Growth Performance and Production Cost of Commercial Microalgae Cultured under Laboratory Conditions with Different Aeration Settings

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ABSTRACT

Microalgae such as the diatom *Chaetoceros calcitrans*, the green algae *Chlorella* spp. and the flagellate *Isochrysis galbana*, are commonly used as important live food for crustacean, fish and bivalve larvae in Thailand, there by they have been produced commercially. In this study, the growth performance and production cost of commercial microalgae cultured under laboratory conditions with different aeration settings (24, 12, 8 and 6 hrs per day) were investigated. Algae were cultured in enriched seawater (28 ppt) in 1 L glass 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 10 days. Results showed that the growth rates of *Chaetoceros* culture grown with 24, 12 and 8 hrs aeration settings were not significantly different ($P>0.05$), but were significantly higher at the 6 hrs aeration setting ($P<0.05$). In addition, the growth rates of *Chlorella* and *Isochrysis* in each treatment were not significantly different ($P>0.05$). Commercial *Chaetoceros* produced with 24 hrs aeration setting had a significantly higher production cost ($P<0.05$) compared with those produced with 12, 8 and 6 hrs aeration settings. In addition, at 12, 8 and 6 hrs aeration settings, *Chaetoceros* production costs could be reduced by 7.96, 14.08 and 20.91%, respectively. Therefore, an alternative option for commercial microalgal production is to set an optimal aeration setting to achieve better profit margin and higher production.

Keywords: commercial microalgae, growth performance, production cost, aeration setting

INTRODUCTION

Commercial culture of microalgae has been done for more than 30 years with the main microalgal species cultured such as *Chlorella* and *Spirulina* for healthy food, *Dunaliella salina* for -carotene and several species for aquaculture (Borowitzka, 1999). Many specific characteristics of microalgae such as cell wall digestibility, cell size and biochemical compositions influence their nutritional value as food. Microalgae are an

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important food source for the larval stages of several crustacean and fish species and used as the main food for bivalve larvaein hatcheries. (Araújo and Garcia, 2005). The common microalgae used in hatcheries in Thailand include *Chlorella*, *Chaetoceros*, *Skeletonema*, *Tetraselmis* and *Isochrysis* (Wongrat, 2000). In Thailand, farmers obtain microalgae seed stock culture from either government agencies or private farms. Seed culture is usually produced by batch culture in 1 L glass bottles in the laboratory. Currently, more people are interested in producing microalgae on a commercial scale for business purposes. One factor which is more important than those affecting the growth of microalgaeis the development of production methods, which should provide the most profit by reducing the costs without affecting the growth and quality of microalgae.

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 (Araujo and Garcia, 2005; Tzovenis *et al*., 1997; Zhu *et al*., 1997). The aeration system management is another important factor for the production of microalgae. Aeration helps to circulate the water, which prevents microalgae to precipitate and helps microalgal cells to get adequate light and grow well (Wongrat, 2000). Krichnavaruk *et al*., (2005) also reported that aeration rates affected algal mass production in the airlift photobioreactor, and that increasing aeration rate (U_{sg}) up to the range of 2-5 cm s^{-1} was found to have influence on the algal growth rate. The maximum cell growth rate occurred at the aeration rate of 3 cm s^{-1} above which the growth rate dropped. Therefore, turning on the air pump all the time might not be necessary and it could cost a lot for power. Another alternative which will help to reduce the production cost of microalgae is to turn on the air pump for a certain time period during the day and then turn it off. We propose the use of different aeration systems management as an alternative cultivation system for commercial microalgae. The objective of this study was to compare growth performance and production cost of microalgae production under laboratory conditions with different aeration system management.

MATERIALS AND METHODS

Experimental design and set-up

The experiment on growth performance of microalgae was conducted at the Phytoplankton Laboratory of Klongwan Fisheries Research Station, Prachuap Khiri Khan Province, Thailand. Stock cultures of the three species of commercial microalgae, the diatom *Chaetoceros calcitrans*, the green algae *Chlorella* spp. and the flagellate *Isochrysis galbana*, were obtained from the Prachuap Khiri Khan Coastal Fisheries Research and Development Center. These microalgae were cultured under laboratory conditions with different aeration settings (Table 1).

Table 1. Time schedule of different aeration settings for microalgae culture in laboratory.

Control (24 hrs/day)	Treatment 1 (12 hrs/day)	Treatment 2 (8 hrs/day)	Treatment 3 (6 hrs/day)
24 hours	6.00 – 8.00 A.M.	6.00 – 8.00 A.M.	6.00 – 8.00 A.M.
	10.00 – 12.00 A.M.	12.00 – 14.00 P.M.	14.00 – 16.00 P.M.
	14.00 – 16.00 P.M.	18.00 – 20.00 P.M.	22.00 – 24.00 P.M.
	18.00 – 20.00 P.M.	24.00 – 02.00 A.M.	
	22.00 – 24.00 P.M.		
	02.00 – 04.00 P.M.		

Cultures were grown in 1 L of sea water (28 ppt) enriched with 1 ml of Conway medium (AQUACOP, 1984), with silicate added only for the monoculture of *Chaetoceros*. All cultures were maintained in a temperature controlled room using an air conditioning unit at 25°C under continuous illumination with cool white fluorescent lamps at a light intensity of about 3,000 Lux, for 10 days. The experiment was performed with four replicates and followed a completely randomized design.

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

$$K = \frac{\ln N_t - \ln N_o}{t}$$

where N_t = cell count at time "*t*", N_o = initial cell count at time "o" and *t* = time (days).

The study on the monthly production cost of the commercial production of *Chaetoceros* was based on data collected from a plankton farm in Kui Buri District, Prachuap Khiri Khan Province. *Chaetoceros* seed stock was produced by batch culture in 1 L glass bottles at 100 L per day. *Chaetoceros* were grown in a temperature controlled room at 25 ± 1°C by 25,000 BTU Central Air conditioning unit under continuous illumination with 40 W Cool White fluorescent lamps at a light intensity of about 3,000 Lux. All treatments were aerated with a 1 HP blower. Production cost was calculated using the formula:

$$\text{Production cost} = \text{Fixed cost} + \text{Variable cost}$$

where Fixed cost = chemical, sea water, public utilities, depreciation, rent of location, labor and other material value, and Variable cost = electricity value.

Statistical analysis

Statistical analysis on growth performance

and production cost was conducted using analysis of variance (ANOVA), while the differences between treatments were compared using Duncan's New Multiple Range Test. Significant differences were considered at a probability level of 0.05. All statistics analysis were performed using SPSS program version 17.0.

RESULTS AND DISCUSSION

Growth performance

Initial cell densities of *Chaetoceros*, *Chlorella* and *Isochrysis* were 1.27 ± 0.15, 17.12 ± 2.05 and 2.89 ± 0.57 x 10^5 cells ml^{-1}, respectively. The cell density of *Chaetoceros* in 24, 12 and 8 hrs aeration settings increased rapidly to 35.93 ± 4.02, 33.43 ± 2.44 and 32.26 ± 0.75 x 10^5 cells ml^{-1}, respectively, on day 3 without any apparent lag phase of growth. However, *Chaetoceros* culture in 6 hrs aeration setting had a gradual increase in cell density (15.30 ± 2.43 x 10^5 cell ml^{-1}) during the corresponding period.

Chlorella culture cell density increased to 34.37 ± 3.06, 51.00 ± 4.84, 35.25 ± 4.19 and 41.84 ± 2.68 x 10^5 cells ml^{-1} on days 8, 9, 8 and 9 for 24, 12, 8 and 6 hrs aeration settings, respectively. Mean while *Isochrysis* culture cell density increased to 27.90 ± 5.23, 27.89 ± 4.55, 35.12 ± 8.33 and 22.75 ± 4.05 x 10^5 cell ml^{-1} on days 10, 10, 10 and 9 for 24, 12, 8 and 6 hrs aeration settings, respectively.

The cell density of *Chaetoceros* culture at different aeration settings decreased considerably between days 6 and 8 until the end of the experimental period. In contrast, the cell densities of *Chlorella* and *Isochrysis* culturedat different aeration settings continued increasing until the end of the experimental period (Figure 1).

The growth performance at 24, 12, 8 and 6 hrs aeration settings revealed that *Chaetoceros* had a growth rate of 0.77, 0.76, 0.76 and 0.44 day^{-1} with maximum cell density of 61.16, 56.93, 57.43 and 44.10 x 10^5 cells ml^{-1}, respectively, where as *Chlorella* had a growth rate of 0.08, 0.12, 0.10 and 0.09 day^{-1} and a maximum cell density of 35.75, 51.00, 42.62 and 41.84 x 10^5 cells ml^{-1}, and *Isochrysis* had a growth rate of 0.24, 0.25, 0.25 and 0.22 day^{-1} and a maximum cell density of 27.90, 27.89, 35.12 and 29.37 x 10^5 cells ml^{-1}, respectively. The growth rates of *Chaetoceros* culture at 24, 12 and 8 hrs aeration settings were not significantly different ($P>0.05$) among each other, but were significantly higher than at 6 hrs aeration setting ($P<0.05$). Mean while the growth rates of both *Chlorella* and *Isochrysis* in all treatments were not significantly different ($P>0.05$) (Table 2).

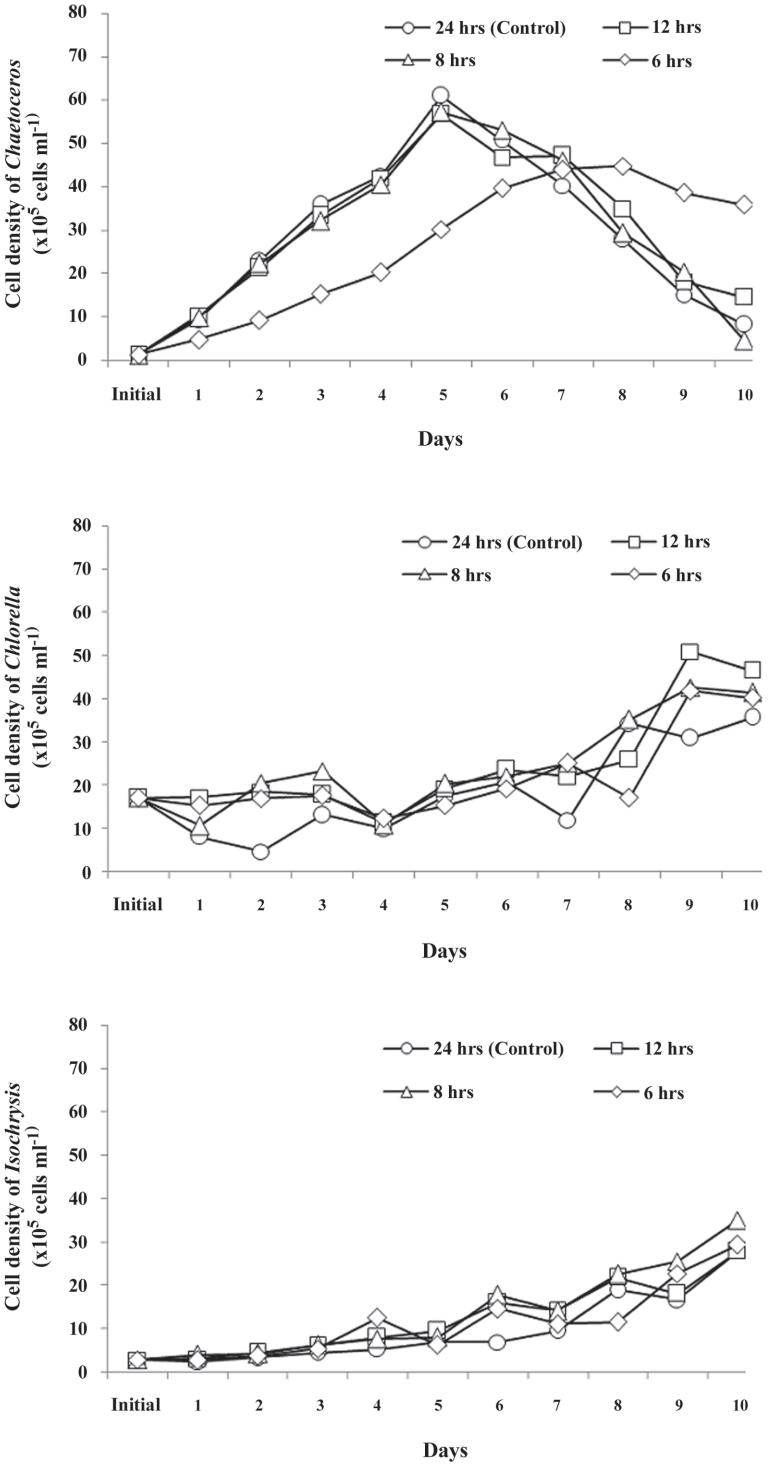

Figure 1. Cell densities of *Chaetoceros calcitrans*, *Chlorella* spp. and *Isochrysis galbana* cultured under laboratory conditions with different aeration settings.

Table 2. Maximum cell density (x10^5 cells ml^{-1}) and growth rate (day^{-1}) of *Chaetoceros calcitrans*, *Chlorella* spp. and *Isochrysis galbana* cultured under laboratory conditions with different aeration settings (n = 4).

Microalgae	Aeration Settings			
	24 hrs (Control)	12 hrs	8 hrs	8 hrs
Chaetoceros				
maximum cell density	61.16 ± 6.45^{a}	56.93 ± 2.70^{a}	57.43 ± 3.53^{a}	44.10 ± 4.55^{b}
growth rate	0.77 ± 0.02^{a}	0.76 ± 0.01^{a}	0.76 ± 0.01^{a}	0.44 ± 0.01^{b}
Chlorella				
maximum cell density	35.75 ± 5.42^{c}	51.00 ± 4.84^{a}	42.62 ± 6.96^{abc}	41.84 ± 2.68^{b}
growth rate	0.08 ± 0.01^{a}	0.12 ± 0.01^{a}	0.10 ± 0.02^{a}	0.09 ± 0.01^{a}
Isochrysis				
maximum cell density	27.90 ± 5.23^{a}	27.89 ± 4.55^{a}	35.12 ± 8.33^{a}	29.37 ± 11.79^{a}
growth rate	0.24 ± 0.04^{a}	0.25 ± 0.01^{a}	0.25 ± 0.01^{a}	0.22 ± 0.02^{a}

Note : Data in the same row with different superscripts are significantly different ($P<0.05$).

Production cost

The average production cost of commercial *Chaetoceros* cultured under laboratory conditions with 24, 12, 8 and 6 hrs aeration settings were 21,091.66 ± 69.50, 19,420.66 ± 97.50, 18,127.33 ± 134.01 and 17,591.00 ± 478.00 Baht/month or 7.03 ± 0.02, 6.47 ± 0.03, 6.04 ± 0.04 and 5.86 ± 0.15 Baht/1 L bottle, respectively. The microalgae produced at 24 hrs aeration setting had a significantly higher cost ($P<0.05$) compared to those produced at 12, 8 and 6 hrs aeration settings. In addition, production cost was reduced by 7.96, 14.08 and 20.91% at 12, 8 and 6 hrs aeration settings, respectively (Table 3).

Table 3. Monthly production cost of microalgae culture in laboratory at 100 L/day production capacity.

Expenditures	**Baht/month**	**Baht/1 L bottle**
Fixed cost		
1) Chemical for Conway Medium	850	
2) Sea water	400	
3) Public utilities (not electricity value)	920	
4) Depreciation	300	
5) Rent of location	5,000	
6) Other material	1,200	
7) Labor	6,000	
Total fixed cost …….(1)	14,670	4.89
Variable cost (electricity cost) …….(2)		
24 hrs aeration setting (Control)	6,421.66	2.14
12 hrs aeration setting (Treatment 1)	4,750.66	1.58
8 hrs aeration setting (Treatment 2)	3,457.33	1.15
6 hrs aeration setting (Treatment 3)	2,921.00	0.97
Production cost (1+2)		
24 hrs aeration setting (Control)	$21,091.66 \pm 69.50^{a}$	7.03 ± 0.02^{a}
12 hrs aeration setting (Treatment 1)	$19,420.66 \pm 97.50^{b}$	6.47 ± 0.03^{b}
8 hrs aeration setting (Treatment 2)	$18,127.33 \pm 134.01^{c}$	6.04 ± 0.04^{c}
6 hrs aeration setting (Treatment 3)	$17,591.00 \pm 478.00^{c}$	5.86 ± 0.15^{c}

Note: Production cost in the same column with different superscripts are significantly different ($P<0.05$), ($n = 3$).

CONCLUSION

The results illustrated that different aeration settings could affect growth performance of *Chaetoceros* but not that of *Chlorella* and *Isochrysis* in the laboratory. Production cost of microalgae applying 24 hrs aeration setting was significantly higher than those produced at 12, 8 and 6 hrs aeration settings. The optimal aeration setting for producing *Chaetoceros* for business was 8 hrs per day and for *Chlorella* and *Isochrysis* were 8 and 6 hrs per day. Therefore, an alternative option for commercial microalgal production is to set an optimal aeration setting to achieve better profit margin and higher production.

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