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สาขาวิชาวิทยาศาสตร์ทางทะเล ภาควิชาวิทยาศาสตร์ทางทะเล
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
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# EFFICIENCY OF CARBON DEPOSITION BY CARBON DIOXIDE ASSIMILATION OF MICROALGAE AND BIVALVES

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A Dissertation Submitted in Partial Fulfillment of the Requirements
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ชมพูนุท ชัยรัตนะ: ประสิทธิภาพการสะสมคาร์บอนโดยคาร์บอนไดออกไซด์แอสซิมิเลชันของสาหร่ายขนาดเล็กและหอยสองฝา (EFFICIENCY OF CARBON DEPOSITION BY CARBON DIOXIDE ASSIMILATION OF MICROALGAE AND BIVALVES) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: ศ.ดร.เปี่ยมศักดิ์ เมนะเศวต, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม: ดร.สรวิศ เผ่าทองศุข, ผศ.ดร.ศิริชัย ธรรมวานิช,106 หน้า.

การศึกษาผลของความเข้มข้นของคาร์บอนไดออกไซด์ต่อประสิทธิภาพการสังเคราะห์ด้วยแสง (Fv/Fm) ของสาหร่ายขนาดเล็ก *Isochrysis galbana* และ *Tetraselmis suecica* พบว่าสาหร่ายที่เลี้ยงโดยให้อากาศผสมคาร์บอนไดออกไซด์ที่ระดับความเข้มข้นร้อยละ 1.5 มีค่า Fv/Fm ประมาณ 0.8 ซึ่งเท่ากับสาหร่ายที่เลี้ยงโดยเป่าอากาศปกติ ในขณะที่สาหร่ายที่เลี้ยงโดยให้คาร์บอนไดออกไซด์สูงกว่าร้อยละ 1.5 มี Fv/Fm ลดลง การเลี้ยงสาหร่ายที่เป่าอากาศผสมคาร์บอนไดออกไซด์ร้อยละ 1.5 พบว่าสาหร่ายมีการเติบโตสูงกว่าการเลี้ยงโดยเป่าอากาศปกติและอากาศผสมคาร์บอนไดออกไซด์ร้อยละ 15 โดยเมื่อเลี้ยงสาหร่ายที่ระดับคาร์บอนไดออกไซด์ร้อยละ 1.5 ทำให้ *I. galbana* มีความหนาแน่นเซลล์สูงสุดเท่ากับ $1.87 \times 10^7$ เซลล์/มิลลิลิตร และ *T. suecica* มีความหนาแน่นเซลล์สูงสุดเท่ากับ $2.39 \times 10^6$ เซลล์/มิลลิลิตรตามลำดับ

การทดลองเลี้ยงหอยในห้องปฏิบัติการเพื่อศึกษาประสิทธิภาพการสะสมคาร์บอนโดยคาร์บอนไดออกไซด์แอสซิมิเลชันของสาหร่ายขนาดเล็กและหอยสองฝาพบว่าหอยนางรม หอยแครงและหอยนางรมปากจีบไม่สามารถเติบโตได้ในการศึกษาครั้งนี้ แต่การเลี้ยงหอยแมลงภู่โดยให้ *T.suecica* เป็นอาหารแบบต่อเนื่องด้วยขวดหยด โดยเลี้ยงหอยแมลงภู่ในห้องปฏิบัติการจำนวน 3 ชุดการทดลอง ได้แก่ ชุดที่ 1 เป็นชุดควบคุมไม่ให้อาหาร ชุดที่ 2 (T-air) ให้ *T. suecica* ที่เลี้ยงโดยเป่าอากาศปกติ เป็นอาหาร และชุดที่ 3 ($T\text{-}CO_2$) ให้ *T. suecica* ที่เลี้ยงโดยเป่าอากาศผสมคาร์บอนไดออกไซด์ร้อยละ 1.5 ผลการทดลองพบว่าชุด T-air มีอัตราการเติบโต 22.33±4.0 มิลลิกรัม/วัน/ตัว ซึ่งต่ำกว่าชุด $T\text{-}CO_2$ ที่มีอัตราการเติบโต 28.9±12.3 มิลลิกรัม/วัน/ตัว อย่างมีนัยสำคัญทางสถิติ ($p \leq 0.05$) โดยการเติบของหอยแมลงภู่ในทั้งสองชุดการทดลองสูงกว่าชุดควบคุมอย่างมีนัยสำคัญทางสถิติ ในชุด T-air มีการถ่ายทอดธาตุคาร์บอนจากสาหร่ายไปยังเปลือกและเนื้อหอยแมลงภู่ร้อยละ 1.95±0.27 และ 9.36±1.24 ตามลำดับ ส่วนชุด $T\text{-}CO_2$ มีการถ่ายทอดธาตุคาร์บอนจากสาหร่ายไปยังเปลือกและเนื้อหอยแมลงภู่ร้อยละ 2.19±0.55 และ 11.22±2.76 ตามลำดับ. และหอยแมลงภู่มีการสะสมคาร์บอนในสัดส่วนที่มากกว่าจากอาหารเพื่อใช้ในกระบวนการแคลซิฟิเคชัน กระบวนทางชีวภาพดังกล่าวนี้ได้ผลผลิตเป็นเนื้อหอย ส่วนเปลือกสามารถเป็นแหล่งเก็บคาร์บอนได้ในระยะยาว ดังนั้นการสะสมคาร์บอนด้วยกระบวนการทางชีวภาพที่ใช้สาหร่ายขนาดเล็กและหอยสองฝาจึงเป็นทางเลือกหนึ่งในการลดการปล่อยก๊าซคาร์บอนไดออกไซด์สู่บรรยากาศ

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| ภาควิชา วิทยาศาสตร์ทางทะเล | ลายมือชื่อนิสิต..... |
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CHOMPUNUT CHAIRATTANA : EFFICIENCY OF CARBON DEPOSITION BY CARBON DIOXIDE ASSIMILATION OF MICROALGAE AND BIVALVES. ADVISOR : PROF. PIAMSAK MENASVETA, Ph.D. CO-ADVISOR: SORAWIT POWTONGSOOK, Ph.D., ASST. PROF. SIRICHAI DHARMVANIJ, Ph.D., 106 pp.

Effect of $CO_2$ concentration on photosynthesis efficiency (Fv/Fm) was studied with *Isochrysis galbana* and *Tetraselmis suecica*. The results showed that the Fv/Fm of both species in pure air culture was similar to microalgae cultured in 1.5% of $CO_2$ (Fv/Fm = 0.8) and 1.5% CO2 clearly enhanced growth of the microalgae. While $CO_2$ concentration higher than 1.5% inhibited photosynthesis in both species. The maximum cell density of *I. galbana* and *T. suecica* cultivated in F/2 medium supplemented with 1.5% $CO_2$ were $1.87x10^7$ and $2.39x10^6$ cells/ml, respectively.

With this study growth rate of oyster blood cockle and hooded oyster could not be detected, biological $CO_2$ assimilation process using marine microalgae 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 microalgae biomass was performed through aeration. The experiment consisted of three treatments which were 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 significant higher than control (mussels without feeding). Growth of mussels in T-Air was significantly lower than T-$CO_2$, ($p \leq 0.05$). 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, 1.93±0.28% and 9.28±1.33% 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 2.21±0.56% and 11.17±2.81% respectively. Larger carbon deposition was from surrounding water through calcification process. As meat of the mussel can be utilized as food and carbon can be long term stored as shell, hence carbon assimilation using microalgae and bivalve could be considered as an alternative way of carbon dioxide mitigation process.

Department : Marine Science ..... Student's Signature .....
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# CHAPTER I

# INTRODUCTION

Climate change and related phenomenon such as rising seawater temperature, unexpected violent monsoon rains and flooding are suspected as the results from global warming which is the biggest environmental issue today. Emission of green house gases from human activities produces excessive amount of carbon dioxide to the atmosphere and being claimed as the course of rising temperature phenomenon. Sources of green house gases are roughly composed of 80% of gases from fossil fuel and the rest is from respiration process of the organisms (Houghton, 2004).

Recently, attempt to reduce $CO_2$ emission to the environment is one of the most popular topics of environmental research. Trapping $CO_2$ by biological process is generally through photosynthesis process in which inorganic carbon ($CO_2$) is fixed into carbohydrate. Parameters such as light, temperature, nutrients etc. are among the most essential components in the $CO_2$ fixation process via photosynthesis. Under the limitation of land use, improvement of the carbon fixation efficiency is essential for the success of $CO_2$ mitigation process. A part of terrestrial plant, marine plant especially seaweed, seagrass and microalgae that have higher capability to assimilate $CO_2$ has been intensively studied (Field *et al.,* 1998). Under natural condition, the "carbon dioxide concentrating mechanism" which is an important mechanism involved with $CO_2$ assimilation process of microalgae allows the microalgae to survive under low $CO_2$ environment (Moroney and Ynalvez, 2007). On the other hand, some microalgal species have the capability of growth under extremely high $CO_2$ concentration even with direct supply of flue gas which contains approximately 15% of $CO_2$ (Maeda *et al*., 1995). Optimization of $CO_2$ assimilation processes in several species of microalgae have been investigated (Hanagata *et al.,* 1992, Hayashi *et al*., 1995; Yue and Chen, 2005). However, the bottleneck of $CO_2$ removal using microalgae is how to utilize or deposit the algal biomass produced from the process. With the most common microalga for $CO_2$ removal research, the green microalga

*Chlorella* has very small cell size (4-5 microns) with thick cell wall. Harvesting *Chlorella* biomass hence cannot be performed by simple filtration but requires the expensive centrifugal process (Sung, 1999)

Although the algal cell is high in protein and nutrition, application of microalgal product is still limited. In fact, use of algal biomass as fertilizer might not be applicable for $CO_2$ removal because decomposition of algal cells finally releases $CO_2$ back to the atmosphere. Extraction of lipids from algal biomass for biodiesel production, which is under investigation elsewhere, is still not economically feasible (Chisti, 2007).

This study is an evaluation of the $CO_2$ assimilation process using microalga and filter feeder bivalve under laboratory conditions. General concept of the process is to trap $CO_2$ using microalgae and use the algal biomass for feeding bivalve. Diagram illustrated the proposed process is shown in Fig 1. In brief, microalgal biomass was produced by autotrophic cultivation with $CO_2$ supplement either from air or $CO_2$ cylinder. Thereafter, microalgal biomass was supplied as feed for marine bivalves. With this process, carbon from $CO_2$ was fixed by microalgae then transferred to be deposited in bivalve. Finally, the conversion rate of carbon transfer from $CO_2$ to microalgae and from microalgae to bivalves were investigated.

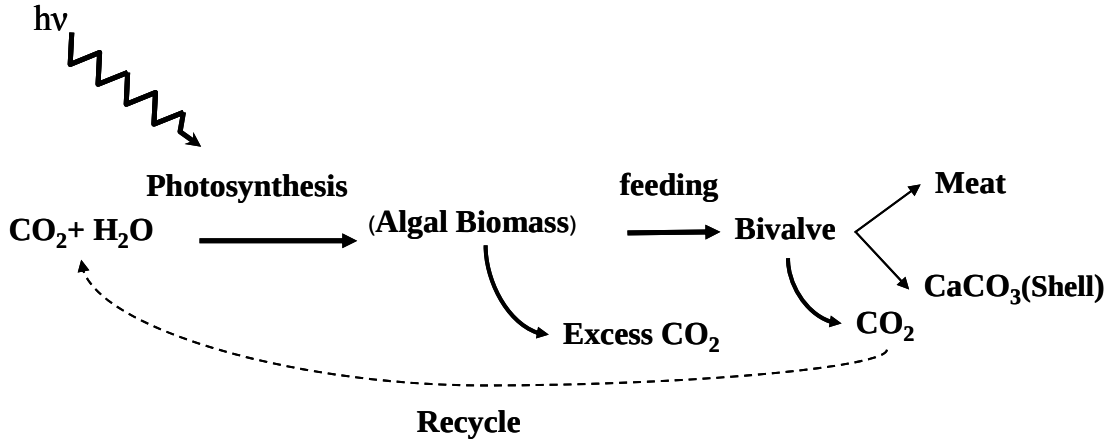



**Figure 1.1** The proposed biological system for $CO_2$ assimilation using microalga and bivalve

## Objectives

1. To determine the optimum $CO_2$ concentration ($VCO_2$/Vair) for growth of marine microalga in batch culture.
2. To determine the growth and feeding rate of marine bivalve under laboratory condition.
3. To evaluated the efficiency of $CO_2$ assimilation process comprise of $CO_2$ fixation process (photosynthesis) in microalgae and carbon deposit in plankton feeder bivalve.

## Scope of study

This study consisted of four parts. The first part was the optimization of carbon dioxide supplement on growth and production yield of the microalga (*Isochrysis galbana* and *Tetraselmis suecica*). The second part was the cultivation of bivalve (*Crassostrea belcheri*, *Anadara granosa*, *Saccostrea cucullata* and *Perna viridis*) fed with the microalga under laboratory condition. The third part was the evaluation of carbon deposition via calcification of bivalve. The final part was the determination of carbon assimilation of microalga and bivalve.

## Expected Outcome

An alternative process of photosynthesis $CO_2$ fixation and carbon deposition in shellfish that could be applied in large scale $CO_2$ assimilation process.

# CHAPTER II

# Literature Review

## 2.1 Global warming

Global warming is the result of rising atmospheric greenhouse gases concentration. The impact of global warming includes variation in precipitate distribution, disease outbreak, climatic disturbance, decrease of food production, seawater level, and many other malignance environmental problems (Bilanovic *et al*., 2009).

Greenhouse gases compose of various gases such as carbon dioxide ($CO_2$), methane ($CH_4$), nitrous oxide ($N_2O$), chlorofluorocarbons (CFCs) and vapor. The most important greenhouse gas is $CO_2$ that has been on an increase in the atmosphere. The main source of $CO_2$ is human activity. The increase of $CO_2$ has contributed about 72% to the enhancement of greenhouse effect to date (Houghton, 2004). The trace gases, principally $CH_4$, $N_2O$, and CFCs, are present at concentrations that are two to six orders of magnitude lower than that of $CO_2$ (Lashof and Ahuja, 1990).

The carbon cycle explains why $CO_2$ attains the dominant position among greenhouse gases. Transfer of carbon between number carbon reservoirs is called carbon cycle. Human and other organisms contributed to the carbon cycle by respiration such as breathing or decomposition in which carbon turned into $CO_2$. While photoautotroph organism is involved in the carbon cycle through photosynthetic process which returns $O_2$ to atmosphere (Odum and Barrett, 2005). However, the excess $CO_2$ emission by human activity disturb the balance of carbon cycle and results in the increasing of $CO_2$ in the atmosphere (Houghton, 2004). The record of carbon dioxide measurements shows the elevated carbon dioxide concentration from the past until now (Fig 2.1).

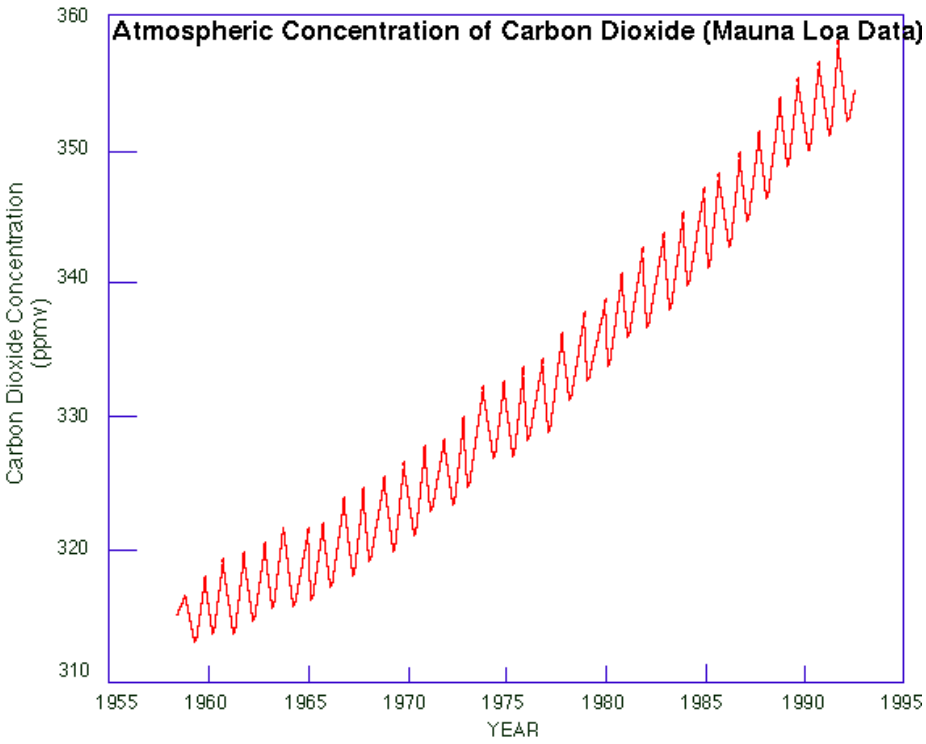



**Figure 2.1** Atmospheric carbon dioxide concentration. Note the steady increase in the concentration of atmospheric cabon dioxide since 1958 when measurement began at the Mauna Loa Observatory, Hawaii. The fluctuations correspond to the annual cycles of plants in the Northern Hemisphere: winter (a high level of carbon dioxide), when plants are not actively growing and absorbing carbon dioxide, and summer (a low level of carbon dioxide) when they are growing and absorbing carbon dioxide (David Keeling and Tim Whorf, Scripps Institution of Oceanography, La Jolla, California quoted by Raven and Berg, 2001)

As $CO_2$ plays an important role in photosynthesis pathway with sunlight and water (Odum and Barrett, 2005) thus biological carbon dioxide fixation is the significant option to fix $CO_2$ from flue gas. In general, $CO_2$ in the atmosphere is removed through natural process roughly $12\times10^9$ tons of $CO_2$ per year (Bilanovic *et al*., 2009). The capacity of natural process is not sufficient to cope with the increasing of $CO_2$ at present. The process to reduce the $CO_2$ emission is the prevalent research topic. Therefore Carbon dioxide mitigation technologies have been developed (Kawa, 1995) and various options for $CO_2$ capture from power plant mixed gases become one of the most widely study issue, nevertheless the waste from that process needs to be disposed or reused (Yeh and Bai, 1999; Diao *et al*., 2004). The $CO_2$ mitigation through photosynthesis process (such as agroforestry and microalgae

cultivation) is able to convert $CO_2$ into biomass, which can be used as biomass fuels or renewable energy such as biodiesel that can replace fossil fuels. However, biomass fuels could shift only little of the current fraction fossil fuel consumption (Benemann, 1992a).

## 2.2 Carbon dioxide mitigation using agroforestry

The violence of global warming impact from $CO_2$ emission influence accelerate the more attempt to find tool for reducing the $CO_2$ emission or enhancing efficiency of atmospheric carbon to biomass and other sinks (Fearnside, 2000). The forest plantation area in the world is 130 million hectare (Allan and Lanly, 1991 quoted by Kraenzel *et al*., 2003). The carbon storage of forest plantation is an estimated 11.8 Pg C (Winjum and Schroeder, 1997). In addition, biomass from agroforestry can be used as fuel directly or modified into other form of fuel. Biomass fuel has the potential to be the source of the renewable energy in the future because of large production economic viability with various social and environmental benefits (Johansson *et a1*., 1992). The $CO_2$ was captured by newly planted or regenerating forests that have been cultivated for 20–50 years or more depending on species, site conditions and the objectives of plantations (Hooda, 2007). Extending rotation and increasing the grow time before harvest can capture more carbon. Carbon could be sequestered in large amount in a short time period by increasing the rotation ages of softwood stands beyond commercially optimal ages (Bravo *et al*., 2008).

However, the nutrients and water available are the limiting factor of concern in $CO_2$ mitigation with agroforestry. Because of higher $CO_2$ concentration results in enhancing the growth of tree (Aber *et al*., 2001). The experiments of Nowak (2004) showed that elevated $CO_2$ levels results in an approximately 12% increase in net primary productivity.

## 2.3 $CO_2$ mitigation using microalgae

Due to the Global warming problem, which caused by the elevation of $CO_2$ in the atmosphere, many research works have been carried out in order to reduce $CO_2$ emission from industries. Among various $CO_2$ mitigation technologies, the biological methods using microalgae has been popularly investigated. The $CO_2$ mitigation by microalgae has many advantages such as high efficiency per unit area, requires only mild condition for $CO_2$ fixation, no requirement for further disposal of recovered $CO_2$ and $CO_2$ fixation can be performed directly with flue gases (Lee and Lee, 2003). Brown (1996) reported that the green alga *Monoraphizium minutum* can tolerate moderate concentration of SOx (200 ppm) and NOx (150 ppm) in laboratory culture, and that a well-engineered outdoor pond can easily achieve in excess of 90% $CO_2$ removal efficiency with pure $CO_2$ experiment. *Euglena glacilis* cultured with directly flue gas supplement in photo-bioreactor (Fig 2.2) was also reported (Chae *et al*., 2006). The other advantages of microalgae culture for $CO_2$ mitigation are 1) potentiality to convert $CO_2$ into useful organic compound 2) $CO_2$ fixation rate 10 times greater than terrestrial plants (Usui and Ikenouchi, 1997) and 3) more appropriateness in involving the $CO_2$ mitigation system into industrial process than higher plants photosynthetic systems (Murakami and Ikenouchi, 1997)

In order to obtain the high $CO_2$ fixation rate by microalgae numerous techniques for microalgae culture have been studied such as $CO_2$ concentration, culture medium reactor type. The three of most popular reactor pattern for microalgae $CO_2$ mitigation are open bioreactor (race way), photobioreactor and membrane photobioreactor.

### Raceway

The conventional design for $CO_2$ mitigation by microalgae reactor is a raceway pond. The pond can be delved and lined or constructed above ground with wall of brick or concrete. Sometime, fiberglass or another material can be used to construct raceway pond. The critical factor for raceway design for both commercial

and bench-scale is pond depth. The raceway pond depth should not exceed 90 cm (2.95 ft) because of mutual shading and microalgal settling which result in decreased growth and fixation productivities. The important device for raceway operation is paddle wheel for water mixing (Fig 2.2) (Borowitzka, 2005)

The $CO_2$ fixation by microalgae *Spirulina* LEB18 and *Chlorella kessleri* study was performed in 6 L of open raceway pond with a paddle wheel for water mixing in different $CO_2$ concentration (0.038-air, 6, 12 and 18% $CO_2$) . The result showed that *C. kesslerri* and *Spirulina* had a maximum mass production in 12% $CO_2$ (2.62 g/L) and pure air (4.82 g/L) respectively. The maximum $CO_2$ fixation was obtained in pure air culture for both of *C. kesslerri* and *Spirulina* (Priscila *et al*., 2011). Raceway for microalgae *Tetraselmis* sp. culture with $CO_2$ supplement was study by Olaizola *et al*. (1991). However, bubbling $CO_2$ gas in the medium is not the most efficient method of incorporating inorganic carbon (Vasquez and Heussler, 1985; Laws and Berning, 1991)

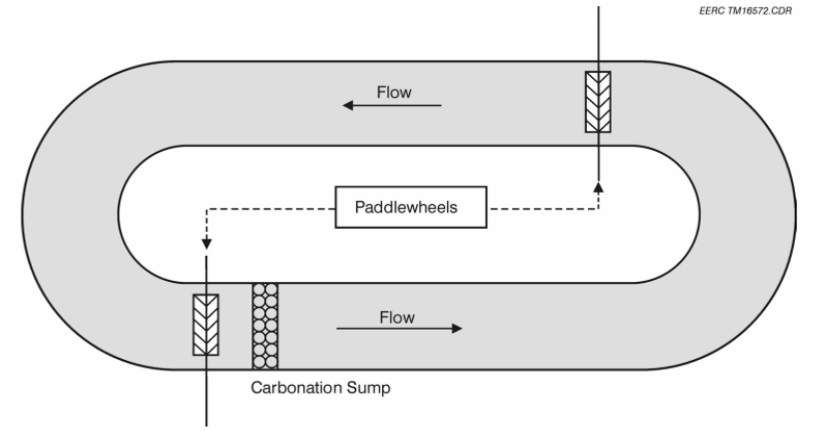



**Figure 2.2** Schematic diagram of raceway reactor (Stepan, 2002)

**Photo-bioreactor**

The photo-bioreactor is a closed (or mostly closed) vessel for phototrophic production with energy supply by electric light. The factors which may be considered for the configuration of photo-bioreactor design are

- how to provide the light

- how to circulate the algae
- the material for construction
- how to provide $CO_2$
- how to remove $O_2$
- how to control pH
- how to control temperature.

As the 100% transparent glass and acrylic are widely use to construct the photo-bioreactor (Behrens, 2005).

The photo-bioreactor is suitable for producing high value biomass with consistent quality product from batch to batch (Behrens, 2005). The useful products from photo-bioreactor operation are hydrogen, oxygen, carbohydrate or high value chemical or bioactive compounds from miroalgae (β-carotenes, polysaccharides, triglycerides, fatty acids, vitamins) (Mata, 2010)

The efficiency of $CO_2$ mitigation by microalgae evaluation can be performed in photo-bioreactor (Fig 2.3) but the $CO_2$ concentration in air streams on microalgal cell growth could be well controlled. Chui *et al*. (2008) studied on reduction of $CO_2$ by a high-density culture of *Chlorella* sp. in a semi continuous photo-bioreactor. The result showed the $CO_2$ removal by microalgae culture with 0, 2, 5, 10 and 15% $CO_2$ was 58, 27, 20 and16% respectively. In order to obtain the high microalgae production, thin layer photo-bioreactor (Fig 2.4) for $CO_2$ mitigation was designed. However, the thin layer bioreactor may have low capacity in dissolving free $CO_2$ due to escape gas from algal culture to atmosphere (Doucha *et al*., 2005).

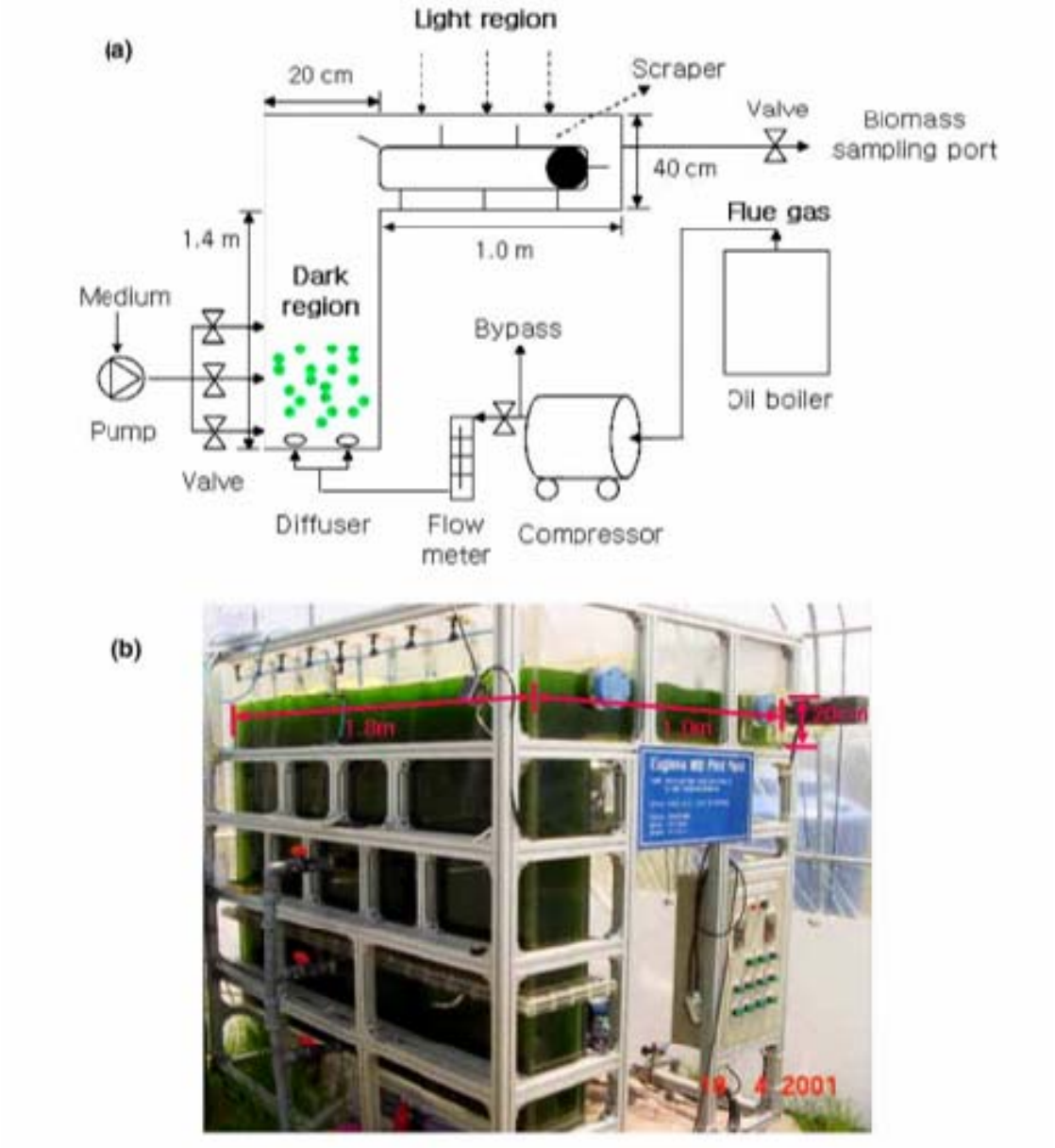



**Figure 2.3** Schematic diagram (a) and cross sectional photograph (b) of an innovative pilot-scale photo-bioreactor (Chae *et al*., 2006).

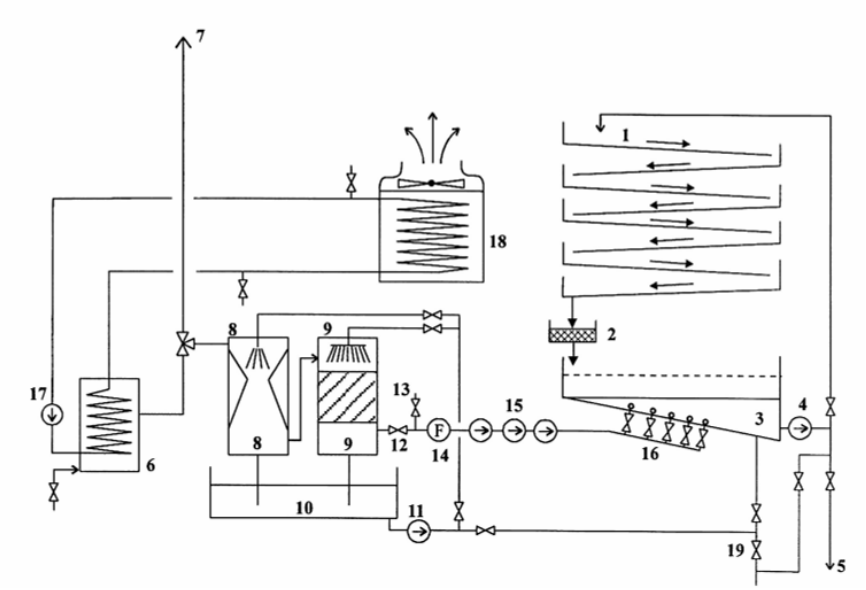



**Figure 2.4** Schematic diagram of an experimental photobioreactor for cultivation of microalgae using flue gas. (1) cultivationlanes; (2) sieve filter; (3)retention tank; (4) circulation pump; (5) harvesting of algae; (6) gas boiler; (7) chimney; (8) flue gas cooler; (9) flue gas scrubber; (10) cooling water tank; (11) water circulation pump; (12) flue gas valve; (13) air valve; (14) gas flow meter; (15) gas blower set; (16) saturation/aeration system; (17) hot water pump; (18) air cooler; (19) water in/out. (Doucha *et al*., 2005)

Another apparatus for gas supply in microalgae culture is a membrane which offer more advantage over the conventional dispersion methods as the result of the high interfacial area in membrane. The $CO_2$ removal by *Chlorella vulgaris* was done with membrane photo-bioreactor (Fig 2.5). In membrane photo-bioreactor the retention time of uniform gas bubbles was more than the conventional dispersal method leading to increased $CO_2$ fixation rate from 80 to 260 mg/L/hr (Cheng, 2006).

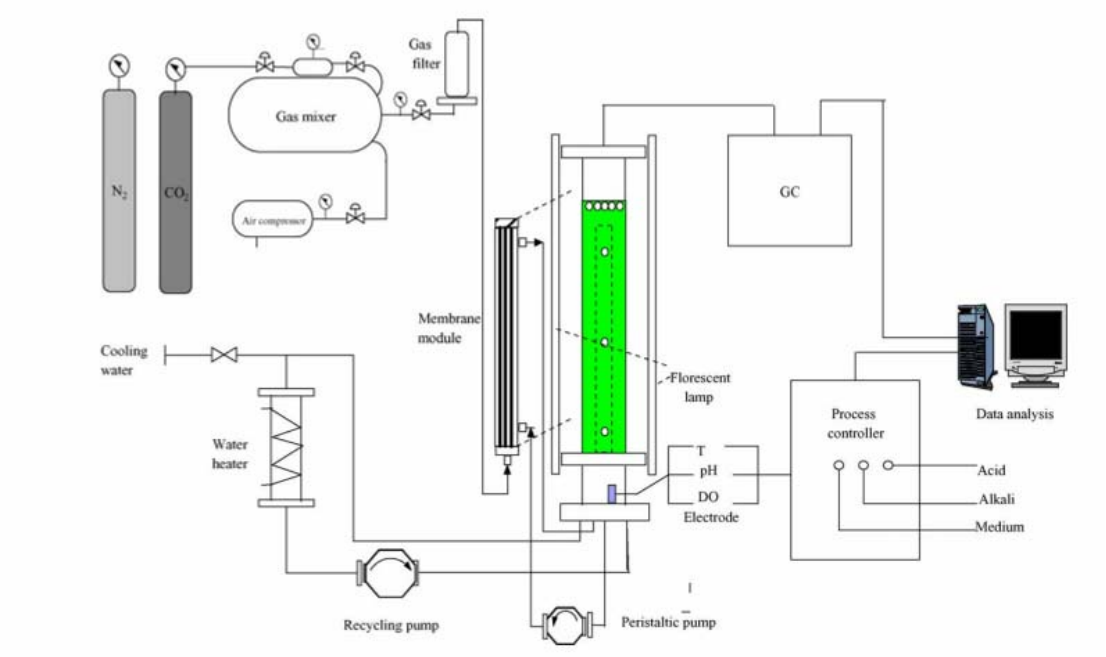



**Figure 2.5** Schematic diagram of membrane photo-bioreactor (Cheng, 2006)

### Microalgae culture under high $CO_2$

Microalgae and another photoautotroph organism which produce their own organic molecule through photosynthesis process**.** Carbon dioxide and water are converted into organic materials by photosynthesis process as shown in the following equation 1

$$6\ CO_2 + 12\ H_2O \rightarrow C_6H_{12}O_6 + 6\ O_2 + 6\ H_2O \qquad (2.1)$$

The process is starting on light reaction in thylakoid membranes. Solar energy is captured by photosystem in membrane to energize electrons then water is splitted and $O_2$ is released. Energized electron is transferred to electron transport chain, where the high molecule NADPH and ATP are produced from energy. Sugar is produced by Calvin cycle, the second stage of photosynthesis. Enzymes of the cycle combine $CO_2$ with RuBP and produce sugar molecule (Fig 2.6) (Campbell, 2006).

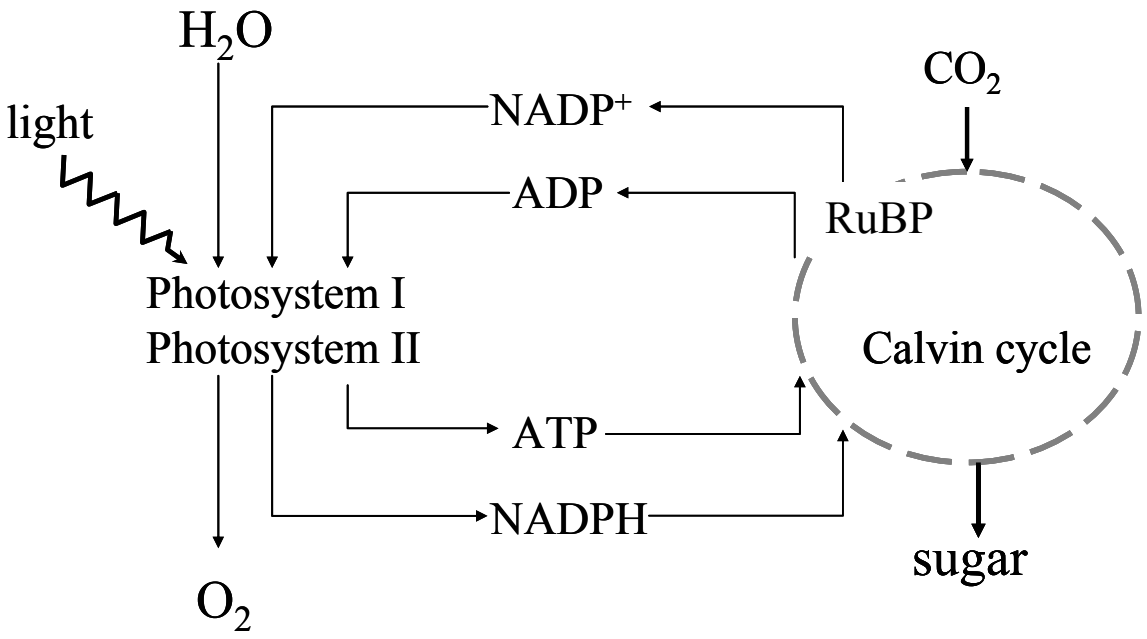



**Figure 2.6** A summary of the chemical processes of photosynthesis (modified from Campbell *et al*., 2006)

Over the last decade many technologies have been developed for microalgae culture. Due to the various usefulness of microalgae cell mass such as human food, animal feed, high value chemical ingredient (Golz-Berner and Zastrow, 2006) or biodiesel (Mata *et al*., 2010). Blue-green alga *Spirulina* sp. in which their cell is rich in protein, vitamins essential amino acids and essential fatty acids. The commercial production of *Spirulina* was worldwidely sold as food supplement (Belay *et al.,* 1993). Not only suitable for human food but microalgae is also used for animal feed. Many species of microalgae is the one of supplements or feed for terrestrial and aquatic animals. Because of high carotenoids content *Haematococus* is the pigment source in animal feed (Spencer, 1989). Marine microalgae such as *Isochrysis galbana*, *Chaetoceros* sp., *Tetraselmis* sp are the major larval food for shrimp and bivalves (Perez-Camacho *et al*., 1994; Chu, 1989). Moreover, mineral and bioactive compounds from marine microalgae are an ingredient in cosmetic products (Kim *et al*., 2008). At present, facing with the increasing of atmospheric $CO_2$, the role of microalgae as $CO_2$ capture organism has been further explored.

In order to obtain the high production rate and to reduce $CO_2$ emission from industrial process, $CO_2$ was applied to microalgae culture. Table 2.1 is summary on the literature survey on the effects of $CO_2$ on growth and production of microalgae. It could be summarized that $CO_2$ supplement can enhance growth of the microalgae but when the $CO_2$ concentration exceeded the optimum level, growth of the microalgae would rapidly be inhibited.

**Table 2.1** Microalgae production from the different $CO_2$ concentration.

| species | $CO_2$ concentration | production | references |
|---|---|---|---|
| *Chlorella vulgaris* UTEX259 | Air, 5, 15% | 1.32, 1.72, 1.49 g D.W./L | Yun (1997) |
| *Chlorella* sp.KR-1 | 10, 30 50, 70% 200 umol/$m^2$/s | 5.5, 4.5, 3.2, 0.5 g D.W./L | Sung (1999) |
| *Chlorella* sp.KR-1 | Air, 2, 5, 10, 15 % | 0.537, 1.21, 0.062, 0.01, 0.009 g D.W./L | Chiu (2008) |
| *Monoraphidium minutum* | Air, Flue gas (13.6 % $CO_2$) | $10^6$, $10^7$ cells/mL | Brown (1996) |
| *Chroococcidiopsis* sp. | 0, 5, 10, 15, 20% | 1.3, 1.3, 1, 0.4, 0.3 g D.W./L | Hayashi *et al*. (1995) |
| *Scenedesmus obliquss* | 0.03, 5, 10, 20, 30, 50% | 1.05, 1.8, 1.84, 1.65, 1.03, 0.82 g D.W./L | Tang *et al.* (2011) |
| *Chlorella pyrenoidsa* | 0.03, 5, 10, 20, 30, 50% | 0.87, 1.44, 1.55, 1.22 0.95, 0.69 g D.W./L | |
| *Chlorella kessleri* | 3% | $2x10^8$ cells / mL | Izumo *et al*. (2007) |
| | 0.04% | 1 $x10^8$ cells / mL | |
| *Chlorella* KR1 | 0.035, 5, 10, 30, 50, 70% | 1.8, 2.7, 3, 2.2, 2, 0.8 g D.W./L | Sung *et al.* (1998) |
| *Thalassiosira weissflogii* | Air, 5, 10 , 20% | 4.2, 4.4, 4.0 ,2.3 ($x10^5$ cells / mL) | Ishida *et al.* (2000) |
| *Littorella uniflora* | 17, 170 uM | 17.9, 57.5 g D.W./$m^2$ | Anderson and Anderson (2006) |

**Table 2.1** Microalgae production from the different $CO_2$ concentration (continued)

| species | $CO_2$ concentration | production | references |
|---|---|---|---|
| *Chlorococcum sp.* | 20% (30$^o$ C) | 0.50 g D.W./L | Hanagata *et al.* (1992) |
| *Eudorina sp.* K17 | 20% (30$^o$ C) | 0.4 g D.W./L | |
| *Eudorina sp.* K29 | 20% (30$^o$ C) | 0.2 g D.W./L | |
| *Scenedesmus sp* | 20% (30$^o$ C) | 0.8 g D.W./L | |
| *Chlorella sp.* | 20% (30$^o$ C) | 0.7 g D.W./L | |
| *Chlorella sp.* | 10, 20, 30, 40, 50, 60, 80% (30$^o$ C) | 0.6, 0.6, 0.5, 0.4, 0.3, 0.05,0.02 g D.W./L | |
| *Scenedesmus sp* | 10, 20, 30, 40, 50, 60, 80% (30$^o$ C) | 0.7, 0.6, 0.5, 0.5, 0.4, 0.3, 0.04 g D.W./L | |
| *Chlorella sp.* | 20% T= 25, 30,35, 40$^o$C | 0.55, 0.6, 0.6, 0.02 g D.W./L | |
| *Scenedesmus sp* | 20% T= 25, 30,35, 40, 45$^o$C | 0.55, 0.55, 0.5, 0.4,0.02 g D.W./L | |
| *Chlorella* ZY-1 | 0.035, 5, 10, 15, 20, 30, 50, 70% 25$^o$C pH 6 | 2, 4.5, 5.8, 5.5, 5.2, 4.5, 2.8, 0.8 g D.W./L | Yue and Chen (2005) |
| | 10% , 25$^o$C Flow rate(L/Min) 0.1, 0.25, 0.5, 1.0 pH 6 | Growth rate 0.9, 1.1,1, 0.9, 0.85 (g D.W./L /day) | |
| | 10% , pH 6 Flow rate 0.25 (L/Min) 20, 25, 30, 35, 40 $^o$C | Growth rate 0.8, 1.09, 1.0, 0.8, 0.4 (g D.W./L /day) | |
| | 10% , 25 $^o$C Flow rate 0.25 (L/Min) pH 3, 4, 5, 6, 7 | Growth rate 0, 1.1, 1.2, 1, 0.8 (g D.W./L /day) | |
| *Chlorella vulgaris* UTEX259 | 6% Constant $CO_2$ conc. | 3 g D.W./L | Yun *et al*. (1996) |
| | 30% Gradual increase of $CO_2$ | 3.5 g D.W./L | |
| *Thermosynechococcus* sp. CL-1 | 10, 20, 40% | 1.5, 2.8, 0 (x10$^3$ mg D.W./L) | Hsueh (2009) |
| *Nannochloropsis oculta* | Air, 5, 8, 10% | 0.45, 1.1,1.4 (x10$^3$ g D.W./L) | |
| *Euglena gracilis* | Flue gas (11 %$CO_2$) | 0.92 g D.W./L | Chae (2006) |

Moreover, numerous researches have been aim to study the $CO_2$ fixation rate of microalgae in order to obtain the suitable strains for direct biofixation of $CO_2$. The $CO_2$ fixation rate in various of microalgae is shown in Table 2.2

**Table 2.2** $CO_2$ fixtion rate in microalgae under laboratory condition

| species | $CO_2$ concentration | $CO_2$ fixation rate | references |
|---|---|---|---|
| *Euglena gracilis* | Flue gas (11 %$CO_2$) | 1g$CO_2$ /0.4 g biomass | Chae (2006) |
| *Scenedesmus obliquss* | 0.03, 5, 10, 20, 30, 50 | 0.15, 0.29, 0.29, 0.25, 0.15, 0.11 g$CO_2$/L/d | Tang *et al.* (2011) |
| *Chlorella pyrenoidsa* | 0.03, 5, 10, 20, 30, 50 | 0.13, 0.24 0.26, 0.22, 0.15, 0.11 g$CO_2$/L/d | |
| *Chlorella vulgaris* UTEX259 | 15% | 26.0 g $CO_2$ /$m^3$/h | Yun *et al.* (1997) |
| *Chlorella sp.* UK 001 | Air with<br>sunlight (0-15.7)<br>xenon lamp(59.9)<br>Florescent lamp<br>(71.4) (W/$m^{-2}$) | <br>31.8<br>728<br>865<br>mg $CO_2$/$dm^{-3}$/$d^{-1}$ | Hirata *et al.* (1996) |
| *Nannochloropsis oculata* | 2, 5, 10, 15% | 0.211, 0.234, 0.35, 0.393 g/h | Chiu *et al*. (2009) |
| *Chlorella littorale* | 20% $CO_2$ in 0.1/4/ 20 L of culture volume | 4, 0.65, 0.8 g$CO_2$/L/d | Kuruno *et al*. (1995) |
| *Apanotheca microscopica* | 15% $CO_2$ different photo period (night/day) (h) 0:24, 4:20, 6:18, 10:14, 12:12, 14:10 | 1.44, 0.96, 0.44, 0.35, 0.56, 0.24 g/L | Jacob-Lopes *et al.* (2009) |

## 2.4 Classification and Biology of *Isochrysis galbana* and *Tetraselmis suecica*

### 2.4.1 Classification and Biology of *Isochrysis galbana*

The marine microalgae *Isochrysis galbana* is classified in Division Chromophyta, Class Prymnesiophyceae, Order Isochrysidales, Family Isochrysidaceae, Genus *Isochrysis* and Spicies *Isochrysis galbana* (Van Den Hoek *et al*., 1995).

*Isochrysis galbana* primarily occurs as a unicellular flagellate (Fig 2.7), but also as palmelloid stages in brackish marine environments. Two long flagella emerge from a gullet-like structure, but no presence of haptonema exist. *Isochrysis galbana* has been widely used as live feed for aquaculture. Cells of *I. galbana* contains essential fatty acids and organic nutrients for the requirement of aquatic animals such as mollusk, fish and crustaceans (Chen, 2003). High nutritional value of *I. galbana* fed to aquatic herbivores hence influences the nutritional value of the animals (Whyte *et al*., 1989 quote by Valenzuela-Espinoza *et al*, 2002)

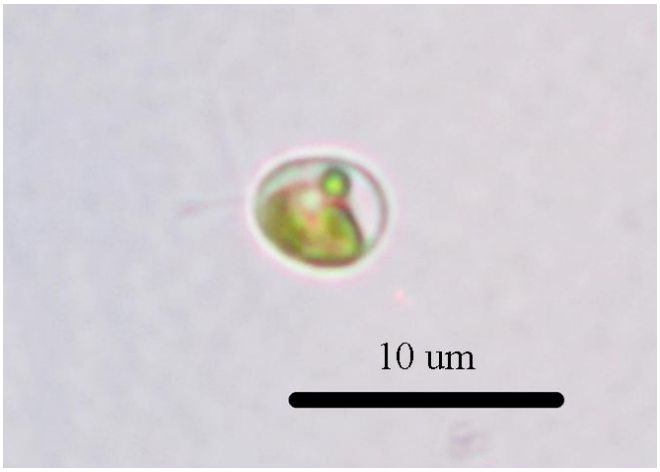



**Figure 2.7** *Isochrysis galbana*

### 2.4.2Classification and Biology of *Tetraselmis suecica*

*Tetraselmis suecica* is a marine green flagellate classified into the Division Chlorophyta, Class: Prasinophyceae. *Tetraselmis* cells are solitary, free-swimming, thecate cells with four flagella. The cells are ovoid and slightly flattened. Cell size is 9-11 micrometers in length (Fig 2.8) (Van Den Hoek *et al*., 1995). *Tetraselmis* is commonly used in aquaculture as feed for bivalve crustacean larva and zooplankton (Benemann, 1992b). *Tetraselmis suecica* has been one of the essential algal foods for hatchery-based seed production of oyster over a 3-week period (Jo, 2004). Although *T. suecica* cell is large, it can be readily ingested by black-lip pearl oyster, *Pinctada margaritifera* (Southgate *et al*., 1998). Mass culture of *Tetraselmis* could be operated in outdoor pond. Moreover, *Tetraselmis* sp. could grow in 14% $CO_2$ (Matsumoto

*et al*., 1995). In addition, Olaizola *et al*.(1991) reported that $CO_2$ supplement could enhance growth of *Tetraselmis* sp. from $1.29x10^5$ to $6.26x10^5$ cells/mL/day which lead to more $CO_2$ removal rate. This $CO_2$ removal is temporary until microalgae died or decomposed.

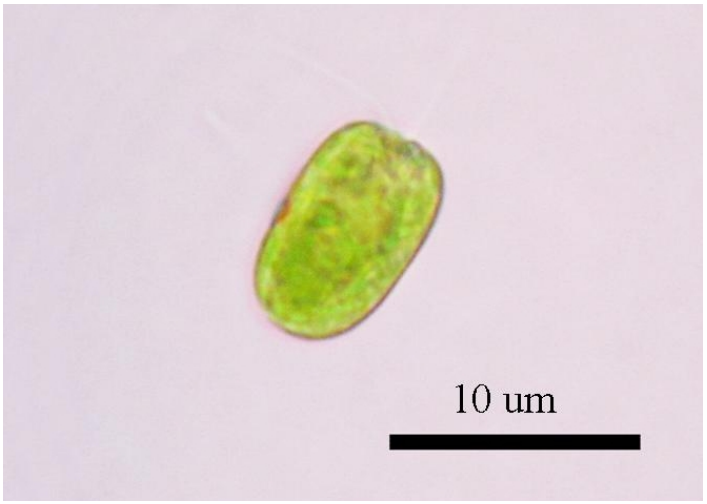



**Figure 2.8** *Tetraselmis suecica*

**2.5 Bivalves**

Bivalves are invertebrate aquatic animal that include numerous species of clams, oysters, mussels and scallops which belong to Class Bivalvia, Phylum Mollusca. The bivalve is the mollusk enclosed in twin shells that hinged together (Garisson, 2007). They consume variety of suspended particles. This method of feeding is called suspension or filter feeding.

The hinge portion of bivalve shell is dorsal. The shell opens on ventral. The bulge on the dorsal surface, adjoining with hinge is termed the umbo. The earliest shell material is located in the umbo. The siphon is projected on the posterior (Fig 2.9) (Pechenik, 1996).

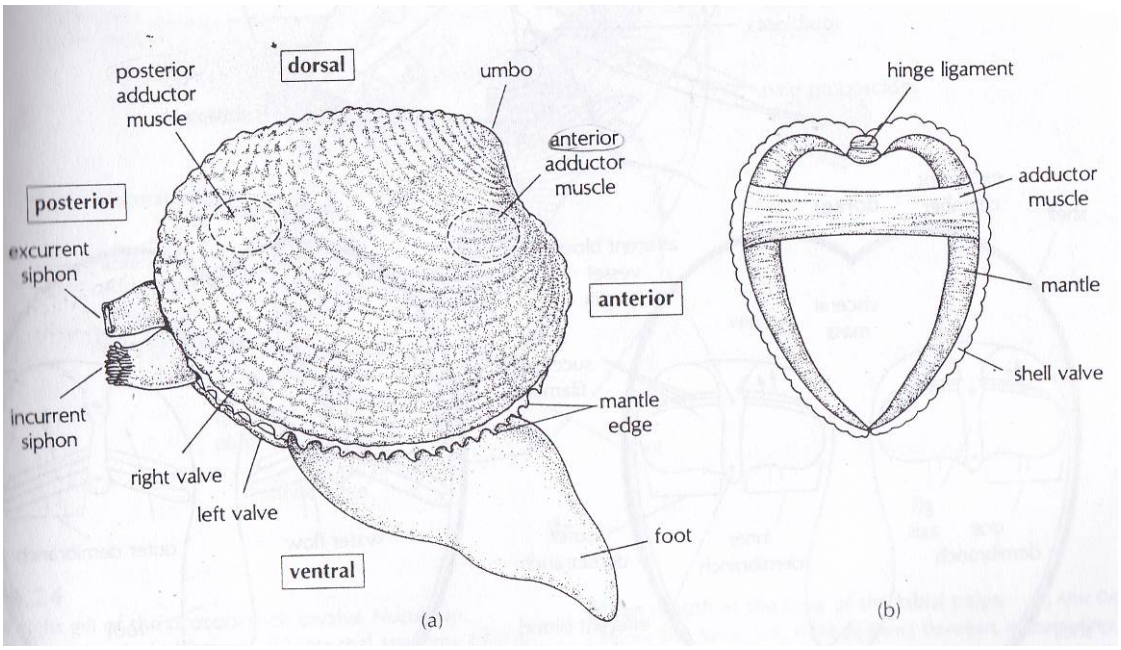



**Figure 2.9** A bivalve, indicating the orientation of the body within the shell valve (a) Lateral view (b) Cross section through shell; siphons would face out of page (Pechenik, 1996)

### 2.5.1 Shell of bivalve

The functions of bivalve shell are for protecting them from predator, acting as a skeleton for muscles attachment and for protecting mantle cavity from sand and mud in burrowing species (Pechenik, 1996)**.** The main component in bivalve shell is calcium carbonate (Bevelander, 1952). The source of calcium in bivalve shell calcification was widely study. From the *Mytilus* shell calcification shows that the calcium from food does not account for the amount deposited in the shell (Fox and Coe, 1943; Bevelander and Benzer, 1948 Quoted by Bevelander, 1952). In addition, from the study of McConnaughey *et al*. (1997); Gillikin *et al*. (2006); Lorrain *et al*. (2004) showed that the carbon source of shell formation in bivalve throughout calcification is both from their feed and surrounding seawater. Calcification process was explained by equation 2.1 (Ware *et al*., 1992). This is the longer term of $CO_2$ removal from the atmosphere than the $CO_2$ assimilation by microalgal cell.

$$Ca^{2+} + 2HCO^{-}_{3} \rightarrow CaCO_3 + CO_2 + H_2O \qquad (2.2)$$

The external characters of bivalve shell such as colour, shape or marking depend on group of bivalve. For example, mussel has the similar size of two valve in oval shape. The concentric ring on mussel shell is used for age determining in mussel. While the oyster shells are approximately circular with off-white, yellowish and cream in colour. Its concentric ring can not be used for age determining in oyster (Gosling, 2003)

The shell growth is one of the indicators for growth measurement in bivalve. The distance from the hinge line to shell margin is termed as height. The widest part across the shell at 90 degree to height is length. The thickest part of two shell valve is measure as width (Fig 2.10) (Dore, 1991 quoted by Gosling, 2003).

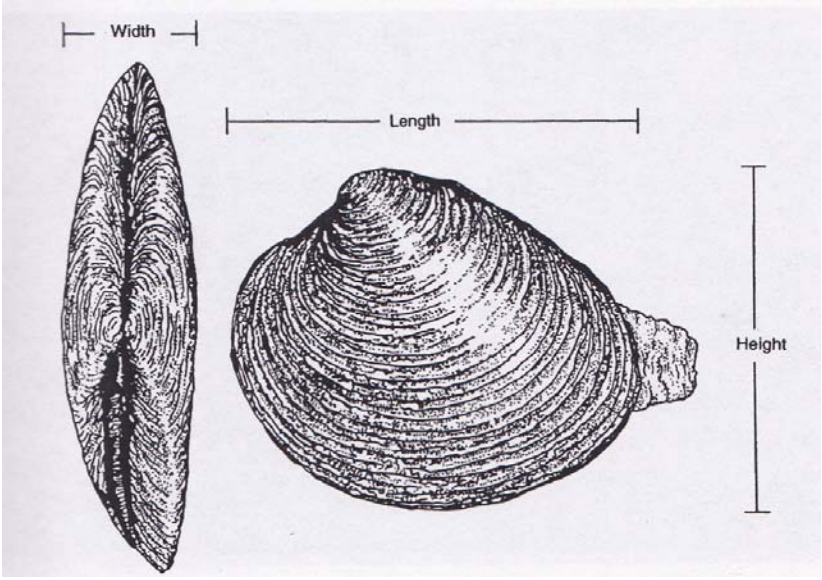



**Figure 2.10** The convention used for the main shell measurements in bivalve (Dore 1991 quoted by Gosling, 2003)

### 2.5.2 Feeding of bivalve

Most of bivalve are filter feeder which filter the water and trap the edible particles. The suspended foods of bivalve are bacteria, phytoplankton, micro-zooplankton, detritus and dissolved organic material (Gosling, 2003). Burrowing species which have a strong muscular foot and use it to dig before embedding themselves into the sediment while their siphon extend to the surface to suction water

and reject waste. The Fig 2.11 showing a bivalve feeding direction, water and food are suctioned into the bivalve with incurrent siphon by the movement of cilia tract on the gill (Garisson, 2007).

The particles is sorted by cilia action on the flattened structure named Labial palp and then food particle is transport to mouth (at the base of labial palp) for ingestion and transferring non food particle to the margin of labial palps. The non food particle (psuedofaeces) is ejected into mantle cavity and expelled (Fig 2.11) (Pechenik, 1996).

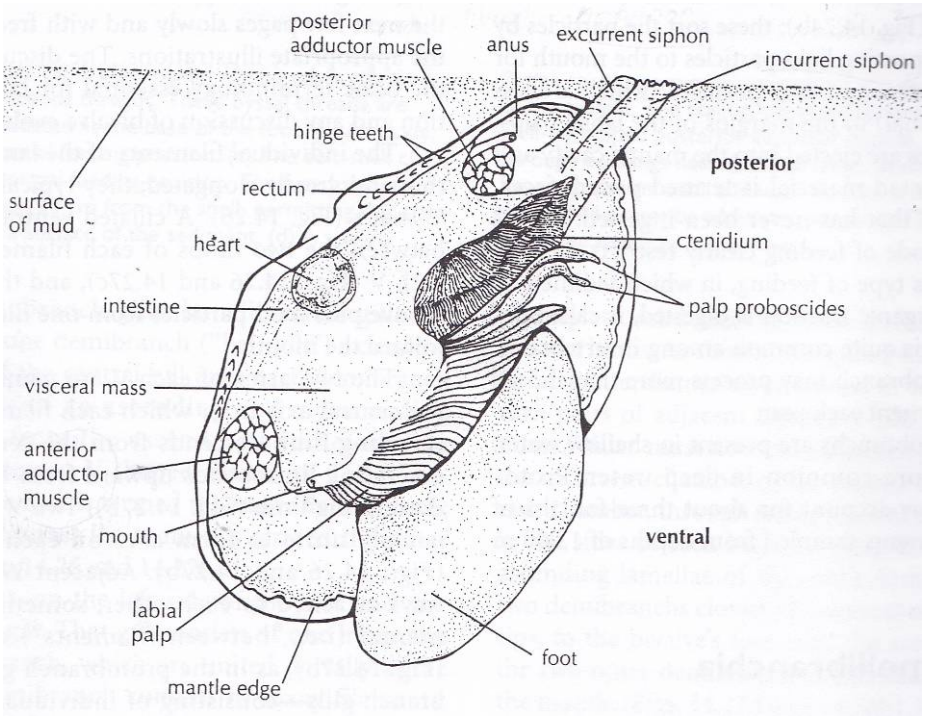



**Figure 2.11** Internal anatomy of the bivalve *Yaldia eightsi* (Pechenik, 1996)

**Factors affecting filtration rate of bivalve**

There are many factors affecting filtration rate of bivalve such as particle concentration, temperature and current speed (Gosling, 2003).

**Particle concentration** Laboratory experiment indicated that mussel pumping rate decreased or stopped when particle-free seawater was used

(Wilson and Seed, 1974 quoted by Gosling, 2003). Bayne *et al*. (1993) reported that feeding with high concentration of seston, mussel *Mytilus edulis* increased their filtration rate. However, high particle concentration probably increased pseudofaeces (rejected particles without passing through digestive tract) production. Pseudofaeces production of *Mytilus edulis* increased rapidly when algae concentration increased from 50-100 cells/ul (Foster-Smith, 1975). Nevertheless, increasing algal lead to increasing filtration rate and ingestion rate until the threshold. The filtration rate will then decrease when particle concentration is higher than the threshold. The highest filtration rate of *Mytilus edulis* on microalgae *Rhodomonas baltica* had been showed with cell density of 3-10 cells/ul but when bivalve was exposed to the gradually increased of *Rhodomonas baltica* cell concentration to 15 cells/ ul valve gape and filtration rate was reduced (Riisgard, 1991). *Perna viridis* and *Crassostrea madrasensis* also show the maximum filtration rate with *Isocrysis galbana* of $10^5$ cell/mL while *Paphia malabarica* of $7.5x10^4$ cell/mL and their filtration rate was rapidly declined with the over threshold concentration (Rajesh *et al*., 2001).

**Temperature** Temperature also affects filtration rate of bivalve. Wong and Cheung (2003) reported that feeding rate of green mussel *Perna viridis* was lowered from late winter to spring (February to April) when temperatures was low. *Mytillus galloprovincialis* Lmk had filtration rate of 0.83 l/h at 18$^{o}$C which was slightly higher than those at 22 $^{o}$C since the mussels are poikilothermic. In general, consumption and growth rate of bivalve increased with temperature up to an optimum and then declined at high temperatures (Okumus *et al*., 2002).

**Current velocity** The current speed is another factor influencing the filtration rate of bivalve. According to Sorbral and Widdows (2000), the clam *Ruditapes decussates* performed maximum filtration rate of 2.5 L/hr with current velocities up to 8 cm $s^{-1}$. Filtration rate declined with increasing current speed, especially above 17 cm $s^{-1}$.

**Size of bivalve** The filtration rate of bivalve depends on the body size within same particle concentration. The experimental result showed that

*Perna viridis* and *Crassostrea madrasensis* with 64-70 mm shell size had the lower filtration rate on *I. galbana* than those in large shell size with 100-105 mm as same as *Paphia malabarica* with 45-47 mm showed the more filtration rate than 30-32 mm (Rajesh *et al*., 2001). The increased of filtration rate with mussel *Mytilus galloprovincial* size was also reported (Okumus *et al*., 2002). On the other hand, small size of fresh water bivalve *Limnoperna fortunei* (15 mm) had the higher filtration of 17.7 mL/mg tissue dry weight/h than the larger size of 23 mm (9.9 mL/mg tissue dry weight/h) when fed with *Chlorella vulgaris* (temperature 15$^{o}$C) (Sylvester, 2005).

### 2.5.3 Bivalve rearing and culture under laboratory condition

As a result of declining of natural stock and more commercial demand, the cultivation under laboratory condition of many kind of bivalves were studied. There are many factors affecting growth of bivalves. Not only food supply but also other environmental factors such as temperature, pH, salinity or stocking density influence growth of bivalve as well (Gosling, 2003).

**Food supply** Food supply is considered to be the most important factors influencing growth of bivalve. The different species or quantity of phytoplankton results in different growth rate of bivalve. The rainbow mussels *Virosa iris* juvenile were cultured with different algae and bacteria species. The maximum shell length of 1.7 cm was the result of rearing with combination of *Neocloris oleoabundans, Bracteacocus grandis* and *Phaeodactylum tricornutum* (Gatenby *et al*., 1997). Moreover, the integrated culture between bivalve filter feeding *Gemma gemma* and polychaete, *Clymenella torquata* was investigated. The result showed that activity of polychaete improved the growth of clam due to transportation of nutrient rich sedimentary pore water into the overlying water by worm-stimulated microfloral populations (Weinberg, 1983). Wikfors *et al.* (1996) examined the growth of oyster *Crassostrea virginica* with nine strain of *Tetraselmis*. Five stains of *Tetraselmis* with high lipid content which contained essential fatty acid 20:5 *n*-3, 24-methylcholesterol and/or 24-methylenecholesterol, supported significantly faster growth of oyster.

**Temperature** *Mytillus edilis* showed maximum growth rate at 20 $^{o}$C and growth at 5 and 10 $^{o}$C decreased under laboratory condition (Brenko and Calabrese, 1969). The pearl oyster also exhibited the higher growth rate at 25 and 30 $^{o}$C than that at 20$^{o}$C. Temperature had a direct influence on growth of bivalve, probably because of the higher energy requirement of organism at high temperature (Bayne, 1973, Widdows, 1978 quoted by Rio-Portilla *et al.,* 1992). In addition, temperature also influenced on both quantitative and quanlitative change in enzyme reaction rate in bivalve (Hawkins and Bayne, 1992 quoted by Gosling, 2003).

**pH** As a result of increasing atmospheric $CO_2$ concentration, reduction of ocean pH will be occurred (Calderia and Wickett, 2003 quoted by Berge *et al*., 2006). The effect of pH (6.7, 7.1, 7.4, 7.6 and 8.1) on bivalve *Mytilus edulis* L was studied in seawater flow-through system. The decreased pH from 8.1 to 6.7 had reduced growth increment of *Mytilus edulis* from 13.18 % to -0.93% with the initial length of 10.8-11.9 mm. While the growth rate of mussel in pH more than 7.6 was not significantly different from the normal pH of 8.1 (Berge *et al*., 2006).

**Salinity** The respond of bivalve to salinity change was by closing their shell valve and adjusting the intracellular ion concentration. By reacting to salinity change, feeding and respiration are depressed and subseqeuently resulted in low growth rate (Almada-Villela, 1984 quoted by Gosling, 2003). The *Mytilus edulis* larva growth is rapid in salinity of 25-35 % in and growth decreased both at high (40%) and low (20%) salinities (Brenko and Calabrese, 1969).

**Stocking density** The high stocking density reduces food availability per individual. The growth rate is reduced in high stocking density because the reduction in space leads to increase in physical contact between individual, with more frequent irritation and retraction of mantle, or valve closure, resulting in less feeding (Cote *et al.*, 1993).

Three species of economically important bivalves in Thailand are oyster, blood cockle and green mussel. All of them are distributed along east and west coast of Thailand, especially in Bandon Bay, Surat Thani province where the experiment was conducted. Oyster *Crassostrea belcheri* is one of the most economically important aquatic species in Surat Thani (Gannarong and Sopakul, 2004). The oyster shell is thick and solid calcium carbonate which can be the candidate for carbon deposition (Gosling, 2003). Blood cockle is another valuable bivalve species in Thailand. Unlike oyster that is usually found attach on hard substrate, the blood cockle lives individually at the sea bottom sediment. Hence, it can be easily collected and placed in the experimental tank. On the other hand, green mussel (*Perna viridis*) is another common bivalve species of Thailand. Among 3 species of bivalve, mussel *P. viridis* has the highest growth rate than other species. Rajagopal *et al.* (1998) reported that growth rate of green mussel *P. viridis* in east coast of India was 0.69 cm/month in length. In contrast, growth rate of blood cockle in Nakornsrithamarath Bay was only 0.58-0.86 mm/month in length (Hannsopa and Thanormkiat, 1991) and growth rate of oyster *Crassostrea belcheri* and hooded oyster *Sassostrea commercialis* in Bandon Bay was 0.17 and 0.049 cm/month in length respectively (Gannarong and Sopakul, 2004).

# CHAPTER III

# MATERIALS AND METHODS

## Overview

The experiments in this study comprise of 4 main sections. The first section (3.1) was the optimization of carbon dioxide supplement on growth and production yield of the microalga. The two main topics in the first section are effect of $CO_2$ concentration on photosynthetic efficiency and growth of microalgae in batch culture. The second section (3.2), bivalve culture consisted of feeding rate evaluation and growth of 4 bivalve species with three feeding patterns *i.e.* continuous feeding, batch feeding and batch feeding with dripping bottle. In the third section (3.3), calcification rate of bivalve in recirculating aquaculture system was evaluated. The final section was the evaluation of carbon assimilation by microalgae and bivalve. The overview of experiments is illustrated in Fig 3.1.

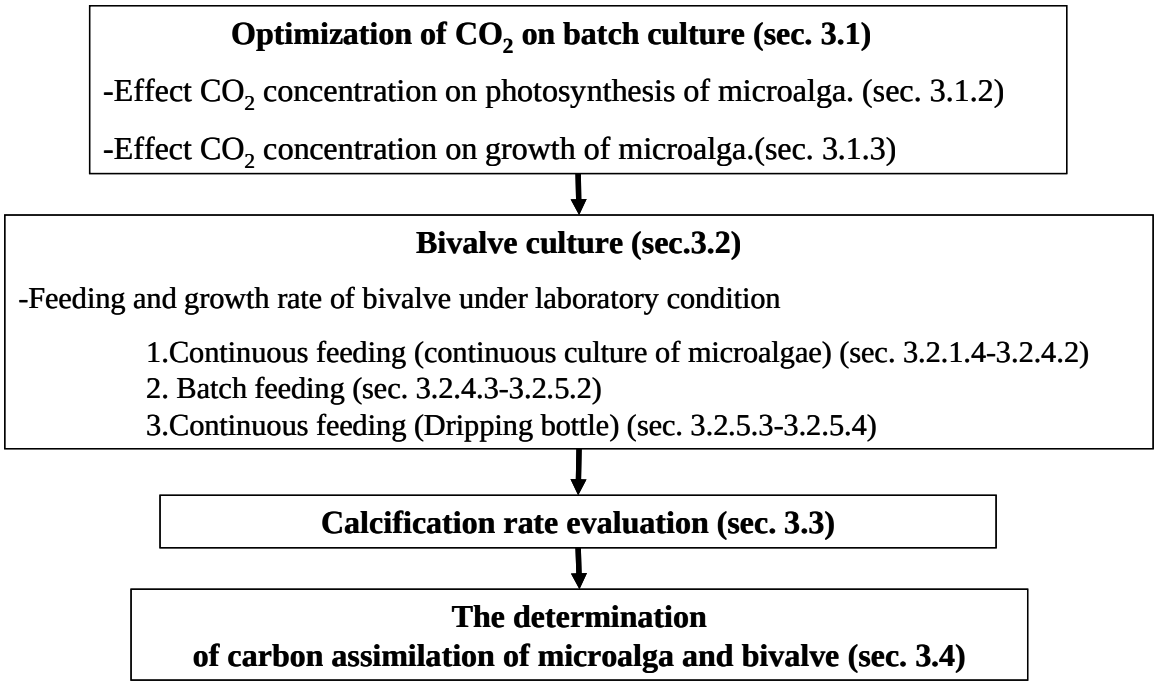



**Figure 3.1** The flow chart of the experiments

## 3.1 Optimization of $CO_2$ on microalgae in batch culture

### 3.1.1 Growth of *Isochrysis gabana* and *Tetraselmis suecica* in batch culture

Cultivation of microalgae was carried out in 1 L Duran glass bottle. Nine-hundred mL of sterilized 30 psu seawater was enriched with F/2 Guillard medium (Appendix A). Stock culture of *I. gabana* and *T. suecica* were inoculated and incubated at 28$^{o}$C at light intensity 5,000 lux continuous illumination. Culture was bubbled with air. Algal cell number was determined every 24 hours by cell count using haemacytometer and all experiments were performed in triplicate.

### 3.1.2 Effect of $CO_2$ concentration on photosynthesis efficiency of *Isochrysis galbana* and *Tetraselmis suecica*

Variable factors: $CO_2$ concentration 0-40 % (V/V) and species of microalgae

In order to study the effect of $CO_2$ concentration on microalgae photosynthesis efficiency, *I. gabana* and *T. suecica* were cultured in 250 mL of 30 psu seawater enriched with F/2 Guillard medium. Aeration was provided with mixture of air and $CO_2$ at 0% [control], 1.5% 5%, 10%, 15%, 20 %, 30% and 40% (V/V). All culture were maintained at 28$^{o}$C with continuous illumination using daylight fluorescence lamps at 5,000 lux. After incubation for 24 hours, *I. gabana* and *T. suecica* from each treatment were filtered with GF/C filter then immediately measured the photosynthesis efficiency. Quantum yield of photosystem II was determined with chlorophyll fluorescence of microalgae cell attached on GF/C filter using Qubit System Chlorophyll Fluorescence Package (Maxwell and Johnson, 2000).

### 3.1.3 Effect of $CO_2$ concentration on growth of *I. galbana* and *T. suecica*

Variable factors: $CO_2$ concentration 0, 1.5 and 15% (V/V) and species of microalgae

Effect of $CO_2$ concentration on growth of *I. galbana* and *T. suecica* in batch culture experiment was conducted in 1 L Duran bottle containing 900 mL of enriched

F/2 Guillard seawater. Stock culture of *I. galbana* and *T. suecica* was inoculated and incubated at 28 $^{o}$C and 5000 lux continuous illumination. Culture was bubbled with air (control) or air mixed with 1.5% $CO_2$ which was the optimum concentration $CO_2$ from previous experiment. Moreover, 15% $CO_2$ was assigned as negative control due to this concentration is the regular $CO_2$ concentration of the flue gas (Matsumoto *et al*., 1995). The experiment was performed in triplicate. Algal cell number and pH was determined every 24 hours by cell count using haemacytometer and pH meter, respectively. At the end of experiment microalgae cell from both treatments was centrifuged and dried for dry weight measurement and analysis for carbon, hydrogen and nitrogen content using CHNS/O Analyzer, Perkin Elmer PE2400 Series II.

## 3.2 Bivalve culture

In this part of research include the study of feeding and growth rate of bivalve under laboratory condition. The maximum feeding rate of each bivalve was used to manipulate the food concentration in bivalve culture.

The experiments in this section were performed in order to develop cultivation techniques for bivalves under laboratory condition. Four species of bivalves which were oyster (*Crassostrea belcheri*), hooded oyster (*Saccostrea cucullata*), cockle (*Anadara granosa*) and green mussel (*Perna viridis*) were chosen as the experimental animals in which feeding rate and growth rate were evaluated.

### 3.2.1 Feeding and growth rate of large oyster

#### 3.2.1.1 Feeding rate of large oyster *C. belcheri* on *I. galbana*

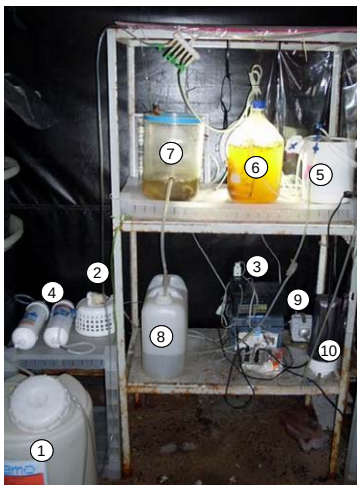
It is realized that microalgae consumption or uptake rate of the oyster is rather depended on microalgae density in the water. Oysters were collected from culture area in Bandon Bay, Surat Thani Province, Thailand. The average oyster shell height and shell length were 10.3±1.5 cm and 7.2±0.7 cm respectively. Average weight of the oysters was 207.5±51.3 g. Oysters were transported to the laboratory where they were cleaned by brushing out epiphytes and debris. Oysters were acclimated for 2 weeks under laboratory condition (28-30 $^{0}$C, 30 psu and pH 8.2) and

then kept in glass aquarium containing 30 psu seawater and temperature during the experiment was 30±1$^{o}$C. Batch cultivation of stock culture microalgae, *I. galbana*, was performed using F/2 algal culture medium. During the experiment, each oyster was weighted and placed in 2.5L glass aquarium containing 2 L of *I. galbana* culture. Density of *I. galbana* was varied by dilution of dense *I. galbana* culture to the desired concentrations between 50-580×10$^{4}$cells/mL. Triplicates of oyster were performed for each *I. galbana* densities. Density of *I. galbana* was determined by counting with haemacytometer. Reduction of *I. galbana* density during the first 100 minutes of each experimental unit was used for feeding rate calculation with linear regression analysis.

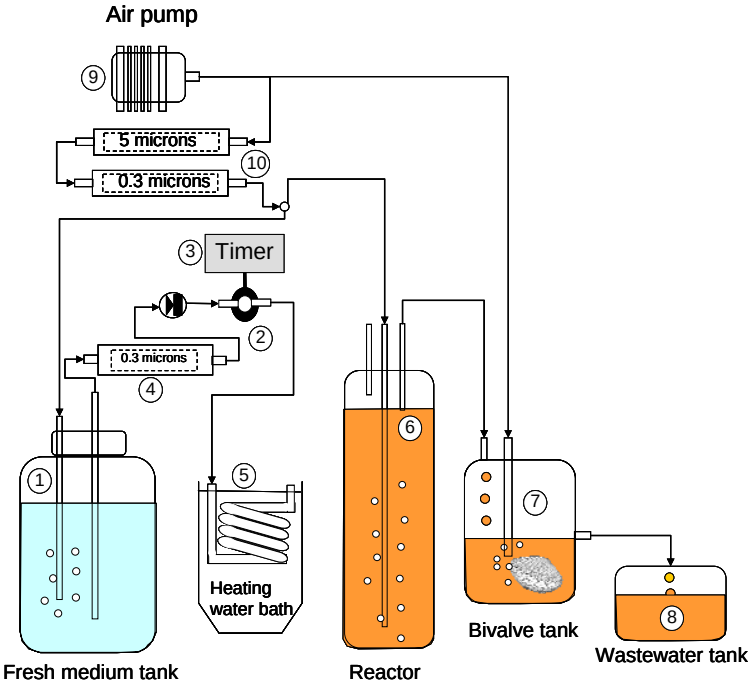
#### 3.2.1.2 Growth of large oyster *C. belcheri* fed by *I. galbana* with continuous feeding

The experimental unit was a continuous culture reactor for *I. galbana* attached to the oyster tank (Fig 3.2). The *I. galbana* continuous culture unit consisted of 5 L Duran bottle as a culture reactor, 50 L plastic tank as a fresh medium storage tank, dosing pump with timer control, and hot water bath as disinfection unit. The algal cells produced from continuous algal reactor were fed directly into 2 L oyster tank. Diagram and photograph of the experimental system are shown in Fig 3.2

Stock culture of *I. galbana* was prepared in 5 L Duran bottle contained 4.5 L of sterilized seawater enriched with Guillard F/2 medium. Inoculum size of *I. galbana* was 500 mL. The culture was incubated at 28$^{o}$C, continuously illuminated with daylight fluorescence lamps at 5,000 lux and aerated with air pump. When cell concentration of *I. galbana* in stock culture reached 1,000×10$^{4}$ cells/mL, algal bottle was then connected to continuous fresh medium supplement to oyster tank whereas continuous culture began.




a




b

**Figure 3.2** Picture (a) and diagram (b) experimental system to grow of oyster *Crassostrea belcheri* continuous fed by *Isochrysis galbana*. Fresh Guillard F/2 medium (1) was transferred by pump (2) controlled by timer (3) to 0.3 uM filter (4), disinfection unit (5) and microalgal reactor (6) respectively. The production from microalgal culture was continuously flow to oyster tank (7) using air pressure. Discharge from oyster tank was removed to wastewater (8) tank by gravity flow.

The oysters were collected from Bandon Bay and prepared as previous described. The average weight of oyster was 177.6±3.83 g. Fresh Guillard F/2 medium for microalgae was prepared by adding nutrient stock solution into chlorine treated seawater. Fresh Guillard F/2 medium form medium tank was transfered to 0.3 uM filter, disinfection unit and microalgal reactor respectively. Microalgal culture

was maintained at 5,000 lux continuously illumination. The production from microalgal culture was continuously fed to oyster tank by air pressure. Discharge from oyster tank was removed to wastewater tank by gravity flow. The oysters were weighed at the initial and the end of experiment. In order to evaluate oyster feeding cell amount cell density of *I. galbana* in microalgal culture and wastewater tank was counted using haemacytometer every 24 hr. The experiment was performed in triplicate.

### 3.2.2 Feeding and growth rate of small oyster

#### 3.2.2.1 Feeding rate of small oyster *C. belcheri* on *I. galbana*

Oysters were collected from culture area in Bandon Bay, Surat Thani Province, Thailand. The average oyster shell height and shell length were 4.28±0.4 cm and 3.62±0.4 cm respectively. Average weight of the oysters was 16.26±2.92 g. Oysters were transported to the laboratory where they were cleaned by brushing out epiphytes and debris. Oysters were acclimated for 2 weeks under laboratory condition (28-30$^{o}$C, 30 psu and pH 8.2) and then they were kept in glass aquarium containing 30 psu seawater and temperature during the experiment was 30±1$^{o}$C. Batch cultivation of stock culture microalgae, *I. galbana*, was performed using F/2 algal culture medium. During the experiment, each oyster was weighted and placed in 500 mL glass aquarium containing 300 mL of *I. galbana* culture. Density of *I. galbana* was varied by dilution of dense *I. galbana* culture to the desired concentrations between 100-800×10$^{4}$cells/mL. Triplicates of oyster were performed for each *I. galbana* densities. Density of *I. galbana* was determined by counting with haemacytometer. Reduction of *I. galbana* density during the first 100 minutes of each experimental unit was used for feeding rate calculation with linear regression analysis.

#### 3.2.2.2 Growth of small oyster *C. belcheri* fed by *I. galbana* with continuous feeding

As the results from previous experiment revealed that large oyster (177.6 g) had poor growth rate, smaller size oysters (12.85±2.46 g) were used instead in the following experiment (3.2.1.2) and all experiments were performed with 3 replicates.

### 3.2.3 Feeding and growth rate of cockle *A. granosa*

#### 3.2.3.1 Feeding rate of cockle *A. granosa* on *I. galbana*

Cockles were collected from culture area in Petchburi Province, Thailand. The average cockle shell height and shell length were 1.49±0.07 cm. and 2.09±0.07 cm respectively. Average weight of the cockle was 3.22±0.43 g. Cockles were transported to the laboratory where they were cleaned by brushing out epiphytes and debris. Cockle were acclimated for 2 weeks under laboratory condition (28-30$^{o}$C, 30 psu and pH 8.2) and then they were kept in glass aquarium containing 30 psu seawater and temperature during the experiment was 30±1$^{o}$C. Batch cultivation of stock culture microalgae, *I. galbana*, was performed using F/2 algal culture medium. During the experiment, each cockle was weighted and placed in glass aquarium containing 150 mL of liquid *I. galbana* culture. Density of *I. galbana* was varied by dilution of dense *I. galbana* culture to the desired concentrations between 100-900×10$^{4}$cells/mL. Triplicates of cockle were performed for each *I. galbana* densities. Density of *I. galbana* was determined by counting with haemacytometer. Reduction of *I. galbana* density during the first 240 minutes of each experimental unit was used for feeding rate calculation with linear regression analysis.

#### 3.2.3.2 Growth of *A. granosa* fed by *I. galbana* with continuous feeding

The experiment was performed using small size of 16 cockles. The condition and apparatus in this experiment were similar to experiment 3.2.1.2 with 3 replicate. Average cockle weight was 4.48±0.87 g with the average shell length and height were 2.18±0.19 and 1.54±0.18 cm respectively. However, since cockles could not survive through the 7 day experiment, another experiment was performed using 4 cockles with average weight of 11.6±1.49 g, shell length of 3.08±0.16 cm and height of 2.49±0.08 cm.

### 3.2.4 Feeding and growth rate of hooded oyster *S. cucullata*

As the poor growth rate of *C. belcheri* and *A. granosa*, *S. cucullata* was used in this experiment.

#### 3.2.4.1 Feeding rate of hooded oyster *S. cucullata.* on *I. galbana*

*S. cucullata* were collected from culture area in Chantaburi Province, Thailand. The average shell height and shell length were 2.3±0.19 cm and 1.9±0.17 cm respectively. Bivalves were transported to the laboratory where they were cleaned by brushing out epiphytes and debris. *S. cucullata* were acclimated for 2 weeks under laboratory condition (28-30 $^{0}$C, 30 psu and pH 8.2) and then they were kept in glass aquarium containing 30 psu seawater and during the experiment was 30±1˚C. Batch cultivation of stock culture microalgae, *I. galbana*, was performed using F/2 algal culture medium. During the experiment, each cockle was weighted and placed in glass aquarium containing 200 mL of *I. galbana* culture. Density of *I. galbana* was varied by dilution of dense *I. galbana* culture to the desired concentrations between 50-800×10$^{4}$cells/mL. Triplicates of hooded oyster were performed for each *I. galbana* densities. Density of *I. galbana* was determined by counting with haemacytometer. Reduction of *I. galbana* density during the first 240 minutes of each experimental unit was used for feeding rate calculation with linear regression analysis.

#### 3.2.4.2 Growth of hooded oyster *S. cucullata* with continuous feeding by *I. galbana*

As growth performance of oyster and cockle in previous experiments was lower than expected, hooded oyster *S. cucullata* was applied in this experiment. The average shell length and height were 1.8±0.65 and 2.09±0.07 respectively. The hooded oyster was cultured with continuous feeding system by *I. galbana*. The condition and apparatus in this experiment was similar to experiment 3.2.1.2.

#### 3.2.4.3 Feeding rate of hooded oyster *S. cucullata* on *T. suecica*

The result from 3.2.1.1-3.2.4.2 showed that *C. belcheri, A. granosa, S. cucullata* could not grow with continuous feeding by continuous culture of *I. galbana*. The next experiment was performed with batch feeding by *T. suecica*, due to larger cell size than *I. galbana. I. galbana* with cell size of 5 micron was suitable for the spat up to 1.0mm (Dharmaraj *et al*., 2004). Although *T. suecica* cell is large, it can be readily ingested by black-lip pearl oyster, *Pinctada margaritifera* (Southgate *et al*., 1998).

*S. cucullata* oysters were collected from culture area in Chantaburi Province, Thailand. The average oyster shell height and shell length were 2.4±0.2 cm. and 2.18±0.2 cm respectively. Bivalves were transported to the laboratory where they were cleaned by brushing out epiphytes and debris. Hooded oysters were acclimated for 2 weeks under laboratory condition (28-30 $^{0}$C, 30 psu and pH 8.2) and then they were kept in glass aquarium containing 30 psu seawater and temperature during the experiment was 30±1˚C. Batch cultivation of stock culture microalgae *T. suecica* was performed using F/2 algal culture medium. During the experiment, each cockle was weighted and placed in glass aquarium containing 200 mL of liquid *T. suecica* culture. Density of *T. suecica* was varied by dilution of dense *T. suecica* culture to the desired concentrations between 50-800×10$^{4}$cells/mL. Triplicates of hooded oyster were performed for each *T. suecica* densities. Density of *T. suecica* was determined by counting with haemacytometer. Reduction of *T. suecica* density during the first 240 minutes of each experimental unit was used for feeding rate calculation with linear regression analysis.

#### 3.2.4.4 Growth of hooded oyster *S. cucullata* with manual batch feeding by *T. suecica*.

Results from the previous experiments exhibited poor growth of bivalves fed with *I. galbana*. In this experiment, another species of microalgae, *T. suecica*, which has two times larger in size than *I. galbana* was chosen instead. Feeding method was changed from continuous to manual feeding once a day. *S. cucullata* were collected from Chantaburi province. Oysters were cleaned and prepared as in the previous experiment. After 2 weeks of acclimation, oysters were placed in 1 and 5 L seawater plastic tank aerated through air stone. Each tank containing 10 hooded oysters and the experiment was carried out with 3 replicates. Feeding was performed by adding 10-30 mL of *T. suecica* culture once a day. After feeding, initial *T. sucica* cell concentration in oyster culture tank was approximately 2.5×10$^{4}$ cells/mL. Seawater in culture tank was changed daily in order to maintain good water quality. Water quality during experiment such as ammonia, alkalinity, pH and temperature was monitored using test kit (AQUA-VBC) and pH meter (Mettler

Toledo-SG2). Cell density of *T. sucica* was counted at before and after feeding in order to evaluated algal consumption of the bivalves

### 3.2.5 Feeding and growth rate of green mussel *P. viridis* on *T. suecica*

As the poor growth rate of *C. belcheri, A. granosa, S. cucullata, P. viridis* was used in this experiment.

#### 3.2.5.1 Feeding rate of *P. viridis* on *T. suecica* with batch feeding

*P. viridis* were collected from farming site, in Surat Thani province. The mussel was washed with seawater, removed the epibionts, then acclimated for 2 weeks under laboratory conditions (28-30 $^{o}$C, 30 psu and pH 8.2). The average mussel shell height and shell length were 3.69±0.07 cm and 1.73±0.05 cm respectively. Average weight of the mussels was 3.97±0.23 g. Stock culture of *T. suecica* was cultured using F/2 algal culture medium. During the experiment, green mussel was placed in 1 L beaker containing 1 L of microalgae (*T. suecica*) culture. The experiment was performed with various density of *T. suecica i.e.* 1-20×$10^4$cells/mL with 3 replicates for each density. Density of *T. suecica* was determined by counting with haemacytometer. Reduction of *T. suecica* density during the first 120 minutes of each experimental unit was used for feeding rate calculation.

#### 3.2.5.2 Growth of mussel *P. viridis* with batch feeding by *T. suecica*

**First experiment at *T. suecica* cell concentration about $1\times10^4$ cells/mL**

Green mussels collected from Bandon Bay, Surat Thani Province, were acclimated and cultured in up-flow seawater system. The average mussel weight was 3.86±0.29 g, the average shell height and length were 3.67±0.29 and 1.73±0.087 cm respectively. Mussels were randomly divided into 2 groups, the first group was used for the estimation of fresh and dry weight relationship and the rest was used for growth rate evaluation. After 2 weeks acclimation, 5 mussels were placed in the 10 L tank containing 10 L of 30 psu seawater. Production of *T. suecica* was accomplished

under batch culture condition. Feeding was provided by an addition of *T. suecica* cells into mussel tank. The initial average *T. suecica* cell concentration in mussel tank was $0.62\pm0.03\times10^4$ cells/mL. Number of microalgae and water quality parameter: pH, temperature, ammonia and alkalinity were monitored on a daily basis by pH meter (Mettler Toledo-SG2) and test kit AQUA-VBC). Shell length and weight were measured every 10 days. Calcification rate of bivalve was evaluated from decreasing of calcium concentration in the water by EDTA titration (APHA, 1998) (Appendix B). At the end of experiment, fresh weight and dry weight of the mussel were determined then dry mass of bivalve were used for CHN analysis (CHNS/O Analyzer, Perkin Elmer PE2400 Series II). The C, H, and N content in bivalve and microalgae were then used for the calculation of biological carbon assimilation process.

**Second experiment at *T. suecica* concentration about $2\times10^4$ cells/mL**

The second experiment was performed at higher concentration of microalgae feeding to bivalve cultures. Green mussels, as described in previous experiment, were acclimated and cultured in an up-flow system. The average mussel weight was 5.14±0.13 g, the average shell height and length were 4.0±0.11 and 1.92±0.07 cm, respectively. The experiment was conducted in plastic tank containing 5 L of 30 psu seawater. Production of *T. suecica* was accomplished under batch culture condition. Feeding was provided by the addition of *T. suecica* cells. The average initial *T. suecica* cell concentration in mussel tank was $1.44\pm0.03\times10^4$ cell/mL. Growth of mussel and environmental monitoring was performed as described in the first experiment.

#### 3.2.5.3 Feeding rate of *P. viridis* on *T. suecica* with continuous feeding by dripping bottle

In order to improved growth of mussel by increase food availability, the continuous feeding by dripping bottle was use in this experiment.

Green mussels were collected from farming site, in Surat Thani province. Mussel was washed with seawater, removed the epibionts, then acclimated

for 2 weeks under laboratory conditions (28-30 $^{o}$C, 30 psu and pH 8.2). The average mussel shell height and shell length were 2.78±0.08 cm. and 1.46±0.06 cm, respectively. Average weight of the mussel was 1.84±0.09 g. Stock culture of *T. suecica*, alga was cultured using F/2 algal culture medium.

During the experiment, 5 green mussels were placed in 10 L plastic container containing 10 L of 30 psu seawater aerated with air stone. Culture of *T. suecica* with cell density approximately $96\times10^{4}$ cells/mL (500 mL) was filled into continuous feeder which was a 1 L plastic bottle hanging over mussel tank. Flow rate of continuous feeder was 2 mL/min. Density of *T. suecica* in mussel tank was determined by counting with haemacytometer. Reduction of *T. suecica* density during 9 hours of each experimental unit was used for feeding rate calculation with linear regression analysis. The experiment was performed with 3 replicates.

#### 3.2.5.4 Growth of mussel *P. viridis* with continuous feeding by *T. suecica*

In order to improve the growth rate of mussel, in this experiment mussels were continuous fed with *T. suecica* cells. Each mussel was affixed on bamboo stick using epoxy glue hence it could be handled and weighted individually during the experiment. Green mussels were collected from culture area in Bandon Bay. The average mussel weight was 1.79±0.1 g, the average shell height and length were 2.82±0.09 and 1.42±0.08 respectively. Some of mussel samples were dried at 80 $^{o}$C for the estimation of relationship between fresh and dry weight. The other mussels was attached on bamboo stick with epoxy glue (Fig 3.3) pinch the 5 bamboo sticks in a piece of foam and then placed them in 100 L plastic container filled with 50 L of 30 psu seawater. Acclimation the mussels under laboratory condition (30 psu seawater, 28 $^{o}$C, fed by *T. suecica* and change seawater everyday) for 2 weeks.

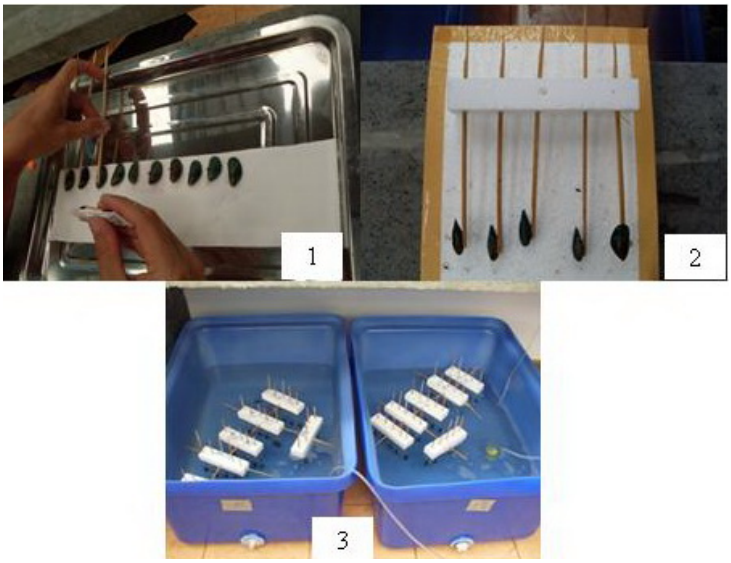



**Figure 3.3** Preparation of mussel before experiment: attached mussel on bamboo stick with epoxy glue (1) pinch bamboo stick in a piece of foam (2) maintained mussel in plastic tank contained 30 psu seawater (3)

After acclimation, experiment was performed in 4 treatments each with 3 replicate as follows:

**Control 1**: Plastic tank filled with 10L of 30 psu seawater without mussel. *T. suecica* in overhang bottle was dripping continuously into mussel tank. This treatment was performed in order to evaluate number of *T. suecica* adding into mussel tank.

**Control 2**: Five mussels were maintained in 10 L plastic tank containing 10 L of 30 psu seawater. This treatment was performed without feeding.

**Treatment 1**: Cultivation of *T. suecica* was performed with pure air (100% air) for the duration of 30 days. At the end of algal batch culture, culture of *T. suecica* was transferred into dripping bottle hanged over the mussel tank. With this treatment, 0.5 L of *T. suecica* cells at 50-100×$10^4$ cells/mL were daily fed into mussel tank. Due to the light intensity of green mussel culturing zone between 0-500 lux, it is assumed that there is no grow of *T. suecica* in dripping bottle and green mussel tank.

**Treatment 2**: This treatment was similar to treatment 3 except the cultivation of *T. suecica* was performed using aeration with the mixture of 1.5% $CO_2$ in air.

Seawater in all experiment units was filtered through 10 microns cartridge filter to eliminate detritus following by chlorine sterilization before use. In control 1, treatment 1 and treatment 2, five hundred milliliters of *T. suecica* cultures with 3 days cultivation period at the concentration approximately 50-100×$10^4$ cells/mL, was fed into mussel tank everyday through dripping bottle (Fig 3.4). Number of microalgae and water quality parameters i.e. pH, temperature, ammonia and alkalinity were monitored on a daily basis. Shell length and weight of mussels were measured every 10 days. The mussels were clean and wipe with tissue paper to remove excess water before weighing. At the end of the experiment, fresh weight and dry weight of the mussel were determined and dry mass of bivalve were further analyzed for carbon, hydrogen, and nitrogen content (CHNS/O Analyzer, Perkin Elmer PE2400 Series II). The CHN data of microalgae and bivalve biomass was then used for the calculation of biological carbon assimilation process.

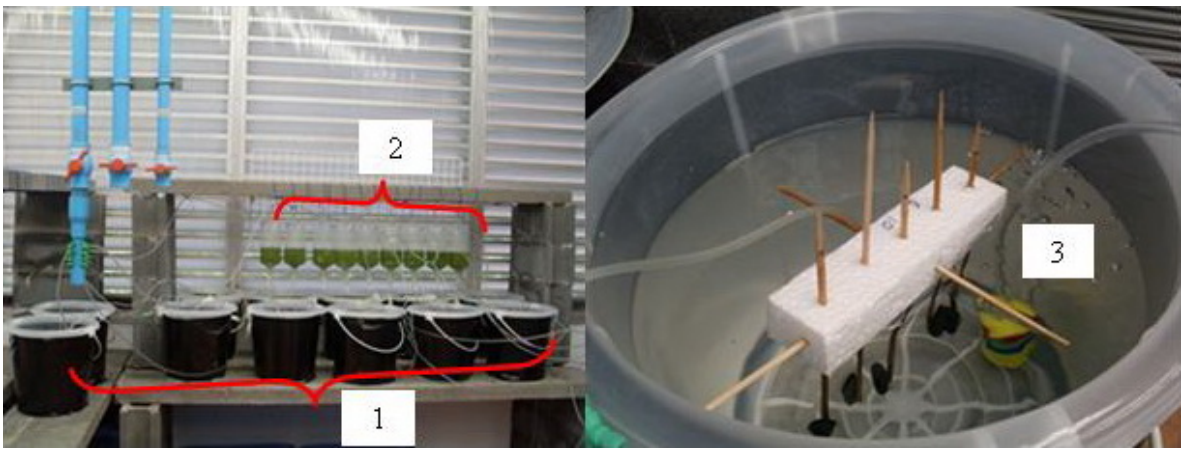



**Figure 3.4** Photographs illustrate mussel culture units with mussel tanks (1), dripping bottle for feeding *T. suecica* to mussel hanging over the mussel tanks (2), and aeration in mussel tank was provided by air stone (3)

### 3.3 Calcification rate evaluation

From the experiment 3.2.5.2 waste water from mussel tank was treated by macroalgae *Gracillaria* sp. before reuse for green mussel culture. Calcification rate of bivalve was evaluated from decreasing of calcium concentration in the water by EDTA titration (APHA, 1998).

### 3.4 Evaluation of $CO_2$ assimilation of the microalgae-bivalve system

According to Redfield's Stoichiomethic equation (3.1), 106 mole of $CO_2$ is converted to 1 mole of organic matter, refer to microalgae. Hence, in theory, carbon content in microalgae cell is 35.8% and 1 g of $CO_2$ is converted to 0.76 g of microalgae. With this study, evaluation of carbon dioxide fixation was based on carbon analysis (CHNS/O Analyzer, Perkin Elmer PE2400 Series II) in biomass of microalgae (*T. suecica*) cells and in shell and meat of the mussel. Initial carbon transferred from microalgae to mussel was estimated from carbon content in dry weight of microalgae cells consumed by mussel throughout 30 days of the experiment (3.2). Thereafter, carbon assimilation in mussel weight gain was evaluated by an increase of carbon content in dry meat (3.3) and in shell (3.4) during the experiment. McConnaughey *et al*. (1997) proposed the assumption that carbon deposition in shell comes from two sources in which 10% is from feed and 90% is from surrounding water (90%). Moreover, Gillikin *et al*. (2006) and Lorrain *et al*. (2004) reported the similar ratio in other species such as *Mytilus edulis* and scallop. Therefore, the dry shell weight gain (g C) from dietary organic carbon and from water was calculated by equation 3.5 and 3.6.

$$106\ CO_2 + 120\,H_2O + 16\,HNO_3 + H_3PO_4 \rightarrow (CH_2O)_{106}(NH_3)_{16}PO_4 + 137\,O_2 \quad (3.1)$$

$$\text{Carbon in } T.suecica \text{ fed by mussel(g)} = \frac{\text{g D.W. of } T.suecica \text{ fed by mussel} \times \%\text{C in cells}}{100} \quad (3.2)$$

$$\text{Carbon gain in mussel's meat (g)} = \frac{\text{g Dry meat weight gain} \times \%\text{C in meat}}{100} \quad (3.3)$$

$$\text{Carbon gain in mussel's shell (g)} = \frac{\text{g Dry shell weight gain} \times \%\text{C in shell}}{100} \quad (3.4)$$

$$\text{Carbon from } T.suecica \text{ deposited in shell (g)} = \frac{\text{g Carbon of shell weight gain} \times 10}{100} \quad (3.5)$$

$$\text{Carbon from water deposited in shell (g)} = \frac{\text{g Carbon of shell weight gain} \times 90}{100} \quad (3.6)$$

# CHAPTER IV

# RESULTS AND DISCUSSIONS

## 4.1 Optimization of $CO_2$ on microalgae in batch culture

### 4.1.1 Basic growth characteristics of *Isochrysis galbana* and *Tetraselmis suecica*

*I. galbana* and *T. suecica* were cultured in 1 L Duran bottle containing Guillard (F/2) enriched seawater. For *I. galbana*, the maximum cell density of $20.87\times10^6$ cells/mL was obtained in 6 days (Fig 4.1) and the specific growth rate was 0.82 $d^{-1}$. Stationary phase was approximately 13 day following by decline phase. The maximum density in this study was higher than $5.19\times10^6$ cells/mL reported by Valenzuela-Espinoza *et al*. (2002).

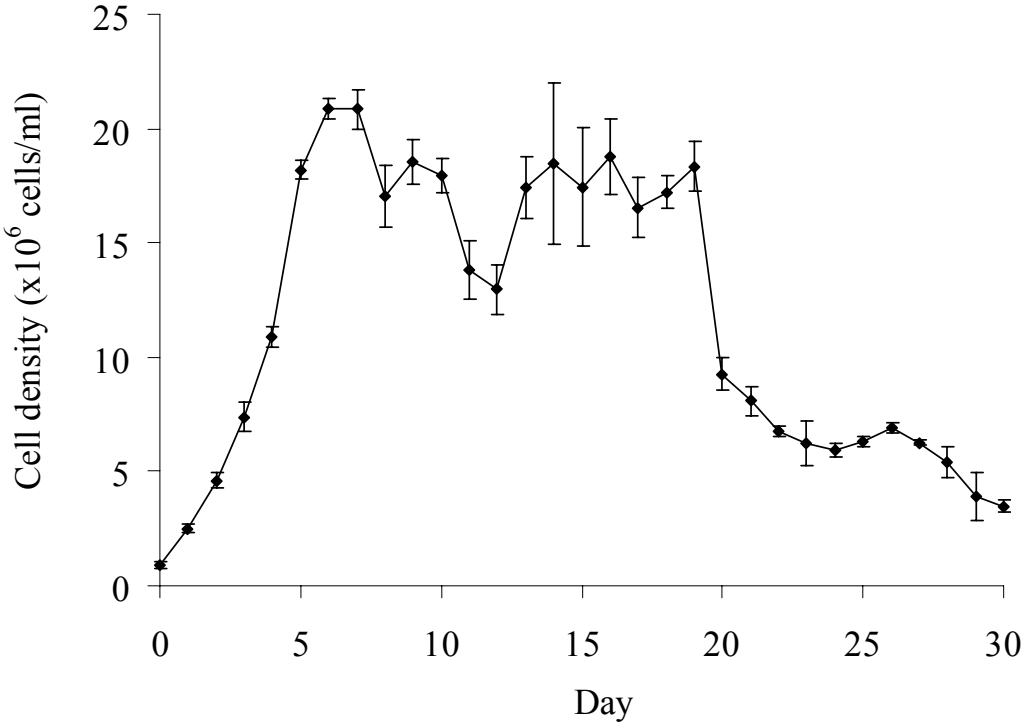



**Figure 4.1** Growth curve of *I. galbana* in batch culture

Batch culture of *T. suecica* exhibited the maximum density of $2.15\times10^6$ cells/mL in day 7 and the specific growth rate was 0.71 $d^{-1}$. This growth performance was rather similar to that reported in Fabregas *et al*. (1995). The stationary phase was after day 7 until the end of the experiment (Fig 4.2).

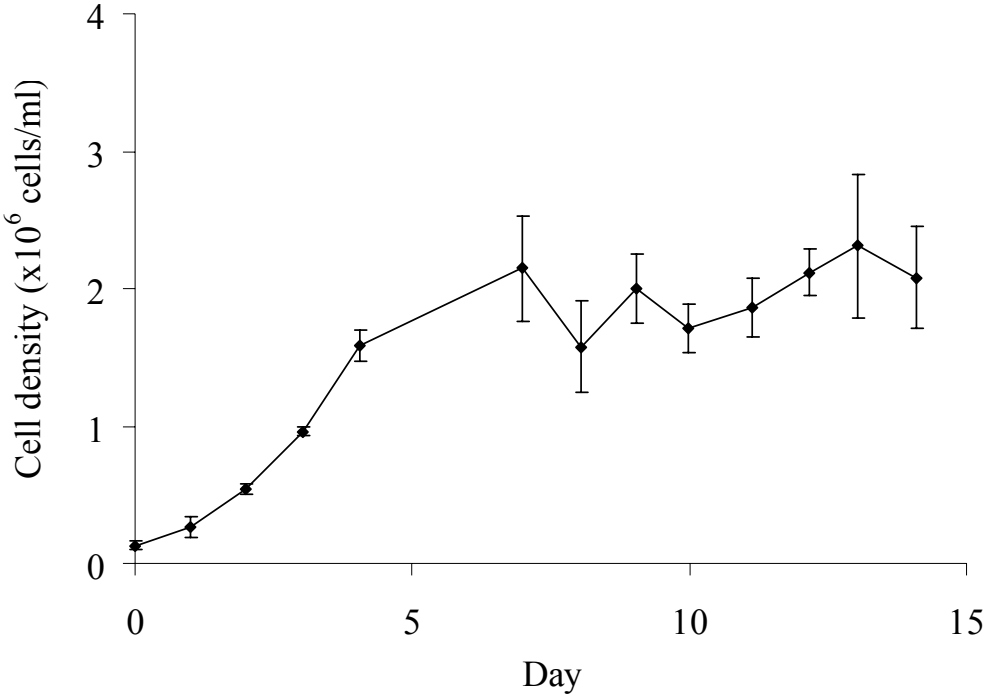



**Figure 4.2** Growth curve of *T. suecica* in batch culture

### 4.1.2 Effect of $CO_2$ concentration on photosynthesis efficiency and growth of *I. galbana* and *T. suecica*

Photosynthesis efficiency (Fv/Fm) of *T. suecica* was determined in order to study the effect of $CO_2$ concentration on microalgae photosynthesis efficiency. When *I. galbana* and *T. suecica* were bubbled with mixture of air and 0 (control), 1.5, 5, 10, 15, 20, 30 and 40 % of $CO_2$, the results in Figure 4.3 indicated that both of *I. galbana* and *T. suecica* had the maximum photosynthetic efficiency (Fv/Fm) of 0.8 and 0.81 respectively when bubbled with mixture of air and 1.5% of $CO_2$. In general, the Fv/Fm value close to 0.8 indicates health and vigor of plant (Bjorkman & Demmig-Adams, 1995 quoted by Akram *et al*., 2006). This study shows that Fv/Fm of *I galbana* and *T. suecica* significantly decreased when supplied with mixture of air and

$CO_2$ at higher than 1.5% $CO_2$ concentration. This data suggested that the photosynthesis process was inhibited with the same manner as photoinhibition (Schreiber *et al*., 1995 quoted by Akram *et al*., 2006). The cell stress caused by $CO_2$ concentrations affected *T. suecica* to a higher extent than *I. galbana*. At the $CO_2$ concentration of flue gas (15% $CO_2$), Fv/Fm of *I galbana* decreased by 88.8% when Fv/Fm of *T. suecica* decreased down to 70.5 %. Hence, it could be denoted that the $CO_2$ concentration more than 1.5% exceeds the optimum level for photosynthesis of both microalgae (Fig 4.3 and Table 4.1).

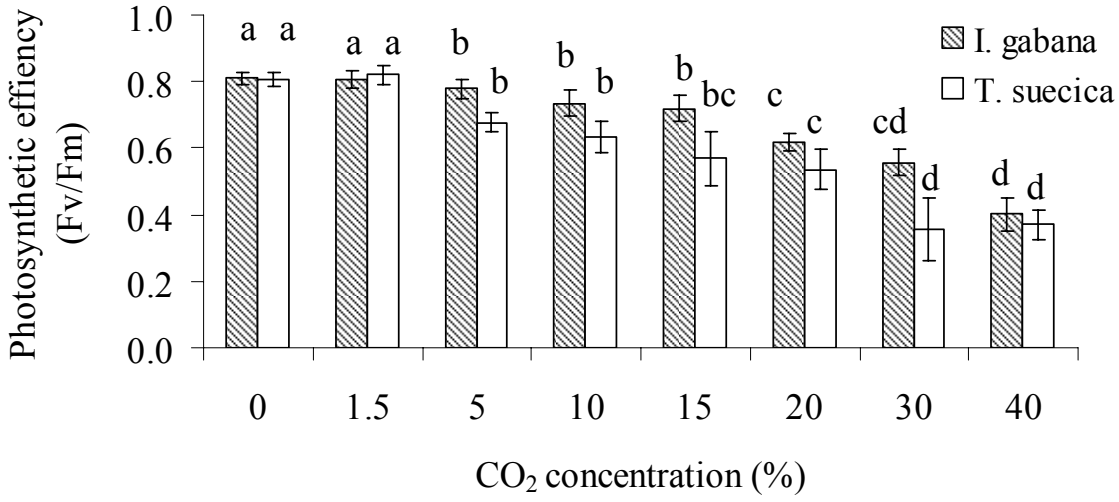



**Figure 4.3** Effect of carbon dioxide concentration on photosynthetic efficiency of microalgae *I. galbana* and *T. suecica*. (Different letters on histogram within the same species = significantly different at $p \leq 0.05$)

**Table 4.1** Photosynthetic efficiency of *I. galbana* and *T. suecica* in various $CO_2$ concentration condition (Different superscript letters within column = significantly different at $p \leq 0.05$)

| $CO_2$ concentration (%) | Photosynthetic effiency | | | |
|---|---|---|---|---|
| | *Isochrysis galbana* | | *Tetraselmis suecica* | |
| | Fv/Fm | Percentage compare with control (%) | Fv/Fm | Percentage compare with control (%) |
| 0 (control) | $0.81 \pm 0.02^{a}$ | 100 | $0.81 \pm 0.02^{a}$ | 100 |
| 1.5 | $0.80 \pm 0.03^{a}$ | 99.3 | $0.82 \pm 0.03^{a}$ | 101.9 |
| 5 | $0.78 \pm 0.03^{b}$ | 96.0 | $0.68 \pm 0.03^{b}$ | 84.0 |
| 10 | $0.73 \pm 0.04^{b}$ | 90.7 | $0.63 \pm 0.05^{b}$ | 78.4 |
| 15 | $0.72 \pm 0.04^{b}$ | 88.8 | $0.57 \pm 0.08^{bc}$ | 70.5 |
| 20 | $0.62 \pm 0.02^{c}$ | 76.2 | $0.54 \pm 0.06^{c}$ | 66.4 |
| 30 | $0.56 \pm 0.04^{cd}$ | 68.7 | $0.36 \pm 0.1^{d}$ | 44.3 |
| 40 | $0.40 \pm 0.05^{d}$ | 49.5 | $0.37 \pm 0.04^{d}$ | 45.8 |

### 4.1.3 Effect of carbon dioxide concentration on growth of microalgae *I. galbana* and *T. suecica*

In this experiment, growth of *I. galbana* and *T. suecica* with air, 1.5% $CO_2$ and 15% of $CO_2$ was evaluated. All cultures were incubated at 28-30 $^{o}$C and continuously illuminated by a fluorescence lamp at 5,000 lux. The results showed that growth rate and maximum cell density increased with increasing $CO_2$ concentrations. The maximum specific growth rate of *I. galbana* and *T. suecica* was found with 1.5% of $CO_2$ supplement. The maximum specific growth rate of *I. galbana* in this study was 1.13 $day^{-1}$ or 118.9% of control when cultured with 1.5% $CO_2$. Maximum cells density, 146.5% of control, was also found with 1.5% CO2 culture. While high $CO_2$ concentration (15% $CO_2$) clearly inhibited growth of *I. galbana* (Table 4.2).

**Table 4.2** Specific growth rate and maximum cell density of *I. galbana* in batch culture aerated by pure air or air mixed with 15% of $CO_2$

| Treatment for ***I.galbana*** | Specific growth rate ( $day^{-1}$) | Percentage of specific growth rate compare with control (%) | Maximum cell density ($\times 10^6$ cells/mL) | Percentage of maximum cell density compare with control (%) |
|---|---|---|---|---|
| Control (pure air) | 0.95 | 100 | 12.74 | 100 |
| air+ 1.5% $CO_2$ | 1.13 | 118.9 | 18.67 | 146.55 |
| air+ 15% $CO_2$ | 0.89 | 93.68 | 11.84 | 92.93 |

The *I. galbana* cultured in pure air, 1.5% and 15 % $CO_2$ had maximum cell density of 12.74, 18.67 and $11.84\times10^6$ cells/mL, respectively (Fig 4.4). This result was similar to Yingying and Changhai (2009) in which 2.6% $CO_2$ supplement can enhance growth of *I. galbana*. Maximum cell density of *I. galbana* in previous and present study was approximately $1.8\times10^7$cells/mL. In general, sensitivity of cells to $CO_2$ varied with algal species. As 15% $CO_2$ inhibited growth of *I. galbana*, however, this $CO_2$ concentration in flue gas could promote growth of *Chlorella* sp (Maeda *et al*., 1995). The result of pH determination was shown in Fig 4.4. In *I. galbana* culture, pH was decreased from pH 8.26 to 7.09 with 1.5% $CO_2$ and from pH 8.19 to 6.9 with 15% $CO_2$.

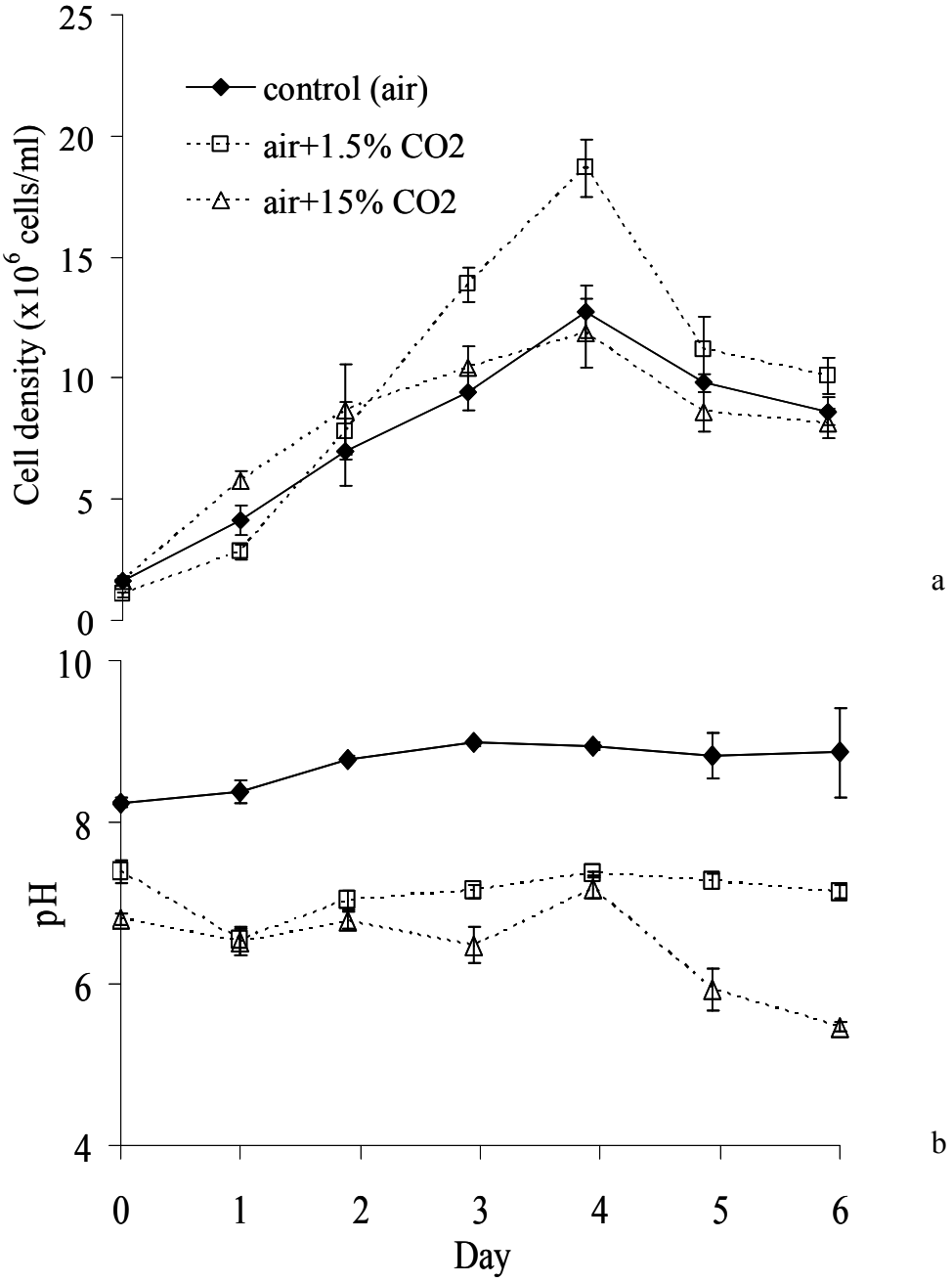



**Figure 4.4** Growth of *I. galbana* (a) and pH (b) in batch culture aerated by mixture of air and 0% (—◆—) 1.5% (- - □ - - -) and 15% (- - - △ - - -) of $CO_2$

For *T. suecica,* the highest specific growth rate of 0.81 $day^{-1}$ or 115.7% of control was found when cultured with 1.5% $CO_2$. On the other hand, *T. suecica* cultured with pure air and 15% of $CO_2$ had the specific growth rate of 0.79 and 0.67, respectively (Table 4.3). The highest cell density, $2.39\times10^6$ cells/mL, was found in 1.5 % $CO_2$ treatment whereas control and 15% of $CO_2$ were 1.27 and $0.43\times10^6$ cells/mL, respectively (Fig 4.5). It indicated that 1.5% of $CO_2$ supplement enhanced growth of *T. suecica* in batch culture while 15% of $CO_2$ inhibited growth of both species. This result was confirmed by the study of Olaizola *et al.* (1991) in which the $CO_2$ concentration (5% $CO_2$) affected growth of *T. suecica*. In addition, several reports *i.e*. Hanagata (1992), Hyashi *et al*. (1995), Yue and Chen (2005), Yun and Yang (1996), Izumo *et al.* (2007) Sung *et al.* (1998) Choosuan (2009) and Ishida *et al*. (2000) illustrated that $CO_2$ supplement encouraged growth of microalgae. This is due to unlimited carbon sources for photosynthesis process. Moreover, high $CO_2$ concentration in the water can reduce the requirement of light energy for Carbon concentrating mechanisms (CCMs) or $HCO_3^-$, therefore growth was promoted (Wu *et al*., 2008). On the other hand, the report of Chiu *et al.* (2009), Hanagata *et al*. (1992), Hayashi *et al.* (1995), Yue and Chen (2005), Ishida *et al* (2000), Sung *et al.* (1998), Sung *et al.* (1999) showed that high concentration of $CO_2$ (more than 10%) inhibited the growth of microalgae.

**Table 4.3** Specific growth rate and maximum cell density of *T. suecica* in batch culture aerated by pure air or air mixed with 1.5% of $CO_2$

| Treatment for ***T.suecica*** | Specific growth rate ($day^{-1}$) | Percentage of specific growth rate compare with control (%) | Maximum cell density ($\times10^6$ cells/mL) | Percentage of maximum cell density compare with control (%) |
|---|---|---|---|---|
| Control (pure air) | 0.79 | 100 | 1.27 | 100 |
| air+ 1.5% $CO_2$ | 0.81 | 102.53 | 2.39 | 187.59 |
| air+ 15% $CO_2$ | 0.67 | 84.81 | 0.43 | 34.09 |

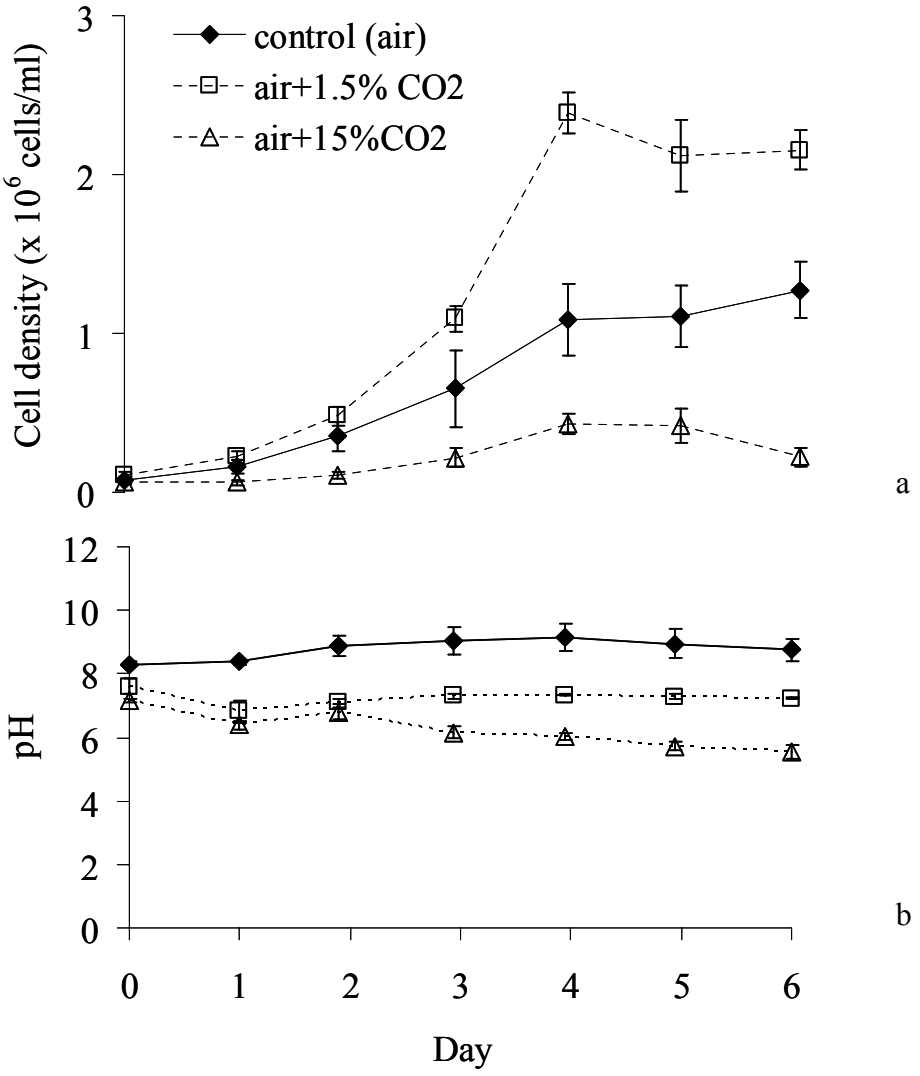



**Figure 4.5** Growth of *T. suecica* (a) and pH (b) in batch culture aerated by mixture of air and 0% (—◆—) 1.5% (- - □ - - -) and 15% (- - - △ - - -) of $CO_2$

The pH of culture medium without $CO_2$ addition was between 8-10 throughout the culture period. On the other hand, increasing of $CO_2$ resulted in decrease of pH in the culture medium. Sharply decreased of pH from 8.23 to 6.32 was found in *T. suecica* culture with 15% $CO_2$. Lower $CO_2$ concentration (1.5%) induced lesser decrease of pH from pH 8.33 to 7.22 (Fig 4.5). In general, low pH reduces the activity of extracellular carbonic anhydrase (Balkos and Colman, 2007) which is a key enzyme of carbon concentration mechanism (Moroney and Ynalvez,

2007). Carbonic anhydrase is the key enzyme that convert $HCO_3^-$ to $CO_2$ and also facilitate $CO_2$ uptake (Tortell *et al*., 2006).

CHN analysis results indicated that the C:H:N ratio of *I. galbana* and *T. suecica* cultured with pure air (control) were 8.2:1.3:1 and 8.9:1.5:1 respectively. On the other hand, growing *I. galbana* and *T. suecica* in 1.5% $CO_2$ resulted in an increase of C:H:N ratio in the algal cells to 9.3:1.6:1 and 17.3:3:1 respectively. This can be implied that both of microalgae exposed to high $CO_2$ concentration had higher carbon assimilation efficiency. This result was somewhat similar to those found in *Skeletonema costatum* (Burkhardt *et al.*, 1999) and *Spirulina platensis* (Gordillo *et al*., 1999). However, Burkhardt and Riebesell (1997) and Andersen and Andersen (2006) showed that the increase of $CO_2$ concentration altered C, N and P content in microalgal cell at different magnitude depending on microalgal species. In this study, the $CO_2$ enrichment seemed to increase carbon content and decrease nitrogen content in *I. galbana* and *T. suecica* cells. Several studies reported that the increase of carbohydrate occurs in response to the rising of $CO_2$ concentrations (Gordillo *et al*., 1999; Giordano, 2001). Moreover, the increase $CO_2$ concentration resulted in the decreased of carbonic anhydrase (CA), and nitrate reductase activities in *Chlamydomonas reinhardtii* and *Chlorella pyrenoidosa* (Xia and Gao, 2005). The declined of nitrate reductase activities diminish nitrogen assimilation efficiency in the microalgae (Xia and Gao, 2005).

## 4.2 Bivalves culture

### 4.2.1 Feeding and growth rate of large oyster

#### 4.2.1.1 Feeding rate of large oyster *C. belcheri* on *I. galbana*

Fifteen oysters with 215±37.3 g average weight were transferred to 2.5 L glass aquarium containing 2L of *I. galbana* cells at various concentrations from $50\times10^4$ to $580\times10^4$ cells/mL. Cell density of *I. galbana* was determined by counting with haemacytometer. Reduction of *I. galbana* density during the first 100 minutes of each experimental unit was used for feeding rate calculation. It was found that feeding rate of the oyster was increased with an increase of food (*I. galbana*)

concentration (Fig 4.6). The maximum feeding rate of $4.9\times10^7$ cells/min was obtained when they exposed to $580\text{x}10^4$ cells/mL of *I. galbana* and the lowest feeding rate of $1.9\times10^7$ cells/min was showed when they were exposed to $50\times10^4$ cells/mL (Table 4.4)

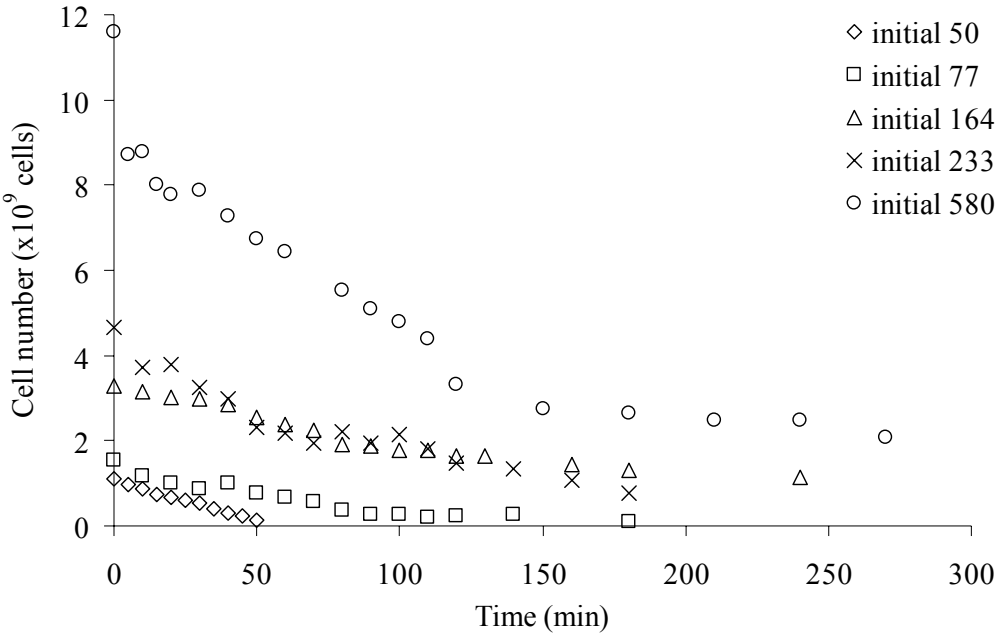



**Figure 4.6** Average cell number reduction of *I. galbana* in glass aquarium with 1 large oyster *C. belcheri* (215±37.3 g). The initial cell density of *I. galbana* were 50 (◇), 77 (□), 164 (Δ), 233 (X) and 580 (○) $\times10^4$ cells/mL

**Table 4.4** Initial cell density of *I. galbana* and large oyster *C. belcheri* feeding rate

| Initial cell density ($\times10^4$ cells/mL) | Total cell ($\times10^7$ cells) | Feeding rate ($\times10^7$ cells/min/individual) |
|---|---|---|
| 50 | 100 | 1.9 |
| 77 | 154 | 1.1 |
| 164 | 328 | 1.6 |
| 233 | 466 | 3.1 |
| 580 | 1,160 | 4.9 |

#### 4.2.1.2 Growth of large oyster *C. belcheri* fed by *I. galbana* with continuous feeding

**First experiment**

Oyster cultured with continuous feeding system consisted of a continuous culture system for microalgae *I. galbana* connecting to an oyster tank.

*I. galbana* was continuously fed to the oyster tank and waste water from the oyster tank was gravity flew to wastewater tank The *I. galbana* suspension was fed to oyster tank and reduction of *I. galbana* cell number was recorded every 24 Hr. The result showed that, after 24 days of experiment, the oyster weight increased from 173.29 g to 174.71 g. The adjustment of flow rate hence resulted in the changes of cell density. The average cell density of *I. galbana* in continuous culture reactor from day 1 to day 7 was 818.2±224.4×$10^4$ cells/mL. While the average flow rate of fresh medium fed into the algal reactor and dilution rate were 2.2±0.3 L $day^{-1}$ and 0.5±0.1 $day^{-1}$, respectively. From day 8 to 15, increasing flow rate to 2.7±0.4 L $day^{-1}$ resulted in an increase of dilution rate to 0.6±0.1 $day^{-1}$ and decrease in cell density to 425.2±67.1×$10^4$ cells/mL. As the cell density decreased, flow rate of fresh medium was adjusted down to 1.6± 0.2 L $day^{-1}$ in day 16-18. During day 21-24, due to the decrease in cells density in algal reactor, flow rate was reduced to 0.27±0.14 L $day^{-1}$ (Fig 4.7). The growth rate of *C. belcheri* in this experiment was 59.16 mg/individual/day or 0.81% of initial weight within 24 days. Ammonia and pH in oyster tank was monitored every 24 Hr. The result showed that ammonia during experiment period was between 0-0.5 mg/l $NH_4^+$and pH was between 8.25-8.43.

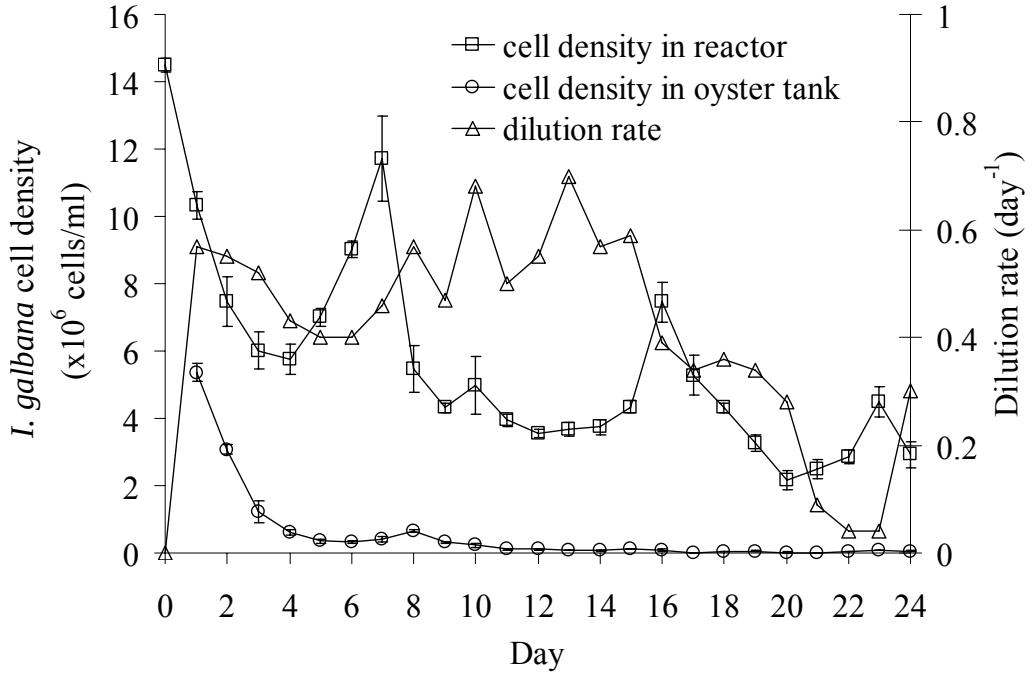



**Figure 4.7** Cell density in algal reactor (□), cell density in oyster tank (○) and dilution rate of *I. galbana* continuous culture (Δ) of first experiment

Feeding rate and *I. galbana* cell density in oyster tank is shown in Fig 4.8. The result illustrated the relationship between feeding rate and *I. galbana* cell density in which the feeding rate increased with increasing *I. galbana* cell density in the algal reactor. The average oyster feeding rate of this experiment was $6.59\pm3.99\times10^6$ cells/min.

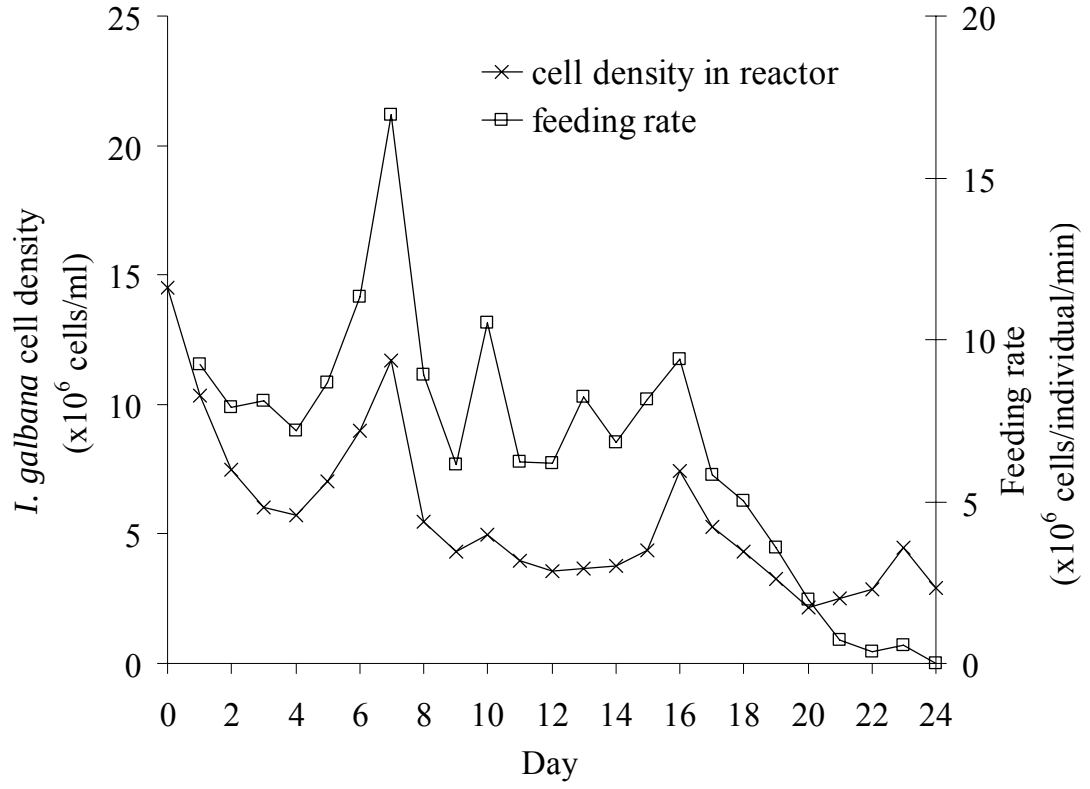



**Figure 4.8** *I. galbana* cell density in algal reactor (x) and oyster feeding rate (□) in the first experiment

**Second experiment**

The oysters were cultured in continuous feeding system for 19 days. No growth of the oyster was detected since the oyster average weight reduced from 180.63 to 178.9 g. The average *I. galbana* cell density in algal reactor, average fresh medium flow rate and dilution rate were $6.0\pm2.2\times10^6$ cells/mL, $2.0\pm0.4$ L $day^{-1}$ and $0.4\pm0.1$ $day^{-1}$ respectively (Fig 4.9). The results of ammonia and pH during experiment period were between 0-0.5 mg/L and pH 8.27-8.39, respectively.

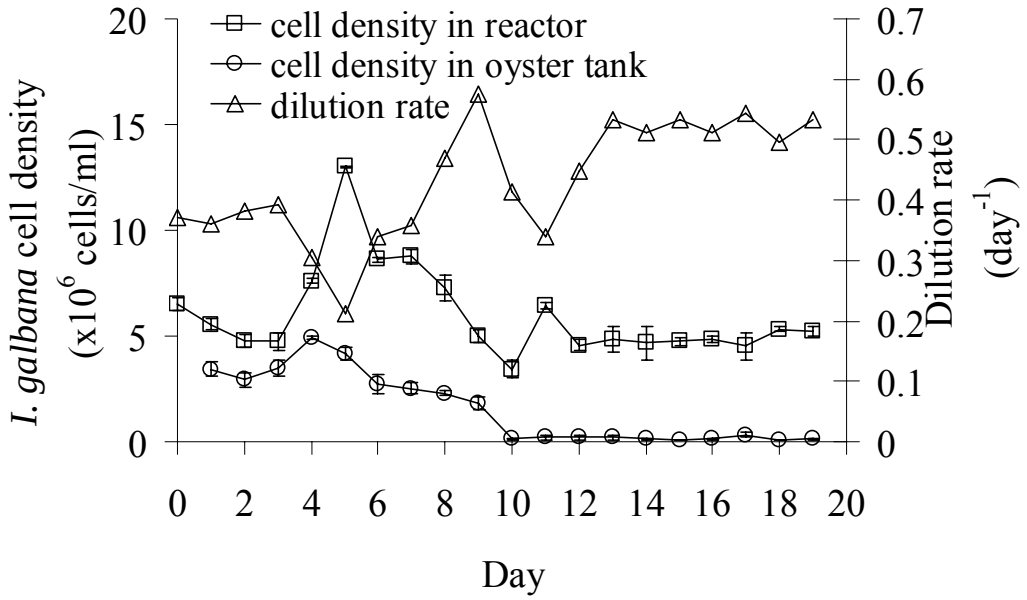



**Figure 4.9** Cell density in algal reactor(□), cell density in oyster tank (○) and dilution rate of *I. galbana* continuous culture (Δ) in the second experiment

The daily cell density in algal reactor and oyster feeding rate are shown in Fig 4.10. The average oyster feeding rate was $6.13 \pm 2.3 \times 10^6$ cells/min. The results showed that the oyster feeding rate depended on *I. galbana* cell density.

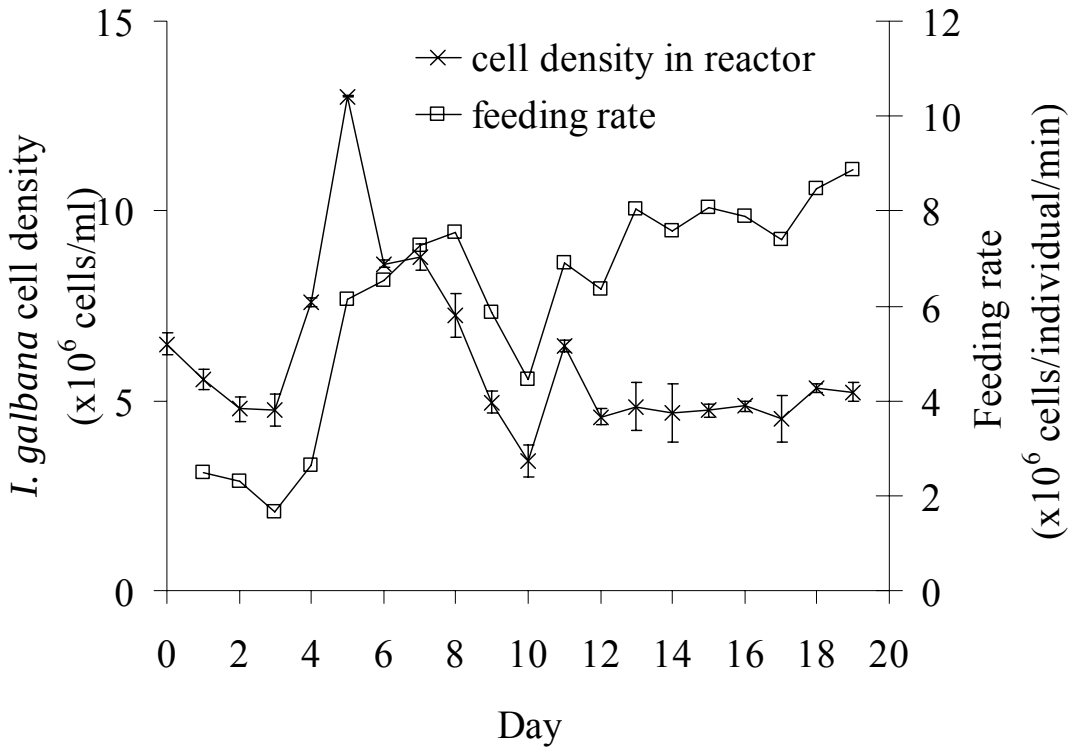



**Figure 4.10** *I. galbana* cell density in algal reactor (X) and oyster feeding rate (□) of second experiment

**Third experiment**

The oyster was continuously fed with *I. galbana.* The results showed that, after 16 days incubation, the oyster fresh weight decreased from 178.88 g to 177.81 g. The average cell density in algal reactor, average fresh medium flow rate and dilution rate were $4.8\pm1.1\times10^4$ cells/mL, 2.2±0.2 L $day^{-1}$ and 0.47±0.4 $day^{-1}$ (Fig 4.11). Ammonia and pH during experimental period were between 0-0.5 mg/L and 8.15-8.25.

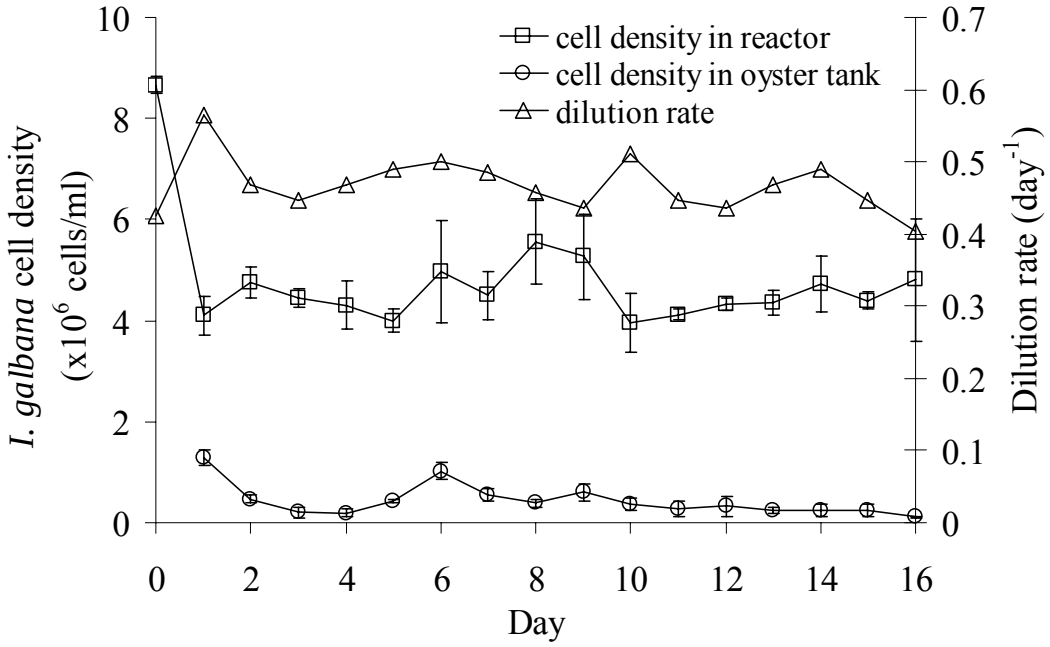



**Figure 4.11** Cell density in algal reactor (□), cell density in oyster tank (○) and dilution rate of *I. galbana* continuous culture (Δ) of third experiment

With the third experiment, the average oyster feeding rate was $624.37 \pm 60.74 \times 10^4$ cells/min. The oyster feeding rate increased with an increase of *I. galbana* cell density (Fig 4.12).

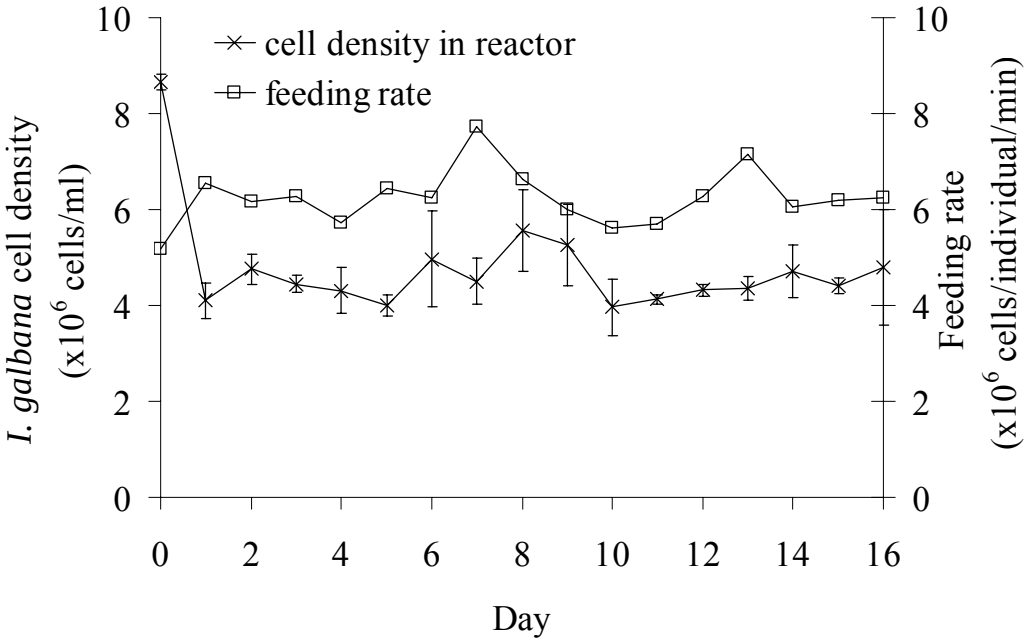



**Figure 4.12** *I. galbana* cell density in algal reactor (X) and oyster feeding rate (□) of third experiment

### 4.2.2 Feeding and growth rate of small oyster

#### 4.2.2.1 Feeding rate of small oyster *C. belcheri* on *I. galbana*

Feeding rate of small oyster with 16.26±2.92 g fresh weight was evaluated with various cell density of *I. galbana* between 109-814×$10^4$ cells/mL. Experiment was performed in 500 mL glass aquarium containing *I. galbana* cultures and the experimental period was 250 minutes. The results showed that the small oyster had the highest feeding rate of 794×$10^4$ cells/min when fed with 246x$10^4$ cells/mL of *I. galbana* (Fig 4.13) while the lowest feeding rate of 178×$10^4$ cells/min was found with 814×$10^4$ cells/mL of *I. galbana*(Table 4.5). In general, feeding rate of small oyster increased with algal cell density. In general, there are many factors influencing on feeding rate or filtration rate of bivalve such as particle concentration, temperature and salinity but particle concentration seemed to be the largest factor (Rajesh *et al*., 2001; Gosling, 2003).

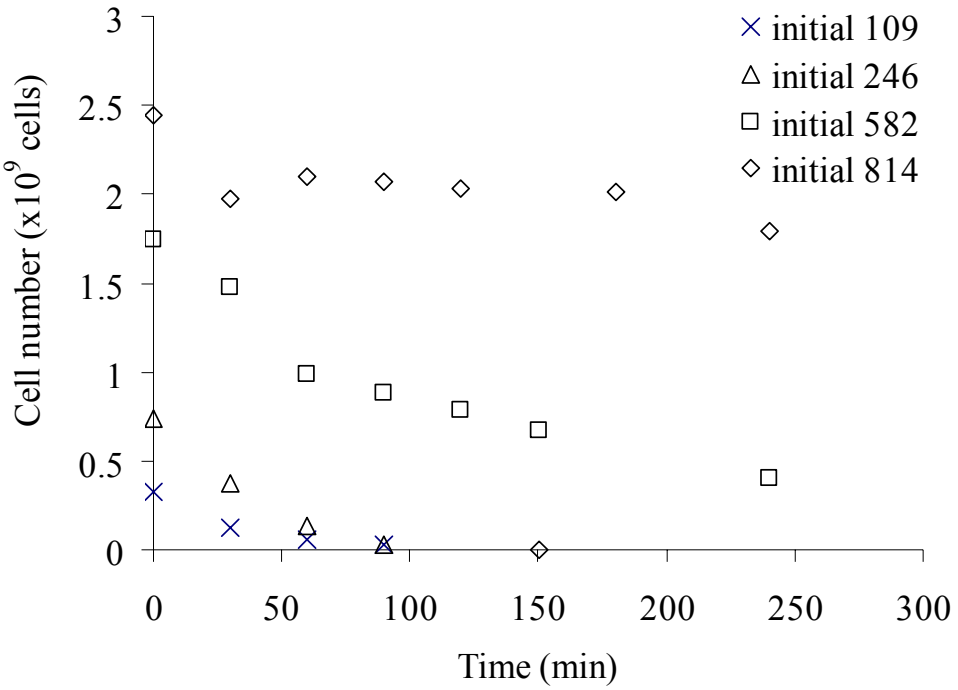



**Figure 4.13** Average cell number reduction of *I. galbana* cells in glass aquarium containing 1 small oyster (16.26±2.92 g). The initial cell density of *I. galbana* were 109(X), 246 (Δ), 582(□) and 814 (◇) ×$10^4$ cells/mL

**Table 4.5** Initial cell density of *I. galbana* and small oyster *C. belcheri* feeding rate

| Initial cell density ($\times 10^4$ cells/mL) | Total cell ($\times 10^7$ cells) | Feeding rate ($\times 10^4$cells/min/individual) |
|---|---|---|
| 109 | 33 | 324 |
| 246 | 74 | 794 |
| 582 | 174 | 540 |
| 814 | 244 | 178 |

#### 4.2.2.2 Growth of small oyster *C. belcheri* fed by *I. galbana* with continuous feeding

According to poor growth rate of large oyster in the previous three experiments, the smaller size of oyster was chosen instead in this experiment. The average weight of small oyster was 12.85±2.46 g and they were cultured with continuous feeding. The results showed that small oyster also had poor growth rate. The final weight of small oyster was 12.22±2.36 g. The results indicated that fresh weight of small oyster decreased by 4.9 % of initial weight or 42.0±39.7 mg/individual/day.

The average cell density of *I. galbana* in algal reactor, fresh medium flow rate and dilution rate was $6.0\pm0.98\times10^6$ cells/mL, 2.0±0.2 L $day^{-1}$ and 0.43±0.4 $day^{-1}$ respectively (Fig 4.14). Daily feeding rate of oyster *C. belcheri* was shown in Fig 4.15. The average feeding rate was $250\times10^4$ cells/individual/min or 0.32 g dry weight of *I. galbana*/individual/day. Along culture period ammonia and pH was evaluated. The result showed that ammonia and pH were between 0-0.5 mg/L and 8.11-8.24.

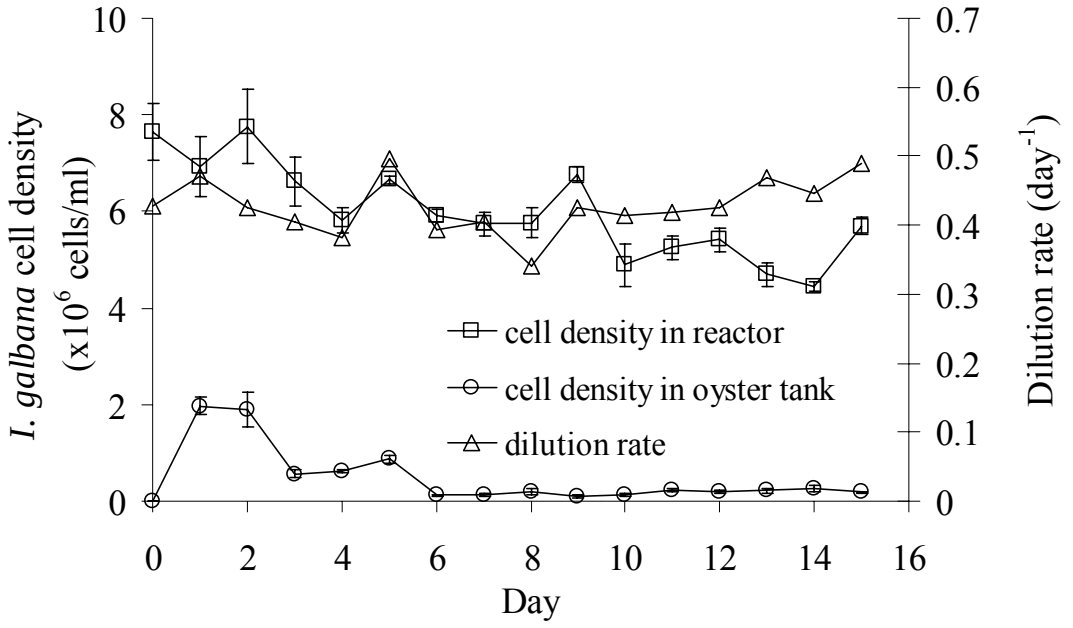



**Figure 4.14** Cell density in algal reactor (□), cell density in small oyster tank (○) and dilution rate of *I. galbana* continuous culture (Δ)

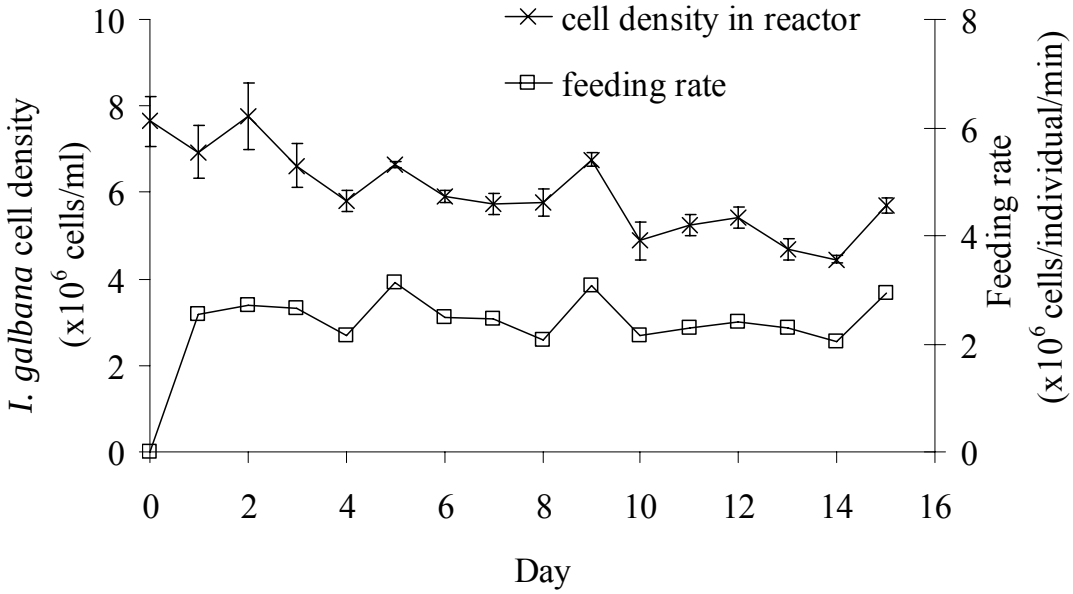



**Figure 4.15** *I. galbana* cell density in algal reactor (X) and small oyster feeding rate (□)

Although smaller size oyster was used but their growth rate was also as poor as the previous experiment. However, growth rate of *C. belcheri* in other report was also low. For example, Tanyaros (2008) reported that growth rate of juvenile

oyster nursed in the intertidal mangrove area for four months was 25.75 mg/individual/day which was even lower than the first experiment of this study. For large oyster, growth rate of *C. belcheri* was 390 mg/individual/day in cultured area, Bandon Bay in Surat Thani province, while shell size increased from 5.97 to 11.6 cm in height and 4.96 to 8.62 in length within 18 month (Gannarong and Sopakul, 2004). Due to low growth of oyster in this experiment, other bivalves such as hooded oyster and blood cockle were used instead in the following experiments.

### 4.2.3 Feeding and growth rate of cockle *A. granosa*

#### 4.2.3.1 Feeding rate of cockle *A. granosa* on *I. galbana*

Feeding rate of blood cockle with 3.22±0.43 g fresh weight was determined with various cell density of *I. galbana* between 128-837×$10^4$ cells/mL in 150 mL glass aquarium. The experimental period was 250 minutes. The results showed that cell concentration was decreased with time (Fig 4.16). The highest feeding rate of was obtained when blood cockle exposed to cell density of 128×$10^4$ cells/mL (Table 4.6).

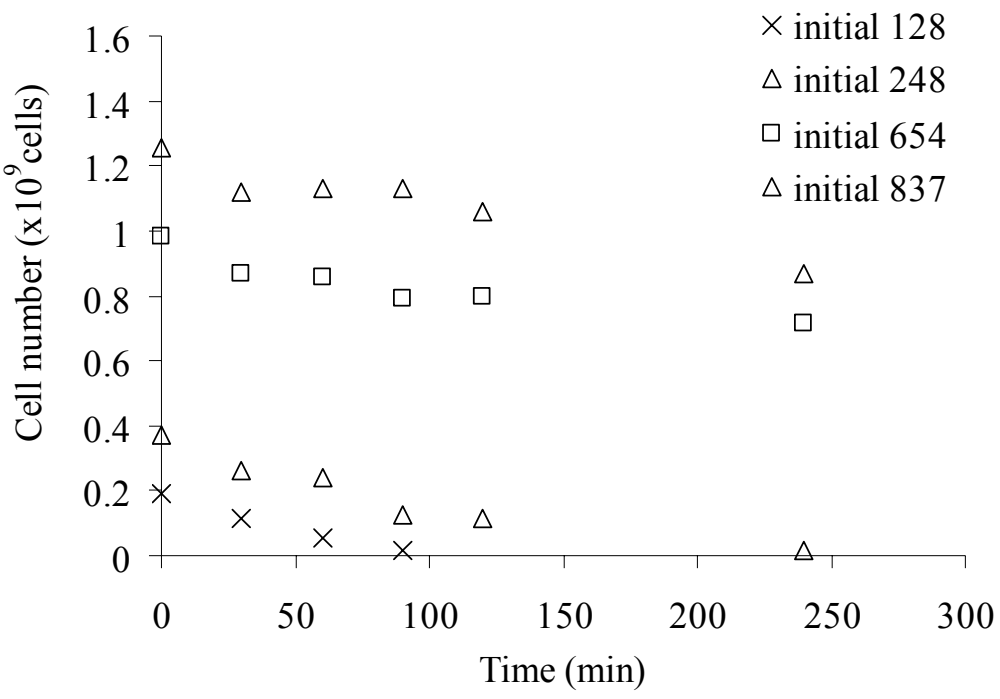



**Figure 4.16** Average cell number reduction of *I. galbana* in glass aquarium with *A. granosa* (3.22±0.43 g). The initial cell density of *I. galbana* were 128 (X), 248 (Δ), 654 (□) and 837 (◇) ×$10^4$ cells/mL

**Table 4.6** Initial cell density of *I. galbana* and *A. granosa* feeding rate

| Initial cell density ($\times 10^4$ cells/mL) | Total cell ($\times 10^7$ cells) | Feeding rate of *A. granosa* ($\times 10^4$ cells/min/individual) |
|---|---|---|
| 128 | 19 | 196 |
| 248 | 37 | 141 |
| 654 | 98 | 96 |
| 837 | 126 | 143 |

#### 4.2.3.2 Growth of *A. granosa* fed by *I. galbana* with continuous feeding

**First experiment**

Blood cockle were continuously fed with *I. galbana* under laboratory condition as described in section 4.5.1. The sixteen blood cockle with average weight, shell length and height of 4.48±0.87 g, 2.18±0.19 and 1.54±0.18 respectively were used. The average cell density, flow rate and dilution rate in continuous culture of *I. galbana* were $8.0\pm3.0\times10^6$ cells/mL, 2.1±0.2 L/day and 0.45±0.06 per day (Fig 4.17). The total ammonia in bivalve tank was between 0-0.5 mg/L along the al period. Feeding rate of blood cockle was shown in Fig 4.18. In this experiment blood cockle died within 7 days so the experiment was terminated.

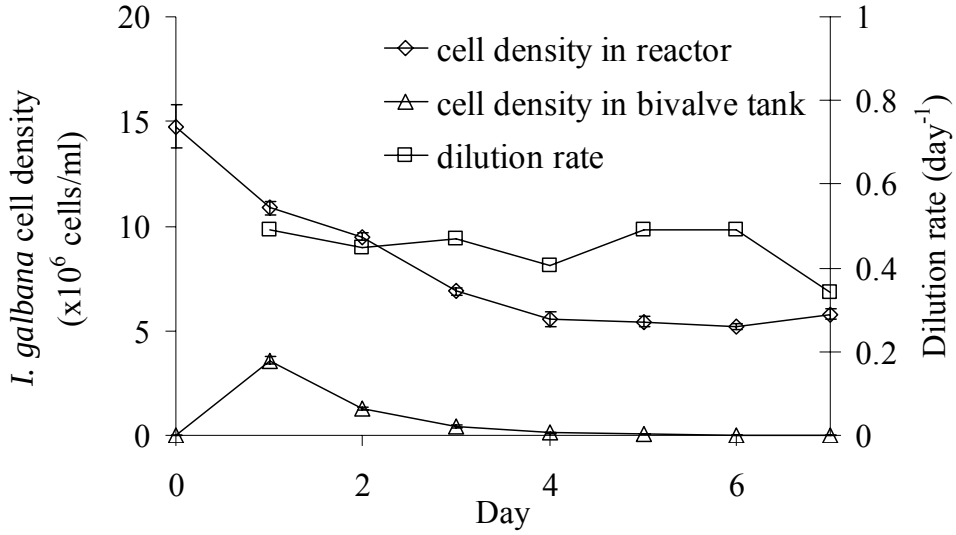



**Figure 4.17** Cell density of *I. galbana* in algal reactor (◇) and in *A. granosa* tank (Δ) and the dilution rate of algal continuous culture system (□) in the *A. granosa* culture (first experiment)

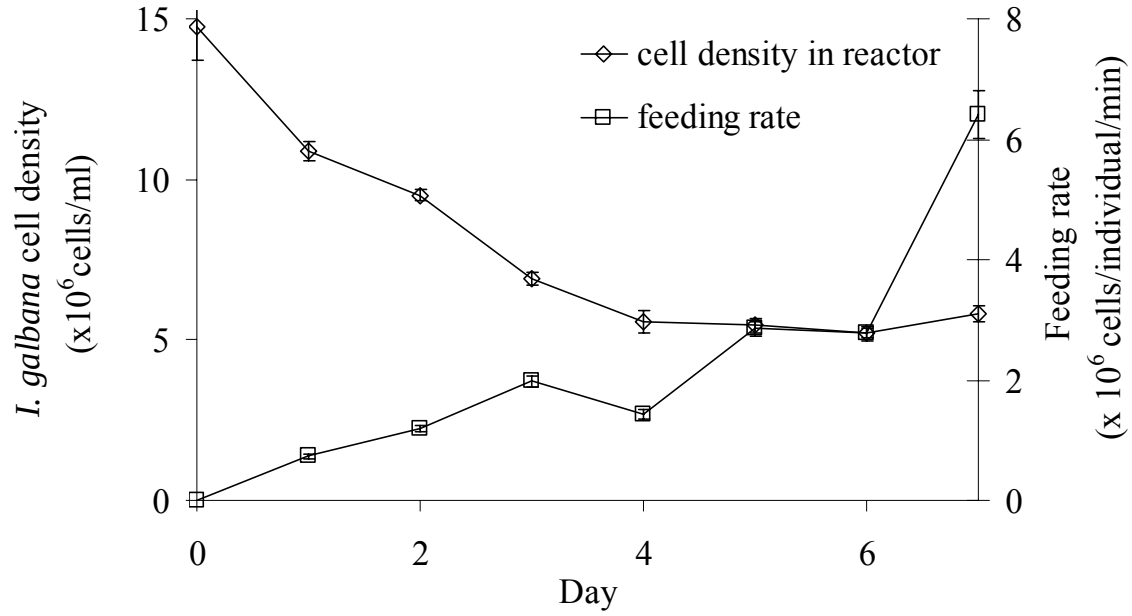



**Figure 4.18** *I. galbana* cell density in algal reactor (◇) and *A. granosa* feeding rate (□) of first experiment

**Second experiment**

In the first experiment of blood cockle could not survive hence the density of cockle was reduced from 16 to 4 individual/tank. The average weight, shell length and height of blood cockle was 11.6±1.49 g, 3.08±0.16 cm and 2.49±0.08 cm respectively. The average *I. galbana* cell density in algal reactor was $7.6\pm3.2\times10^6$ cells/mL. The flow rate and dilution rate in *I. galbana* continuous culture system was 2.3 ± 0.3 L/day and 0.4±0.18 per day (Fig 4.19). The results showed that during experiment total ammonia was between 0-0.5 mg/L. Feeding rate of blood cockle was shown in Fig 4.20. Unfortunately, blood cockle could not survive under laboratory condition and died within 6 days.

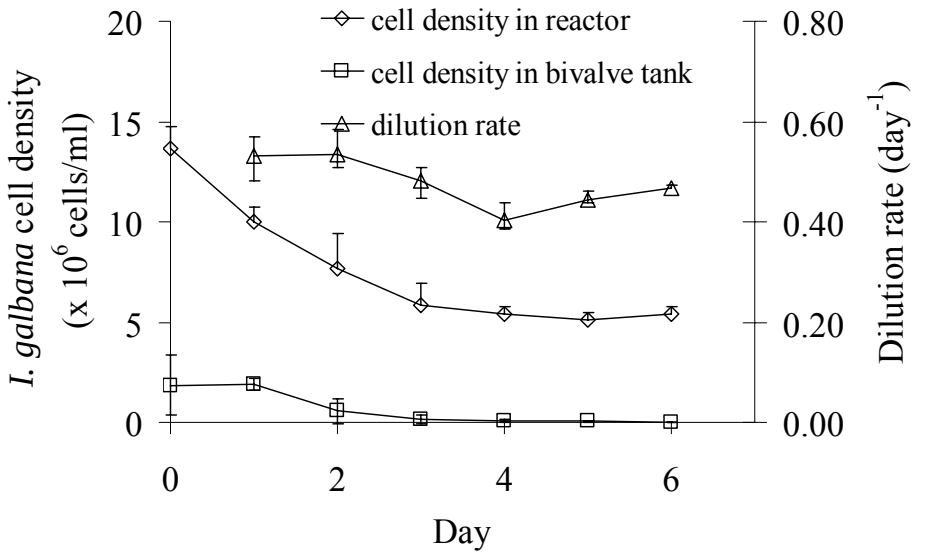



**Figure 4.19** Average cell density of *I. galbana* in algal reactor (◇) and in *A. granosa* tank (□) and the dilution rate of algal continuous culture system (Δ) in the *A. granosa* culture (second experiment).

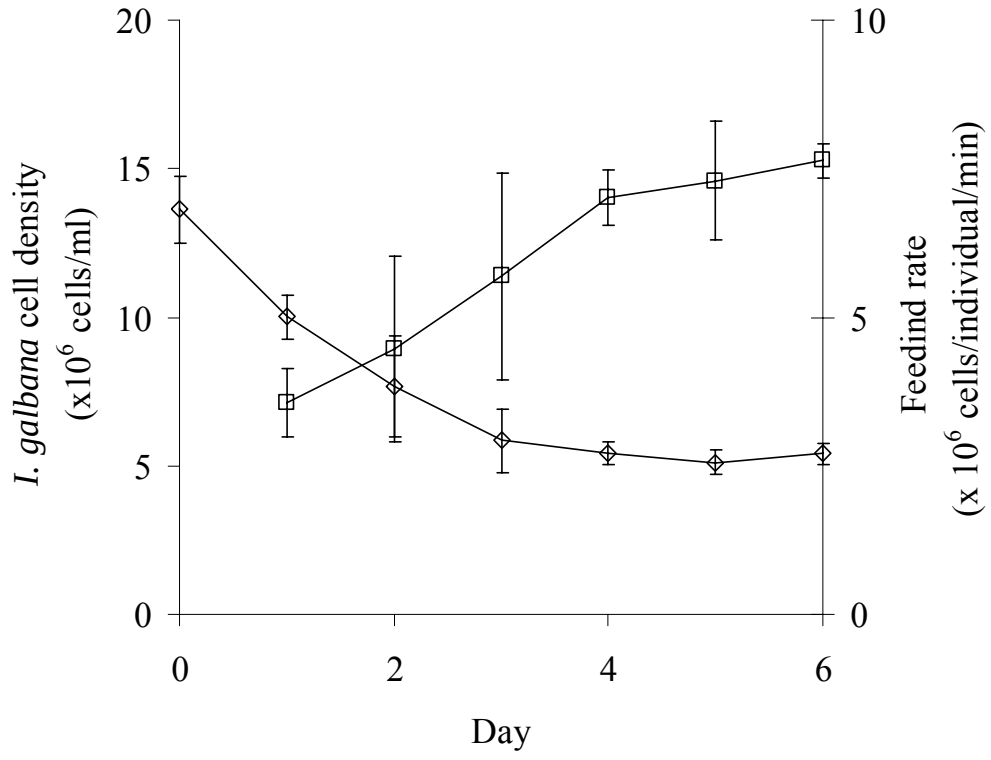



**Figure 4.20** *I. galbana* cell density in algal reactor (◇) and *A. granosa* feeding rate (□) of the second experiment.

### 4.2.4 Feeding and growth rate of hooded oyster *S. cucullata*

As the poor growth rate of *C. belcheri* and *A. granosa*, *S. cucullata* was used in this experiment.

#### 4.2.4.1 Feeding rate of hooded oyster *S. cucullata.* on *I. galbana*

Hooded oyster with average oyster shell length and shell height of 2.3±0.19 cm and 1.9±0.17 cm respectively were transfered to 200 mL beaker containing 200 mL of suspended *I. galbana*. The cell density of microalgae was between 47-738×$10^4$ cells/mL. Reducing of cell density in bivalve tank was evaluated by counting with haemacytometer. The result showed that feeding rate of hooded oyster was increased with initial cell density (Fig 4.21). The highest feeding rate of 378×$10^4$ cells/min was found along with the highest initial cell density of 738×$10^4$ cells/mL. The lowest feeding rate of 58×$10^4$ cells/min was also found with 47×$10^4$ cells/mL (Table 4.7).

**Table 4.7** Initial cell density of *I. galbana* and hooded oyster *S. cucullata* feeding rate

| Initial cell density (×$10^4$ cells/mL) | Total cell (×$10^7$ cells) | Feeding rate of *Saccostrea* sp (×$10^4$ cells/min) |
|---|---|---|
| 47 | 8.6 | 58 |
| 117 | 21.3 | 128 |
| 338 | 61.5 | 219 |
| 738 | 134.2 | 378 |

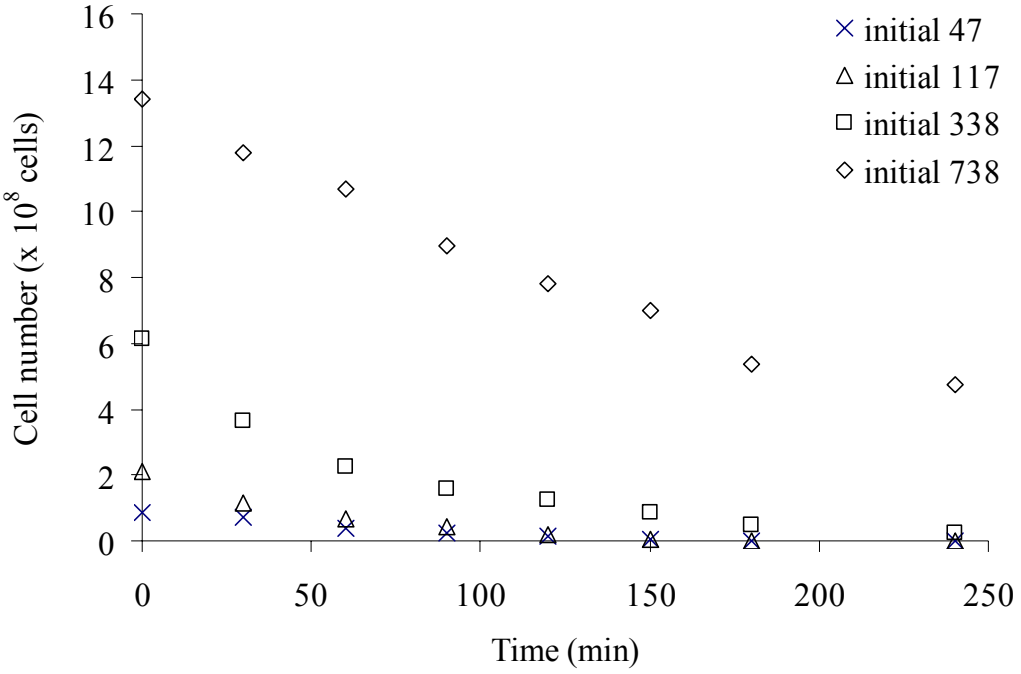



**Figure 4.21** Average cell number reduction of *I. galbana* in glass aquarium with a *S. cucullata* (2.3±0.19 cm in length and 1.9±0.17 cm in height). The initial cell density of *I. galbana* 47 (X), 117 (Δ), 338 (□) and 738 (◇)×$10^4$ cells/mL

#### 4.2.4.2 Growth of hooded oyster *S. cucullata* with continuous feeding by *I. galbana*

The results of previous experiments indicated that blood cockle fed with *I. galbana* could not survive under laboratory condition, hence, sixteen hooded oyster *S. cucullata*., with average shell height and length of 2.09±0.7 and 1.8±0.65 cm respectively, was used in this experiment. The culture system was described in 4.5.1. The result showed that the *I. galbana* cell density, flow rate and dilution rate in algal continuous culture was 7.03±1.8×$10^6$ cells/mL, 1.9±0.3 L/day and 0.4±0.18 per day (Fig 4.22). Total ammonia in oyster tank during experiment period was between 0-0.5 mg/L.

The average initial weight of hooded oyster (body weight plus substrate) was 7.09±1.09 g. After 25 day the survival rate and average weight was 40% and 6.63±0.62 g. The *I. galbana* cell amount consumed by hooded oyster 5.5×$10^{10}$ cells/individual/25 days or 0.07 g dry weight/day/individual. The hooded

oyster feeding rate shown in Fig 4.23 and the average feeding rate of hooded oyster was $153\times10^4$ cells/min/individual.

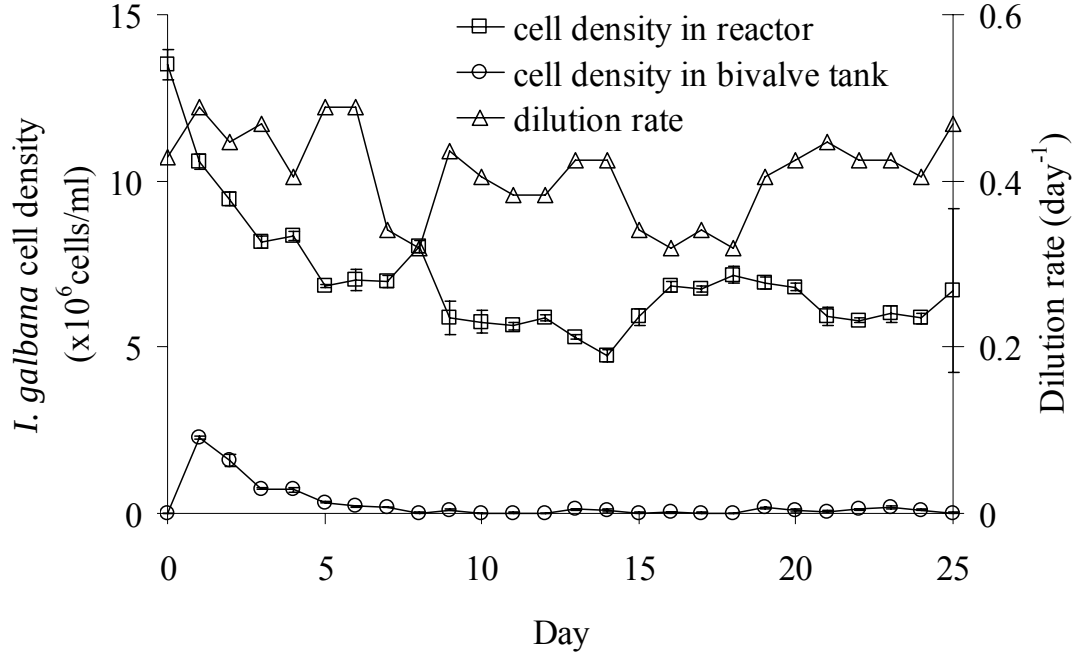



**Figure 4.22** Average cell density of *I. galbana* in algal reactor (□) and in *S. cucullata* tank (○) and the dilution rate of algal continuous culture system (Δ) in the *S. cucullata* culture.

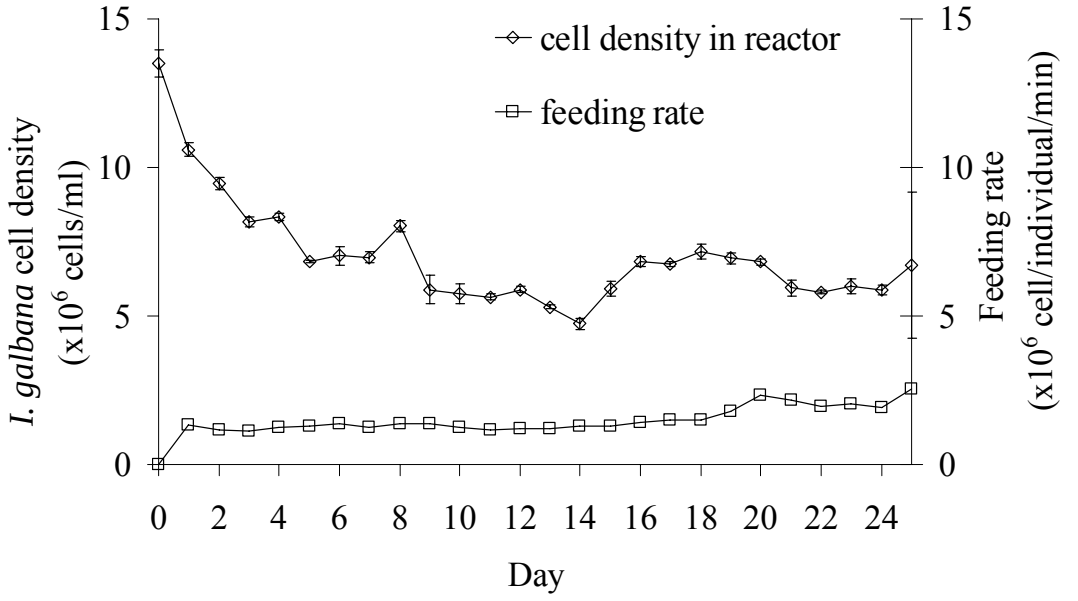



**Figure 4.23** *I. galbana* cell density in algal reactor (◇) and feeding rate (□) of *S. cucullata*

The bivalve culture experiments indicated that oyster, blood cockle and hooded oyster could not grow and survive in this culture system with continuous feeder. One of the possible reasons was that the seawater in bivalve tank was not suitable for bivalve growth since continuous feeding of algae, accordingly, supplied large amount of algal medium into bivalve tank. High concentration of trace metals such as iron, copper and zinc from F/2 Guillard medium in bivalve tank may cause of mortality of bivalve in this culture system. According to Knezovich *et al.* (1981), 10 ug/L of copper could decrease embryo survival of Pacific oyster, *Crassstrea gigas*. Moreover, Martin *et al* (1981) reported that the lowest acute copper concentration for Pacific oyster was 7.8 ug/L. While the 96-h LC50 of copper for *Anadara granosa* was 0.23 mg/L Cu (Ong and Din, 2001). In addition, the 50% reduction in shell growth rate of juvenile *Mytilus edulis* was found with 4 ug/l of copper (Widdows and Donkin, 1992). Moreover, in natural blood cockles usually bury themselves in muddy bottoms but there is no sediment in present study, it may resulted in mortality of blood cockle.

#### 4.2.4.3 Feeding rate of hooded oyster *S. cucullata* on *T. suecica*

The result from previous experiment showed that *C. belcheri, A. granosa, S. cucullata* could not grow with continuous feeding by continuous culture of *I. galbana*. The next experiment was performed with batch feeding by *T. suecica*, due to larger cell size than *I. galbana.*

Hooded oyster with average oyster shell length and shell height of 2.4±0.2 cm and 2.18±0.2 cm respectively were transfered to 200 mL beaker containing 200 mL of suspended *I. galbana*. The cell density of microalgae was between 1-50×$10^4$ cells/mL. Reducing of cell density in bivalve tank was evaluated by counting with haemacytometer. The result showed that feeding rate of hooded oyster was increased with initial cell density (Fig 4.24). The highest feeding rate of 5.9×$10^4$ cells/min was found along with the highest initial cell density of 50×$10^4$ cells/mL. The lowest feeding rate of 0.89×$10^4$ cells/min was also found with 1×$10^4$ cells/mL (Table 4.8).

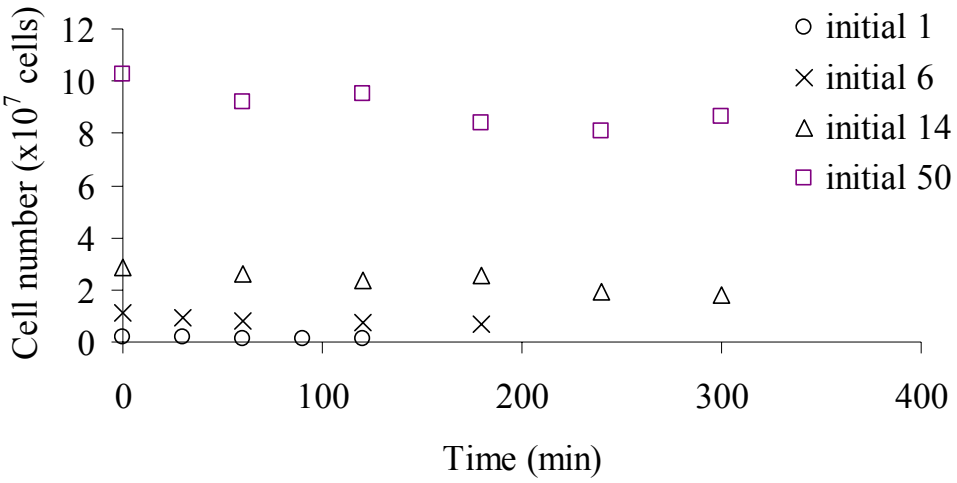



**Figure 4.24** Average cell number reduction of *T. suecica* in glass aquarium with a *S.cucullata* (2.4±0.2 cm in length and 2.18±0.2 cm in height). The initial cell density of *T. suecica* 1(○), 6(X), 14(Δ), and 50(□)×$10^4$ cells/mL

**Table 4.8** Initial cell density of *T. suecica* and *S. cucullata*. feeding rate

| Initial cell density (×$10^4$ cells/mL) | Total cell (×$10^7$ cells) | Feeding rate of *S. cucullata* (×$10^4$ cells/min) |
|---|---|---|
| 1 | 0.2 | 0.89 |
| 6 | 1.2 | 2.2 |
| 14 | 2.8 | 3.4 |
| 50 | 10 | 5.9 |

#### 4.2.4.4 Growth of hooded oyster *S. cucullata* with batch feeding by *T. suecica*.

Due to the problems of continuous feeding in previous experiments, this experiment was performed in the plastic container and fed with high concentration of microalgae at the lower frequency of once a day. Moreover, in order to maintain the optimum water quality, the water in bivalve tank was changed every 24 Hr. The experiment was performed with 2 treatments (1 L and 5 L of seawater), each with triplicate. The filtered 30 psu seawater was filled into 1 and 5 L plastic container with 5 hooded oysters per each plastic container. The initial weight and shell of hooded oyster in 1L treatment was 7.88±0.86 g 2.26±0.38 cm in height and 2.03±0.54 in length, respectively. The initial weight and shell of hooded oyster in

another treatment (5L) was 8.15±0.86 g 2.14±0.41 cm in shell height and 1.87±0.47 in shell length, respectively. The result showed that the fresh weight of hooded oyster from both treatments was lower than the initial fresh weight. Due to the poor growth of hooded oyster in this experiment, green mussel *Perna viridis* which has less shell thickness was therefore chosen for the following experiment.

### 4.2.5 Feeding and growth rate of green mussel *P. viridis* on *T. suecica*

As the poor growth rate of *C. belcheri, A. granosa, S. cucullata*, *P. viridis* was used in this experiment.

#### 4.2.5.1 Feeding rate of *P. viridis* bivalve on *T. suecica* with batch feeding

Feeding rate was study in *P. viridis* with average weight 3.79±0.23 g. The initial cell density of *T. suecica* in mussel tank was 1, 5, 10 and $20\times10^4$ cells/mL. The result showed that number of *T. suecica* decreased with time (Fig 4.25). The highest feeding rate of $173\times10^4$ cells/min/individual was achieved at the initial cell density of $20\times10^4$ cells/min (Table 4.9). Feeding rate of green mussel increased with cell density, this was similar to that of Wong and Cheung (1999) who reported that clearance rate of *P. viridis* is a function of seston concentration and organic content of seston.

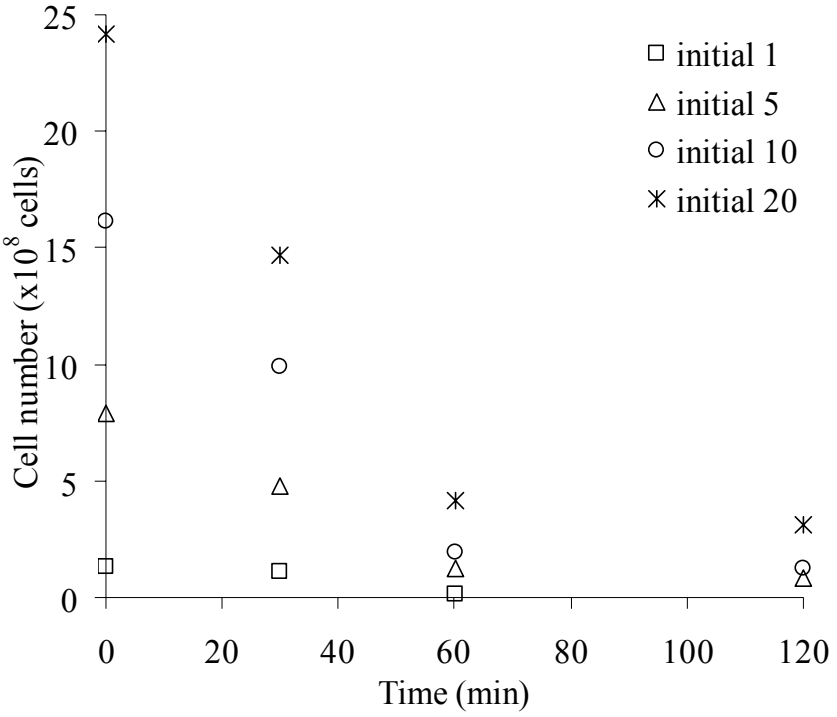



**Figure 4.25** Average cell number reduction of *T. suecica* in glass aquarium with *P. viridis* . (3.79±0.23 g). The initial cell density of *T. suecica* 1 (□), 5 (Δ), 10 (○) and 20(∗)×$10^4$ cells/mL

**Table 4.9** Initial cell density of *T. suecica* and feeding rate of *P. viridis*

| Initial cell density (×$10^4$ cells/mL) | Total cell (×$10^7$ cells) | Feeding rate of *P. viridis* (×$10^4$ cells/min) |
|---|---|---|
| 1 | 1.3 | 20 |
| 5 | 8 | 58 |
| 10 | 16 | 124 |
| 20 | 24 | 173 |

#### 4.2.5.2 Growth of mussel *P. viridis* with batch feeding by *T. suecica*

**First experiment at *T. suecica* cell concentration about 1×$10^4$ cells/mL**

Green mussels were used in this experiment as a result from the poor growth of hooded oysters. Green mussels, as described in previous experiment, were acclimated and cultured in an up-flow system. The average mussel weight was

3.86±0.29 g (30 individuals) the average shell height and length were 3.67±0.29 and 1.73±0.087cm respectively.

Mussels were divided into 2 groups. The first group was used for an estimation of fresh and dry weight relationship. Another group was used for growth rate evaluation. The relationship between fresh weight and dry weigh of *P. viridis* is shown in Fig 4.26.

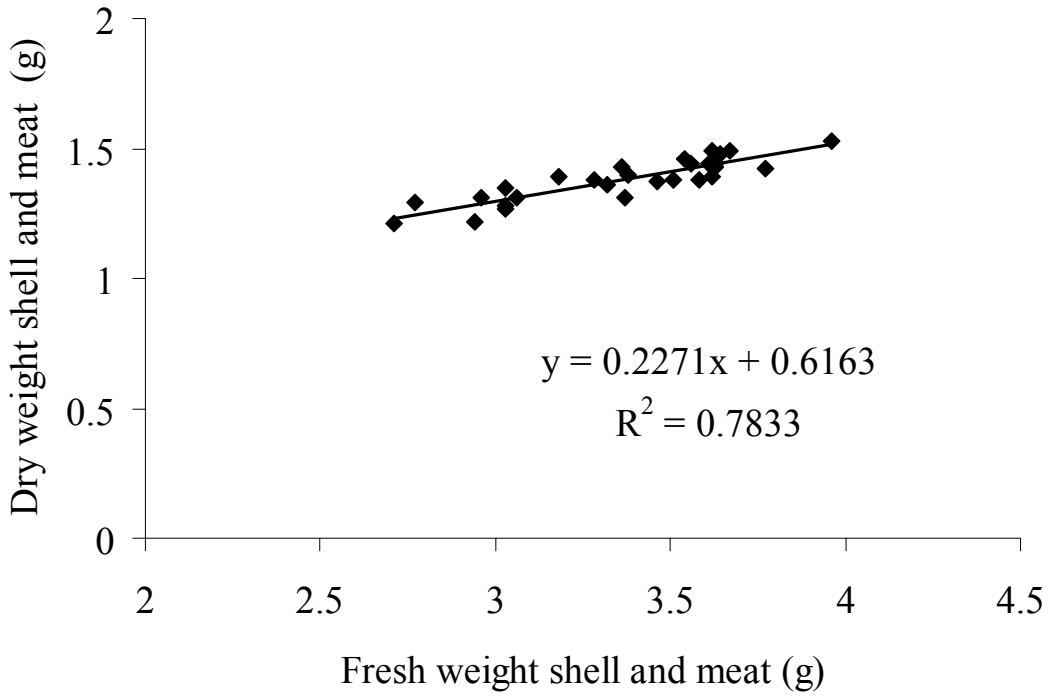



**Figure 4.26** Relationship between fresh and dry weight of green mussel in first experiment

After 2 weeks pre-acclimation, 10 mussels were placed in the 10 L tank containing 10 L of 30 psu seawater. Production of *T. suecica* was accomplished under batch culture condition. Feeding was provided by the addition of *T. suecica* cells once a day. The initial average *T. suecica* cell concentration in mussel tank was $0.62 \pm 0.03 \times 10^4$ cell/mL. At the end of experiment growth of bivalve was evaluated. The result showed that average weight, shell height and length of the bivalve were 4.1±0.16 g, 3.72±0.08 cm and 1.76±0.05 cm, respectively (Fig 4.27). The average weight of mussel significantly increased by 0.2 g/individual within 39 days ($p \leq 0.01$)

and the growth rate was 6.15 mg/individual/day. Cell quantity of *I. galbana* consumed by green mussel was $2.6\times10^8$ cells/individual.

Calcification was evaluated at the end of experiment by EDTA method. The result showed that the average calcification rate of green mussel was 1.94±0.2 mgCa/individual/day

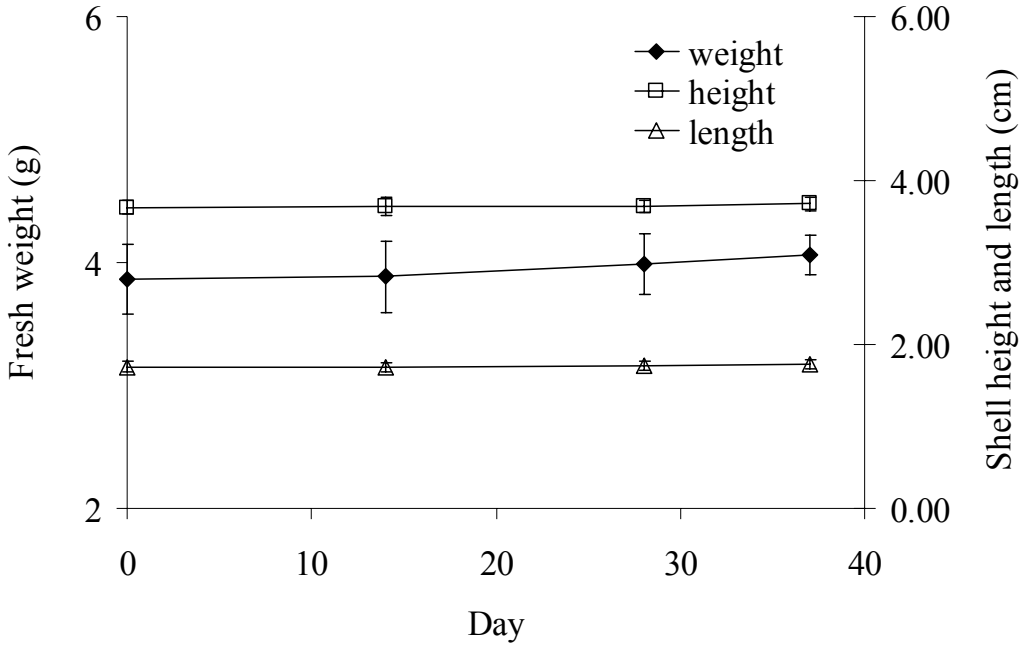



**Figure 4.27** Growth of green mussel *P. viridis* under laboratory condition (first experiment) measured in fresh weight (◆), shell height (□) and length (△)

**Second experiment at *T. suecica* cell concentration about $2\times10^4$ cells/mL**

Green mussel with average weight, shell height and shell length of 5.14±0.13 g, 4.0±0.11 cm and 1.93±0.07 cm, respectively, was cultured under laboratory condition. Green mussel was pre-acclimated before the experiment, similar to the previous experiment. The Relationship between fresh and dry weight of green mussel in second experiment is shown in Fig 4.28. In this experiment, 5 mussels were cultured in plastic container with 5 L of 30 psu sea water. Feeding was provided by the addition of *T. suecica* cells once a day. The initial average *T. suecica* cell concentration in mussel tank was $1.24\pm0.23\times10^4$ cells/mL. After 54 days, average weight, shell height and length of green mussel was 5.49±0.08 g, 4.05±0.07

cm and 1.99±0.05 cm respectively (Fig 4.29). The average weight of green mussel significantly increased at 0.35 g/individual and the growth rate of green mussel was 6.48 mg/individual/day. Cell quantity of *I. galbana* eaten by green mussel was $6.8\times10^8$ cells/individual.

Calcification was evaluated at the end of experiment by EDTA method. The result showed that the average calcification rate of green mussel was 1.54±0.08 mgCa/individual/day

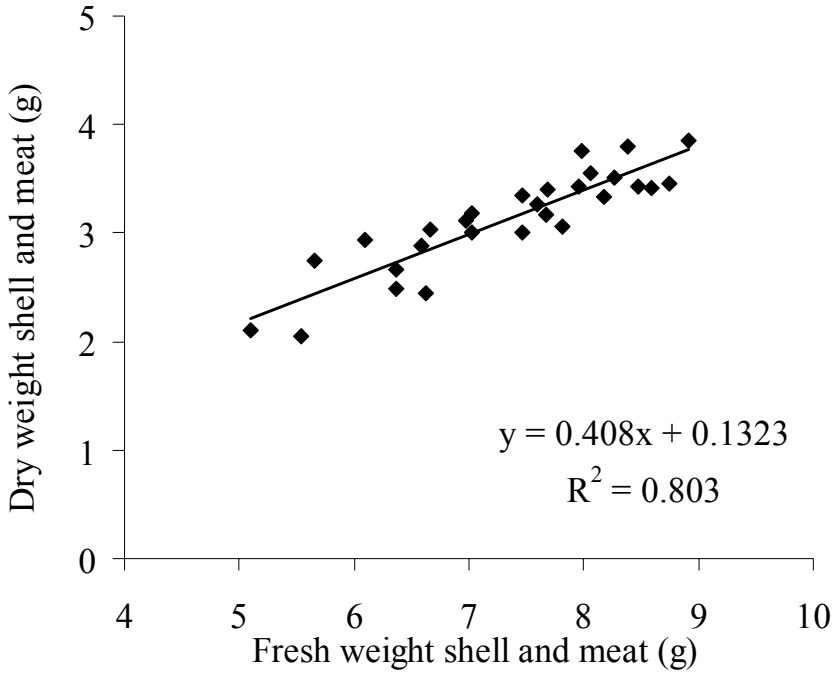



**Figure 4.28** Relationship between fresh and dry weight of *P. viridis* in second experiment

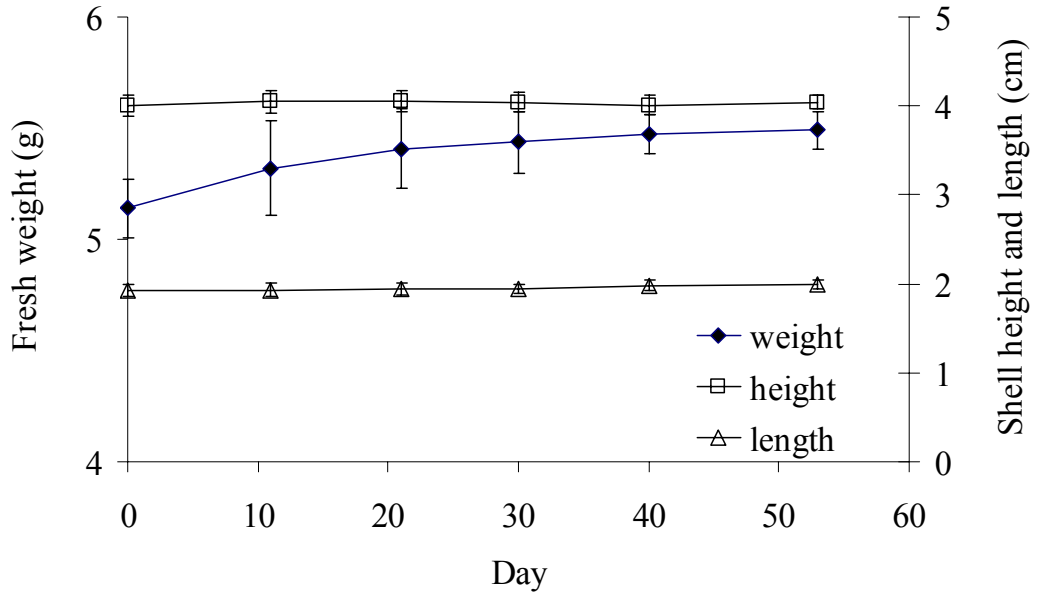



**Figure 4.29** Growth of *P. viridis* under laboratory condition (second experiment) measured in fresh weight (◆), shell height (□) and length (△)

#### 4.2.5.3 Feeding rate of *P. viridis* bivalve on *T. suecica* with continuous feeding by dripping bottle

Feeding rate in continuous feeding system was studied in *P. viridis* with average weight of 1.84 ± 0.09 g. The initial cell density in feed bottle was $96.43\times10^4$ cells/mL. Since continuous feeding system was operated only from hour 0-5, it was found that *T. suecica* cell density in mussel tank increased during the first 5 hours. This was following by the decrease of *T. suecica* cell in hours 5-9 which resulted from feeding (Fig 4.30). It could be assumed that green mussel feeding rate in continuous system was $10.92\times10^6$ cells/ individual/hr.

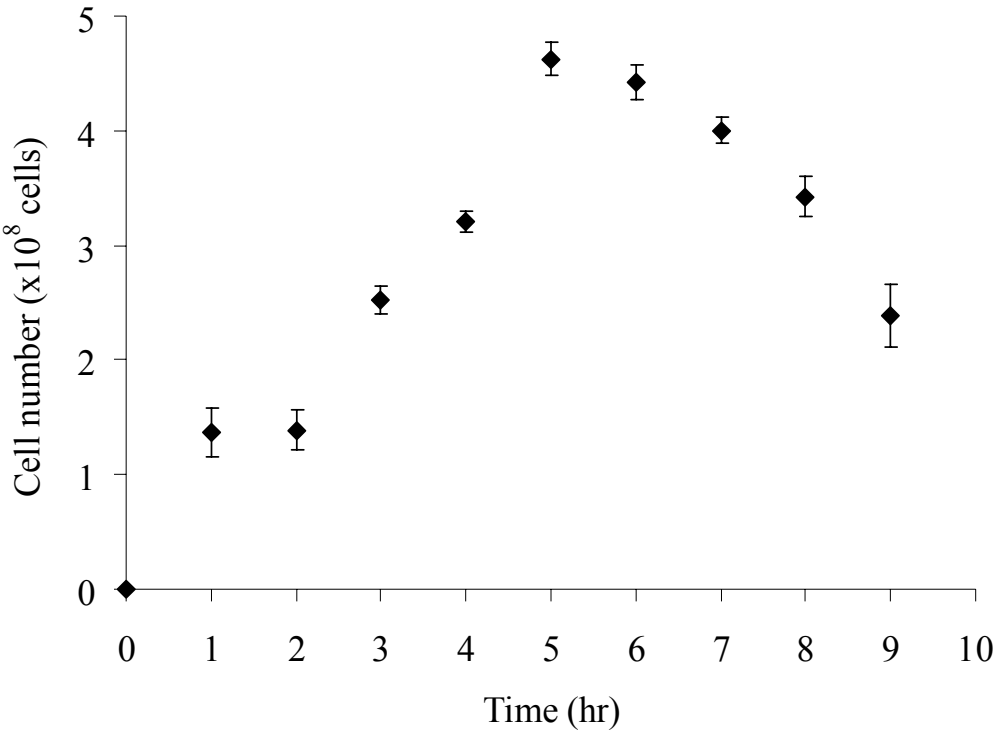



**Figure 4.30** Change of *T. suecica* cell number in *P. viridis* (1.84 ± 0.09 g) tank with continuous feeding system by dripping bottle

The plankton clearance rate of 4 species of bivalve *i.e. C. belcheli, Saccostrea* sp., *A. granosa* and *P. viridis* was evaluated with different food concentrations. The results indicated that, in general, high clearance rate was found with high food concentration likewise filtration rate of *Mytilus edulis* increased with an increase in algal cell density (Bayne *et al*., 1993). However, the present experiment showed that filtration rate of small oyster and blood cockle decreased with an increase of food concentration. This might be because the bivalve was exposed with higher food concentration than the threshold concentration of food. According to Riisgard (1991), filtration rate of bivalve *Mytilus edulis* on microalgae *Rhodomonas baltica* was highest with cell density of 3-10 cells/ul but the gradually increased of *Rhodomonas baltica* cell concentration to 15 cells/ul led to the reduction of valve gape and filtration rate. In addition, there are other factors influencing the filtration rate such as temperature, current velocity or bivalve size (Gosling, 2003).

#### 4.2.5.4 Growth of mussel *P. viridis* with continuous feeding by *T. suecica*

Green mussels were cultured under laboratory condition. Unlike previous experiments, feeding was provided with different cultures of *T. suecica*. The algal cultivation was performed with air (T-air) or with the mixture of air and 1.5% of $CO_2$ (T-$CO_2$). Green mussel without feeding was assigned as control. The relationship between fresh weight and dry weight of mussel was shown in Fig 4.31.

Growth performance of green mussels measured every 10 days is illustrated in Fig 4.32. The initial average 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 $^{o}$C, pH 8.1-8.3, 0-0.5 mg-$NH_4^+$/L and 110-120 mg/L alkalinity. Along with 30 days of the experimental period, weight of green mussel in T-air and T-$CO_2$ was significantly increased to 2.46±0.17 and 2.63±0.36 g, respectively. In control without feeding, weight of green mussels increased only in the first 12 days, thereafter growth was clearly declined. This indicated that, as the negative control, *T. suecica* green mussel could not grow without feeding in our experimental system.

With 30 days cultivation, growth rate of green mussel without feeding (control) was only 7.4±3.5 mg/individual/day which was significantly lower ($p<0.01$) than treatments. Statistical analysis revealed that growth of green mussels in T-air (22.4±4.0 mg/individual/day) was lower than T-$CO_2$ (28.9±12.3 mg/individual/day) ($p<0.05$). 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, microalgae, micro zooplankton or detritus (Gosling, 2003). The results in our study suggested that the culture condition was appropriate for growth of the green mussel, at least equally with the flow-through system.

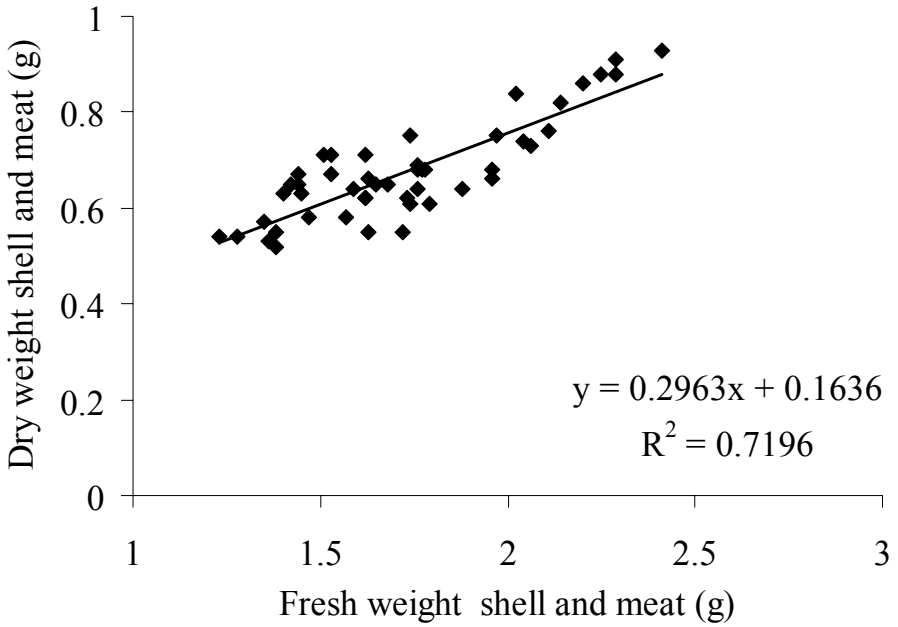



**Figure 4.31** Relationship between fresh and dry weight of *P. viridis* in third experiment

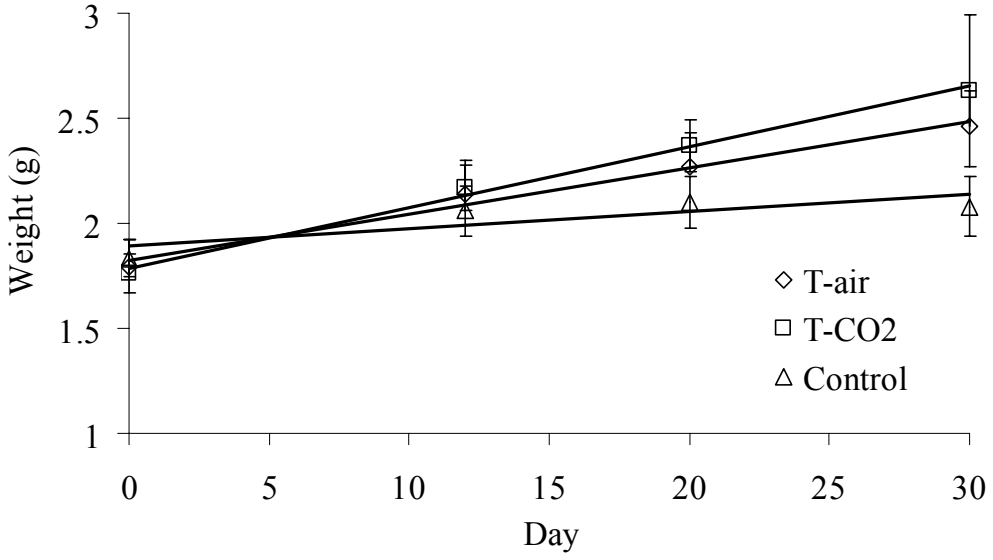



**Figure 4.32** Growth of *P. viridis* fed with *T. suecica* cultured in air (T-Air) (◇), 1.5% $CO_2$ (T-$CO_2$) (□) and control without feeding (Δ), under laboratory condition (third experiment)

Carbon hydrogen and nitrogen content in meat and shell of green mussels and in *T. suecica* cells are shown in Table 4.10. In green mussel, carbon content was varied according to culture condition. Several factors affecting biochemical composition of marine bivalve such as diet, water temperature, maturation and culture area have been mentioned (Li *et al*., 2007; Dunstan *et al*., 1999; Linehan *et al.*, 1999). Variation of biochemical composition in *P. viridis* depends on their reproductive cycle and food availability (Mohan and Kalyani, 1989; Li *et al*., 2007). Protein content is increased before spawning and gradually decreased during spawning while decline in lipid is detected due to intermittent spawning (Mohan and Kalyani, 1989). With our results, 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. Altering in carbon, hydrogen, and nitrogen content in mussel was probably due to different food sources since mussel in laboratory culture consumed only the microalgae provided while mussel collected from nature generally consumed various types of suspended solid in the water. Moreover, aging of mussel was another factor influencing C:H:N content since mussels from nature were collected and immediately analyzed for C:H:N but the experiment cultured mussels had been grow for another month under laboratory condition. It was found that the highest C:H:N ratio in mussel shell was found in mussel of T-$CO_2$ treatment. This was possibly a result from high C:H:N ratio in *T. suecica* cultured with 1.5% $CO_2$ supplement.

**Table 4.10** CHN composition of *P. viridis*, air grow cell of *T. suecica* and $CO_2$ grow cell of *T. suecica.* (Different superscript letters within column = significantly different at $p \leq 0.05$)

| Sample | CHN composition (g/100g dry weight) | | | C:H:N |
|---|---|---|---|---|
| | C | H | N | |
| Green mussel **meat** (fed by *T. suecica*-air :T-air) | $38.28 \pm 1.72^{d}$ | $6.46 \pm 0.37^{cd}$ | $9.82 \pm 0.62^{f}$ | 3.9: 0.7: 1 |
| Green mussel **meat** (fed by *T. suecica*-$CO_2$: T-$CO_2$) | $36.61 \pm 0.43^{c}$ | $6.29 \pm 0.35^{c}$ | $9.44 \pm 0.15^{e}$ | 3.9: 0.7: 1 |
| Green mussel **meat** (natural) | $42.11 \pm 1.01^{e}$ | $6.93 \pm 0.42^{e}$ | $10.23 \pm 0.28^{g}$ | 4.1: 0.7: 1 |
| Green mussel **shell** (fed by *T. suecica*-air: T-air) | $15.59 \pm 0.57^{b}$ | $1.19 \pm 0.2^{b}$ | $1.81 \pm 0.3^{b}$ | 8.6: 0.7: 1 |
| Green mussel **shell** (fed by *T. suecica*-$CO_2$: T-$CO_2$) | $14.2 \pm 0.47^{a}$ | $0.84 \pm 0.16^{a}$ | $1.14 \pm 0.23^{a}$ | 12.4: 0.7: 1 |
| Green mussel **shell** (natural) | $16.31 \pm 0.74^{b}$ | $1.28 \pm 0.34^{b}$ | $2.05 \pm 0.44^{b}$ | 7.9: 0.6: 1 |
| *T. suecica* (air) | $38.73 \pm 0.22^{d}$ | $6.64 \pm 0.18^{d}$ | $4.37 \pm 0.12^{d}$ | 8.9: 1.5: 1 |
| *T. suecica* ($CO_2$) | $42.72 \pm 0.28^{e}$ | $7.4 \pm 0.22^{f}$ | $2.47 \pm 0.14^{c}$ | 17.3: 3:1 |

## 4.3 Calcification rate evaluation

From the experiment 3.2.5.2 waste water from mussel tank was treated by macroalgae *Gracillaria* sp. before reuse for green mussel culture. Calcification rate of bivalve was evaluated from decreasing of calcium concentration in the water by EDTA titration (APHA, 1998). The result showed that calcification rate of green mussel in control without feeding was 5.04±4.36 mgCa/individual/day. This value was significantly lower ($P<0.01$) than 17.63±4.36 and 23.5±4.07 mg Ca/individual/day for T-air and T-$CO_2$, respectively. Due to the fact that the main composition of bivalve shell is calcium carbonate, Bevelander (1952) demonstrated that radioactive labeled calcium (as calcium chloride) 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 carbon dioxide assimilation process.

## 4.4 Evaluation of $CO_2$ assimilation of the microalga-bivalve system

Biological carbon dioxide assimilation process was evaluated with carbon deposition from microalgae into bivalve. As illustrated in Fig 4.33, from 1 g of $CO_2$, 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 4.34), carbon deposited in mussels was 13.41% (11.22% in meat and 2.19% in shell). In general, it could be presumed that approximately 11.31-13.41% of carbon supplied to *T. suecica* culture was incorporated into mussel biomass. Larger carbon deposition was from surrounding water through calcification process. However, carbon from water must be included when considering the overall $CO_2$ assimilation process of microalgae and bivalve. Other unaccounted carbon was presumably referred to respiration and other processes. The energy loss through trophic level food chain is 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 microalgae culture

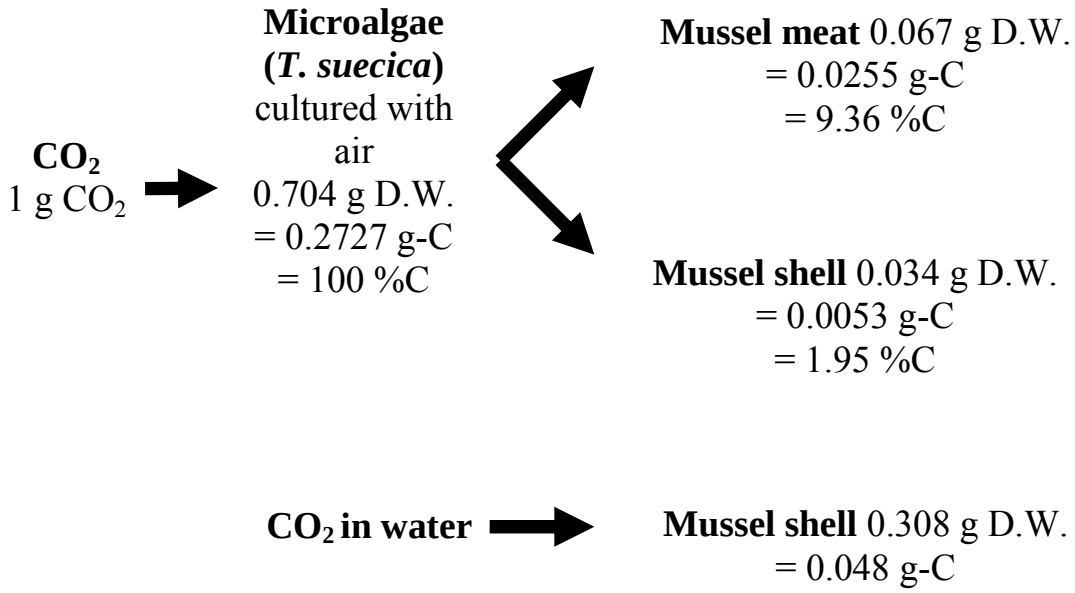



**Figure 4.33** Carbon assimilation in microalgae and bivalve fed with aerated cultivation of *Tetraselmis suecica* (T-air), *carbon content in comparison with carbon in microalgae

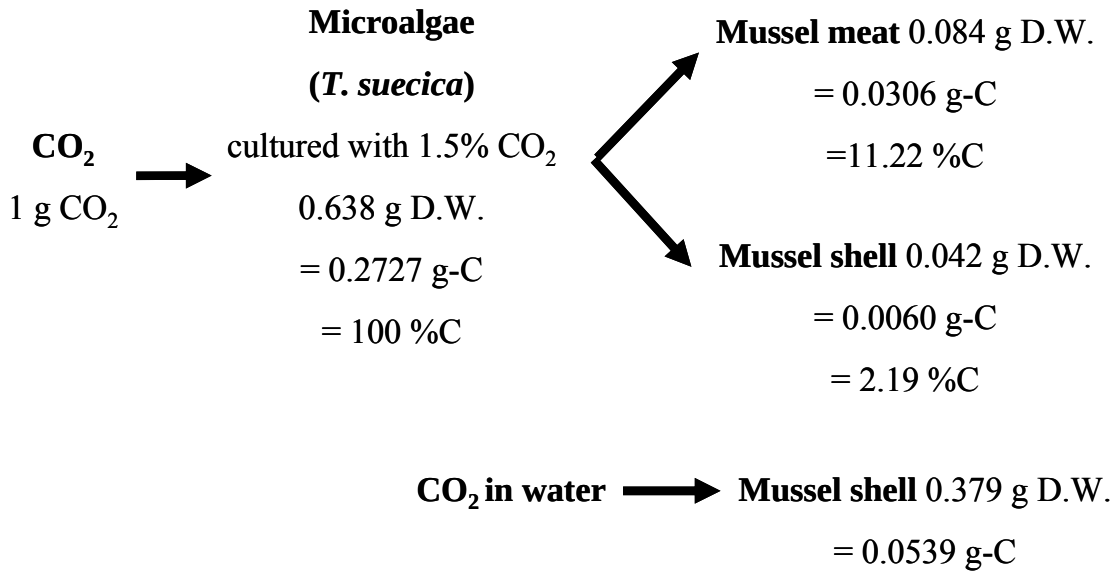



**Figure 4.34** Carbon assimilation in microalgae and bivalve fed with 1.5% $CO_2$ cultivation of *Tetraselmis suecica* (T-$CO_2$), *carbon content in comparison with carbon in microalgae

# CHAPTER V

# CONCLUSIONS AND SUGGESTION FOR FUTURE WORK

## 5.1 Conclusions

The bottleneck of using microalgae for $CO_2$ removal process is how to utilize or deposit the microalgae biomass produced from the process. This study illustrated the process of $CO_2$ fixation process (photosynthesis) then carbon was 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.

The first part of this study involved the growth and carbon deposit enhancement of microalgae, *Isochrysis galbana* and *Tetraselmis suecica* supplement. It was found that growth of *I. galbana* and *T. suecica* was enhanced by $CO_2$ supplement. In the present study, 1.5% $CO_2$ was the optimum concentration for both species and supplying 1.5% $CO_2$ with aeration increased the carbon content of *T. suecica*. The $CO_2$ concentration higher than 1.5% significantly affected photosynthesis efficiency as illustrated by the reduction of Fv/Fm ratio. With 1.5% $CO_2$, maximum cell density of *I. galbana* and *T. suecica* were 18.67 and $2.39\times10^6$ cells/mL respectively which were significantly higher than control with air supplied. The specific growth rate of *I. galbana* and *T. suecica* in 1.5% $CO_2$ culture was increased to 118.9 and 102.53% comparing with the control.

The second part was the evaluation of carbon deposition in bivalve fed with cultured microalgae. Several experiments were performed with various bivalve species. Unfortunately, experiments on culturing bivalve such as oyster, hooded oyster and blood cockle under laboratory condition using both batch and continuous feeding were not success. Finally, green mussel was chosen as it could be cultured in a recirculating system. 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 came from surrounding water through 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 microalgae and bivalve could be considered as an alternative way of carbon dioxide mitigation process.

**5.2 Suggestions for future work**

Recently, cultivation of bivalve culture under laboratory condition, especially with the recirculating culture system, is still limited. Several problems concerning food supply and environmental control are among the most important techniques that need further intensively studies. This will lead to the optimum growth of bivalve which is the maximum carbon assimilation into bivalve biomass.

Calcification rate of bivalve is another topic that need to be evaluated. In general, the calcification process in bivalve was influenced not only by certain elements dissolved in surrounding water but also the requirement of energy for calcification process. Unfortunately, the results from this study were not accurate enough to verify calcification rate of the bivalve. Further study with more precise technique such as radioactive labeled calcium and carbon is therefore recommended.

Although the carbon assimilation in bivalve was successfully illustrated in this study but it was only the startup of an alternative process of carbon mitigation. The experimental unit is needed to be scaling up to the larger size with continuous processes of algal and bivalve culture systems. With larger bivalve tanks with high performance microalgal production units, the evaluation of carbon assimilation process would be evaluated with higher accuracy.

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# APPENDICES

# APPENDIX A

**Guillard medium (F/2)** (Guillard and Ryther, 1962)

| | | |
|---|---|---|
| $NaNO_3$ | $8.82x10^{-4}$ | M |
| $NaH_2PO_4$ $H_2O$ | $3.62x10^{-5}$ | M |
| $Na_2SiO_3$ $9H_2O$ | $1.06x10^{-4}$ | M |
| $FeCl_3$ $6H_2O$ | $1.17x10^{-5}$ | M |
| $Na_2EDTA$ $2H_2O$; | $1.17 \times 10^{-5}$ | M |
| $CuSO_4$ $5H_2O$ | $3.93x10^{-8}$ | M |
| $Na_2MoO_4$ $2H_2O$ | $2.60x10^{-8}$ | M |
| ZnSO4 $7H_2O$ | $7.65x10^{-8}$ | M |
| $CoCl_2$ $6H_2O$ | $4.20 \times 10^{-8}$ | M |
| $MnCl_2$ $4H_2O$ | $9.10 \times 10^{-7}$ | M |
| thiamine HCl (vit. $B_1$) | $2.96 \times 10^{-7}$ | M |
| biotin (vit. H) | $2.05 \times 10^{-9}$ | M |
| cyanocobalamin (vit. $B_{12}$) | $3.69 \times 10^{-10}$ | M |

# APPENDIX B

## Calcium analysis (APHA, 1998)

### Reagent

1. Sodium Hydroxide 1N
2. Murexide indicator

   Prepare by mixing 200 mg of merexide with 100 g solid Nacl.
3. Standard EDTA 0.01 M (titrant)

### Procedure

1. Use 50 mL sample, or smaller portion diluted to 50 mL so that calcium content is about 5-10 mg.
2. Add 2.0 mL of NaOH solution or a volume sufficient to produce a pH of 12.
3. Add 0.1 -0.2 g of indicator.
4. Add EDTA tritrant slowly, with continuous stirring to the proper end point.

### Calculation

$$\text{mg Ca/L} = \frac{A \times B \times 400}{\text{mL sample}}$$

where:

A = mL titrant for sample

B = mg $CaCO_3$ equivalent to 1 mL EDTA titrant at the calcium indicator end point.

# APPENDIX C

## Fv/Fm value of *Isochrysis galbana* ad *Tetraselmis suecica* bubbled by mixture of air and different $CO_2$

| $CO_2$ concentration (%) | Fv/Fm of *I. galbana* | | | | |
|---|---|---|---|---|---|
| | Replicate 1 | Replicate 2 | Replicate 3 | $\bar{x}$ | SD |
| 0 (pure air) | 0.83 | 0.79 | 0.82 | 0.81 | 0.02 |
| 1.5 | 0.78 | 0.83 | 0.8 | 0.8 | 0.03 |
| 5 | 0.77 | 0.75 | 0.81 | 0.78 | 0.03 |
| 10 | 0.78 | 0.73 | 0.7 | 0.73 | 0.04 |
| 15 | 0.69 | 0.71 | 0.76 | 0.725 | 0.04 |
| 20 | 0.62 | 0.59 | 0.64 | 0.62 | 0.02 |
| 30 | 0.55 | 0.6 | 0.52 | 0.56 | 0.04 |
| 40 | 0.37 | 0.37 | 0.46 | 0.4 | 0.05 |

| $CO_2$ concentration (%) | Fv/Fm of *T. suecica* | | | | |
|---|---|---|---|---|---|
| | Replicate 1 | Replicate 2 | Replicate 3 | $\bar{x}$ | SD |
| 0 (pure air) | 0.81 | 0.78 | 0.82 | 0.81 | 0.02 |
| 1.5 | 0.81 | 0.85 | 0.8 | 0.82 | 0.03 |
| 5 | 0.7 | 0.69 | 0.64 | 0.68 | 0.03 |
| 10 | 0.68 | 0.63 | 0.59 | 0.63 | 0.05 |
| 15 | 0.65 | 0.58 | 0.48 | 0.56 | 0.08 |
| 20 | 0.54 | 0.59 | 0.47 | 0.54 | 0.06 |
| 30 | 0.25 | 0.43 | 0.39 | 0.36 | 0.09 |
| 40 | 0.32 | 0.39 | 0.4 | 0.37 | 0.04 |

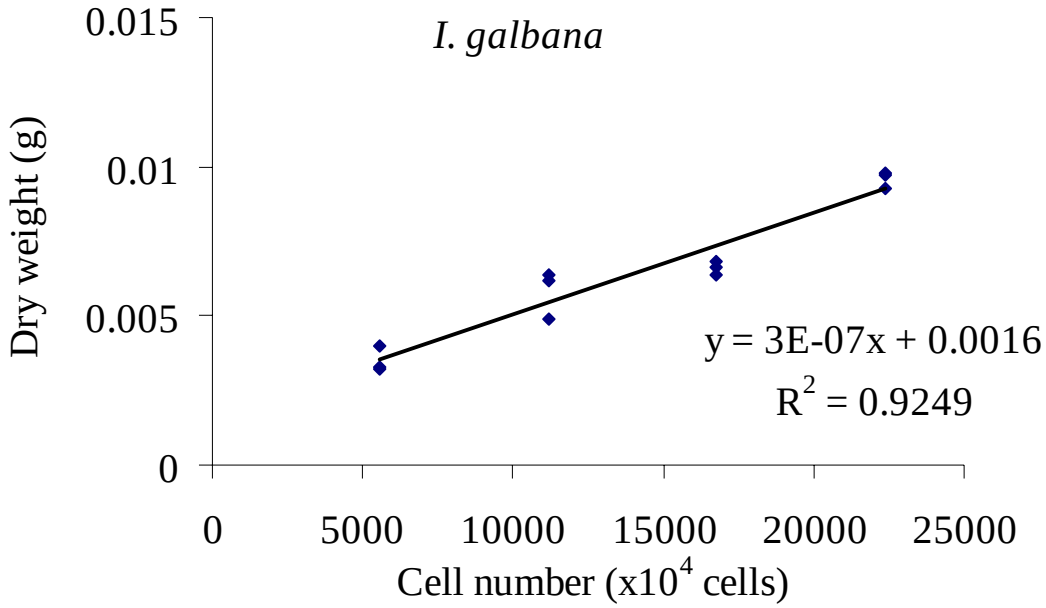
# APPENDIX D

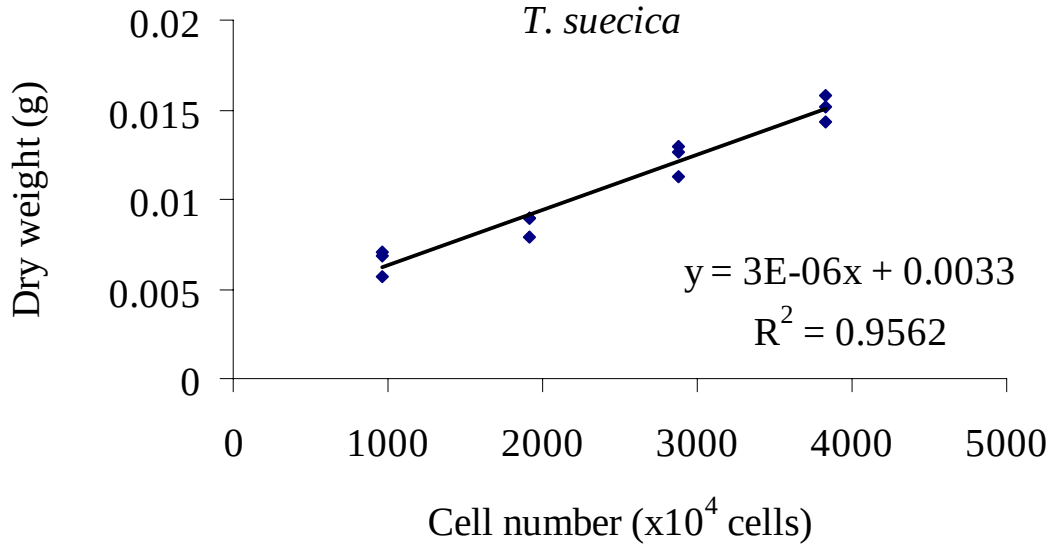
**Dry weight determination of microalgae** (Bougaran, 2003)

1. Cell density of *I. galbana* or *T. suecica* culture was evaluated by counting with haemacytometer.
2. A filter (GF/C Whatman filter, 47 mm in diameter) was dried at 70 $^{o}$C and weighed.
3. Suspension of each species 5, 10, 15 and 20 ml was filtered onto tared GF/C Whatman filters. Three replicates were performed for each volume.
4. The filters with microalgae was dried at 70 $^{o}$C for 24 hr before weighing.
5. The plot of dry weight of microalgae vursus cell number was regression analyzed

**APPENDIX E**

**Regression analysis of dry weight and cell number of microalgae**



# APPENDIX F

## Growth of mussel *P. viridis* with continuous feeding by *T. suecica*

Control: without feeding

| Day | Weight of mussel in control (g) | |
|---|---|---|
| | $\bar{x}$ | SD |
| 0 | 1.83 | 0.09 |
| 12 | 2.06 | 0.12 |
| 20 | 2.1 | 0.12 |
| 30 | 2.08 | 0.1 |

T-air: fed by air grown cell of *T. suecica*

| Day | Weight of mussel in control (g) | |
|---|---|---|
| | $\bar{x}$ | SD |
| 0 | 1.78 | 0.13 |
| 12 | 2.14 | 0.16 |
| 20 | 2.27 | 0.16 |
| 30 | 2.46 | 0.17 |

T-$CO_2$: fed by *T. suecica* aerated by mixture of air and 1.5%$CO_2$

| Day | Weight of mussel in control (g) | |
|---|---|---|
| | $\bar{x}$ | SD |
| 0 | 1.76 | 0.09 |
| 12 | 2.17 | 0.11 |
| 20 | 2.37 | 0.12 |
| 30 | 2.63 | 0.36 |

## APPENDIX G

## Feeding rate of bivalves

**Table A** Cell number of *I. galbana* in glass aquarium with 1 large oyster *C. belcheri*: The initial cell density of *I. galbana* were 50 (D1), 77 (D2), 164(D3), 233 (D4) and 580 (D5) $\times 10^4$ cells/mL

| | Cell number of *I. galbana* in glass aquarium with 1 large oyster *C. belcheri* ($\times 10^9$ cells) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| min | D1 | ±SD | D2 | ±SD | D3 | ±SD | D4 | ±SD | D5 | ±SD |
| 0 | 1.09 | 0.06 | 1.54 | 0.10 | 3.28 | 0.18 | 4.65 | 0.49 | 11.60 | 0.43 |
| 5 | 0.96 | 0.09 | | | | | | | 8.73 | 0.55 |
| 10 | 0.87 | 0.08 | 1.17 | 0.18 | 3.15 | 0.33 | 3.73 | 0.61 | 8.77 | 1.23 |
| 15 | 0.73 | 0.16 | | | | | | | 8.01 | 0.77 |
| 20 | 0.65 | 0.23 | 0.99 | 0.21 | 3.01 | 0.32 | 3.80 | 0.52 | 7.77 | 0.83 |
| 25 | 0.62 | 0.28 | | | | | | | | |
| 30 | 0.54 | 0.32 | 0.88 | 0.22 | 2.97 | 0.34 | 3.24 | 0.79 | 7.86 | 0.77 |
| 35 | 0.40 | 0.25 | | | | | | | | |
| 40 | 0.29 | 0.28 | 0.99 | 0.19 | 2.85 | 0.40 | 2.99 | 0.88 | 7.26 | 0.46 |
| 50 | 0.23 | 0.30 | | | | | | | | |
| 60 | 0.14 | 0.19 | 0.79 | 0.20 | 2.56 | 0.50 | 2.30 | 1.00 | 6.74 | 0.80 |
| 80 | | | 0.66 | 0.32 | 2.40 | 0.67 | 2.18 | 1.20 | 6.42 | 1.35 |
| 90 | | | 0.57 | 0.30 | 2.25 | 0.68 | 1.94 | 0.75 | 0.00 | 0.00 |
| 100 | | | 0.38 | 0.26 | 1.90 | 0.86 | 2.21 | 1.14 | 5.52 | 1.68 |
| 110 | | | 0.25 | 0.19 | 1.88 | 0.68 | 1.95 | 0.88 | 5.11 | 1.90 |
| 120 | | | 0.27 | 0.19 | 1.77 | 0.64 | 2.15 | 0.93 | 4.78 | 1.94 |
| 130 | | | 0.19 | 0.17 | 1.78 | 0.80 | 1.80 | 0.96 | 4.38 | 2.66 |
| 140 | | | 0.24 | 0.16 | 1.64 | 0.78 | 1.47 | 1.02 | 3.33 | 2.47 |
| 150 | | | | | 1.65 | 0.82 | | | | |
| 160 | | | 0.26 | 0.22 | | | 1.34 | 0.92 | | |
| 180 | | | | | | | | | 2.75 | 2.01 |
| 210 | | | | | 1.43 | 0.99 | 1.06 | 0.59 | | |
| 240 | | | 0.11 | 0.14 | 1.32 | 0.85 | 0.78 | 0.55 | 2.66 | 2.82 |
| 270 | | | | | | | | | 2.50 | 2.79 |

**Table B** Cell number of *I. galbana* cells in glass aquarium containing 1 small oyster. The initial cell density of *I. galbana* were 109 (D1), 246 (D2), 582 (D3) and 814 (D4)$\times 10^4$ cells/mL

| | Cell number of *I. galbana* in glass aquarium with 1 small oyster *C. belcheri* ($\times 10^9$ cells) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| min | D1 | ±SD | D2 | ±SD | D3 | ±SD | D4 | ±SD |
| 0 | 2.44 | 0.01 | 1.75 | 0.01 | 0.74 | 0.05 | 0.33 | 0.01 |
| 30 | 1.98 | 0.08 | 1.47 | 0.13 | 0.37 | 0.11 | 0.13 | 0.02 |
| 60 | 2.10 | 0.15 | 0.99 | 0.04 | 0.13 | 0.12 | 0.06 | 0.02 |
| 90 | 2.07 | 0.08 | 0.89 | 0.38 | 0.03 | 0.01 | 0.03 | 0.01 |
| 120 | 2.04 | 0.08 | 0.78 | 0.14 | 0.00 | | | |
| 150 | 0.00 | 0.00 | 0.67 | 0.10 | | | | |
| 180 | 2.01 | 0.10 | | | | | | |

**Table C** Cell number of *I. galbana* in glass aquarium with *A. granosa*. The initial cell density of *I. galbana* were 128 (D1), 248 (D2), 654 (D3) and 837(D4)$\times 10^4$ cells/mL

| | Cell number of *I. galbana* in glass aquarium with *A. granosa*($\times 10^9$ cells) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| min | D1 | ±SD | D2 | ±SD | D3 | ±SD | D4 | ±SD |
| 0 | 0.19 | 0.04 | 0.37 | 0.06 | 0.98 | 0.04 | 1.26 | 0.03 |
| 30 | 0.11 | 0.02 | 0.26 | 0.06 | 0.87 | 0.04 | 1.12 | 0.17 |
| 60 | 0.06 | 0.02 | 0.24 | 0.13 | 0.86 | 0.04 | 1.13 | 0.10 |
| 90 | 0.02 | 0.00 | 0.13 | 0.09 | 0.79 | 0.05 | 1.13 | 0.04 |
| 120 | | | 0.11 | 0.00 | 0.80 | 0.01 | 1.06 | 0.04 |
| 240 | | | 0.02 | 0.01 | 0.72 | 0.09 | 0.87 | 0.06 |

**Table D** Cell number of *I. galbana* in glass aquarium with a *S. cucullata*. The initial cell density of *I. galbana* 47 (D1), 117 (D2), 338 (D3) and 738 (D4)$\times 10^4$ cells/mL

| | Cell number of *I. galbana* in glass aquarium with a *S. cucullata*($\times 10^8$ cells) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| min | D1 | ±SD | D2 | ±SD | D3 | ±SD | D4 | ±SD |
| 0 | 0.9 | 0.1 | 2.1 | 0.1 | 6.2 | 0.2 | 13.4 | 0.4 |
| 30 | 0.7 | 0.1 | 1.1 | 0.1 | 3.6 | 0.7 | 11.8 | 0.9 |
| 60 | 0.4 | 0.1 | 0.7 | 0.1 | 2.3 | 0.2 | 10.7 | 0.4 |
| 90 | 0.3 | 0.0 | 0.4 | 0.1 | 1.6 | 0.2 | 9.0 | 0.5 |
| 120 | 0.1 | 0.0 | 0.2 | 0.1 | 1.2 | 0.2 | 7.8 | 0.4 |
| 150 | | | 0.1 | 0.0 | 0.9 | 0.1 | 7.0 | 0.5 |
| 180 | | | | | 0.5 | 0.1 | 5.4 | 0.5 |
| 240 | | | | | 0.2 | 0.1 | 4.8 | 0.4 |

**Table E** Cell number of *T. suecica* in glass aquarium with a *S. cucullata*. The initial cell density of *T. suecica* 1 (D1), 6(D2), 14(D3) and 50 (D4)×$10^4$ cells/mL

| | Cell number of *T. suecica* in glass aquarium with *S.cucullata*(×$10^7$ cells) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| min | D1 | ±SD | D2 | ±SD | D3 | ±SD | D4 | ±SD |
| 0 | 0.21 | 0.22 | 1.11 | 0.91 | 2.87 | 2.45 | 10.24 | 0.98 |
| 30 | 0.18 | 0.28 | 0.91 | 0.75 | | | | |
| 60 | 0.15 | 0.53 | 0.78 | 0.85 | 2.62 | 2.66 | 9.18 | 1.79 |
| 90 | 0.11 | 0.44 | | | | | | |
| 120 | 0.11 | 0.49 | 0.72 | 0.76 | 2.38 | 3.46 | 9.50 | 1.49 |
| 180 | | | 0.67 | 1.13 | 2.57 | 2.67 | 8.38 | 1.39 |
| 240 | | | | 1.32 | 1.92 | 2.17 | 8.10 | 0.88 |
| 300 | | | | | | | 8.65 | 1.47 |

**Table F** Cell number of *T. suecica* in glass aquarium with *P. viridis.* The initial cell density of *T. suecica* 1(D1), 5 (D2), 10 (D3) and 20(D4)×$10^4$ cells/mL

| | Cell number of *T. suecica* in glass aquarium with *S.cucullata* (×$10^8$ cells) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| min | D1 | ±SD | D2 | ±SD | D3 | ±SD | D4 | ±SD |
| 0 | 1.3 | 0.2 | 7.9 | 1.1 | 16.1 | 1.7 | 24.2 | 4.0 |
| 30 | 1.1 | 0.1 | 4.8 | 1.7 | 9.9 | 1.1 | 14.7 | 1.7 |
| 60 | 0.2 | 0.1 | 1.3 | 0.2 | 1.9 | 0.7 | 4.2 | 0.6 |
| 120 | | | 0.8 | 0.1 | 1.2 | 0.1 | 3.1 | 0.8 |

**Table G** Change of *T. suecica* cell number in *P. viridis* tank with continuous feeding system by dropping bottle

| Time (hr) | *T. suecica* cell number (×$10^8$ cells) | |
|---|---|---|
| | $\bar{x}$ | SD |
| 0 | 0 | 0 |
| 1 | 1.37 | 0.21 |
| 2 | 1.39 | 0.17 |
| 3 | 2.52 | 0.12 |
| 4 | 3.21 | 0.10 |
| 5 | 4.62 | 0.14 |
| 6 | 4.42 | 0.16 |
| 7 | 4.00 | 0.11 |
| 8 | 3.43 | 0.18 |
| 9 | 2.39 | 0.27 |

# BIOGRAPHY

Chompunut Chairattana was born in Surat Thani Province on 1977. After graduating from high school from Suratthani School, in 1994 she enrolled at Prince of Songkhla University. In May 1998 she graduated with Bachelor of Science degree in Aquatic Science. After that she studied in Marine Science Department and graduated (with one publication) in master degree of Science (Marine Science) in 2000 and was given an outstanding graduate award from Professor Dr. Tab Nilaniti Foundation. Between October 2000-March 2003 she worked as a research assistant at National Center for Genetic engineering and Biotechnology, National Science and Technology Development Agency, Thailand. From April 2003 until now she has been working as lecturer at Surat Thani Rajabhat University. In 2007, she enrolled for the Degree of Doctor of Philosophy Program in Marine Science, Faculty of Science, Chulalongkorn University.

**Publication**

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