The Effect of Sodium Bicarbonate Concentrations on Growth and Biochemical Composition of *Chaetoceros gracilis* Schutt

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ABSTRACT

The unicellular marine diatom, *Chaetoceros gracilis* Schutt, has been commonly used as an important live food for crustacean larvae in Thailand. In this study, the effects of sodium bicarbonate concentrations (0.05, 0.50 and 5.00 g L^{-1}) on growth and biochemical composition of *C. gracilis* were investigated. *C. gracilis* was cultured with modified f/2 medium at 25 ± 1°C and continuous illumination (5,000 Lux). *C. gracilis* cells were harvested only once after the culture period at 60 h to analyze carbohydrates, protein and total lipid contents. The results showed that the accumulation of carbohydrates and total lipid in *C. gracilis* was highest in 0.05 g L^{-1} sodium bicarbonate while cell density was unaffected as it was not different ($P>0.05$) from that of the control group (no sodium bicarbonate added). These results can be applied for nutritional improvement of this diatom used as live feed for marine animal larviculture.

Key words: *Chaetoceros gracilis* – Sodium bicarbonate – Lipids - Biochemical composition

INTRODUCTION

Microalgae are an important food source for the larval stage of several crustacean and fish species and are used as the main food for bivalve larvae and spat in hatcheries. Many specific characteristics of microalgae such as cell wall digestibility, cell size and biochemical compositions influence their nutritional value as food (Araújo and Garcia, 2005). In general, the biochemical composition of microalgae varies with their growth rate and the phase of their life cycle (Ranga Rao *et al*., 2007). Diatoms such as *Chaetoceros calcitrans* have high long chain polyunsaturated fatty acid (PUFA content especially arachidonic acid (20:4n6; AA), eicosapentaenoid acid (20:5n3; EPA) and decosahexaenoic acids (22:6n3; DHA) with 9.4, 20.8 and 4.5 % of

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total fatty acids, respectively (Natrah *et al*., 2007). These PUFAs are essential to the growth and development of young aquatic animals (Ben-Amotz *et al*., 1987; Whyte *et al*., 1989). With this study, a diatom *Chaetoceros gracilis* was selected due to its fast growth rate and suitable size for feeding crustacean larvae. Moreover, *C. gracilis* has been widely used as food during nursing of many aquatic animal larvae of shrimps, crabs and bivalves in Thailand.

The main environmental factors influencing microalgal growth and chemical composition are light, nutrients, temperature and pH (Rousch *et al*., 2003). Carbon source is also considered an important factor that affects cell growth because it is a source of energy and skeleton for cell growth (Wen and Chen, 2003). Previous studies reported that carbon sources affect growth and fatty acid compositions in numerous microalgal species (Wood *et al*., 1999; Wen and Chen, 2003). Generally, the carbon source of micro algae in culture systems is carbon dioxide (CO_2) which is naturally present in the air at approximately 300 ppm. Mixture of air and CO_2 gas can significantly enhance microalgal growth but CO_2 supplement also increases the cost of microalgal production. On the other hand, an alternative inorganic carbon source which can be used with microalgal cultivation is sodium bicarbonate (Wen and Chen, 2003). Since the use of sodium bicarbonate as the carbon source for diatom culture was rarely studied, the aim of this study was to evaluate the effect of sodium bicarbonate concentration on growth and biochemical composition of *C. gracilis* under laboratory conditions.

MATERIALS AND METHODS

The marine diatom *Chaetoceros gracilis* used in this study was obtained from the National Institute of Coastal Aquaculture, Songkhla (southern) Thailand. The stock culture was kept in sterilized seawater (25 PSU) enriched with standard f/2 medium (Guillard and Ryther, 1962). The medium was composed of (units in mg L^{-1}) 168.3 $NaNO_3$, 12 $Na_2HPO_4.H_2O$, 5.8 $FeCl_3$. $6H_2O$, 20 Na_2EDTA. $2H_2O$, 66 Na_2SiO_3. $9H_2O$, 1.96 $CuSO_4$. $5H_2O$, 4.40 $ZnSO_4$. $7H_2O$, 1.26 Na_2MoO_4. $2H_2O$, 36 $MnCl_2$. $4H_2O$, 2.0 $CoCl_2$. $6H_2O$, 0.4 vitamin B_1, and (µg L^{-1}) 2 vitamin B_{12} and 100 biotin.

In order to test the effects of sodium bicarbonate on growth and biochemical compositions of *C. gracilis*, the experiment was performed in 1 L Erlenmeyer flasks with 25 PSU modified f/2 medium (Krichnavaruk *et al*., 2005) that had two fold of silica, phosphorus and vitamins concentrations. Continuous illumination was provided to the culture through cool white fluorescent lamps at 5000 Lux while temperature was controlled at 25 ± 1°C. Log phase stock culture was inoculated to the initial cell concentration of 0.55 x 10^6 cells ml^{-1} and four concentrations of sodium bicarbonate i.e. 0, 0.05, 0.50 and 5.00 g L^{-1} were supplied in the culture medium. The experiment was performed with five replicates and followed a completely randomized design.

Cell samples were collected every 12 h to measure cell density and nutrient concentrations. Cell concentrations were counted using a haemacytometer under an optical microscope (Olympus CHS No.2B1199)

at 40x magnification. The specific growth rate of *C. gracilis* was calculated by the following equation (Guillard and Ryther, 1962):

$$\mu = \frac{\ln N_2 - \ln N_1}{t_2 - t_1}$$

where μ = specific growth rate (h^{-1}),
N_1 = cell concentration at t_1 (cells mL^{-1}),
N_2 = cell concentration at t_2 (cells mL^{-1}),
t_1 = the first sampling time (h)
t_2 = the second sampling time (h)

For biochemical analysis, *C. gracilis* cells were gently harvested by centrifugation at 6,000 g for 3 minutes. Cell paste was then washed with 5-6 ml of 0.5 M ammonium formate solution to eliminate salt. The dry biomass (DW) of the harvested cells was determined by oven drying at 100°C then weighed using four decimal electronic balances (Hu and Richmond, 1994). Protein, total carbohydrate and total lipid contents were analyzed using the procedures described in Lowry *et al*. (1951), Dubois *et al*. (1956) and Blight and Dyer (1959), respectively.

Concentrations of orthophosphate and silicate in the culture medium at 12, 24, 36, 48 and 60 hours were analyzed using the method mentioned in Strickland and Parson (1972). Nitrate concentration was determined by UV screening method (Greenberg *et al*., 1992). Statistical analysis was performed using analysis of variance (ANOVA) and the differences between treatments were compared using Duncan's Multiple Range Test. Significant differences were considered at a probability level of 0.05. All statistics were analyzed using SPSS Version 11.

RESULTS AND DISCUSSION

This study confirmed that the marine diatom *C. gracilis* could grow well in modified f/2 medium under laboratory conditions. The growth curve in Figure 1 illustrates that only the highest concentration of sodium bicarbonate (5.00 g L^{-1}) significantly reduced growth rate and maximum cell density of *C. gracilis* while other concentrations e.g. 0.05 and 0.50 g L^{-1} gave similar results with that of control (Table 1, Figure 1). The high pH (9.3) in the culture medium caused by a high concentration of sodium bicarbonate (Figure 2) could have affected growth.

Biochemical compositions were quantified from the harvested biomass at the end of experiment. Table 2 shows that the highest carbohydrate (13.79 % dry weight) and total lipid (18.71 % dry weight) contents were found in the treatment with 0.05 g L^{-1} sodium bicarbonate. In general, microalgae use nutrient for support growth and synthesis of biochemical compositions (Lewin and Hellebust, 1978; Khoi *et al*., 2006). Growth of microalgae is therefore related with the nutrient concentration in the culture medium. Nutrient analysis in Figure 2 revealed that nitrate, orthophosphate and silicate continuously decreased during *C. gracilis* cultivation. Nitrate concentration in the algal culture medium at 60 h remained higher than 18.76 mg L^{-1} hence nitrate was probably not the limiting factor for *C. gracilis* growth. Similar results were also reported in other microalgal species (Harrison *et al*., 1990; Cruz *et al*., 2006; Niraula *et al*., 2007). Silicate concentrations were depleted alongside with growth of *C. gracilis* due to the requirement of silica for frustule (shell)

formation (Harrison *et al*. 1980). Consequently, orthophosphate concentrations in all treatments decreased rapidly within 24 h related with the decrease in cell density of *C. gracilis* (Fig 2). Since phosphate is a composition of reducing agents (ATP, NADPH) for providing a source of energetic electron (Krichnavaruk *et al*., 2005), limitation of phosphate is therefore an important factor influencing growth of the microalgae.

Table 1. Maximum cell density (x10^6 cells ml^{-1}) and specific growth rate (h^{-1}) of *Chaetoceros gracilis* cultured in different sodium bicarbonate ($NaHCO_3$) concentrations

Treatment	Maximum cell density (x10^6 cells ml^{-1})	Specific growth rate (h^{-1})
Control	8.59 ± 0.45^{b}	$9.81 \times 10^{-2} \pm 0.01^{b}$
0.05 g L^{-1}	8.49 ± 0.89^{b}	$10.73 \times 10^{-2} \pm 0.01^{b}$
0.50 g L^{-1}	8.44 ± 0.27^{b}	$10.82 \times 10^{-2} \pm 0.01^{b}$
5.00 g L^{-1}	5.05 ± 0.23^{a}	$6.91 \times 10^{-2} \pm 0.02^{a}$

Data are expressed as mean ± SD (*n*=5).
Values within the same column sharing a common superscript are not significantly different (P>0.05) and a<b<c.

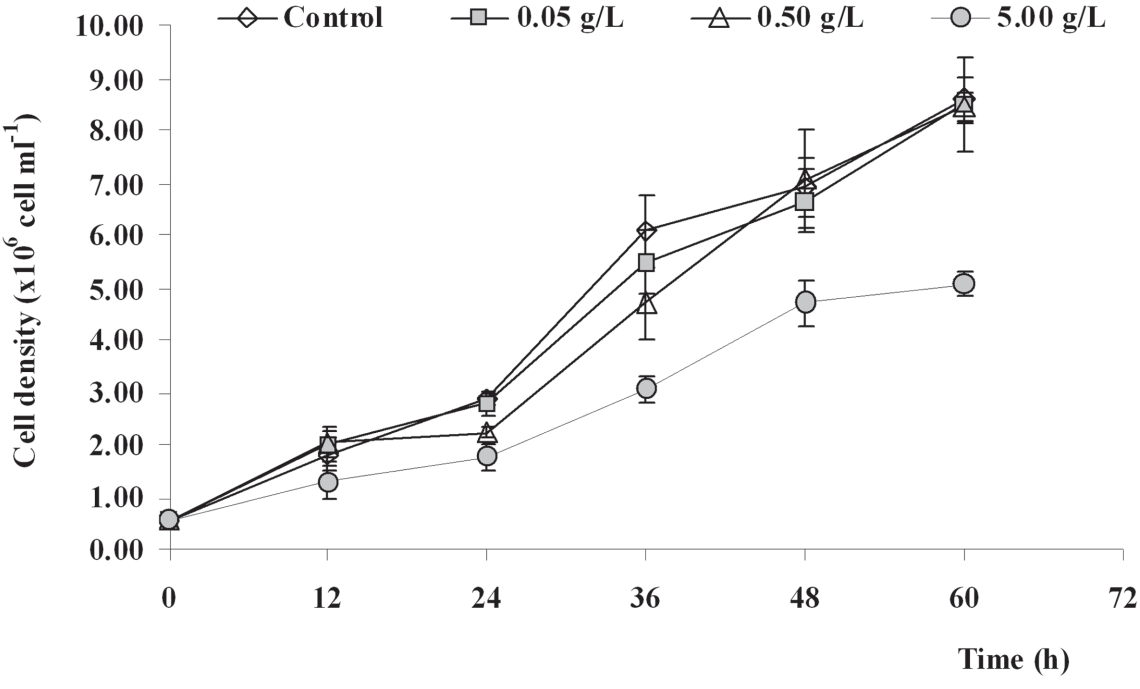

Figure 1. Cell density of *Chaetoceros gracilis* cultured in different sodium bicarbonate ($NaHCO_3$) concentrations

Table 2. Biochemical composition (% dry matter) of *Chaetoceros gracilis* cultured in different sodium bicarbonate ($NaHCO_3$) concentrations

Treatment	Carbohydrate	Protein	Total lipids
Control	7.47 ± 0.90 ^{b}	53.96 ± 0.72 ^{d}	12.40 ± 0.66 ^{c}
0.05 g L^{-1}	13.79 ± 0.94 ^{d}	50.81 ± 0.75 ^{c}	18.71 ± 0.57 ^{d}
0.50 g L^{-1}	9.08 ± 0.76 ^{c}	46.08 ± 0.94 ^{b}	11.47 ± 0.98 ^{b}
5.00 g L^{-1}	5.21 ± 0.84 ^{a}	39.37 ± 0.66 ^{a}	8.83 ± 0.41 ^{a}

Data are expressed as mean ± SD percentage of dry matter (n=5).
Values within the same column sharing a common superscript are not significantly different (P>0.05) and a<b<c.

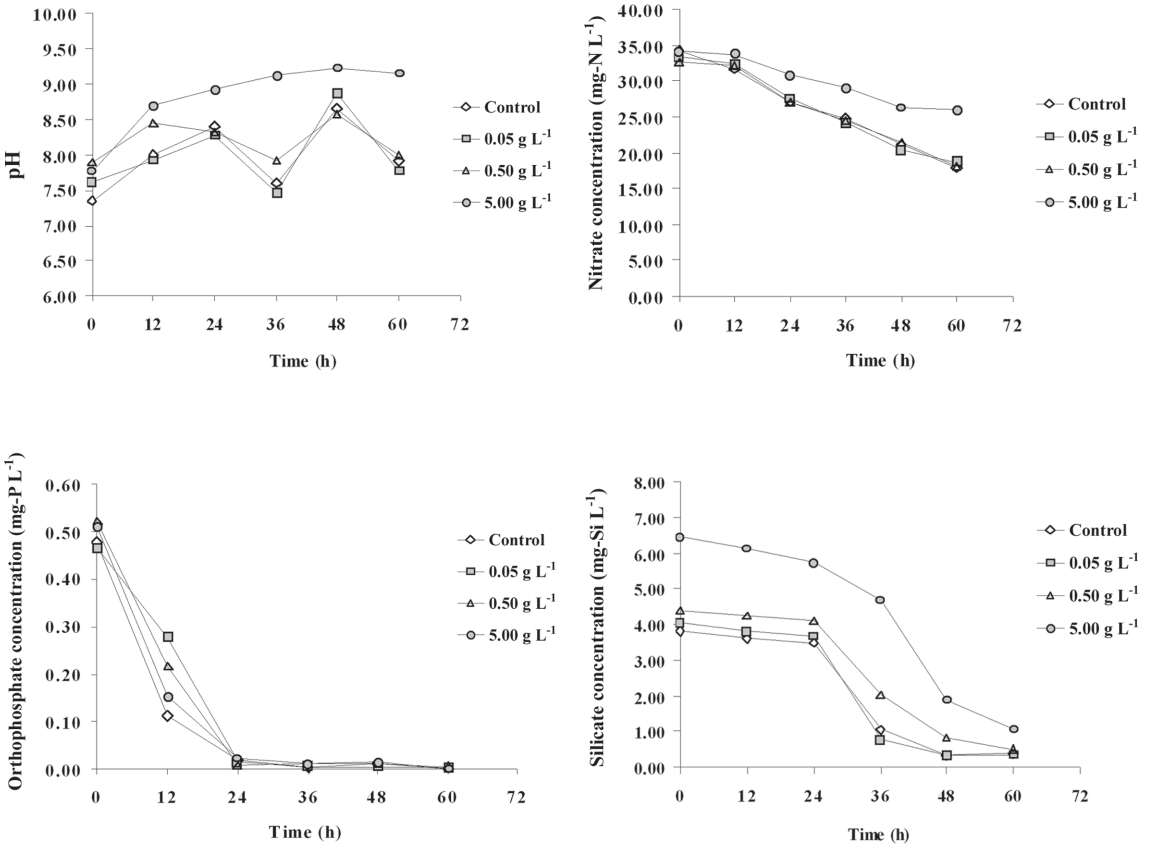

Figure 2. Nutrients (nitrate, orthophosphate and silicate) and pH in culture medium of *Chaetoceros gracilis* with different sodium bicarbonate ($NaHCO_3$) concentrations

Carbon is the main element in organic matter that is required for energy and carbon skeleton for growth of the microorganisms (Wen and Chen, 2003). Addition of inorganic carbon (CO2) in microalgae culture system has been reported to increase growth (Talling, 1974). Carbohydrate and lipid contents in the diatom Nitzschia inconspicud (Chu *et al*., 1996) as CO_2 are required for photosynthesis. This study used sodium bicarbonate because it is easier to apply in hatchery or farm conditions without the requirement of expensive gas bottle and sophisticated gas mixing equipment for CO_2. In this study, the addition of sodium bicarbonate at the concentration of 0.05 g L^{-1} resulted in the highest accumulation of carbohydrate and total lipids in *C. gracilis*. Lipids are essential in aquatic animal nutrition. Lipids in shrimp diets do not only serve as an energy source, but also enhance growth of the organisms as they facilitate absorption of other fat soluble nutrients such as sterol and vitamins (Bottino *et al*., 1980). Lipids promote renovation of damaged tissue as they are significant components of cell and subcellular membranes (Bautista *et al*., 1991). However, a high concentration of sodium bicarbonate (5.00 g L^{-1}) was hazardous due to high pH which increased from 7.78 to 9.16 (Fig 2). This finding was supported by Chen and Durbin (1994), who reported that as the microalgae took up carbon in HCO^-_3 form and excreted OH^- ion, this would elevate the pH in culture medium. According to Dickman (1973), the addition of sodium bicarbonate in culture medium could increase the growth of the microalgae, while growth reduction (osmotic effect) was found at high concentrations (> 1 g L^{-1}). Results from this study suggested that the growth of *C. gracilis* was not affect by sodium bicarbonate between 0.05 – 0.50 g L^{-1} (Table 1). Rather at these concentrations sodium bicarbonate could enhance carbohydrate and total lipids composition of *C. gracilis* .

CONCLUSIONS

The addition of sodium bicarbonate at 0.05 g L^{-1} in modified f/2 medium could enhance carbohydrate and total lipid contents in *C. gracilis* but did not affect growth rate and maximum cell density. This report presented the potential of sodium bicarbonate supplement to improve nutritional values of microalgal live feed for marine animal larvae cultures.

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