized as one of the most potential photo autotrophic producers of pigment such as chlorophylls and carotenoids (Lubián et al. 2000, Hosikian et al. 2010). The purpose of this study was to evaluate the effect of salinity on cell growth and total chlorophyll production of *Nannochloropsis* sp. BJ17.

MATERIALS AND METHODS

Algae cultures: *Nannochloropsis* sp. BJ17 was obtained from Institute of Brackish water Aquaculture, Jepara, Indonesia. Stock was cultured under laboratory conditions with the temperature of 29±2°C and light intensity of 4,500 lux in Walne medium (1 ml L^{-1}) without vitamin supplementation.

Experimental culture conditions: Logarithmic phase cell of *Nannochloropsis* sp. BJ17 was used as inoculum. Initial cell concentration of 5×10^5 cells mL^{-1} was applied in all treatments. The inoculum was cultured into 2.5 L bottle containing 1.5 L of Walne medium at four different salinities (5, 15, 25 and 35‰). All treatments were performed in triplicates. Algae cultures were mixed by the air pump and illu-

minated by the tube lamp at a light intensity of 4,500 lux, photoperiod of 24:0 (light:dark) and temperature of 29±2°C. The experiment was carried out for 6 days.

Growth analysis: Cell count using Neubauer haemocytometer (BOECO, Hamburg, Germany) was used to evaluate growth of *Nannochloropsis* sp. BJ17. The specific growth rate (μ) was determined according to the equation (Levasseur et al. 1993).

$$\mu = \frac{\ln (x_2) - \ln (x_1)}{t_2 - t_1} \quad ...(1)$$

Where μ represents the growth rate per unit amount of cell concentration, x_1 and x_2 are cell concentration at time 1 (t_1) and time 2 (t_2), respectively.

The doubling time (day) of growth rate was determined from the following equation (Fogg & Thake 1987):

$$td = \frac{\ln 2}{\mu} \quad ....(2)$$

Chlorophyll analysis: Chlorophyll *a* content was evaluated using the spectrophotometric method. 10 mL algal cultures at logarithmic and stationary phases were centrifuged at 6,000 rpm for 10 min. The pellet was rinsed twice with distilled water and was extracted with 10 mL methanol absolute. Then the extract was incubated in the water bath at 70°C for 30 minutes, followed by centrifugation at 6,000 rpm for 10 min. The extract was incubated at dark condition overnight. The supernatant was measured at the wavelength of 650 and 665 nm using a Spectroquant Pharo 300 spectrophotometer. Chlorophyll concentrations were calculated according to the following formula: Total chlorophyll (mg L^{-1}) = $25.8Abs_{650} + 4.0Abs_{665}$ (Hipkins & Baker 1986).

Statistical analysis: Statistical analysis was performed using SPSS 20.0. Data were analysed using one way analysis of variance (ANOVA) to evaluate the existence of significant differences between treatments. Levels of significance are tested at 95% level.

RESULTS

Cell concentration of *Nannochloropsis* sp. BJ17 cultured under batch cultivation at different salinities is shown in Fig. 1. The species showed similar growth pattern in all salinities and maximum cell concentration was obtained on day 6. Moreover, the growth curve showed that no lag phase was found in all treatments and microalgae was entered into the logarithmic phase rapidly. The maximum growth rates in all salinities were achieved between day 1 and day 2.

The maximum cell concentration of 17.88×10^6 cells mL^{-1} ($p<0.05$), the highest specific growth rate of 1.36 per day ($p<0.05$) and the fastest doubling time of 12.24 hours were obtained at the salinity of 15‰ (Table 1). *Nannochloropsis* sp. BJ17 showed significantly lower cell concentration when cultivated at the salinity of 5, 25 and 35‰. However, the microalgae can grow well in a wide range of salinity.

Chlorophyll content of *Nannochloropsis* sp. BJ17 in response to different salinities in the logarithmic and stationary phases is given in Fig. 2. Increasing salinity from 15‰ to 35‰ resulted in decreasing chlorophyll content of *Nannochloropsis* sp. BJ17. The highest chlorophyll concentration of *Nannochloropsis* sp. BJ17 was observed in salinity of 15‰ ($p<0.05$) in both phases. Under optimum salinity of 15‰, algae cultures contained higher chlorophyll in the stationary phase (8.76 mg L^{-1}) than in the logarithmic phase (5.77 mg L^{-1}).

DISCUSSION

Salinity has been known to influence the cell growth and biochemical content of microalgae (Adenan et al. 2013, Ak et al. 2008). This study showed that no lag phase was observed in all treatments, indicating that the cell of *Nannochloropsis* sp. BJ17 could adapt and grow well in all salinities (Fig. 1). This is probably due to the inoculum of culture has taken from the exponential phase of growth. Spencer (1954) explained that the duration of the lag phase is least when the inoculum is in its logarithmic phase of growth. Khan et al. (1998) and Shah et al. (2003) have found similar results in algal culture.

The present study demonstrated that *Nannochloropsis* sp. BJ17 grew much faster at low salinities as compared with high salinities (Fig. 1). This result agreed with Gu et al. (2012), who reported that *N. oculata* could grow better in

Table 1: Specific growth rates, doubling times, and maximum cell concentrations at different salinities of *Nannochloropsis* sp. BJ17.

Salinity (‰)	Specific growth rate (day^{-1})	Doubling time (hours)	Maximum cell concentration ($\times 10^6$ cells mL^{-1})
5	1.18	14.16	12.00
15	1.36	12.24	17.88
25	1.23	13.44	16.00
35	1.22	13.68	15.00

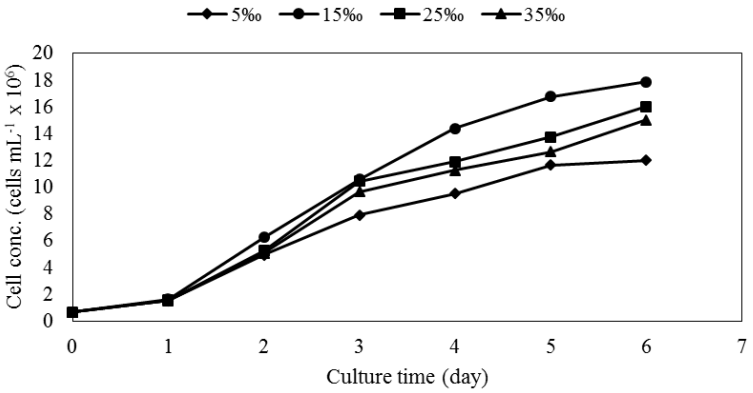

Fig. 1: Increase in cell concentration (n=3) of *Nannochloropsis* sp. BJ17 under different salinities (5, 15, 25 and 35‰) during the culture period.

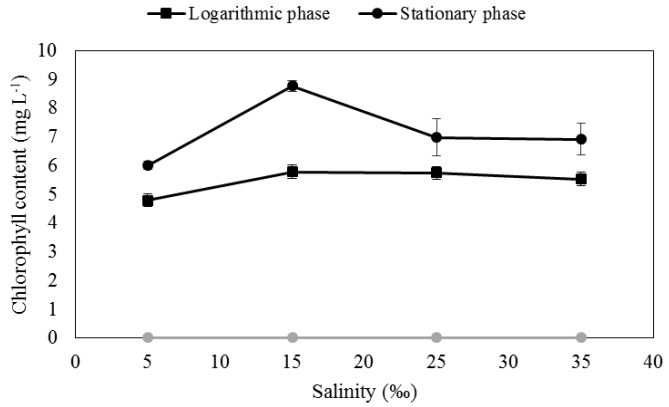

Fig. 2: Chlorophyll content of *Nannochloropsis* sp. BJ17 under different salinities in logarithmic and stationary phases.

low salinity and had slow growth when cultivated in high salinities. Kirst (1990) showed that the microalgae would expend energy while attempting to maintain the turgor pressure and this resulted in a decrease in algal growth. Hart et al. (1991) also explained that decrease in photosynthetic rate led to a reduction of marine microalgal growth at high salinities.

In this study, the optimum salinity for *Nannochloropsis* sp. BJ17 was 15‰. In contrast to our results, Gu et al. (2012) reported that the optimum growth of *N. oculata* CS 179 was obtained in salinity of 25‰. Moreover, Fakhri et al. (2015) also reported that the optimum salinity for *Nannochloropsis* sp. growth was 10‰. This result indicated that salinity effect on microalgae is species-specific (Richmond 1986). In general, the suitable salinity range of *N. oculata* has been known between 10‰ and 35‰ (Renaud & Parry 1994). Lower growth was observed when microalgae cultured in high salinities (25 and 35‰).

Increasing salinity led to a decrease in the concentration of total chlorophyll (Fig. 2). This phenomenon agreed with Gu et al. (2012), who found that chlorophyll concentration decreased when the salinity was increased from 25‰ to 35‰. Ghezelbash et al. (2008) also reported that low salinity resulted in the highest chlorophyll-*a* content in *Tetraselmis chuii*. Chlorophyll content in the stationary phase was higher than in the logarithmic phase. This result in accordance with Fabregas et al. (1986), who reported that the highest chlorophyll content of *Dunaliella tertiolecta* was obtained in the stationary phase. We suggest that chlorophyll content was related to the cell concentration of microalgae, where high cell concentration resulted in high chlorophyll value (Danesi et al. 2011).

CONCLUSION

Salinity is one of the significant factors that influence the growth and chlorophyll content of *Nannochloropsis* sp. BJ17. The algae show a similar growth pattern in response to different salinity during the culture period. The cell grows best and produces the highest chlorophyll content under salinity of 15‰. However, the algae cultures can grow well in a wide range of salinity.

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