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Original Article

Growth performance and proximate composition of mixed cultures of marine microalgae (*Nannochloropsis* sp. & *Tetraselmis* sp.) with monocultures

Wasana Arkronrat[1]*, Prapapron Deemark[2], and Vutthichai Oniam[1]

[1] *Klongwan Fisheries Research Station, Faculty of Fisheries, Kasetsart University, Mueang, Prachuap Khiri Khan, 77000 Thailand.*

[2] *Aquaculture Development and Certification Centre, Department of Fisheries, Chatuchak, Bangkok, 10900 Thailand.*

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Abstract

The growth performance and proximate composition of two marine microalgae, *Nannochloropsis* sp. and *Tetraselmis* sp., in monoculture and mixed culture of both species under laboratory conditions were compared. Microalgae were cultured in enriched seawater (28 ppt) in 8 L plastic bottles using Conway medium. Microalgae were grown in a temperature-controlled room (25°C) under continuous illumination at a light intensity of about 3,000 Lux for 240 hours (10 days). Results showed that maximum cell density and growth rate of both species ($45.06\pm2.67\times10^5$ cells ml^{-1} and 0.43 ± 0.02 day^{-1}, reps.) in mixed culture had a significantly higher than *Tetraselmis* sp. ($5.09\pm0.99\times10^5$ cells ml^{-1} and 0.16 ± 0.03 day^{-1}, reps.), but not significantly different with *Nannochloropsis* sp. ($29.25\pm22.70\times10^5$ cells ml^{-1} and 0.37 ± 0.14 day^{-1}, reps.) in monoculture. For proximate composition, protein content of both species in mixed culture and *Nannochloropsis* sp. in monoculture was significantly higher than for *Tetraselmis* sp. in monoculture after 120 and 240 hours of cultivation. Carbohydrate and lipid contents of each treatment were found to be significantly different after 120 hours of cultivation but not significantly different after 240 hours of cultivation. Therefore, this study indicates that an alternative option for commercial microalgal production is to use a mixed culture to obtain higher production.

Keywords: microalgae, mixed culture, growth performance, proximate composition

1. Introduction

Commercial culture of microalgae has been done for more than 30 years with the main microalgal species cultured such as *Chlorella* sp. and *Spirulina* sp. for healthy food, *Dunaliella salina* for β-carotene and several species for aquaculture (Borowitzka, 1999; Araújo and Garcia, 2005). Marine microalgae such as the green algae *Chlorella* sp., *Tetraselmis* sp. and *Nannochloropsis* sp., the diatom *Chaetoceros* sp. and *Thalassiosira* sp., and the flagellate *Isochrysis* sp., were commonly used as important live food for crustacean, fish and bivalve larvae in Thailand, and therefore they have been produced commercially (Wongrat, 2000; Arkronrat and Oniam, 2012a; 2012b; 2012c). The success of commercial laboratory-scale production of microalgae depends on many factors, mainly those which control microalgae growth, such as temperature, nutrients, light, salinity and pH (Tzovenis *et al*., 1997; Zhu *et al*., 1997; Araujo and Garcia, 2005). Currently, mixed cultures of microalgae are another important factor for the increase of the production of microalgae (Huang *et al*., 2011; Ahmad *et al*., 2012; Brito *et al*., 2013; Arkronrat and Oniam, 2014; Sureshkumar *et al*., 2014). *Nannochloropsis* sp. and *Tetraselmis* sp. are widely used in aquaculture industries as they are comprised of nutritional values suitable for rearing of marine animal larvae. However, the growth characteristics and biochemical

* Corresponding author.
Email address: ffiswna@ku.ac.th

composition of microalgal species could also be altered when two or more microalgal species were cultured together in the same culture medium (Cai and Duan, 2008; Huang *et al.*, 2011). Therefore, the aims of this study were to compare the cell density, growth rate and proximate composition of *Nannochloropsis* sp. and *Tetraselmis* sp. in monoculture and mixed culture of both species under laboratory conditions as an alternative for commercial microalgal production.

2. Materials and Methods

2.1 Experimental design and set-up

The experiment on growth performance of microalgae was conducted at the Phytoplankton Laboratory of Klongwan Fisheries Research Station (KFRS), Prachuap Khiri Khan Province, Thailand. Stock cultures of the two species of commercial microalgae, the green algae *Nannochloropsis* sp. and *Tetraselmis* sp., were obtained from the Prachuap Khiri Khan Coastal Fisheries Research and Development Center, Department of Fisheries.

These microalgae were cultured under laboratory conditions, 1) monoculture of *Nannochloropsis* sp., 2) monoculture of *Tetraselmis* sp., and 3) mixed culture of both species. Microalgae were cultured in enriched seawater (28 ppt) in 8 L plastic bottles using Conway medium without silicate (AQUACOP, 1984), grown in a temperature-controlled room (25°C) under continuous illumination with cool white fluorescent lamps at a light intensity of about 3,000 Lux, for 240 hours (10 days). All cultures were started with equal inoculums (about 10^5 cell ml^{-1}) of respective species while the inoculum of mixed culture consisted of half the number of each species. The experiment was performed with four replicates and followed a completely randomized design.

During the growth experiment, algal cell samples were collected every 12 hours for estimation of cell density and growth pattern. Cells were fixed with 5% formalin then counted using a haemacytometer under a compound microscope at 40× magnification. Growth rate (K) of the culture was calculated by the following equation (Phatarpekar *et al*., 2000):

$$K = \frac{InN_t - InN_o}{T}$$

where N_t is the cell count at time "*t*", *N*o is the initial cell count at time "o" and *T* is the time (day).

For proximate composition analysis, the dry biomass was evaluated on the same dates, concentrating known volumes of each culture in precalibrated Whatman GF-C glass fiber filters. These were washed with 5-6 ml of a 3% ammonium formate solution to eliminate salt and dried to constant weight at 60°C (Sánchez-Saavedra and Voltolina, 2006). Protein, total carbohydrate and total lipid were determined according to the methods of Lowry *et al.* (1951), Dubois *et al.* (1956) and Bligh and Dyer (1959), respectively.

2.2 Statistical analysis

At the end of the experiments, data on the growth performance (cell density and growth rate) and proximate composition (protein, carbohydrate and lipid) were analyzed using analysis of variance and the difference between means was tested using Duncan's multiple range test at the 95% level of confidence using the SPSS program.

3. Results

3.1 Cell density and growth rate

Initial cell densities of *Nannochloropsis* sp., *Tetraselmis* sp. in monoculture and both species in mixed culture were 1.12±0.09, 1.18±0.14 and 1.13±0.13×10^5 cells ml^{-1}, respectively. The cell density of both species in mixed culture increased rapidly to 15.87±1.76×10^5 cells ml^{-1}, at hour 60 of cultivation without any apparent lag phase of growth. *Nannochloropsis* sp. culture cell density increased to 17.13 ±26.24×10^5 cells ml^{-1} at hour 156 of cultivation (lag phase). Meanwhile *Tetraselmis* sp. culture cell density increased to 3.90±1.35×10^5 cells ml^{-1} at hour 132 of cultivation (lag phase). The cell density of both species in mixed culture and *Nannochloropsis* sp. in monoculture decreased considerably at hour 216 and 180 of cultivation, respectively, until the end of the experimental period. In contrast, the cell densities of *Tetraselmis* sp. in monoculture continued increasing until the end of the experimental period. In addition, growth patterns of *Nannochloropsis* sp. and *Tetraselmis* sp. improved appreciably in the mixed culture than momoculture (Figure 1).

Maximum cell density of both species in mixed culture had a significantly higher than maximum cell density of *Tetraselmis* sp. in monoculture ($P<0.05$), but not significantly different with *Nannochloropsis* sp. in monoculture ($P>0.05$). Also, the growth rates of both species in mixed culture had a significantly higher than growth rates of *Tetraselmis* sp. in monoculture ($P<0.05$), but not significantly different with *Nannochloropsis* sp. in monoculture ($P>0.05$) (Table 1).

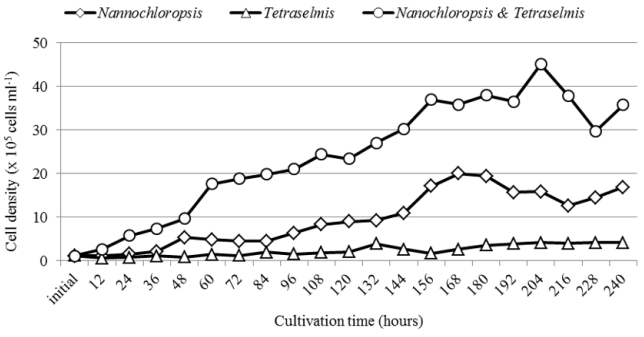

Figure 1. Growth of *Nannochloropsis* sp. and *Tetraselmis* sp. in monoculture and mixed culture of both species under laboratory conditions.

Table 1. Maximum cell density, growth rate and proximate composition of *Nannochloropsis* sp., *Tetraselmis* sp. in monoculture and mixed culture of both species under laboratory conditions (n = 4).

Parameter	*Nannochloropsis*	*Tetraselmis*	Mixed culture of both species
Maximum cell density($\times 10^5$ cells ml^{-1})	29.25 ± 22.70^{ab}	5.09 ± 0.99^{b}	45.06 ± 2.67^{a}
Growth rate (day^{-1})	0.37 ± 0.14^{a}	0.16 ± 0.03^{b}	0.43 ± 0.02^{a}
120 hours of cultivation			
Protein (% dw)	35.8 ± 2.6^{a}	25.7 ± 1.3^{b}	33.7 ± 0.9^{a}
Carbohydrate (% dw)	19.9 ± 1.1^{a}	16.6 ± 1.2^{b}	17.5 ± 1.5^{b}
Lipid (% dw)	11.7 ± 0.5^{a}	9.4 ± 0.9^{b}	10.8 ± 1.0^{ab}
240 hours of cultivation			
Protein (% dw)	32.6 ± 3.2^{a}	21.7 ± 0.7^{b}	29.7 ± 3.0^{a}
Carbohydrate (% dw)	17.6 ± 1.7^{a}	14.5 ± 1.6^{a}	16.2 ± 1.2^{a}
Lipid (% dw)	11.1 ± 0.9^{a}	9.4 ± 0.8^{a}	10.0 ± 1.4^{a}

Note: Data in the same row with different superscripts are significantly different ($P<0.05$), dw = dry weight.

3.2 Proximate composition

After 120 hours of cultivation, protein content of both species in mixed culture and *Nannochloropsis* sp. in monoculture were found to be significantly higher than protein content of *Tetraselmis* sp. in monoculture ($P<0.05$). However, carbohydrate and lipid contents of both species in mixed culture and *Tetraselmis* sp. in monoculture were not significantly different ($P>0.05$). For *Nannochloropsis* sp. in monoculture, carbohydrate content had a significantly higher than *Tetraselmis* sp. in monoculture and both species in mixed culture ($P<0.05$), and lipid content was not significantly different with both species in mixed culture ($P>0.05$) but had a significantly higher than *Tetraselmis* sp. in monoculture ($P<0.05$) (Table 1).

After 240 hours of cultivation, protein content of both species in mixed culture and *Nannochloropsis* sp. in monoculture had a significantly higher than protein content of *Tetraselmis* sp. in monoculture ($P<0.05$), but not significantly different were found in carbohydrate and lipid contents ($P>0.05$) (Table 1).

4. Discussion

Nannochloropsis sp. and *Tetraselmis* sp. were widely used in aquaculture as a source of protein, lipid and carbohydrate (Khatoon *et al*., 2014). The growth performance and the growth patterns of *Nannochloropsis* sp. and *Tetraselmis* sp. in monoculture of this studied were different. The monoculture of *Nannochloropsis* sp. grew rapidly, while *Tetraselmis* sp. had a relatively slow and steady growth. This is because of the size difference of the microalgae had a affect growth rate (*Nannochloropsis* sp. – 2 μm diameter, *Tetraselmis* sp. – 8 μm diameter), smaller size species grow faster than larger ones because of greater surface or volume ratio of smaller sized cells which facilitates assimilation of nutrients at relatively faster rate (Phatarpekar *et al*., 2000). In the mixed culture, the growth performance and the growth patterns of both species were better than monoculture. The results suggest that mixed culture of microalgae can increased the production of microalgae as was similarly reported by Cai and Duan (2008), Huang *et al.* (2011), Ahmad *et al.* (2012), Arkronrat and Oniam (2014) and Sureshkumar *et al*. (2014). In order to support this result, Brito *et al*. (2013) reported that mixed culture of microalgae was not simply a mixture of individual monocultures; the growth characteristics, biochemical compositions, nutritional compositions and other growth factors of mixed culture can be substantially different from those of monocultures.

For proximate composition in this study, protein content of *Nannochloropsis* sp. and *Tetraselmis* sp. in mixed culture had a significantly higher than protein content of *Tetraselmis* sp. in monoculture after 120 and 240 hours of cultivation. Carbohydrate and lipid contents of each treatment were found to be significantly different after 120 hours of cultivation, which exhibited the similar trends as other microalgal species. Phatarpekar *et al.* (2000) compared the growth performance, biochemical and nutritive values of *Isochrysis galbana* and *Chaetoceros calcitrans* in mixed culture with those in monoculture under laboratory condition and found that cellular concentrations of chlorophyll a, protein, carbohydrate, lipid and particulate organic carbon were significantly higher in mixed culture when compared with monocultures during all the growth phases. Huang *et al.* (2011) reported that chlorophyll a and protein content of mixed culture of *Dunaliella salina* and *Phaeodactylum tricornutum* were increased with the increasing age of the culture when compared with that obtained with monoculture of *D. salina* and *P. tricornutum*. However, the biochemical content of microalgae can vary with changes in the environ-

mental conditions. For example, Khatoon *et al*. (2014) reported that *Nannochloropsis* sp. and *Tetraselmis* sp. had signicantly higher cell density, lipid and carbohydrate contents under control condition at 30 ppt. High cell density, protein, lipid and carbohydrate content was obtained when cultured at pH 7.5 and 8.5 for both species. In addition, the microalgae growth was limited by several factors such as the type and concentration of nutrients, light intensity, photoperiod, genetic deficiency and CO_2 all this modifies the biochemical composition of microalgae (Tzovenis *et al*., 1997; Zhu *et al*., 1997; Phatarpekar *et al*., 2000; Araujo and Garcia, 2005).

5. Conclusions

The results indicated that the growth performance and the growth patterns of *Nannochloropsis* sp. and *Tetraselmis* sp. improved appreciably in the mixed culture than monoculture. Additionally, protein, carbohydrate and lipid contents were significantly higher in mixed culture when compared with monocultures. Therefore, an alternative option for commercial microalgal production is to mixed culture to higher production. Moreover, there is a possibility to enhance growth performance and proximate composition by environmental factor optimization. This is an interesting point for future study.

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