EnvironmentAsia
The international journal published by the Thai Society of Higher Education Institutes on Environment

Available online at www.tshe.org/EA
EnvironmentAsia 5(1) (2012) 63-69

Biological Carbon Dioxide Assimilation Process using Marine Phytoplankton *Tetraselmis suecica* and Bivalve *Perna viridis*

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Abstract

The Biological CO_2 assimilation process using marine phytoplankton and marine bivalve was evaluated by carbon assimilation of the green mussel *Perna viridis* fed with *Tetraselmis suecica* under laboratory condition. Incorporation of carbon dioxide into phytoplankton biomass was performed through aeration. The experiment consisted of three treatments *i.e.* mussels without feeding (Control), mussels fed with *T. suecica* cultured with air (Treatment 1: T-Air), and mussels fed with *T. suecica* cultured with 1.5% CO_2 in air (Treatment 2: T-CO_2). The results showed that growth of mussels in T-Air and T-CO_2 was 22.4±4.0 mg/individual/day and 28.9±12.3 mg/individual/day, respectively, which was significantly higher than control (mussels without feeding). Growth of mussels in T-Air was significantly lower than in T-CO_2. Carbon content in shell (15.59±0.57% D.W.) and meat (38.28±1.72% D.W.) of mussels fed with aerated *T. suecica* (T-Air) was significantly higher than that found in mussels fed with 1.5% CO_2 *T. suecica* (14.2±0.47 and 36.61±0.43% D.W. in shell and in meat, respectively) ($p \leq 0.05$). With T-Air, 1.95±0.27 and 9.36±1.24% of carbon from *T. suecica* cells was assimilated into shell and meat of the mussel, respectively, while in T-CO_2, carbon assimilation from *T. suecica* cells in shell and meat was 2.19±0.55 and 11.22±2.76% respectively.

Keywords: CO_2 assimilation; *Tetraselmis suecica*; *Perna viridis*

1. Introduction

Attempts to reduce CO_2 emission to the environment is presently one of the most popular topics of climate change environmental research (Houghton, 2004). Trapping CO_2 by biological processes is generally through photosynthesis in which CO_2 is fixed into carbohydrate. Apart from terrestrial plant, phytoplankton have been intensively studied for CO_2 removal process (Field *et al*., 1998). However, the bottleneck of this process is how to utilize or deposit the phytoplankton biomass produced from the process (Hayashi *et al*., 1995; Yue and Chen, 2005). The green phytoplankton *Chlorella*, the most common phytoplankton for CO_2 removal research, has a very small cell size (4-5 microns). Harvesting *Chlorella* biomass hence cannot be performed by simple filtration but requires the expensive centrifugal process. Moreover, utilization of phytoplankton produced is still limited. In fact, use of phytoplankton biomass as fertilizer might not applicable because decomposition of phytoplankton finally releases CO_2 back to the atmosphere while extraction of lipids from algal biomass for biodiesel production is still not economical feasible (Chisti, 2007).

The purpose of this study is to evaluate the CO_2 assimilation process using phytoplankton and bivalve under laboratory conditions. The general concept of the process is to trap CO_2 by a CO_2 fixation process (photosynthesis), then carbon is further transferred to deposit in plankton feeding bivalve which is the next level in aquatic food chain. Finally, the conversion rate of carbon transfer from CO_2 to bivalve was evaluated.

2. Materials and Methods

2.1. Algal medium and culture conditions

T. suecica strain was obtained from Plankton collection, Marine Science Department, Chulalongkorn University. Guillard medium (F/2) for *T. suecica* cultivation was 30 psu seawater enriched with 8.82×10^{-4} M $NaNO_3$, 3.62×10^{-5} M NaH_2PO_4 H_2O, 1.06×10^{-4} M Na_2SiO_3 $9H_2O$, 1.17×10^{-5} M $FeCl_3$ $6H_2O$, 1.17×10^{-5} M

$Na_2EDTA\,2H_2O$, $3.93x10^{-8}$ M $CuSO_4\,5H_2O$, $2.60x10^{-8}$ M $Na_2MoO_4\,2H_2O$, $7.65x10^{-8}$ M ZnSO4 $7H_2O$, $4.20x10^{-8}$ M $CoCl_2\,6H_2O$, $9.10x10^{-7}$ M $MnCl_2\,4H_2O$, $2.96x10^{-7}$ M thiamine HCl (vit. B_1), $2.05x10^{-9}$ M, biotin (vit. H), and $3.69x10^{-10}$ M cyanocobalamin (vit. B_{12}) (Guillard and Ryther, 1962).

Stock culture of *T. suecica* was maintained under continuous illumination at 5,000 lux with aeration. The temperature was 29±1°C. Scaling up of *T. suecica* culture to 5 L was performed using aeration by either air or 1.5% CO_2 in air. Biomass of *T. suecica* was finally used as live food for green mussel culture. Carbon, hydrogen and nitrogen (CHN) contents of *T. suecica* dried biomass were determined after cell drying at 80°C for 24 hr and analyzed with CHN analyzer (CHNS/O Analyzer, Perkin Elmer PE2400 Series II) at Scientific and Technological Research Equipment Center, Chulalongkorn University, Thailand.

2.2. Green mussel culture system

Green mussel (*Perna viridis*) was collected from Bandon Bay, Suratthani province, Thailand, and transferred to Suratthani Rajabhat University where the experiments were conducted. Mussels were separated from clumps by cutting their byssus thread to obtain individuals. Each mussel was affixed on a bamboo stick using epoxy glue, hence it could be handled and weighted individually during the experiment. Green mussels were acclimated for one week under laboratory conditions (29±1°C, 30 ppt. salinity seawater and pH 8.2±0.04). The experimental unit was the closed recirculating aquaculture system made from plastic tanks, each tank contained 10 L of 30 psu seawater and 5 mussels. Production of *T. suecica* as live food for mussels was accomplished under batch culture in 5 L bottles as previously described. At late exponential growth phase, *T. suecica* cells were transferred to a dripping bottle that hanged over the mussel tank. Algal cells, 0.5-1.1x10^6 cells/ml, were continuously dripped into the mussel tank at approximately 120 ml/hr. Wastewter from mussel tank was removed and treated in a 50 L seaweed tank (*Gracilaria* sp.) to eliminate nitrogen waste before being returned back to the mussel tank. Calcification rate of the bivalves was evaluated from decreasing of calcium in the water using EDTA titration (APHA, 1998).

With the experiment, treatments consisted of green mussel culture tanks fed with *T. suecica* from an air-culture system (T-air) and a 1.5% CO_2 culture system (T-CO_2) while green mussel without *T. suecica* feeding was assigned as control (C), all with 3 replicates. Number of phytoplankton and water quality *i.e.* pH, temperature, ammonia and alkalinity were monitored on a daily basis. Shell length and weight of the mussels were measured every 10 days. At the end of experiment, fresh weight, dry weight and C:H:N content in dry mass of the mussels were analyzed.

2.3. Evaluation of CO_2 assimilation of the phytoplankton-bivalve system

According to Redfield's Stoichiomethic equation (1), 106 mole of CO_2 is converted to 1 mole of phytoplankton. Hence, in theory, the carbon content in phytoplankton cell is 35.8% and 1 g of CO_2 is converted to 0.76 g of phytoplankton. With this study, evaluation of carbon dioxide fixation was based on carbon analysis (CHN analyzer) in biomass of phytoplankton (*T. suecica*) cells and in shell and meat of the mussel. Initial carbon transferred from phytoplankton to mussel was estimated from carbon content in dry weight of phytoplankton cells consumed by mussel throughout 30 days of the experiment (2). Thereafter, carbon assimilation in mussel weight gain was evaluated by an increase of carbon content in dry meat (3) and in shell (4) during the experiment. Due to the fact that carbon deposit in shell came from two sources in which 10% is from feed and 90% is from surrounding water (90%) (McConnaughey *et al*., 1997; Gillikin *et al*., 2006), so the dry shell weight gain (g C) from dietary organic carbon and from water was calculated by equation 5 and 6.

$$106\ CO_2 + 120\,H_2O + 16\,HNO_3 + H_3PO_4 \rightarrow (CH_2O)_{106}(NH_3)_{16}PO_4 + 137\,O_2 \quad (1)$$

$$\text{Carbon in } T.suecica \text{ fed by mussel (g)} = \frac{\text{g D.W. of } T.suecica \text{ fed by mussel} \times \%\text{C in cells}}{100} \quad (2)$$

$$\text{Carbon gain in mussel's meat (g)} = \frac{\text{g Dry meat weigh t gain} \times \%\text{C in meat}}{100} \quad (3)$$

$$\text{Carbon gain in mussel's shell (g)} = \frac{\text{g Dry shell weight gain} \times \%\text{C in shell}}{100} \quad (4)$$

$$\text{Carbon from } T.suecica \text{ deposited in shell (g)} = \frac{\text{g Carbon of shell weight gain} \times 10}{100} \quad (5)$$

$$\text{Carbon from water deposited in shell (g)} = \frac{\text{g Carbon of shell weight gain} \times 90}{100} \quad (6)$$

The experiment data were presented as mean and standard deviation of three replicates. Significant different of means was determined by either t-test or ANOVA with Duncan's multiple range test at 95% confidence interval.

3. Results and Discussion

3.1. Growth and biomass of Tetraselmis suecica

Growth of *T. suecica* cultured with air or 1.5% CO_2 is shown in Fig 1. We found that growth performance of *T. suecica* dramatically increased by 187% with maximum cell density of $2.39x10^6$ cells/ml or 0.71 g dry weight/L when supplemented with 1.5% CO_2. In contrast, *T. suecica* cultured with pure air had significantly lower density of $1.27x10^6$ cells/ml or 0.38 g dry weight/L. Hence, CO_2 fixation rate of *T. suecica* could be enhanced by 315% as the carbon fixation rate increased from 3.54 g-$CO^2/m^3/h$ to 11.16 g-$CO_2/m^3/h$ when 1.5% CO_2 was applied instead of pure air.

3.2. Growth of Perna viridis under laboratory condition

Growth performance of green mussels measured every 10 days is illustrated in Fig 2. The average initial weight of green mussel of control, T-air and T-CO_2 condition was 1.83±0.09, 1.79±0.13 and 1.76±0.09 g respectively. Environmental parameters during the experiment were 26.3-28.3 ^oC, pH 8.1-8.3, 0-0.5 mg-NH_4^+/L and 110-120 mg/L alkalinity. During the 30 days experiment, the weight of mussels in T-air and T-CO_2 was increased to 2.46±0.17 and 2.63±0.36 g, respectively. In control without feeding, the weight of mussels increased only in the first 12 days, thereafter growth was clearly declined.

With 30 days cultivation, the growth rate of mussels without feeding (control) was only 7.4±3.5 mg/individual/day which was significantly lower ($p \leq 0.01$) than treatments. Statistical analysis revealed that growth of green mussels in T-air (22.4±4.0 mg/individual/day) was significantly lower ($p \leq 0.05$) than T-CO_2 (28.9±12.3 mg/individual/day). This growth rate was rather similar to 29 mg/individual/day of *P. viridis* in flow-through system supplemented with *Chaetoceros, Skeletonema* and microencapsulated feed (Havanont and Chaikul, 1999). In fact, bivalve in nature feed on a variety of suspended particles such as bacteria, phytoplankton,

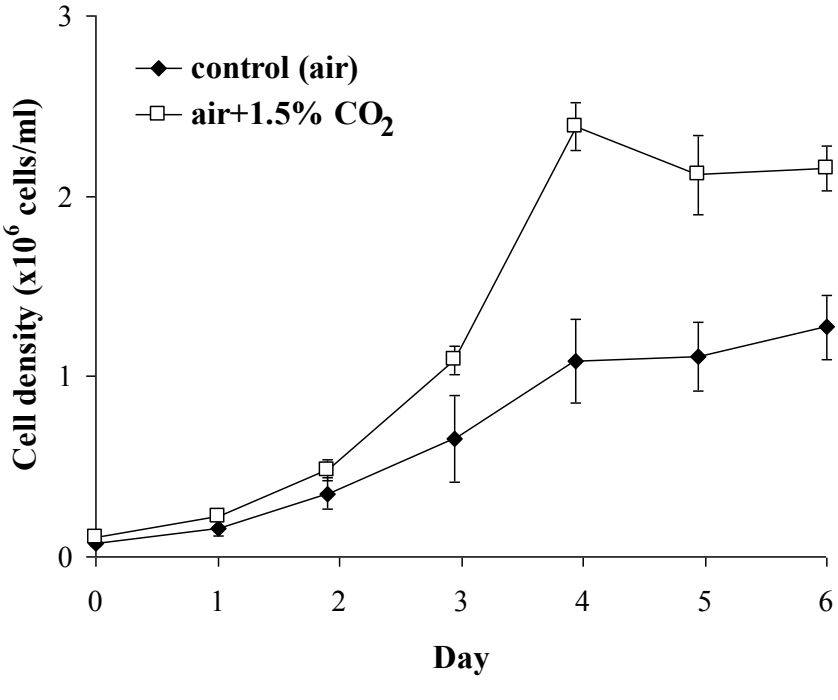

Figure 1. Growth of *Tetraselmis suecica* cultured with air (◆) or 1.5% CO_2 (□) under laboratory condition. Each data point was mean ± SD from 3 replicates.

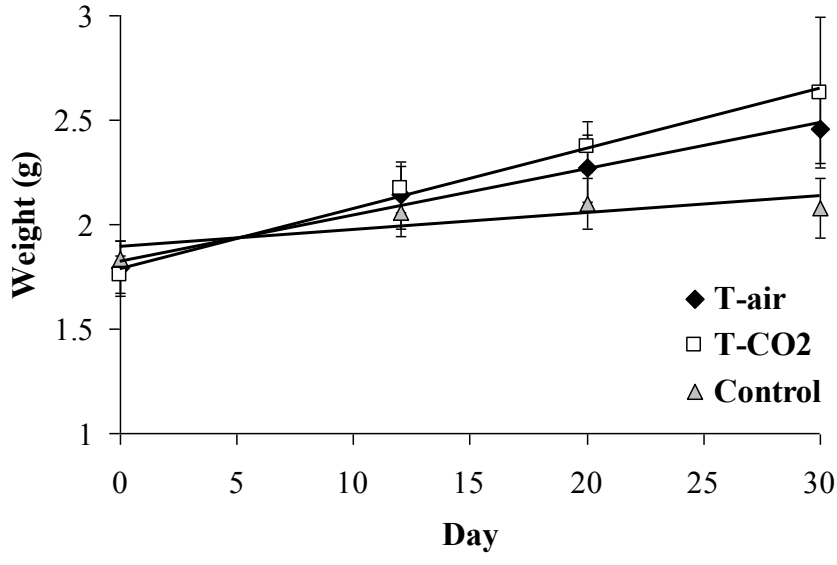

Figure 2. Growth of mussels fed with *T. suecica* cultured in air (T-Air), 1.5% CO_2 (T-CO_2) and control without feeding under laboratory condition. Each data point was mean± SD from 3 replicates. Trend lines were derived from regression analysis.

micro zooplankton or detritus (Gosling, 2003). The results in our study suggested that the culture condition was appropriate for growth of the mussel, at least equally with the flow-through system.

Due to the fact that the main composition of bivalve shell is calcium carbonate, Bevelander (1952) demonstrated that calcium in the water could be rapidly incorporated into the mineral component of the marine and freshwater bivalve shell. Deposition of carbonate ion in bivalve shell, as described in Furuhashi *et al*. (2009), could be another carbon sink for the carbon dioxide assimilation process. The results from this study showed that the calcification rate of green mussel without feeding was 5.04±4.36 mg-Ca/individual/day. This value was significantly lower ($P \leq 0.01$) than 17.63±4.36 and 23.5±4.07 mg-Ca/individual/day for T-air and T-CO_2, respectively.

Carbon hydrogen and nitrogen content in *T. suecica* cells and in mussels are shown in Table 1. We found that the carbon content of *T suecica* cells was 38.73% and 42.72% for T-air and T-CO_2, respectively. These values were higher than theoretical value of 35.8% according to Redfield's equation (1). CHN analysis results indicated that the C:H:N ratio of *T. suecica* cultivated

Table 1. C,:H, and:N content (Means± SD) of *Tetraselmis suecica* and green mussel (*Perna viridis*) under various culture conditions.

Sample	CHN composition (g/100g dry weight)		
	C	H	N
T. suecica (cultured with air)	38.73±0.22^{d}	6.64±0.18^{d}	4.37±0.12^{d}
T. suecica (culture with 1.5% CO_2)	42.72±0.28^{e}	7.4±0.22^{f}	2.47±0.14^{c}
Green mussel **meat** from nature	42.11±1.01^{e}	6.93±0.42^{e}	10.23±0.28^{g}
Green mussel **meat** fed with *T. suecica*-air [T-air]	38.28±1.72^{d}	6.46±0.37^{cd}	9.82±0.62^{f}
Green mussel **meat** fed with *T. suecica*-1.5% CO_2 [T-CO_2]	36.61±0.43^{c}	6.29±0.35^{c}	9.44±0.15^{e}
Green mussel **shell** from nature	16.31±0.74^{b}	1.28±0.34^{b}	2.05±0.44^{b}
Green mussel **shell** fed with *T. suecica*-air [T-air]	15.59±0.57^{b}	1.19±0.2^{b}	1.81±0.3^{b}
Green mussel **shell** fed with *T. suecica*-1.5% CO_2 [T-CO_2]	14.2±0.47^{a}	0.84±0.16^{a}	1.14±0.23^{a}

Remark: Different superscript letters in each column indicate significant different ($P \leq 0.05$) after ANOVA-Duncan analysis.

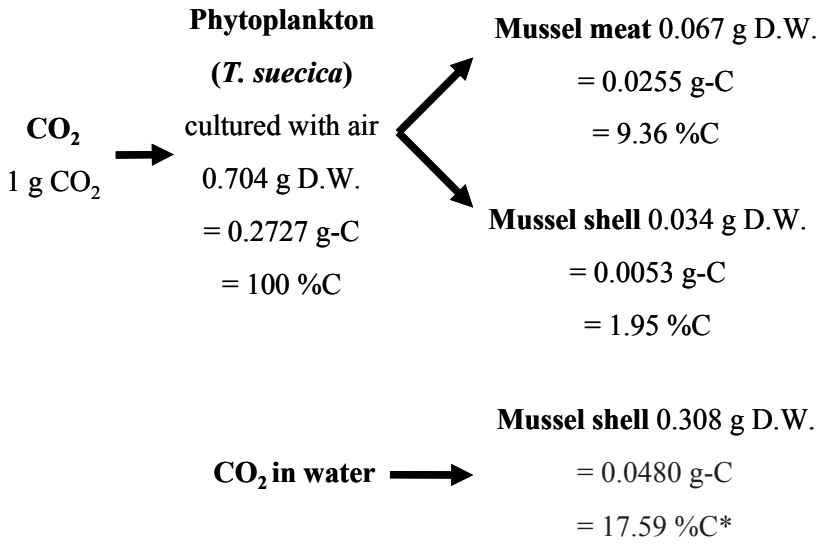

Figure 3. Carbon assimilation in phytoplankton and bivalve fed with aerated cultivation of *Tetraselmis suecica* (T-air), *carbon content in comparison with carbon in phytoplankton

in air was 8.9:1.5:1 while growing *T. suecica* in 1.5% CO_2 resulted in an increase of C:H:N ratio to 17.3:3:1. This could be implied that phytoplankton exposed to high CO_2 concentration had higher carbon assimilation efficiency. This results were somewhat similar to those found in *Skeletonema costatum* (Burkhardt *et al*., 1999) and *Spirulina platensis* (Gordillo *et al*., 1999). Moreover, Burkhardt and Riebesell (1997) and Andersen and Andersen (2006) suggested that the increase of CO_2 concentration altered C, N and P content in microalgal cells at different magnitude depending on microalgal species.

In green mussels, several factors affecting the biochemical composition such as diet, water temperature, maturation and culture area have been mentioned (Li *et al*., 2007; Dunstan *et al*., 1999; Linehan *et al*., 1999). With our results, the highest carbon content in meat, 42.11±1.01 g/100g dry weight, was found in green mussel collected from natural source. This was following by carbon content in T-air (38.28±1.72 g/100g dry weight) and T-CO_2 (36.61±0.43 g/100g dry weight) respectively (Table 1).

3.3. Efficiency of biological CO_2 assimilation using phytoplankton and bivalve

The biological carbon dioxide assimilation process was evaluated with carbon deposition from phytoplankton into bivalve. As illustrated in Fig. 3, from 1 g of CO_2, the percentage of carbon assimilation in mussel was 11.31%, of which 9.36% was incorporated in meat and 1.95% was in shell. With T-CO_2 (Fig. 4), carbon

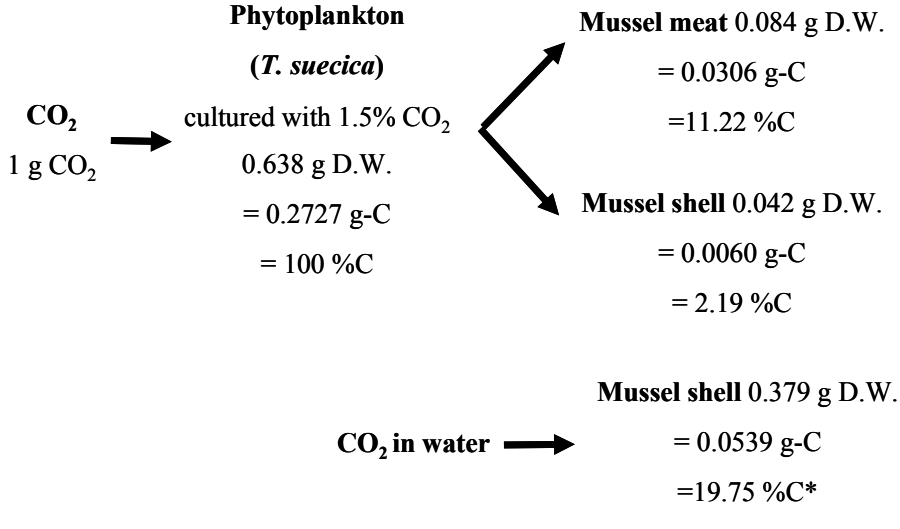

Figure 4. Carbon assimilation in phytoplankton and bivalve fed with 1.5% CO_2 cultivation of *Tetraselmis suecica* (T-CO_2), *carbon content in comparison with carbon in phytoplankton

deposited in mussels was 13.41% (11.22% in meat and 2.19% in shell). In general, it could be presumed that approximately 11.21-13.38% of carbon supplied to *T. suecica* culture was incorporated into mussel biomass.

However, source of carbon assimilated in the shell was not only came from feeding but it also came from water through calcification process. Results illustrated that 17.59-19.75% of carbon in the shell was assigned as unaccounted source in which the major part was came from calcification process. The carbon from water must be included when considering the overall CO_2 assimilation process of phytoplankton and bivalve. Other unaccounted carbon was presumably referred to respiration and other processes. The energy loss through trophic levels of the food chain are rather similar to the ordinary food chain which is approximately 90% (Odum and Barett, 2005). In addition, carbon loss as CO_2 from shellfish respiration might be recycled as CO_2 supply in phytoplankton culture.

In conclusion, our study illustrated that supplying 1.5% CO_2 with aeration increased the carbon content of *T. suecica*. Green mussel was successfully cultivated in the recirculating system and growth of the mussel indicated the success of carbon fixation process. It was found that the percentage of carbon assimilation from *T. suecica* into mussel was 11.31-13.41%. Within the mussel biomass, approximately 9.36-11.22% of carbon was deposited in meat while 1.95-2.19% was found in shell. Larger carbon deposition, 17.59-19.75%, was from surrounding water through the calcification process. As meat of the mussel can be utilized as food and carbon can be stored long-term as shell, hence carbon assimilation using phytoplankton and bivalve could be considered as an alternative way of carbon dioxide mitigation process.

Acknowledgement

This research is supported by Ratchadaphiseksomphot Endowment Fund-Climate Change Cluster, Chulalongkorn University (CU-CLUSTER-Climate-1-16-53). Authors also thank Suratthani Rajabhat University, Center of Excellence for Marine Biotechnology, Chulalongkorn University and National Center for Genetic Engineering and Biotechnology for partial in kind support and Assoc. Prof. Somkiat Piyatiratitivorakul for useful suggestions. A Ph.D. scholarship from Faculty Development Scholarship, Office of the Higher Education Commission, Royal Thai Government for C. Chairattana is also appreciated.

References

Andersen T, Andersen FØ. Effects of CO_2 concentration on growth of filamentous algae and *Littorella uniflora* in a Danish Softwater lake. Aquatic Botany 2006; 84: 267-71.

APHA. Standard methods for the examination of water and wastewater. 20th ed. APHA, AWWA, WPCF, Washington DC, USA. 1998; 3-64.

Bevelander G. Calcification in mollusk III. Intake and deposition of Ca45 and P32 in relation to shell formation. The Biological Bulletin (Woods hole) 1952; 102: 9-15.

Burkhardt S, Riebesell U. CO_2 availability affects elemental composition (C: N: P) of the marine diatom *Skeletonema costatum*. Marine Ecology Progress Series 1997; 155: 67-76.

Burkhardt S, Zondervan I, Riebesell U. Effect of CO_2 concentration on C: N: P ratio in marine phytoplankton: A species comparison. Limnology and Oceanography 1999; 44: 683-90.

Chisti Y. Biodiesel from phytoplankton. Biotechnology Advances 2007; 25: 294-306.

Dunstan GA, Olley J, Ratkowsky DA. Major environmental and biological factors influencing the fatty acid composition of seafood from Indo-Pacific to Antarctic waters. Recent Research Developments in Lipid 1999; 3: 63-86.

Field CB, Beherfeld MJ, Randerson JT, Falkowski P. Primary production of the biosphere: Integrating terrestrial and oceanic components. Science 1998; 281: 237-40.

Furuhashi T, Schwarzinger C, Miksik I, Smrz M, Beran A. Molluscan shell evolution with review of shell calcification hypothesis. Comparative Biochemistry and Physiology, Part B 2009; 154: 351-71.

Gillikin DP, Lorrain A, Bouillon S, Willenz P, Dehairs F. Shell carbon isotopic composition of *Mytilus edulis* shells: relation to metabolism salinity, $\delta^{13}C_{DIC}$ and phytoplankton. Organic Geochemistry 2006; 37: 1371-82.

Gosling E. Bivalve molluscs: biology, ecology and culture. Blackwell Publishing, Oxford, UK. 2003; 118-22.

Gordillo FJ, Jiménez C, Figueroa FL, Niell FX. Effects of increased atmospheric CO_2 and N supply on photosynthesis, growth and cell composition of the cyanobacterium *Spirulina platensis* (Arthrospira). Journal of Applied Phycology 1999; 10(5): 461-69.

Guillard RRL, Ryther JH. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* Cleve. Canadian Journal of Microbiology 1962; 8: 229-39.

Havanont V, Chaikul SL. Effect of salinity on growth and survival of green mussel (*Perna viridis*, Linnaeus). Technical paper 12/1999. Department of fisheries, Thailand 1999; 1-14.

Hayashi NR, Ishida T, Peerapornpaisal Y, Igarashi Y, Kodama T. Effect of carbon dioxide concentration on the growth and rubisco activity of a thermophilic Cyanobacterium, *Chroococcidiopsis* sp. strain TS-821. Journal of Fermentation and Bioengineering 1995; 80(5): 507-9.

Houghton JT. 2004. Global warming: the complete briefing. Cambridge University Press, Cambridge, UK. 2004; 14-30.

Li D, Zhang Y, Sinclair AJ. Seasonal variations of lipid content and composition in *Perna viridis*. Lipids 2007; 42: 739-47.

Linehan LG, O'Connor TP, Burnell G. Seasonal variation in the chemical composition and fatty acid profile of Pacific oysters (*Crassostrea gigas*). Food Chemistry 1999; 64: 2111–214.

McConnaughey TA, Burdett T, Whelan JF, Paull PK. Carbon isotopic in biological carbonates: respiration and photosynthesis. Geochimica et Cosmochimica Acta 1997; 61: 611-22.

Odum EP, Barrett GW. Fundamentals of ecology. 5th ed. Thomson Brooks Cole, Ontario, Canada. 2005; 77-137.

Yue L, Chen W. Isolation and determination of cultural characteristics of a new highly CO_2 tolerant fresh water phytoplankton. Energy Conversion and Management 2005; 46: 1868-76.

Received 6 August 2011
Accepted 12 September 2011

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