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Original Research Paper

Effect of Salinity and Photoperiod on Growth of Microalgae *Nannochloropsis* sp. and *Tetraselmis* sp.

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ABSTRACT

In this present study, the effect of salinity and photoperiod on the growth of *Nannochloropsis* sp. and *Tetraselmis* sp. was examined to optimize microalgal growth. Different salinities (5, 10, 15 and 20‰) for *Nannochloropsis* sp. and (10, 15, 20 and 25‰) for *Tetraselmis* sp. were studied. Both microalgae were cultivated at two different photoperiod cycles (24:0 and 12:12 h light:dark). Maximum growth rates and cell concentrations for *Nannochloropsis* sp. and for *Tetraselmis* sp. were achieved at salinities of 10 and 15‰, respectively. In terms of photoperiod, the maximum growth rates and cell concentrations for both microalgae were obtained at photoperiod of 24:0 h light:dark. This study shows that the cell concentrations and growth rates of both microalgae are highly dependent on salinity and photoperiod.

INTRODUCTION

Microalgae are photosynthetic organisms that are one of the most promising primary producers (Pruvost et al. 2009). Marine microalgae are used for many purposes, such as for animal feed (De Pauw & Persoone 1988), industrial applications (Singh & Gu 2010) and biofuel production (Hossain et al. 2008). *Nannochloropsis* sp. and *Tetraselmis* sp. are known to be an important food source for aquaculture (De Pauw & Persoone 1988, Borowitzka 1997) because of their rapid growth rates and useful chemical properties (Singh & Gu 2010). Fast growth rate of microalgae generally depends on their abilities to double their cells during exponential phase within 24 h or in a short time (210 min) (Chisti 2007).

Adjusting the environmental conditions could become an effective way to optimize the growth rates of microalgae (Mata et al. 2010). A combination of environmental factors such as salinity, light intensity, temperature (Ak et al. 2008), photoperiod and nutrient composition in the culture system are known to affect the microalgal growth rates (Kitaya et al. 2008). Photoperiod cycle is one of the main factors that influence the growth rate of photoautotrophic microalgae culture (Parmar et al. 2011). Besides that, variations in salinity also affect the growth rate of marine microalgae (Adenan et al. 2013).

A study on the effect of photoperiod on growth of *Nannochloropsis* sp. and *Tetraselmis* sp. have been reported (Wahidin et al. 2013, Alsull & Omar 2012). Moreover, the effect of salinity on *Nannochloropsis* sp. and *Tetraselmis suecica* growth have also been studied (Pal et al. 2011, Alsull & Omar 2012). However, algal growth varies from one species to another and depends on the environmental conditions and the species origin (Banerjee et al. 2011).

The purpose of this study was to determine cell concentration and the cell growth rate of *Nannochloropsis* sp. and *Tetraselmis* sp. under different salinities and different photoperiod regimes (light:dark cycles).

MATERIALS AND METHODS

Algal cultures: *Nannochloropsis* sp. and *Tetraselmis* sp. were obtained from the Laboratory of Fish Nutrition, Faculty of Fisheries and Marine Sciences, University of Brawijaya. Stock cultures were cultivated under the laboratory conditions (29±2°C, 4,500 lux) in a Walne medium.

Experimental culture conditions: Four-day old culture of *Nannochloropsis* sp. and *Tetraselmis* sp. was applied as inoculum. Initial cell concentration in all treatments was adjusted at 5×10^5 cells/mL. The inoculum was cultivated into 0.5 L flasks containing 0.35 L of Walne medium at four different salinities (5, 10, 15 and 20‰) for *Nannochloropsis* sp. and (10, 15, 20 and 25‰) for *Tetraselmis* sp. and at two different photoperiod regimes (24:0 and 12:12 h light:dark cycles). All treatments were conducted in triplicates. Cul-

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

Photoperiod (L:D cycle) h	Salinity (‰)	Specific growth rate (day^{-1})	Doubling time (day)	Maximum cell concentration ($\times 10^6$ cell mL^{-1})
24:0	5	0.82	0.85	7.75
	10	1.16	0.59	11.80
	15	0.85	0.81	7.33
	20	0.76	0.91	6.92
12:12	5	0.75	0.92	6.67
	10	0.90	0.77	9.58
	15	0.78	0.88	7.08
	20	0.74	0.93	6.75

Table 2: Specific growth rates, doubling times, and maximum cell concentrations at different salinities and photoperiods of *Tetraselmis* sp.

Photoperiod (L:D cycle) h	Salinity (‰)	Specific growth rate (day^{-1})	Doubling time (day)	Maximum cell concentration ($\times 10^6$ cell mL^{-1})
24:0	10	0.53	1.31	4.08
	15	0.63	1.09	5.25
	20	0.53	1.30	4.25
	25	0.48	1.44	3.25
12:12	10	0.44	1.56	3.33
	15	0.55	1.24	4.67
	20	0.47	1.46	3.75
	25	0.41	1.51	2.75

tures were mixed by an air pump and illuminated by fluorescent lamp at a light intensity of 4,500 lux with temperature of 29±2°C. The experiment was conducted for 6 days.

Growth analysis: Cell count using a 0.1 mm deep Neubauer Haemocytometer (BOECO, Hamburg, Germany) was used to monitor algal growth. At a logarithmic growth phase, the increase in cell concentration (*dx*) is proportional to the amount (*x*) present and to the time interval (*dt*). The specific growth rate (μ) was calculated from the following equation:

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

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

Doubling time (*td*) of cell represents the mean of the generation time of biomass. The doubling time (day) of growth rate was calculated according to the equation:

$$td = \frac{\ln 2}{\mu} = \frac{0.693}{\mu} \quad ...(2)$$

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

RESULTS

Cell growth of *Nannochloropsis* sp. and *Tetraselmis* sp. at different salinities and photoperiods is given in Figs. 1 and 2. Both the species showed similar growth pattern and the highest cell concentration was achieved in day 6. In *Nannochloropsis* sp., the highest specific growth rate of 1.16 per day ($p<0.05$) and the maximum cell concentration of 11.80×10^6 cell mL^{-1} ($p<0.05$) on the 6th day of culture period was obtained at salinity of 10‰ under continuous illumination. Meanwhile, *Tetraselmis* sp. achieved the highest growth rate of 0.63 per day ($p<0.05$) and the maximum cell concentration of 5.25×10^6 cell/mL ($p<0.05$) on day 6 at salinity of 15‰ under a photoperiod of 24:0 light/dark cycle. The fastest doubling time resulted from *Nannochloropsis* sp. and *Tetraselmis* sp. was 0.59 day and 1.09 day, respectively (Tables 1 and 2).

Salinity and photoperiod clearly influenced the cell concentration in *Nannochloropsis* sp. and *Tetraselmis* sp. Increasing the photoperiod from light:dark of 12:12 to 24:0h resulted in increasing the specific growth rate and maximum cell concentration in both the microalgal species (Tables 1 and 2). Tables 1 and 2 also show that *Nannochloropsis* sp. and *Tetraselmis* sp. grew much faster at low salinities as compared with high salinities.

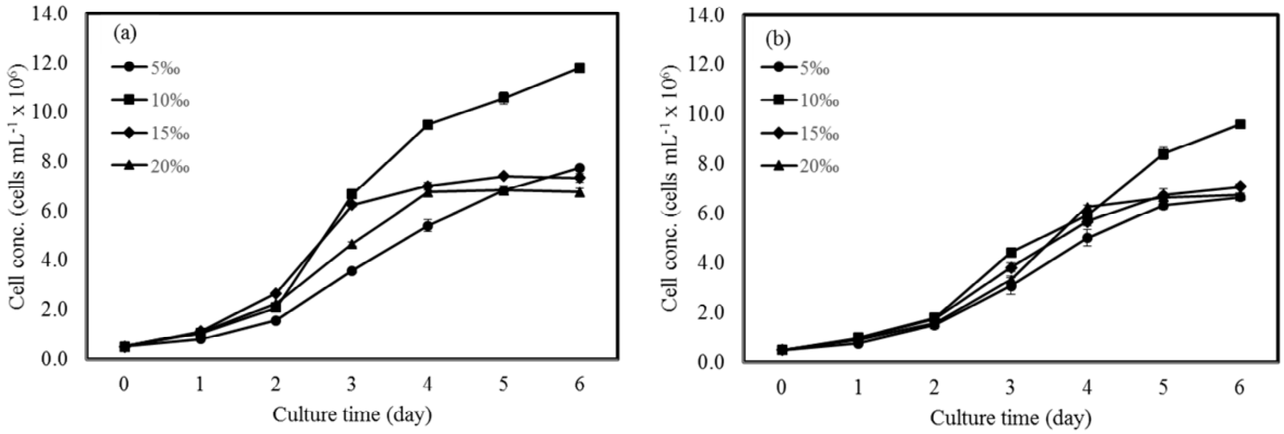

Fig. 1: Increase in cell concentration (n = 3) of *Nannochloropsis* sp. under different photoperiod lengths (a) 24:0 and (b) 12:12 light and dark cycles and different salinities (5, 10, 15 and 20‰) during the culture period.

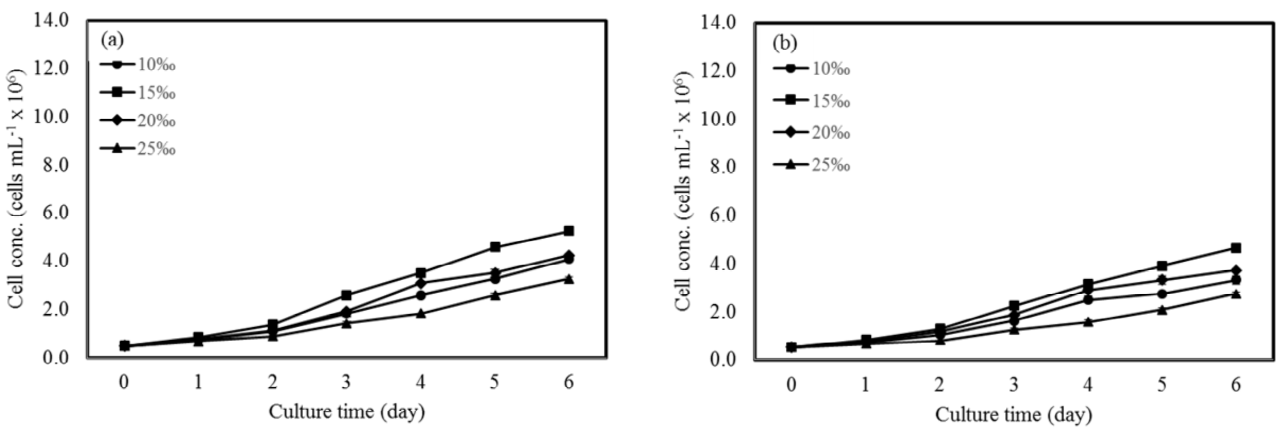

Fig. 2: Increase in cell concentration (n = 3) of *Tetraselmis* sp. under different photoperiod lengths (a) 24:0 and (b) 12:12 light and dark cycles and different salinities (10, 15, 20 and 25‰) during the culture period.

DISCUSSION

Environmental parameters such as salinity, light, photoperiod and temperature have been reported to affect the growth of microalgae (Wahidin et al. 2013, Adenan et al. 2013, Banerjee et al. 2011, Ak et al. 2008). In the present study, the effect of salinity and photoperiod on growth of *Nannochloropsis* sp. and *Tetraselmis* sp. was determined. This study showed that a specific growth rate increased with the increasing salinity in both the species with optimum values of 10‰ for *Nannochloropsis* sp. and 15‰ for *Tetraselmis* sp. (Tables 1 and 2). However, the growth rates turned to drop significantly ($p<0.05$) when salinity was above 10‰ and 15‰ for *Nannochloropsis* sp. and *Tetraselmis* sp., respectively. This phenomenon agreed with Adenan et al. (2013), who reported that increasing salinity from 20 to 25‰, increased the growth rate of *Chlorella* sp., however, increasing salinity to 30‰ decreased the specific growth rate. On the contrary to our results, Alsull and Omar (2012) reported that the optimum salinity for growth of both species was 33‰. On the other hand, Pal et al. (2011) found that *Nannochloropsis sp.* grew best at salinity of 13‰. We suggest that the results are probably different because each species has a different growth response to salinity (Sudhir & Murthy 2004, Ak et al. 2008).

The maximum specific growth rates of *Nannochloropsis* sp. and *Tetraselmis* sp. obtained in this study were 1.16 day^{-1} and 0.63 day^{-1}, respectively at the optimum salinity. The specific growth rate of *Nannochloropsis* sp. in this study was significantly higher than the values of 0.80 and 0.86 day^{-1} as reported by Pal et al. (2011) and Alsull & Omar (2012), respectively. In *Tetraselmis* sp., the growth rate in this study is in agreement with that of (Michels et al. 2014), who found that the highest growth rate of *Tetraselmis suecica* was 0.68 day^{-1}.

In the present study, the growth rates of both microalgal species increased with increasing photoperiod (Tables 1 and 2). The optimum photoperiod for the growth of both species was 24:0 h light:dark cycle. This result agrees with the findings of Wahidin et al. (2013), who found that photoperiod of 24:0 light:dark cycle resulted the highest growth rate of *Nannochloropsis* sp. In addition, Alsull & Omar (2012) showed that *Tetraselmis* sp. grew best under continuous illumination. This is probably due to the fact that insufficient light causes photolimitation of microalgal growth (Wahidin et al. 2013).

CONCLUSION

Salinity and photoperiod are two major factors that significantly affect the growth of *Nannochloropsis* sp. and *Tetraselmis* sp. Both microalgae show a similar growth pattern under different salinity and different photoperiod during the culture period. Both species grow best under continuous illumination, however, each species has a specific optimum salinity. These findings are very important in order to prepare a mass culture of these two species of microalgae.

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