สภาวะการเติบโตที่เหมาะสมสำหรับการเพาะเลี้ยงไดอะตอม *Entomoneis* sp. ในถังปฏิกรณ์ชีวภาพเชิงแสง

นางสาวกรกนก สหัสทัศ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิศวกรรมศาสตรมหาบัณฑิต
สาขาวิชาวิศวกรรมเคมี ภาควิชาวิศวกรรมเคมี
คณะวิศวกรรมศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2552

OPTIMAL GLOWTH CONDITION FOR DIATOM *ENTOMONEIS* SP. CULTIVATION IN PHOTOBIOREACTOR

Miss Konkanok Sahasthat

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Engineering Program in Chemical Engineering
Department of Chemical Engineering
Faculty of Engineering
Chulalongkorn University
Academic Year 2009

Thesis Title	OPTIMAL GLOWTH CONDITION FOR DIATOM *ENTOMONEIS* SP. CULTIVATION IN PHOTOBIOREACTOR
By	Miss Konkanok Sahasthat
Field of Study	Chemical Engineering
Thesis Advisor	Associate Professor Prasert Pavasant, PhD.
Thesis Co-Advisor	Sorawit Powtongsook, Ph.D.

Accepted by the Faculty of Engineering, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

.....Dean of the Faculty of Engineering
(Associate Professor Boonsom Lerdhirunwong, Dr.Ing.)

THESIS COMMITTEE

.....Chairman
(Associate Professor Muenduen Phisalaphong, Ph.D.)

.....Thesis Advisor
(Associate Professor Prasert Pavasant, Ph.D.)

.....Thesis Co-Advisor
(Sorawit Powtongsook, Ph.D.)

.....Examiner
(Associate Professor Artiwan Shortipruk, Ph.D.)

.....External Examiner
(Assitant Professor Marasri Ruengjitchatchawalya, Ph.D.)

กรกนก สหัสทัศ: สภาวะการเติบโตที่เหมาะสมสำหรับการเพาะเลี้ยงไดอะตอม *Entomoneis* sp. ในถังปฏิกรณ์ชีวภาพเชิงแสง (OPTIMAL GROWTH CONDITION FOR DIATOM *ENTOMONEIS* SP. CULTIVATION IN PHOTOBIOREACTOR) อ.ที่ปรึกษาวิทยานิพนธ์: รศ.ดร. ประเสริฐ ภวสันต์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ดร. สรวิศ เผ่าทองศุข, 79 หน้า

การศึกษาสภาวะการเติบโตที่เหมาะสมของไดอะตอม *Entomoneis* sp. ในถังสัมผัสแบบธรรมดาขนาด 2.5 ลิตร ใช้สูตรอาหาร F/2 แบบปรับปรุงเป็นสารอาหารมาตรฐานในงานวิจัยนี้ จากการศึกษาพบว่าซิลิกอนเป็นตัวกำหนดการเจริญเติบโตของไดอะตอมชนิดนี้ สามารถลดขีดจำกัดของซิลิกอนต่อการเจริญเติบโตของเซลล์ได้โดยการเติมซิลิกอนแบบช่วง (ที่ปริมาณ 6.7 มิลลิกรัมซิลิกอนต่อลิตร) ในวันที่ 3, 4 และ 5 ของการเลี้ยง การเจริญเติบโตที่ดีที่สุดเกิดขึ้นในสภาวะการเลี้ยงที่ให้ปริมาณความเข้มแสง 270 ไมโครโมลโฟตอนต่อตารางเมตรต่อวินาทีและอัตราการให้อากาศ 0.5 เซนติเมตรต่อวินาที ได้ความเข้มข้นเซลล์สูงสุดเท่ากับ 41.39×10^4 เซลล์ต่อมิลลิลิตรและอัตราการเจริญเติบโตจำเพาะเท่ากับ 0.48 ต่อวัน ต่อมาได้ศึกษาการเลี้ยง *Entomoneis* sp. ในถังปฏิกรณ์ชีวภาพเชิงแสงแบบอากาศยกชนิดท่อในแบนขนาด 17 ลิตร ที่สภาวะการเลี้ยงภายใต้แสงอาทิตย์ในตอนกลางวันและกลางคืนให้แสงโดยหลอดฟลูออเรซเซ้นที่ความเข้มแสง 43 ไมโครโมลโฟตอนต่อตารางเมตรต่อวินาที อัตราการให้อากาศ 0.5 เซนติเมตรต่อวินาที พบว่าในการเลี้ยงแบบกะได้การเจริญเติบโตสูงสุด เท่ากับ 31.05×10^4 เซลล์ต่อมิลลิลิตรและอัตราการเจริญเติบโตจำเพาะ เท่ากับ 0.59 ต่อวัน ในขณะที่การเลี้ยงแบบกึ่งต่อเนื่องที่ใช้ระยะเวลาการเก็บเกี่ยวเซลล์ประมาณ 3 วัน ความเข้มข้นของเซลล์สูงสุดเท่ากับ 37.49×10^4 เซลล์ต่อมิลลิลิตรและอัตราการเจริญเติบโตจำเพาะ เท่ากับ 0.54 ต่อวัน สุดท้ายได้ทำการศึกษาเบื้องต้นของผลคาร์บอนไดออกไซด์ต่อการเจริญเติบโตของไดอะตอม *Entomoneis* sp. โดยศึกษาช่วงความเข้มข้นของคาร์บอนไดออกไซด์ในอากาศ ตั้งแต่ 1 ถึง 7 เปอร์เซ็นต์ ความเข้มข้นของเซลล์สูงสุดเกิดที่ปริมาณความเข้มข้นคาร์บอนไดออกไซด์ 5 เปอร์เซ็นต์ ได้ความเข้มข้นเซลล์และอัตราการเจริญเติบโตจำเพาะเท่ากับ 53.89×10^4 เซลล์ต่อมิลลิลิตร และ 0.68 ต่อวัน ตามลำดับ

ภาควิชา ..วิศวกรรมเคมี.. ลายมือชื่อนิสิต..กรกนก สหัสทัศ..

สาขาวิชา ..วิศวกรรมเคมี.. ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

ปีการศึกษา..2552.. ลายมือชื่อ อ.ที่ปรึกษาร่วม.....

5170548621 : MAJOR CHEMICAL ENGINEERING
KEY WORD: PHOTOBIOREACTOR / *Entomonies* sp. / OPTIMAL CONDITION / SILICON LIMITATION / BATCH CULTURE / SEMI-CONTINUOS CULTURE / CARBON DIOXIDE

KONKANOK SAHASTHAT: OPTIMAL GROWTH CONDITION FOR DIATOM *ENTOMONEIS* SP. CULTIVATION IN PHOTOBIOREACTOR. THESIS ADVISOR: ASSOC. PROF. PRASERT PAVASANT, Ph.D., THESIS CO-ADVISOR: SORAWIT POWTONGSOOK, Ph.D., 79 pp.

The optimal conditions for the growth of a diatom *Entomoneis* sp. were investigated in a 2.5 L bubble column. A modified standard F/2 medium was selected as a standard medium. Silicon was the limiting factor for the growth of this alga, and a periodic addition of Si (fixed 6.7 mg L^{-1}) at the cultivating Days 3, 4 and 5 could, to some extent, overcome this limitation. The light intensity for the best growth was found at around 270 µmol photons m^{-2} s^{-1} at the aeration rate (in terms of superficial velocity) of 0.5 cm s^{-1}. At this condition, the alga exhibited the highest growth rate of 41.39 x10^4 cells mL^{-1} and a maximum specific growth rate of 0.48 d^{-1}. The cultivation of *Entomoneis* sp. in the 17 L flat plate airlift photobioreactor was exposed to sunlight during the day time and fluorescent light during the night. The light intensity from fluorescent light was about 43 µmol photon m^{-2} s^{-1}. The aeration was supplied at u_{sg} of 0.5 cm s^{-1} and this obtained a maximum specific growth rate of 0.59 d^{-1} with a maximum cell concentration of 31.05×10^4 cells mL^{-1} in a batch culture. A semi-continuous culture could be achieved where the 3-day harvesting interval provided the maximum cell concentration of 37.49x10^4 cell mL^{-1} and the specific growth rate of 0.54 d^{-1}. Lastly, the pre-evaluation of the effect of CO_2 for the cultivation of *Entomoneis* sp. operated with CO_2 from 1 to 7% in 17 L flat plate airlift in semi-continuous mode demonstrated that the best growth occurred at 5% CO_2 where the maximum cell concentration and specific growth rate were 53.89x10^4 cell mL^{-1} and 0.68 d^{-1}, respectively.

Department : Chemical Engineering Student's Signature กกนก สหัสทัศ์
Field of study : Chemical Engineering Advisor's Signature
Academic year 2009 Co–Advisor's Signature

ACKNOWLEDGEMENTS

This thesis would have never been completed without the help and supports of many people who are gratefully acknowledge here. I would like to express my thankfulness for all of them.

Firstly, I would like to express my deepest sincere appreciation to all the support and help given from my Associate Professor Dr. Prasert Pavasant, my advisor. This thesis would not have been accomplished without him and his excellent supervision.

Secondly, I would like to thank Dr. Sorawit Powtongsook who is an thesis co-advisor for their valuable suggestions, guidance, warm encouragement and generous supervision throughout my master program. I am grateful to Associate Professor Dr. Muenduen Phisalaphong, chairman of the committee, Associate Professor Dr. Artiwan Shotipruk, and Assistant Professor. Dr. Marasri Ruengjitchatchawalya, members of the committees for many valuable suggestions. In addition, I would also like to say thank you to Assistant Professor Dr.Worapon Kiatkittipong who is an my advisor in Bachelor Degree of Engineering (from Silpakorn University) for his valuable recommendation me to continually study in the Master Degree and influent me to meet the best advisor, Associate Professor Dr. Prasert Pavasant.

Thanks must go to all of my special person, whom I met in the Environmental and Biochemical Engineering Research Laboratory. I would also like to say thank you to P’Max, P’Big, P’note, P’Toey, P’Tik, P’Fa, P’Moo, P’Mod, P’Mode, P’Kae, Dump, Aom, Pud and Yot who rescued me and thanks for all kind of help of them. Moreover, special thanks should be made for the Graduate School, Chulalongkorn University to give the place as warm house for two years of my master degree research.

Most of all, I would like to express my love and say thank you to my family (my father, my sister, my brother and grandmother) especially my mother for their precious love, inspiration and supports that have propelled me to the completion of this work and worthy supports at all times.

CONTENTS

Page

LIST OF TABLES

LIST OF FIGURES

Page

CHAPTER I

INTRODUCTION

1.1 Motivations

Microalgae have attracted various commercial attention due to their versatile application potentials particularly in agricultural and aquaculture industries, and several other applications such as human consumption, mainly as nutraceutical foods, feed, cosmetic, chemical industry and etc. In addition, the global energy crisis and climate change problem induced by an abrupt increase in greenhouse gases have raised urgent environmental concerns. Research issue arised whether algae could be used as alternative fuel. Not only that, microalgae uptake carbon dioxide from the atmosphere for their photosynthesis, they also have potential to produce biomass or other useful chemicals such as lipid and fatty acid which can then be further converted to biofuel. Several species of microalgae have been reported to have high CO_2 uptake capacity, e.g. *Chlorella* sp. = 4.4 g CO_2 L^{-1} day^{-1} (Douskova et al., 2009), *Nannochloropsis oculata* = 9.4 g CO_2 L^{-1} day^{-1} (Chiu et al., 2009)., whereas some of the species contain lipid at as much as 20-50% by weight (dry), e.g. *Botryococcus braunii* (25-75 % dry weight), *Schizochytrium* sp. (50-77% dry weight) (Chisti, 2007).

Some advantages of cultivating algae can be summarized below (Amin, 2009):

a. Algae are considered to be a very efficient biological system for harvesting solar energy for the production of organic compounds via the photosynthetic process.

b. Algae are non-vascular plants, lacking complex reproductive organs, making the entire biomass available for harvest and use.

c. Many species of algae can be induced to produce particularly high concentrations of chosen, commercially-valuable compounds, such as proteins, carbohydrates, lipids, and pigments (Cohen, 1986).

d. Algae are microorganisms that undergo a simple cell division cycle, in most cases without a sexual type stage, enabling them to complete their cell cycle within a

few hours and making genetic selection and strain screening relatively quick and easy. This also allows much more rapid development and demonstration of production processes than with other agricultural crops.

e. For many regions suffering low productivity due to poor soils or the shortage of sea water, the farming of microalgae that can be grown using sea or brackish water may be almost the only way to increase productivity and secure a basic protein supply.

f. Algal biomass production systems can easily be adapted to various levels of operational or technological skills, from simple, labor-intensive production units to fully automated systems requiring high investments.

g. Microalgae are easy to culture and less area occupation for employed high yield oil, 58,700 L/ha compared to Corn (172 L/ha), Jatropha (1,892 L/ha), Coconut (2,689 L/ha), oil palm (5,950 L/ha) (Chisti, 2007).

In generally, there are 2 steps in the production of microalgae; the first step is the cultivation of highly concentrated microalgal culture. Growth parameters such as light intensity, photoperiod, temperature, salinity, culture medium, pH, carbon dioxide concentration, aeration rate and the system of cultivation need to be optimized to achieve this. The second step is to harvest the cells which could be done by several techniques such as filtration, centrifugation, flocculation and sedimentation, and solvent extraction.

Entomoneis sp. is a typical alga species often found to bloom in shrimp ponds. Our preliminary study indicated that this alga, under some stress conditions, was able to induce lipid and fatty acids at significant level and this is attractive in terms of a future option for alternative energy. Moreover, being a relatively large cell, the cultivation of *Entomoneis* sp. does not seem to encounter similar harvesting problem with other algae, and the harvest can only be achieved through a simple physical sedimentation. Todate, there have been no evidences of mass production of *Entomoneis* sp. Knuckey et al., 2002, was the only report available. In this report, *Entomoneis cf. punctulata* was cultivated in 75-ml F/2 media -125-ml flasks with condition at 17.5±2^{o}C on a 12:12 h light/dark cycle light intensity of 45 µmol photons m^{-1} s^{-1}. The results revealed the growth rate of 1.73 day^{-1} and the maximum cell concentration of 3.1x10^{6} cells mL^{-1}. There is therefore a clear need to investigate the

cultivation of such alga in a larger scale cultivation system which became the main objective of this study.

1.2 Objectives

1.2.1 The cultivation of diatom *Entomoneis* sp. in 2.5 L bubble column photobioreactor was investigated where the optimal conditions for the growth of such alga were examined.

1.2.2 The cultivation of diatom *Entomoneis* sp. in 17 L flat-plate airlift photobioreactor was investigated. The search for optimal cultivating condition started from the conditions obtained from Section 1.2.1, and manipulated to best suit the growth in the large scale.

1.3 Working Scopes

1.3.1 The optimal growth condition for *Entomoneis* sp. was investigated in the 2.5 L bubble photobioreactor, and this was subject to the following parameters:

- The influences of different culture media, i.e. standard F/2 (Guillard's) medium, F/2 modified WC medium, Chu's freshwater medium, and Walne medium.
- The influences of the concentration of silicon, phosphorus and nitrogen were examined in the selected mediums.
- The range of superficial gas velocity (u_{sg}) employed in this work was between 0.3-2.0 cm s^{-1}.
- The light intensity employed in this work ranged between 81-270 µmol photons m^{-2} s^{-1} or 6,000-20,000 Lux.
- The growth of the culture in normal nutrient was compared to that with the addition of 2% CO_2 to preliminarily investigate the effect of CO_2 on algal growth.

1.3.2 The cultivation in 17 L flat-plate airlift photobioreactor was investigated in a large scale system. This system was exposed to sunlight during the day time and fluorescent light during the night. The light intensity from fluorescent

light was about 43 μmol photon m^{-2} s^{-1}. The aeration was supplied at u_{sg} of 0.5 cm s^{-1} and the temperature was controlled in an evaporative room at 27±2°C. The operation of this system was subject to the following parameters:

- batch and semi-continuous modes
- aeration mixed with 1-7% CO_2

Chapter II

BACKGROUNDS AND LITERATURE REVIEW

2.1 Microscopic algae

Algae are a large and diverse group of simple, typically autotrophic organisms, ranging from unicellular to multicellular forms. The largest and most complex marine forms are called seaweeds. They are photosynthetic, like plants, and "simple" because they lack the many distinct organs found in land plants.

The terms algal refers to both macroalgae and a highly diversified group of microorganisms known as microalgae. The profound diversity of size ranging from picoplankton only 0.2-2 μm in diameter to giant kelps with fronds up to 60 m in length, ecology and colonized habitats, cellular structure, levels of organization and morphology, pigments for photosynthesis, reserve and structural polysaccharides, and type of life history reflect the varied evolutionary origins of this heterogeneous assemblage of organisms. The number of algal species has been estimated to be one to ten million, and most of them are microalgae (Barsanti and Gualtieri, 2006).

2.1.1 Diatoms

Diatoms are aquatic, single-celled algae which can be unicellular or multicellular organisms. Most diatoms are unicellular. Diatoms are a widespread group and can be found in the oceans, in soils, on damp surfaces atmospheric conditions, in freshwater, and salt water environments as long as some moisture is available. Diatoms are a major group of eukaryotic algae and are one of the most common types of phytoplankton. Scientific classification of diatoms in Kingdom: *Chromophyta*, Phylum: *Heterokontophyta* and Class: *Bacillariophyceae*. A characteristic of diatom cells are usually between 2 and 200 microns in diameter. Diatom cells are contained within a unique silicate (silicic acid) cell wall comprising two separate valves (or shells). The biogenic silica that the cell wall is composed of is synthesised intracellularly by the polymerisation of silicic acid monomers. This

material is then extruded to the cell exterior and added to the wall. Diatom cell walls are also called frustules. energy or valuable chemicals. Some species of diatom contain oil at as much as 20-50% by weight (dry) compare with general microalgae (Chisti, 2007) shown in Table 2.1.

2.1.2 *Entomoneis* sp.

Entomoneis sp. is a unicellular floating diatom organism/ phytoplankton, Domain: *Eukaryota,* Kingdom: *Chromista,* Subkingdom: *Chromobiota,* Infrakingdom: *Heterokonta,* Phylum: *Ochrophyta,* Subphylum: *Diatomeae,* Class: *Bacillariophyceae,* Subclass: *Bacillariophycidae,* Order: *Surirellales* , Family: *Entomoneidaceae,* Genus: *Entomoneis.*

Generally, cell growth and reproduction is accomplished by dividing into two cells. The size of the cell is about 55-160 µm in length and about 30-60 µm in width (Akulut, 2003). It has a slit along the valves (the raphae) and inside the valves the cytoplasm forms a relatively thin lining surrounding a large vacuole filled with cell sap; the nucleus is central in position with cytoplasmic strands extending across the vacuole. *Entomoneis* sp. is comprised of highly-unsaturated fatty acids. Example the composition of *Entomoneis cf. punctulata* expressed as a percentage of dry weight shown in Table 2.2.

2.2 Conditions for cultivation of algae

The most important parameters controlling algal growth are nutrient quantity and quality, light, temperature, salinity, pH, carbon dioxide and aeration, etc. Also, the various factors may be interdependent and a parameter that is optimal for one set of conditions is not necessarily optimal for the others.

2.2.1 Culture medium/ Nutrients

Concentrations of cells in phytoplankton cultures are generally higher than those found in nature. Algal cultures must therefore be enriched with nutrients to make up for the deficiencies in the seawater. Macronutrients include nitrate, phosphate, and silicate. Specifically, silicate is used for the growth of diatoms which

utilize this compound for production of an external shell. Micronutrients consist of various trace metals and the vitamins, thiamin (B_1), cyanocobalamin (B_{12}) and sometimes biotin. The culture media have been used extensively and are suitable for the growth of most diatom are the Freshwater WC medium, (Guillard and Lorenzen, 1992) Chu's medium (Bold and Wynne, 1978), the Walne medium (Laing, 1991), the standard Guillard's F/2 medium (Guillard, 1975), and the modified standard Guillard's F/2 medium (Smith et al., 1993), etc. The culture media for this experimental see in Appendix A. Commercially available nutrient solutions may reduce preparation labor. In large-scale extensive systems, alternative enrichment media that are suitable for mass production of micro-algae contain only the most essential nutrients and are composed of agriculture-grade rather than laboratory-grade fertilizers (Lavens and Sorgeloos, 1996).

2.2.2 Light intensities

As with all plants, micro-algae photosynthesize, e.g. they assimilate inorganic carbon for conversion into organic matter. Light is the source of energy which drives this reaction and in this regard, intensity, spectral quality and photoperiod need to be considered. Light intensity plays an important role, but the requirements vary greatly with the culture depth and the density of the algal culture: at higher depths and cell concentrations the light intensity must be increased to be able to penetrate through the culture (e.g. 1,000 luxes is suitable for Erlenmeyer flasks, 5,000-10,000 luxes is required for larger volumes). Light may be natural or supplied by fluorescent tubes. Too high light intensity (e.g. direct sun light, small container close to artificial light) may result in photo-inhibition. Also, overheating due to both natural and artificial illumination should be avoided. Fluorescent tubes emitting either in the blue or the red light spectrum should be preferred as these are the most active portions of the light spectrum for photosynthesis (Glover et al., 1987; Brown, 1980; Jeffrey et al., 1977). Irradiance may be measured and expressed energetically in units of watts meter^{-2}. Irradiance measurements are given in SI units as micromoles meter^{-2} second^{-1} whereas a non-SI unit of microeinsteins meter^{-2} second^{-1} is also frequently used. Irradiance and photon flux density (PFD) may be readily interconverted as: (Lunning, 1981)

$$1\ \mu mol\ (m^{-2}s^{-1}) \quad = \quad 1\ \ \mu E\ m^{-2}s^{-1} = \quad \frac{119.7}{\lambda} \quad W\ m^{-2} \quad = \quad 74\ \ luxes \ldots. (2.1)$$

where λ is the wavelength of the light in nanometer ($\approx$ 1.6 nm).

Most algae require a photoperiod of alternating light and dark (Rebolloso Fuentes et al., 1999 and Lunning, 1981). The photoperiod is defined on a light/dark (LD) cycle of 24 hours duration. By the suggestion of Grima et al., (1996), this was representing the factor of the LD cycles due to three elements occurring simultaneously. These are: (a) photon capture starting the chain of biochemical reactions and leading to biomass synthesis; (b) reversible loss of photon trap activity due to high light intensity, and (c) photon trap recovery, which also occurs in the dark. However, cultivation *Entomoneis* sp. in inoculated plates were maintained at 12:12 h light/dark cycle with cool white fluorescent lighting of 100 µmol photon $m^{-2}s^{-1}$ was found in previous (Knuckey et al., 2002).

2.2.3 pH

The pH range for most cultured algal species is between 7 and 9, with the optimum range being 8.2-8.7. Complete culture collapse due to the disruption of many cellular processes can result from a failure to maintain an acceptable pH (Lavens, and Sorgeloos, 1996).

2.2.4 Salinity

Marine phytoplanktons are extremely tolerant to changes in salinity. Most species grow best at a salinity that is slightly lower than that of their native habitat, which is obtained by diluting sea water with tab water. Salinity of 20-40 g L^{-1} has been found to be optimal (Knuckey et al., 2002).

2.2.5 Temperature

The optimal temperature for phytoplankton cultures is generally between 20 and 30°C (Fernando et al., 2009; Oncel et al., 2009; Ranjbar et al., 2008, Marcelo et

al., 2008), although this may vary with the composition of the culture medium, the species and strain cultured. Most commonly cultured species of microalgae tolerate temperature between 20-32 °C. If necessary, algal cultures can be cooled by a flow of cold water over the surface of the culture vessel or by controlling the air temperature with refrigerated air-conditioning units.

2.2.6 Aeration/mixing

Mixing is necessary to prevent sedimentation of the algae, to ensure that all cells of the population are equally exposed to the light and nutrients, to avoid thermal stratification (e.g. in outdoor cultures) and to improve gas exchange between the culture medium and the air. The latter is of primary importance as the air contains the carbon source for photosynthesis in the form of carbon dioxide. For very dense cultures, the CO_2 originating from the air (the natural CO_2 concentration in air (0.03%) bubbled through the culture is limiting the algal growth and pure carbon dioxide may be supplemented to the air supply (e.g. at a rate of 1% of the volume of air). Depending on the scale of the culture system, mixing is achieved by stirring daily by hand (test tubes, erlenmeyers), aerating (bags, tanks), porous gas sparger (airlift), or using paddle wheels and jet pumps (ponds). However, it should be noted that not all algal species can tolerate vigorous mixing. (Lavens, and Sorgeloos, 1996).

A supply of CO_2 enriched air may be necessary to maintain exponential growth in dense cultures but high concentrations may have inhibitory effects. The excessive addition of CO_2 will move the pH to the acid range (as a result of the CO_2/HCO_3^- balance) and this may bring the growth to an end.

2.2.7 Carbon dioxide (CO_2)

Like any other autotrophically plant, algae require an inorganic carbon source to perform photosynthesis, CO_2 is a principal carbon source for the algae. The supplying rate of CO_2 therefore controls the growth rate of the algae. In dense cultures, the rate of diffusion of carbon dioxide into the culture from the air becomes limiting more rapidly than the system with low population density. Improvement of

the rate of aeration by shaking, stirring, or bubbling air through the culture will prolong exponential growth.

2.3 Algal culture techniques

Algae can be produced using a wide variety of methods, ranging from closely controlled laboratory methods to less predictable methods in outdoor tanks. The terminology used to describe the type of algal culture includes:

2.3.1 Indoor/Outdoor: Indoor culture allows control over illumination, temperature, nutrient level, contamination with predators and competing algae, whereas outdoor algal systems make it very difficult to grow specific algal cultures for extended periods.

2.3.2 Open/Closed: Open cultures such as uncovered ponds and tanks (indoors or outdoors) are more readily contaminated than closed culture vessels such as tubes, flasks, carboys, bags, etc.

2.3.3 Batch: Batch culture systems are widely applied because of their simplicity and flexibility, allowing to change species and to remedy defects in the system rapidly. Although often considered as the most reliable method, batch culture is not necessarily the most efficient method. Batch cultures are harvested just prior to the initiation of the stationary phase and must thus always be maintained for a substantial period of time past the maximum specific growth rate. Also, the quality of the harvested cells may be less predictable than that in continuous systems and for example vary with the timing of the harvest (time of the day, exact growth phase). Another disadvantage is the need to prevent contamination during the initial inoculation and early growth period. Because the density of the desired phytoplankton is low and the concentration of nutrients is high, any contaminant with a faster growth rate is capable of outgrowing the culture. Batch cultures also require a lot of labour to harvest, clean, sterilize, refill, and inoculate the containers.

2.3.4 Continuous: The continuous culture method, permits the maintenance of cultures very close to the maximum growth rate. Two categories of continuous cultures can be distinguished:

- turbidostat culture, in which the algal concentration is kept at a preset level by diluting the culture with fresh medium by means of an automatic system.
- chemostat culture, in which a flow of fresh medium is introduced into the culture at a steady, predetermined rate. The latter adds a limiting vital nutrient (e.g. nitrate) at a fixed rate and in this way the growth rate and not the cell density is kept constant.

The disadvantages of the continuous system are its relatively high cost and complexity. The requirements for constant illumination and temperature mostly restrict continuous systems to indoors and this is only feasible for relatively small production scales. However, continuous cultures have the advantage of producing algae of more predictable quality. Furthermore, they are amenable to technological control and automation, which in turn increases the reliability of the system and reduces the need for labor.

2.3.5 Semi-Continuous: The semi-continuous technique prolongs the use of large tank cultures by partial periodic harvesting followed immediately by topping up to the original volume and supplementing with nutrients to achieve the original level of enrichment. The culture is grown up again, partially harvested, etc. Semi-continuous cultures may be indoors or outdoors, but usually their duration is unpredictable. Competitors, predatorsand/or contaminants and metabolites eventually build up, rendering the culture unsuitable for further use. Since the culture is not harvested completely, the semi-continuous method yields more algae than the batch method for a given tank size.

The major advantages and disadvantages of the various algal culture techniques can be summarized in Table 2.3.

2.4 Cultivation in bioreactors

2.4.1 Bubble column photobioreactor

Bubble column bioreactor is basically a column, in which the reaction medium is kept mixed and aerated by the introduction of air into the bottom. Bubble columns have been recently used as bioreactor, however, this type of reaction might not be suitable for high viscosity and three phase cultures as effective mixing in such systems is difficult to obtain.

2.4.2 Air-lift photobioreactor

Airlift photobioreactors are similar to bubble column reactors, but differ by the fact that they contain a draft tube which improves circulation and oxygen transfer and equalizes shear forces in the reactor. It consists of a liquid pool divided into two distinct zones only one is usually sparged by gas. Airlift photobioreactors can be classified into two major types as the internal loop, which is the cylindrical column with a vertical draft tube where a loop channel for the fluid is formed in the airlift, and external loop which has two separated columns operating as riser and downcomer with connection tubes connecting the two columns together near the top and the bottom (Sriouam, 2007). One advantage of airlift reactors is the elimination of attrition effects generally encountered in mechanical agitated reactors. It is ideally suited for aerobic cultures since oxygen mass transfer coefficient are quite high in comparison to stirred tank reactors**.**

2.5 Literature Reviews

Many microalgae have a potential to produce biomass, protein, carbohydrate, lipid and fatty acid and other valuable chemicals suitable for agricultural and aquaculture industries, and several other applications such as *Chaetoceros calcitrans* (Feed for shrimp larvae) (Krichnavarak et al., 2005), *Chlorella vulgaris* (biofuel) (Powell et al., 2009), *Haematococcus pluvialis* (a pigmentation, additive for nutraceutical food) (Kaewpintong et al., 2007), *Scenedesmus almeriensis* (lutein) (Sanchez et al., 2008), *Spirulina platensis* (nutritional content and valuable

biochemicals) (Oncel et al., 2009), *Nitzschia* sp., *Amphora* sp., *Amphora* spp., *Navicula* sp., *Nitzschia ovalis* and *Cylindrotheca closterium* (biofilm).

Growth parameters (such as light intensity and photoperiod, temperature, salinity, culture medium, pH, carbon dioxide concentration, aeration rate and the system of cultivation) are important for the cultivation of highly concentrated microalgal. Literature as summarized in Table 2.4 suggests the range of condition for the culture of microalgae in the photobioreactors i.e. light intensity at range 40-350 μmol photons m^{-2} s^{-1} (Oncel et al., 2009; Ranjbar et al., 2008; Krichnavarak et al., 2005, 2007; Knuckey et al., 2002), photoperiod 12:12 (Oncel et al., 2009, Sanchez et al., 2008), temperature at range 20-30^{o}C (Fernando et al., 2009; Oncel et al., 2009; Ranjbar et al., 2008, Marcelo et al., 2008), culture medium i.e. F1 medium (Kaewpintong et al., 2007), modified F/2 medium (F/2M) (Fernando et al., 2009; Krichnavarak et al., 2005; Yim et al., 2003), Walne's nutrient (Hsieh and Wu, 2009), Mann and Myers, Bioprocess and Hemmerick medium (Sanchez et al., 2008), Modified Ukeles medium (Mirón et al., 2002), salinity at range 3-50 g L^{-1} (Alvarez et al., 2006), carbon dioxide concentration at range 1-15% (Chiu et al., 2009; Powell et al., 2009), aeration rate: usg = 0.4-3 cm s^{-1} (Kaewpintong et al., 2007; Krichnavarak et al., 2005; Mirón et al., 2002).

Types and the systems of cultivation are also important for the algal growth. There are various types of photobioreactors reported to be promising for mass production of algae. Algae are grown either in open culture systems or closed systems (photobioreactors). Given the advantages of closed systems over open ponds, closed photobioreactors have attracted much interest because they allow a better control of the cultivation conditions than open systems. With closed photobioreactors, higher biomass productivities are obtained and contamination can be easily prevented. Algal culture systems can be illuminated by artificial light, solar light or by both. Naturally illuminated algal culture systems with large illumination surface areas include open ponds (Hase et al., 2000), flat-plate (Huet al., 1996), horizontal/serpentine tubular airlift (Camacho Rubio et al., 1999), and inclined tubular photobioreactors (Ugwu et al., 2002). Generally, laboratory-scale photobioreactors are artificially illuminated (either internally or externally) using fluorescent lamps or other light distributors. Some of these photobioreactors include bubble column photobioreactors (Fernando et

al., 2009; Oncel et al., 2009; Ranjbar et al., 2008; Sanchez et al., 2008; Kaewpintong et al., 2006; Krichnavarak et al., 2005; Ogbonna et al., 2002; Degen et al., 2001), airlift column photobioreactors (Oncel et al., 2009; Kaewpintong et al., 2006; Krichnavarak et al., 2005; Harker et al., 1996), Bristles photobioreactor (Fernando et al., 2009), Tubular photobioreactor (Converti et al., 2006), Fed-batch (Matsudo et al., 2008).

Todate, there have been no evidences of mass production of *Entomoneis* sp. Knuckey et al., 2002, was the only report available. In this report, *Entomoneis cf. punctulata* was cultivated in 75-ml F/2 media -125-ml flasks with condition at 17.5±2 ^{o}C on a 12:12 h light/dark cycle light intensity of 45 µmol photons $m^{-1}s^{-1}$. The results revealed the growth rate of 1.73 day^{-1} and maximum cell concentration of $3.1x10^{6}$ cells mL^{-1}.

Table 2.1 Oil content of some microalgae (Chisti, 2007)

Microalga	**Oil content (% dry weight)**
Chlorella sp.	28-32
Botryococcus braunii	25-75
Isochysis sp.	25-33
Nannochloris sp.	20-25
Nannochloropsis sp.	31-68
Nitzchia sp.	45-47
Phaeodactylum tricornutum	20-30
Schizochytrium sp.	50-77

Table 2.2 Biochemical composition of *Entomoneis cf. punctulata* expressed as a percentage of ash free dry weight (Hong et al., 2008)

Biochemical Composition	**% Dry weight**
Protein	27.3
Carbohydrate	25.1
Total lipids	47.1

Table 2.3 Summary of the major advantages and disadvantages of various algal culture techniques **(**Ugwu et al., 2008**)**

Culture type	Advantages	Disadvantages
Indoors/ Close	a high degree of control (predictable)/ Contamination less likely	Expensive
Outdoors/ Open	Cheaper	Little control (less predictable)/ Contamination more likely
Continuous	Efficient, provides a consistent supply of high-quality cells, automation, highest rate of production over extended periods	Difficult, usually only possible to culture small quantities, complex, equipment expenses may be high
Semi-continuous	Easier, somewhat efficient	Sporadic quality, less reliable
Batch	Easiest, most reliable	Least efficient, quality may be inconsistent

Table 2.4 Literature reviews

Algae	Type of reactor Volume (L)	Conditions	Cell or Biomass conc. x10^4 (cells mL^{-1})	Biomass conc. (g L^{-1})	μ (day^{-1})	Remark	Applications	References
Nannochloropsis oculata	-cylindrical glass 800 ml	- Temperature =26±1 ^{O}C - usg = 200 mL min^{-1} - Light intensity 300 μE m^{-2} s^{-1} - Medium: modified standard F/2 -CO_2 concentrations: 2%, 5%, 10%, 15%	10,000	1.277 ±0.043	0.571	Lipid accumulation and CO_2 utilization of *Nannochloropsis oculata* in response to CO_2 aeration	High lipid content. microalgal-based biodiesel	Chiu et al., 2009
1. *Nitzschia* sp. 2. *Amphora* sp. 3.*Amphora* sp2 4. Navicula sp. 5. *Nitzschia ovalis* 6.*Cylindrotheca closterium*	-Bristles photobioreactor (PBB) -Bubble column Photobioreactor (PBC) 20 L	- Temperature = 20±2 ^{O}C - light: dark = 12:12 - cultured for 10 days -Light intensity = 100 μE m^{-2} s^{-1} - Medium: F/2M (sodium metasilicate)	(PBC) 259.33 (PBB) 210.00 (PBB) 115.00 (PBB) 277.00 (PBB) 167.67 (PBC) 350.00	2.26 1.27 1.16 1.56 0.83 1.60	-	Comparisons of the growth of six diatom species between two configurations of photobioreactors	Production of specific diatom-bateria biofilm. are potentially valuable for use the food cosmetics, pharmaceutic and alagricultural	Fernando et al., 2009
Chlorella sp.	rectangular photobioreactor 1 L	- Temperature = 27±1 ^{O}C - Light intensity =320 μE m^{-2} s^{-1} - Medium: Walne's nutrient - CO_2 concentrations: 2%	-	2.027	1.42	Cultivation of microalgae for oil production with a cultivation strategy of urea limitation	Lipid productivity for bio-fuel production	Hsieh and Wu, 2009

Algae	Type of reactor Volume (L)	Conditions	Cell or Biomass conc. x10^4 (cells mL^{-1})	Biomass conc. (g L^{-1})	μ (day^{-1})	Remark	Applications	References
Artrospira platensis (Spirulina platensis).	-Internal loop airlift -bubble column photobioreactors 1.5 L	- Temperature = 25±1 ^{O}C - light:dark = 12:12 - Light intensity = 54 μE m^{-2} s^{-1} - cultured for 17 days - Medium: Zarrouk	-	3.24	0.45 0.33	Comparison of two different pneumatically mixed column photobioreactors for the cultivation of *Artrospira platensis (Spirulina platensis)*	-Nutritional Content -Valuable biochemical	Oncel et al., 2009
Chlorella vulgaris	1.0 L vessels	- Temperature = 22.5 ^{O}C - initial cell concentration = 3 mg L^{-1} - concentration of CO_2 in feed air = 10% by volume - Total flow rate =200 ml min^{-1} - Medium: a modified Bolds Basic media	-	-	3.6 mg/L-h	Growth kinetics of *Chlorella vulgaris* and its use as a cathodic half cell	The microbial fuel cell (MFC)	Powell et al., 2009
Arthrospira (Spirulina) platensis	Bioreactor, Fed-bacth mode 5 L	- Temperature = 28 ^{O}C - light: dark = 12:12 - Light intensity=108 μE m^{-2} s^{-1} - cultured for 16 days - Medium: Schlosser with $NaNO_3$	-	2.44	0.6±0.09	Repeated fed-batch cultivation of *Arthrospira* (*Spirulina*) *platensis* using urea as nitrogen source	Human consumption, mainly as a nutarceutical	Matsudo et al., 2008

Algae	Type of reactor Volume (L)	Conditions	Cell or Biomass conc. x10^4 (cells mL^{-1})	Biomass conc. (g L^{-1})	μ (day^{-1})	Remark	Applications	References
Haematococcus pluvialis	Bubble column Photobioreactor 1 L	- Temperature = 28 ^{O}C -Light intensity=48.5 μE m^{-2} s^{-1} - cultured for 900 hour - Medium: Standard inorganic	350	-	-	High efficiency production of astaxanthin in an airlift photobioreactor	Production of antioxidant, astaxantin	Ranjbar et al., 2008
Scenedesmus almeriensis	bubble column photobioreactors 2 L	- Temperature = 20^{O}C - PH = 8 - light: dark = 12:12 - Light intensity=1700μE m^{-2} s^{-1} - Medium: 1.Mann and Myers 2.Bioprocess 3.Hemmerick	-	3.1	0.63	Influence of culture conditions on the productivity and lutein content of the new strain *Scenedesmus almeriensiss*	Production of Lutein use for - a food coolant - additive	Sanchez et al., 2008
Chaetoceros calcitrans	-Airlift bioreactor -Bubble column 170 L	- Temperature =30^{O}C - usg = 3 cm s^{-1} -Light intensity =350 μE m^{-2} s^{-1} - Medium: modified standard F/2	29.6 25.1	-	0.62 0.59	Enhanced productivity in airlift photobioreactors	Feed for shrimp larvae	Krichnavaruk et al., 2007
Spirulina platensis	Tubular photobioreactor 5.5 L	- Temperature = 30 ^{O}C - Light intensity 120 μE m^{-2} s^{-1} - Medium: medium of Schlosser	-	10.6	0.19	Cultivation of *Spirulina platensis* in a combinedairlift-tubular reactor system	-Nutritional Content -valuable biochemical	Converti et al., 2006

Algae	Type of reactor Volume (L)	Conditions	Cell or Biomass conc. x10^4 (cells mL^{-1})	Biomass conc. (g L^{-1})	μ (day^{-1})	Remark	Applications	References
Haematococcus pluvialis	-Airlift bioreactor -Bubble column 3 L	- Temperature = 27±1 ^{O}C - usg =0.4 cm s^{-1} - light: dark = 12:12 - Light intensity=20 μE m^{-2} s^{-1} - Medium: F1	79.5 42.0	-	0.45 0.36	Photoautotrophic high-density cultivation of vegetative cells of *Haematococcus pluvialis* in airlift bioreactor	One of the best sources of red secondary carotenoid astaxanthin, used as - a pigmentation - additive - nutraceutical food	Kaewpintong et al., 2006
Chaetoceros calcitrans	-Airlift bioreactor -Bubble column 17 L	- Temperature =30^{O}C - initial cell concentration = 5x10^5 cell mL^{-1} - A_d/A_r = 2.63 - usg = 3 cm s^{-1} -Light intensity = 130 μE m^{-2} s^{-1} - Medium: modified standard F/2	888 588	-	1.78 0.91	Optimal growth conditions and the cultivation of *Chaetoceros calcitrans* in airlift photobioreactor	Feed for shrimp larvae	Krichnavarak et al., 2005
Phaeodactylum tricornutum	-Airlift bioreactor -Bubble column 20 L	- Temperature =22 ^{O}C - usg = 3 cm s^{-1} -Light intensity =230 μE m^{-2} s^{-1} - Medium: Modified Ukeles medium	-	-	2.16 1.92	Growth and biochemical characterization of microalgal biomass produced in bubble column and airlift photobioreactors: studies in fed-batch culture	Produced as essential aquaculture feed for shellfish and fish juveniles.	Mirón et al., 2002

CHAPTER III

MATERIALS AND METHODS

3.1 Experimental Set up

3.1.1 Bubble column system

The growth of *Entomoneis* sp. was investigated in the bubble column with the setup as detailed in Mongkolsit (2008). This system was a 2.5 L clear acrylic plastic bubble column with a diameter of 9.4 cm and height of 60 cm (see Fig. 3.1 for a schematic of the bubble column photobioreactor). Compressed air was supplied through a 0.2 μm Gelman filter and measured with a flowmeter before entering the reactor at the bottom. The aeration rate was controlled by adjusting the superficial gas velocity in the riser at 0.3-2 cm s^{-1}. Light was supplied through fluorescent lamp (18 W) located at the side along the length of the column, and was measured by "Digicon LX-50 lux meter" around the column. This was then converted into μ mol photon m^{-2} s^{-1} using Equation 3.1. The temperature was controlled at 27-32^{o}C.

3.1.2 Flat-plate airlift system

The growth of *Entomoneis* sp. was investigated in a 17 L flat-plate airlift photobioreactor in similar fashion with that reported in Issarapayup (2007). The bioreactor and draft tube were made from clear acrylic plastic with the thickness of 5 and 2 mm, respectively. The bioreactor was 20 cm wide, 50 cm high and 20 cm long. This made the volume of 17 L. Table 3.1 shows geometric details of such reactor. The ratio between the downcomer and riser cross section area (A_d/A_r) was 0.4. The schematic diagram of experimental setup in flat-plate airlift photobioreactor was shown in Figure 3.2.

The liquid culture in the airlift photobioreactor was agitated by supplying air bubbles at the bottom of the airlift column through three gas spargers which help distribute gas bubbles within the contactor. Air from an air compressor was metered

through a flowmeter, sterilized with a 0.20 μm Gelman filter, and passed into the culture at the bottom of the reactor. The aeration rate was controlled by adjusting the superficial gas velocity in the riser at 0.5 cm s^{-1}. Light for the airlift photobioreactor was supplied from the vertical 18 W fluorescent lamps during the night time and during the day time was supplied by sunlight. The light intensity from fluorescent light was about 43 μmol photon m^{-2} s^{-1} (measured by "Digicon LX-50 lux meter" around the column). The temperature was controlled in an evaporative room at 27±2^{o}C.

3.2 Experimental Methods

3.2.1 Concentrated seawater preparation

Concentrated seawater used in this investigation is obtained from salt farms with the original concentration of around 100-120 ppt (parts per thousand). Seawater is prepared step by step as follows:

1. Dilute the concentrated seawater to 30 ppt with tap water

2. Disinfect the seawater with 50 ppm (parts per million) of chlorine (as sodium hypochloride)

3. Sparge air through the sea water for 2–3 h to remove residual chlorine in seawater. (adding sodium thiosulfate to neutralize chlorine if aeration cannot eliminate the chlorine) Check with potassium iodide whether chlorine is exhausted, seawater will turn yellow if the remaining chlorine reacts with potassium iodide.

3.2.2 Preparation of culture medium

Standard F/2(Guillard's) medium, modified F/2 (F/2M), WC medium, Chu's freshwater medium, and Walne medium are prepared according to the composition as shown in Tables 3.1-3.5.

3.2.3 Preparation of stock culture

1. Select inoculums of diatom, *Entomoneis* sp. from the Center of Excellence form Marine Biotechnology. Faculty of science, Chulalongkorn University, Thailand

2. Inoculate cells into 50 mL Erlenmeyer flask with the sterilize (in autoclave at 121 ^{o}C for 20 min) culture medium, and then scale up into 250 mL Erlenmeyer flask

3. Inoculate cells from the flask into 2000 mL vessel and supply continuous aeration, temperature control at 27-30^{o}C and, light intensity of 20 μmol photon m^{-2} s^{-1} to the surface of the culture vessel.

3.2.4 Bubble column photobioreactor culture systems

1. Setup the bioreactor as described in Section 3.1.1

2. Sterilize Bubble column and the seawater with 50 ppm chlorine (as sodium hypochloride). Sparge air through the porous sparger at the bottom of the column for about 1 day, check residual chlorine in seawater by adding potassium iodide in seawater if chlorine is not exhausted, seawater is turned yellow

3. Fill in the column with sterilized culture medium together with the pure culture with initial cell concentration $1x10^{4}$ cells mL^{-1}, adjust the total volume to 2.5 L

4. Cover the column with a plastic funnel to minimize airborne contamination

5. Supply sterilized compressed air (sterilized with a 0.2 μm Gelman autoclave filter) through a porous sparger and adjusts the superficial gas velocity to 0.3-2.0 cm s^{-1}

6. Supply both sides of column with 18 W fluorescent light bulbs, placed along the column height (6,000 – 20,000 luxes or 81-270 μmol photon $m^{-2}s^{-1}$) as shown in Figure 3.1

7. Take samples daily and count for the cell density using Haemacytometer (mentioned in Section 3.3.2) and measure the concentration of nutrient content in the medium (see Appendix A)

8. Calculate the specific growth rate using Equation 3.3, the productivity using Equation 3.4 and the specific productivity using Equation 3.6.

3.2.5 Feeding strategy experiment

1. Follow Steps 1-4 in Section 3.2.4

2. Periodically replenish silicon and/or phosphorus and/or nitrogen in the F/2M medium (the frequency of the replenishment will be determined from this experiment)

3. Take samples and count for the cell density using Haemacytometer (mentioned in Section 3.3.2) and measure the concentration of each nutrient content in the medium on a daily basis until stationary phase (see Appendix A for the detail of measurement)

4. Calculate the specific growth rate using Equation 3.3, the productivity using Equation 3.4, and the specific productivity using Equation 3.5.

3.2.6 Cultivation with air/CO_2 mixture in 2.5 L bubble column

1. Follow Steps 1-4 in Section 3.2.4

2. Sparge a mixture of air and CO_2 through the reactor bottom at superficial gas velocity at 0.5 cm s^{-1} and adjust the flow rate of CO_2 at 2% v/v

3. Supply both sides of column with fluorescent light bulbs, placed along the column height (20,000 luxes or 270 μmol photon $m^{-2}s^{-1}$)

4. Take samples daily and count for the cell density using Haemacytometer (mentioned in Section 3.3.2)

5. Calculate the specific growth rate using Equation 3.3, the productivity using Equation 3.4 and the specific productivity using Equation 3.5.

3.2.7 Flat-plate airlift photobioreactor culture systems

1. Setup the airlift system according to the description in Section 3.1.2

2. Sterilize airlift bioreactor and the seawater with 50 ppm chlorine (as sodium hypochloride). Sparge air through the porous sparger at the bottom of the column for about 1 day, check for residual chlorine in seawater.

3. Fill in the column with sterilized fresh medium together with the pure culture with initial cell concentrations of $1x10^4$ and $10x10^4$ cells mL^{-1} in batch and semi-continuous mode, adjust the total volume to 17 L

4. For cultivation with air/CO_2 mixture, sparge a mixture of air and CO_2 through the reactor bottom at superficial gas velocity of 0.5 cm s^{-1} and adjust the flow rate of CO_2 at 1, 3, 5 and 7% v/v

5. Cover the column with a plastic funnel to minimize airborne contamination

6. Supply sterilized compressed air (sterilized with a 0.2 μm Gelman autoclave filter) through a porous sparger and adjust the superficial gas velocity to 0.5 cm s^{-1}

7. Expose the culture with sunlight during the day time and fluorescent light during the night. (The light intensity from fluorescent light was about 43 μmol photon m^{-2} s^{-1})

8. Take samples daily and count for the cell density using Haemacytometer

9. Calculate the specific growth rate using Equation 3.3, the productivity using Equation 3.4 and the specific productivity using Equation 3.5.

3.3 Analyses

3.3.1 Determination of light intensity

The light intensity (measured by "Digicon LX-50 lux meter") can be calculated from Equation 3.1 as follows:

$$I = \frac{E}{74} \tag{3.1}$$

where

I = light intensity (μmol photon $m^{-2}s^{-1}$)

E = light intensity (lux)

3.3.2 Determination of cell concentration

The cell concentration can be determined using a normal blood cell counting slide, Haemacytometer. The depth of the counting grid and the medium area are 0.1 mm and 1 mm^2, respectively. The cell concentration can be determined as follows:

1. Clean the counting slide and cover glass
2. Fill the slide with sample

3. Cover the slide with cover glass, avoid the presence of bubbles
4. Count the cell in 9 medium squares on the grid
5. Calculate the cells number, using Equation 3.2:

$$N = \frac{n}{9} \times 10^4 \qquad (3.2)$$

where

N = cells concentration (cells mL^{-1})

n = number of cells on 9 squares in upper or lower grid (cells)

3.3.3 Determination of specific growth rate

The specific growth rate can be calculated from Equation 3.3 as follows:

$$\mu = \frac{\ln(N_2) - \ln(N_1)}{t_2 - t_1} \qquad (3.3)$$

where

μ = specific growth rate (h^{-1})

N_1 = cells concentration at t_1 (cells mL^{-1})

N_2 = cells concentration at t_2 (cells mL^{-1})

t_1 = first sampling time (h)

t_2 = second sampling time (h)

3.3.4 Determination of productivity

The productivity of the diatom was calculated from Equation 3.4 as follows:

For batch and semi-continuous cultivation:

$$P = \frac{N_2 - N_1}{t_2 - t_1} \times \frac{V \times 1000}{3600} \qquad (3.4)$$

where

P = productivity (cells s^{-1})

N_1 = cells concentration at t_1 (cells mL^{-1})

N_2 = cells concentration at t_2 (cells mL^{-1})

t_1 = first sampling time (h)

t_2 = second sampling time (h)

V = harvest volume (L)

3.3.5 Determination of specific productivity

The specific productivity for the cultivation of the diatom in the photobioreactor can be calculated from Equation 3.5:

$$SP = \frac{P}{V} \tag{3.5}$$

where

SP = specific productivity (cells L^{-1} s^{-1})

P = productivity (cells s^{-1})

V = harvest volume (L)

3.3.6 Determination of remaining chlorine in the solution

The remaining chlorine in the solution is determined by adding a small amount of potassium iodide into the solution. Should the color of the mixture turn to yellow, there is still chlorine remaining in the solution in the level that is harmful for the diatom.

3.3.7 Determination of salinity

The salinity of the seawater was determined by using a “Refractometer”. The salinity of the saline water should be adjusted to 30 ppt (parts per thousand).

3.3.8 Nutrient Analysis

Procedure of measurement (see Appendix A for the detail)

1. Determination of reactive silicate (modified from Strickland and Parson, 1972)
   - Add 0.5 ml of Sample to 0.2 ml of molybdate solution, mix for 10 min
   - Add 0.3 ml of reducing agent, allow 2- 3 hr for reaction time
   - Measure the solution by Spectrophotometer at wavelength of 810 nm
2. Determination of reactive phosphate (modified from Strickland and Parson, 1972)
   - Add 0.1 ml of mixed reagent in 1 ml sample, allow 10 min for reaction time. Measure by Spectrophotometer at wavelength of 885 nm
3. Determination of Nitrate by Screening method (APHA, 1998)
   - Measure the sample by Spectrophotometer at a wavelength of 220 nm to obtain NO_3^- reading and a wavelength of 275 nm to determine interference due to dissolved organic matter.

3.3.9 The calculation of conversion of CO_2 by *Entomoneis* sp.

The conversion of CO_2 by *Entomoneis* sp. can be calculated from Equation 3.6

$$Conversion = \frac{CO_2\ uptake\ to\ cell\ mass}{mass\ of\ CO_2 feed\ into\ reactor} \times 100 \qquad (3.6)$$

Note: see Appendix B for detail and example of the calculation of conversion of CO_2 by *Entomoneis* sp.

Table 3.1 Geometric details of flat-plate airlift photobioreactor

	Symbol	17 L Flat plate
volume (L)		17
Column height (cm)	H_R	50
Column length (cm)	L_R	20
Column width (cm)	W_R	20
Draft tube height (cm)	H_r	30
Bottom clearance (cm)	H_B	10

Table 3.2 Composition of modified standard F/2 (Guillard's) (modified from Smith et al., 1993)

Nutrient	Final concentration (mg L^{-1} seawater)
$NaNO_3$	75
$NaH_2PO_4.H_2O$	10
$Na_2SiO_3.9H_2O$	60
Trace	
$CoCl_2.6H_2O$	0.01
$CuSO_4.5H_2O$	0.01
$MnCl_2.4H_2O$	0.18
$Na_2MoO_4.2H_2O$	0.006
$ZnSO_4.7H_2O$	0.022
Na_2EDTA	4.36
$FeCl_3.6H_2O$	3.15
Vitamins	
Thiamin HCl	0.1
Biotin	0.0005
B_{12}	0.0005

Table 3.3 Composition of standard F/2 (Guillard, 1975)

Nutrient	Final concentration (mg L^{-1} seawater)
$NaNO_3$	75
$NaH_2PO_4.H_2O$	5
$Na_2SiO_3.9H_2O$	30
Trace (1 ml)	
$CoCl_2.6H_2O$	0.01
$CuSO_4.5H_2O$	0.01
$MnCl_2.4H_2O$	0.18
$Na_2MoO_4.2H_2O$	0.006
$ZnSO_4.7H_2O$	0.022
Na_2EDTA	4.36
$FeCl_3.6H_2O$	3.15
Vitamin (1 ml)	
Thiamin HCl	0.1
Biotin	0.0005
B_{12}	0.0005

Table 3.4 Composition of "WC" medium (Guillard & Lorenzen,1972)

Nutrient	Final concentration (mg L^{-1} seawater)
$CaCl_2.2\ H_2O$	36.76
$MgSO_4.7\ H_2O$	36.97
$NaHCO_3$	12.60
$NaNO_3$	85.01
K_2HPO_4	8.71
$Na_2SiO_3.9H_2O$	28.42
Trace (1 ml)	
$CoCl_2.6H_2O$	0.01
$CuSO_4.5H_2O$	0.01
$MnCl_2.4H_2O$	0.18
$Na_2MoO_4.2H_2O$	0.006
$ZnSO_4.7H_2O$	0.022
Na_2EDTA	4.36
$FeCl_3.6H_2O$	3.15
H_3BO_3	1.0
Vitamins (1 ml)	
Thiamin HCl	0.1
Biotin	0.0005
B_{12}	0.0005

Table 3.5 Composition of Chu's medium (Bold & Wynne, 1978)

Nutrient	Final concentration (mg L^{-1} seawater)
$CaCl_2.2\ H_2O$	36.70
$MgSO_4.7\ H_2O$	36.90
$NaHCO_3$	12.60
$NaNO_3$	85.00
K_2HPO_4	8.70
$Na_2SiO_3.9H_2O$	28.40
Citric acid	33.5
Ferric citrate	33.5
Trace (1 ml)	
$CoCl_2.6H_2O$	20
$CuSO_4.5H_2O$	19.6
$MnCl_2.4H_2O$	36
$Na_2MoO_4.2H_2O$	12.6
$ZnSO_4.7H_2O$	44
Na_2EDTA	50
H_3BO_3	618

Table 3.6 Composition of Walne medium (modified from Laing, 1991)

Nutrient	Final concentration (mg L^{-1} seawater)
Nutrient solution (1 ml)	
$MnCl_2.4H_2O$	0.36
$FeCl_3.6H_2O$	1.30
H_3BO_3	33.60
EDTA (disodium salt)	45
$NaH_2PO_4.2H_2O$	20
$NaNO_3$	200
$Na_2SiO_3.9H_2O$	40
Trace metal solution (1 ml)	
$ZnSO_4$	21
$(NH4)_6Mo7O_{24}.4H_2O$	9
$CoCl_2.6H_2O$	20
$CuSO_4.5H_2O$	20
Concentrated HCl	10 ml
Vitamins	
Thiamin HCl	1
Biotin	0.002

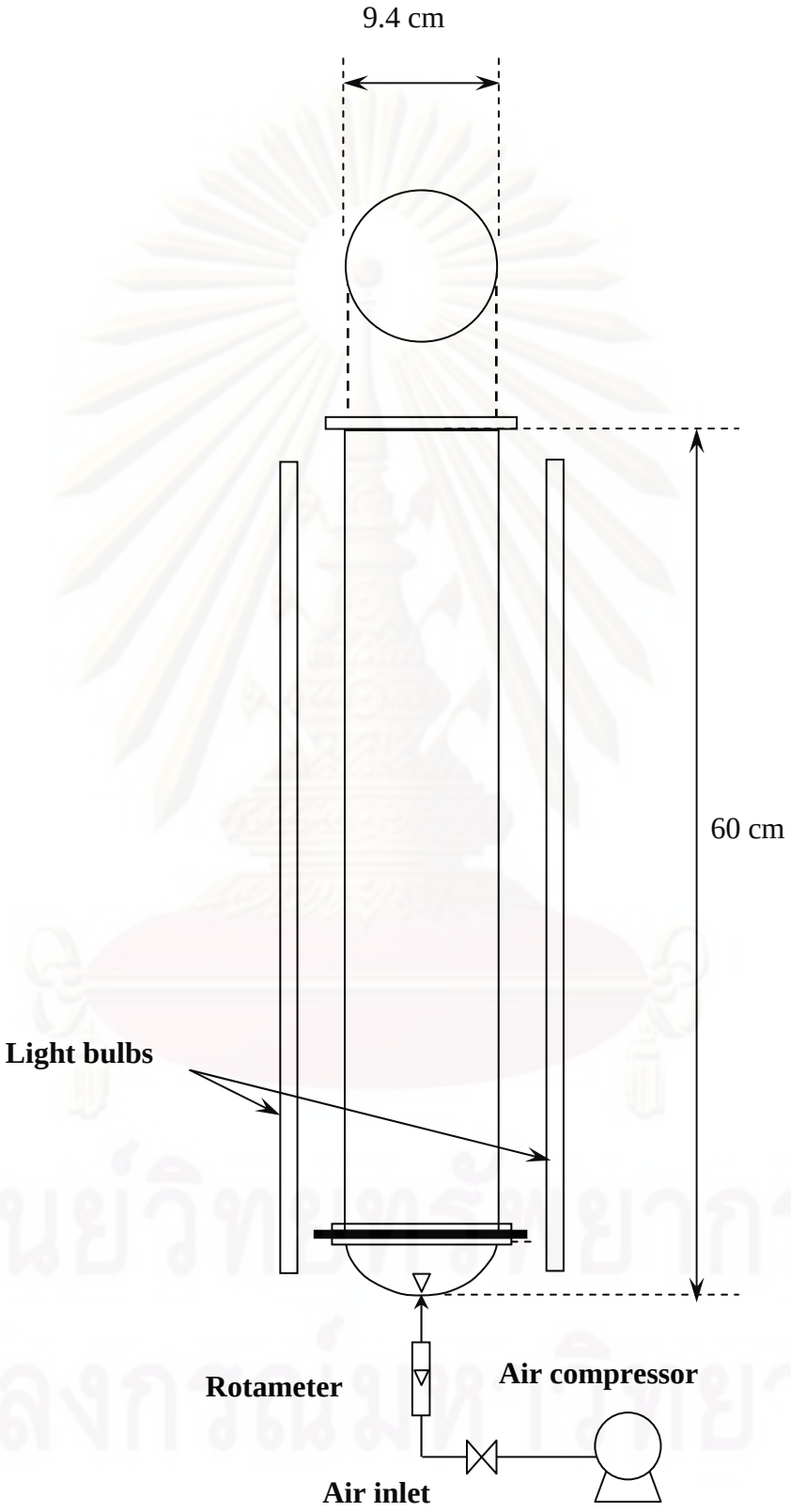


Figure 3.1 The schematic diagram of experimental setup for cultivation of *Entomoneis* sp. in bubble column.

Figure 3.2 Experimental setup for cultivation of *Entomoneis* sp. in bubble column.

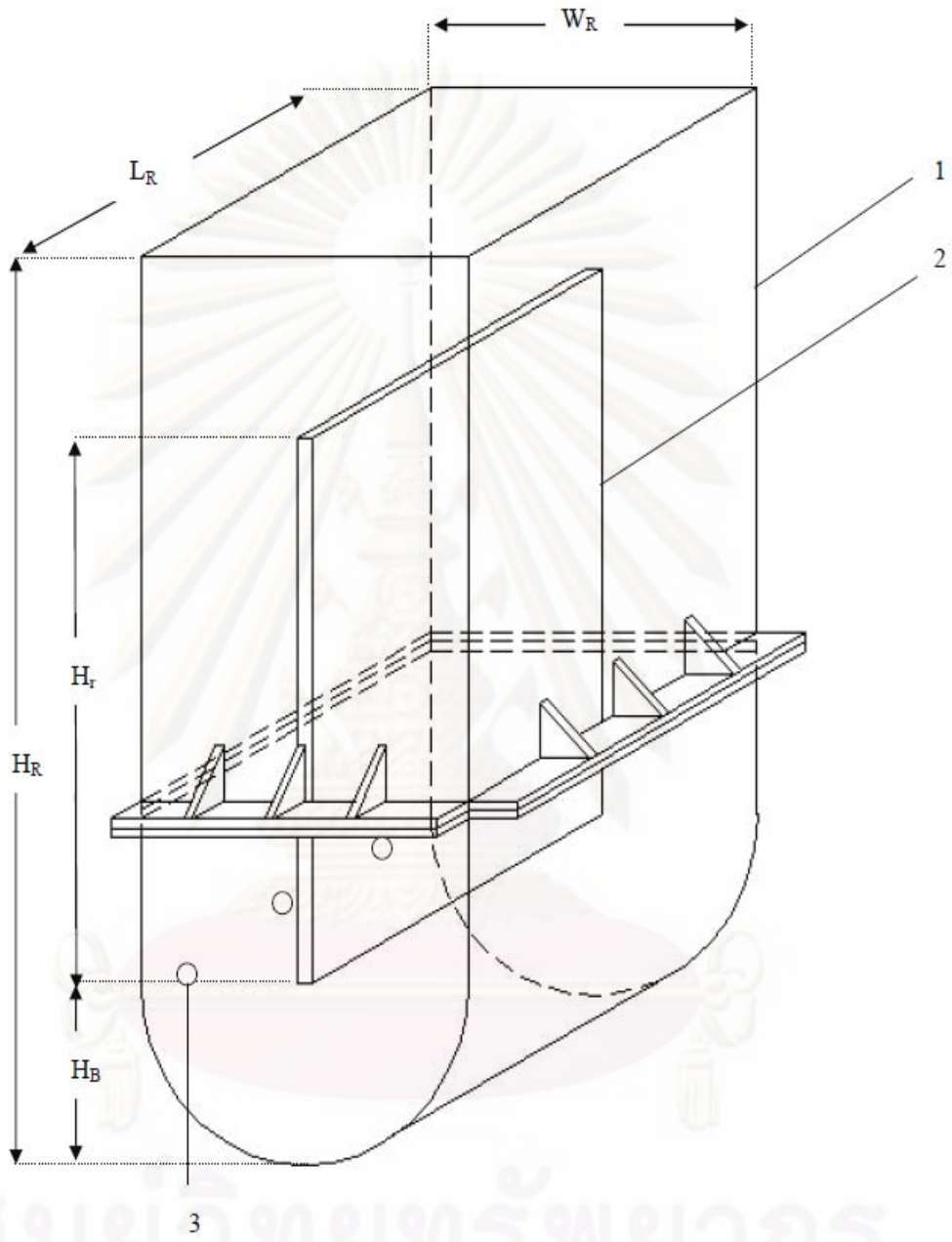


Figure 3.3 Schematic diagram of flat-plate airlift photobioreactor.

Symbol, 1 = reactor column; 2 = vertical plate; 3= spargers; H_R = column height; L_R = column length; W_R = column width; H_r = plate height; H = bottom clearance

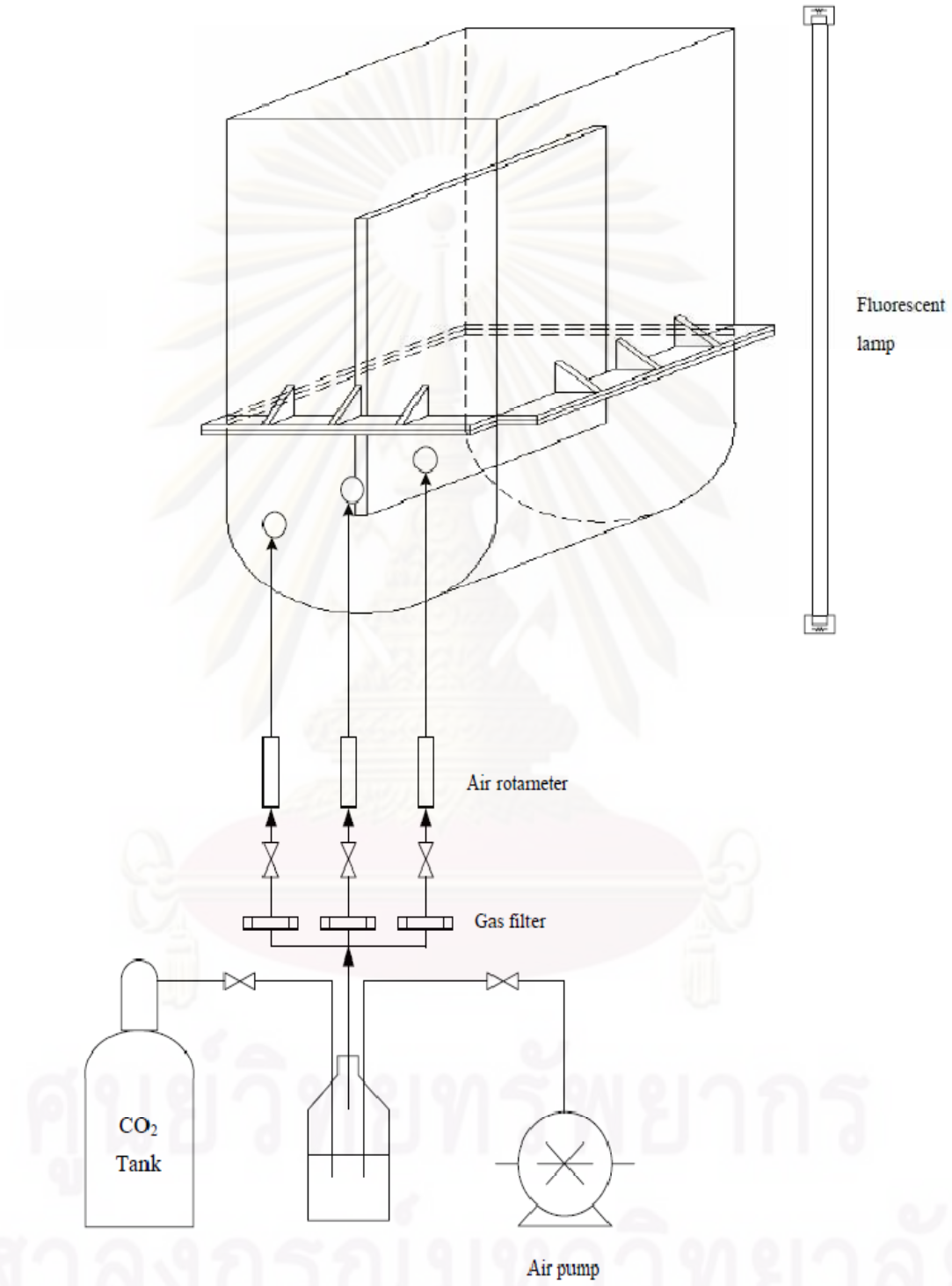


Figure 3.4 Experimental setup drawing for cultivation of *Entomoneis* sp. in flat-plate airlift photobioreactor.

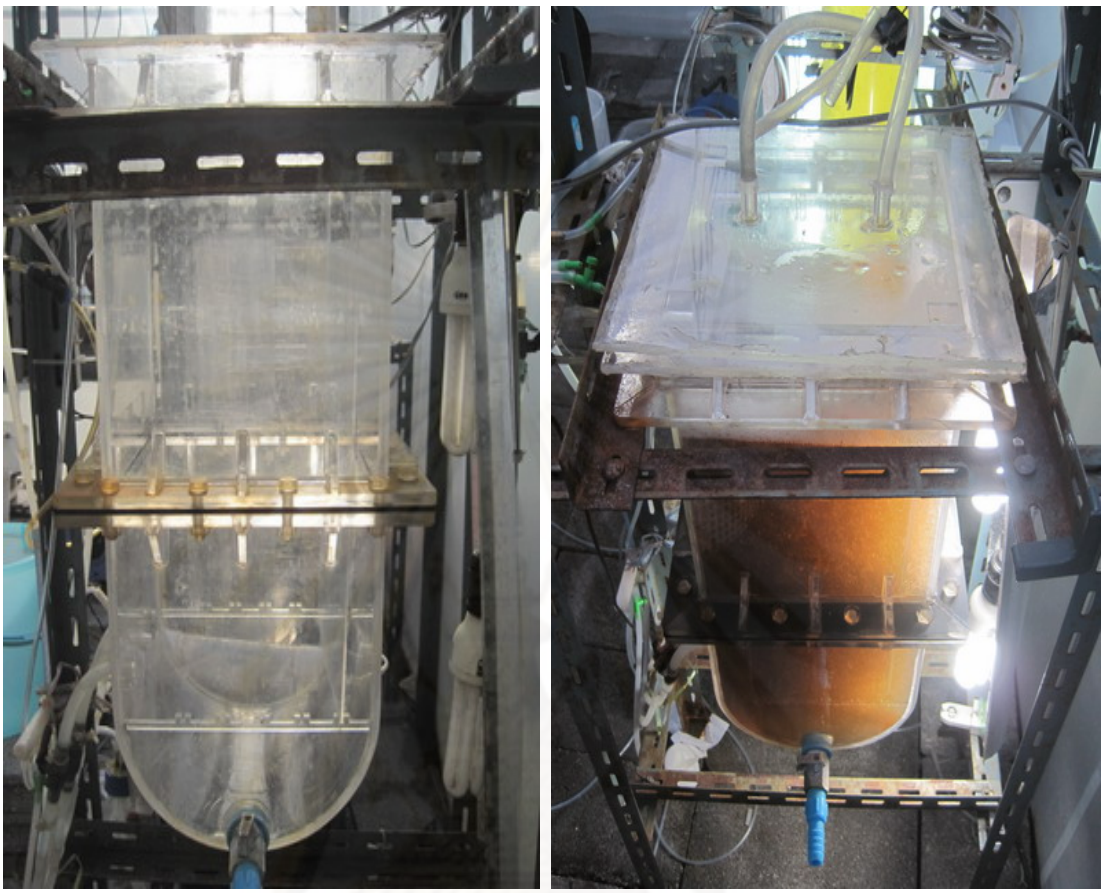


Figure 3.5 Experimental setup for the cultivation of *Entomoneis* sp. in 17 L flat-plate airlift Photobioreactor

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Growth of *Entomoneis* sp. in different culture media

Five different recipes of media were examined for their effectiveness in the cultivation of *Entomoneis* sp. These include: standard F/2 (Guillard's) medium, modified F/2 (F/2M), WC medium, Chu's freshwater medium, and Walne medium. The experiments were tested in the reactor system running at a light intensity of 81 µmol photons m^{-2} s^{-1} and superficial velocity (u_{sg}) of 0.5 cm s^{-1}, temperature of 30±2^{o}C. All experiments in this section were run in batch mode. Figure 4.1 illustrates that F/2M and WC were the two most effective media for this particular strain. Table 4.1 demonstrates that the maximum cell concentrations were 11 x10^{4} cell mL^{-1} for F/2M and 10.5 x 10^{4} cell mL^{-1} for WC with specific growth rates of 0.30 d^{-1} and 0.32 d^{-1}, respectively. In both cases, cells took approximately 6 days to reach their stationary stage. The F/2M medium was finally selected due to its ease of handling and preparation, and economical reasons. Table 4.2 displays the comparison between the two media in terms of cost.

4.2 Super-rich nutrient for enhanced growth

4.2.1 Preliminary assessment

The experiments of super-rich nutrient for the growth of *Entomoneis* sp. were tested in 2.5 L bubble column photobioreactor system running at a light intensity of 81 µmol photons m^{-2} s^{-1} and superficial velocity (u_{sg}) of 0.5 cm s^{-1}, and with high concentrations of major components such as silicon (Si), nitrogen (N) and phosphorus (P). The F/2M medium was selected as a starting medium for this cultivation. All experiments in this section were run in batch mode.

Time profiles of the nutrient concentrations during the growth of the diatom were monitored and reported in Figure 4.2. During the initial lag phase where very slow growth was observed, nutrients concentration remained at the concentration

level in F/2M medium. Once cells entered the exponential phase, all nutrients were being consumed quite quickly. Si concentration seemed to be consumed significantly and the level of Si in the medium went down to almost zero (at the beginning of Day 3). The reason for the significant uptake of Si could be that Si is an important constituent in cell wall, and it is definitely needed for cell division, and this is why a significant reduction in the concentration of Si was observed. Furthermore, P was also being consumed quite rapidly and the level of P went down to zero at Day 8. It could be that cells accumulated energy during the growth in the form of adenosine triphosphate (ATP) which required the uptake of P. N is one important element for protein synthesis and is conventionally supplied to the culture in nitrate form. Nevertheless, N was still left in the medium at a reasonably high content.

As a preliminary assessment, the results in this section led to the conclusion that Si and P might be rate limiting factors for the growth of *Entomoneis* sp. This issue is discussed further in the next section.

4.2.2 Effect of silicon concentration and feeding strategy

Silicon (Si) is one of the main constituents of the diatom shell and needed in the cell-wall formation. In addition, Si is required for the synthesis of DNA, protein, and chlorophyll halts (Lesley et al., 1997). It is generally known that the assimilation of Si into cell is directly connected with the formation of new cell wall, therefore there was a definite low concentration of Si below which a population could not survive. Figure 4.3 presents the results of Si consumption by *Entomoneis* sp. coupled with microalgal cell growth. It was found that Si was consumed upto a certain level and the cell growth seemed to level off after that. Si concentration at Day 3 (0-2.2 mg L^{-1}) seemed to have significant influence to the growth as below this level the alga could hardly grow. Hence, Si at Day 3 was treated as the critical or threshold Si concentration for which, for all subsequent experiments, Si concentration was maintained such that it did not fall below this critical level. The effects of Si feed concentration and feeding schedule on microalgal growth were investigated in batch cultivation, see Table 4.3 for detailed information.

Figure 4.3a illustrates that the maximum cell concentration of the culture without the addition of Si (typical Si in modified F/2) was 11 x10^4 cells mL^{-1} (specific growth rate of only 0.30 d^{-1}). In Experiments B-1, B-2 and B-3, Si was added into the medium at Day 3 at various concentrations, i.e. 4.8, 6.7 and 13.7 mg (Si) L^{-1}, respectively. Figure 4.3b demonstrates the results from the adding of Si at 4.8 mg L^{-1} where the growth increased significantly when compared with the growth with F/2M and a maximum cell concentration of as high as 32x10^4 cells mL^{-1} could be achieved at Day 8^{th}. Figure 4.3c shows that adding of Si (at 6.7 mg L^{-1}) further exerted slight positive influence for the growth and the attainable maximum cell concentration of 32.75x10^4 cells mL^{-1} was obtained at Day 7^{th}. However, Figure 4.3d illustrates that a further increase in Si to 13.7 mg L^{-1} cause a slight drop in the growth where the maximum cell concentration of 26.5x10^4 cells mL^{-1} could only be reached at Day 8^{th}. From the results, the most suitable Si concentration was at 6.7 mg L^{-1} (Experiment B-2) and this was then used in subsequent batch experiments in this work. In Experiment B-4, Si was added in excess (30.6 mg L^{-1}) into the medium at the beginning of the run, but no clear positive effect to the growth could be observed as illustrated in Figure 4.3e. In this case, the maximum cell concentration was 17 x10^4 cells mL^{-1}.

This study demonstrated that extra amounts of Si, when fed at appropriate manner, could improve cell growth. A large supply of Si, on the other hand, could decrease cell growth. The optimal makeup Si concentration was observed at 6.7 mg L^{-1} when added into the medium on Day 3. This concentration yielded the highest cell concentration and became an optimized supply level of silicon concentration for the growth this microalga.

4.2.3 Effect of phosphorus concentration

Phosphorus (P) plays an important role in most cell metabolic activities, and it is an important component of cell membrane. P is instrumental in the storage of energy as adenosine triphosphate (ATP) molecules, Nicotinamide adenine dinucleotide phosphate (NADPH), etc. Inadequate supply of P could have negative effect on the growth of the culture (Berg et al., 2002). For this work, the effects of P

concentration on the growth of *Entomoneis* sp. were investigated. Figure 4.4 shows the results of P consumption by *Entomoneis* sp. Note that, in this Figure, 1xP means the case with typical P concentration (in F/2M), 2xP is the case where F/2M was prepared with P at double concentration and 3xP is when F/2M was prepared with P at 3 times original concentration. It was found that P in the medium was totally taken up at Day 8, whereas using media with 2x and 3x concentrations, P still remained in the medium. Figure 4.5 presents the results of the effects of P concentration on the growth of *Entomoneis* sp. The results indicated surprisingly that although P (1x) was depleted, *Entomoneis* sp. could still grow continually. This could be because when phosphorus in the medium was depleted, phosphorus accumulated in the cells was transformed, released and transferred to the new generation. Cells should therefore continue to grow but with the minimum energy storage mechanism (Nelson et al., 1979). The maximum cell concentrations obtained from the media with 1x, 2x and 3x of P were comparable at 32.75, 33.26 and 34.26 $x10^4$ cells mL^{-1}, respectively. For this reason, the nutrient used in the cultivation of this alga was still maintained at 1x P content.

4.2.4 Effect of nitrogen concentration

Nitrogen (N) is essential for the microbial growth and the availability of N is often one of the major factors influencing the growth and chemical composition of the algae as inadequate N could lead to a rapid reduction in photosynthetic performance. The following description is based on the cultivation in the F/2M medium. Figure 4.6 illustrates that the maximum cell concentration of $32.75x10^4$ cells mL^{-1} could be reached at Day 7 whereas N remained in the F/2M (3.09 mg L^{-1}). The results indicated that N seemed to remain adequately in the medium even after the experiment. Therefore it was concluded at this point that the nutrient used in the cultivation should still be maintained at original N content.

4.2.5 Analysis of nutrient requirements

4.2.5.1 Analysis of Si requirement

Analysis for the yield of cell with Si or $Y_{X/Si}$ is as follows:

$$Y_{X/Si} = \frac{\Delta X}{\Delta Si} \quad \text{..... (4.1)}$$

where X cell concentration (mg cell L^{-1})

Si Si concentration (mg Si L^{-1})

The culture with the F/2M medium with various feeding Si concentrations (based on the result in Section 4.2.2) gave the yields: as displayed in Table 4.4.

This reveals that cellular yield on Si was not constant but depends on Si concentration.

4.2.5.2 Analysis of Phosphorus requirement

Analysis for the yield of cell with phosphorus or $Y_{X/P}$ is as follows:

$$Y_{X/P} = \frac{\Delta X}{\Delta P} \quad \text{..... (4.2)}$$

where X cell concentration (g cell L^{-1})

P phosphorus concentration (g P L^{-1})

This yield is reported in Table 4.5, and the results, illustrated very clearly that $Y_{X/P}$ decreased significantly with P. This suggested that increasing P could adversely affect the growth. This suggested that increasing P could adversely affect the growth.

4.2.5.3 Analysis of Nitrogen requirement

The results from Section 4.2.4 indicate that 1x N content at original in F/2M medium was adequately maintained for the cultivation of this alga. Analysis for the yield of cell with N or $Y_{X/N}$ is as follows

$$Y_{X/N} = \frac{\Delta X}{\Delta N} \quad \text{..... (4.3)}$$

where X cell concentration (g cell L^{-1})

N nitrogen concentration (g N L^{-1})

and in this case, $Y_{X/N}$ = 56.7 g cell/g N.

4.3 Effect of aeration rate

The effect of aeration rate on the growth of *Entomoneis* sp. was investigated using 2.5 L bubble column photobioreactor at the light intensity of 81 μmol photons m^{-2} s^{-1}. The aeration rate (which was measured in terms of superficial gas velocity, u_{sg}) was varied in the range of 0.3 to 2 cm s^{-1}. The resulting best growth performances are compared in Figure 4.7 where Table 4.6 shows the results on the maximum cell concentration, specific growth rate and specific productivity. The maximum cell concentration (32.75×10^4 cell mL^{-1}) occurred at the aeration rate of 0.5 cm s^{-1} above and below which the growth rate dropped.

It was observed that aeration rate at low speed (u_{sg} < 0.5 cm s^{-1}) led to a small cell density and specific growth rate (18.47×10^4 cell mL^{-1} and 0.30 d^{-1}, respectively). This could be due to an inadequate level of circulation in the system. In bubble column systems, aeration induced mixing, liquid circulation and mass transfer between gas and liquid phases in the systems (Krichnavaruk and Pavasant, 2002) and therefore increasing aeration could result in a better mixing in the system (Xu et al., 2002) which then imposed positive effect on the growth. This was obvious as when u_{sg} increased to 0.5 cm s^{-1}, an increase in both cell concentration and specific growth rate to 32.75×10^4 cell mL^{-1} and 0.43 d^{-1} could be observed. However, increasing the aeration rate further to u_{sg} = 1 cm s^{-1}, although the growth rate seemed to be relatively high, the maximum cell concentration tended to decrease to 30.87×10^4 cell mL^{-1}. A further increase in u_{sg} to 2 cm s^{-1} induced quite a number of negative effects on growth, where the maximum cell concentration attainable went down to only 26.94×10^4 cell mL^{-1}. This might be due to the excessive shear force induced at high aeration. This indicated that the cell of *Entomoneis sp.* was highly shear sensitive and even the shear caused by aeration could deteriorate the growth. This explanation was supported by several past reports. For instance, Gudin and Chaumont (1991) stated that the key problem in the cultivation of microalgae in photobioreactors was cell damage due to shear stress. Hata et al. (2001) illustrated that the culture of the algae cell in exponential growth phase required a low liquid velocity due to its fragility.

In conclusion, *Entomoneis* did not seem to exhibit different growth pattern at aeration rates, u_{sg}, of 0.5 and 1 cm s^{-1} and therefore u_{sg} of 0.5 cm s^{-1} was, from economical point of view, selected for subsequent experiments.

4.4 Growth under different light conditions

4.4.1 Growth of *Entomoneis* sp. under fluorescent lamp

The effect of light intensities on the growth of *Entomoneis* sp. was investigated using 2.5 L bubble column photobioreactor at the aeration rate of 0.5 cm s^{-1}. The light source from fluorescent lamp was varied to give light intensities in the range of 81 to 270 μmol photons m^{-2} s^{-1}. The resulting best growth could be achieved performances as illustrated in Figure 4.8 where Table 4.7 illustrates that the maximum cell concentration and specific growth rate ($41.29x10^4$ cell mL^{-1}, 0.474 d^{-1} respectively) occurred at light intensity of 270 μmol photons m^{-2} s^{-1}. Note that the light intensity of 270 μmol photons m^{-2} s^{-1} was the upper limit that could be achieved in the existing laboratory.

As micro-algae photosynthesize, they assimilate inorganic carbon into organic matter. Light is the source of energy which drives this reaction and thus plays an important role for the growth of this cell. It seemed that an increase in light intensity from 81 to 135 and 270 μmol photons m^{-2} s^{-1} helped induce photosynthesis resulting in a continuous increase in maximum cell concentration, i.e. the maximum cell concentration at the three light intensities were $32.75x10^4$ at Day 7, $33.33x10^4$ at Day 6, and $41.29x10^4$ cell mL^{-1} at Day 6, respectively.

4.4.2 Growth of *Entomoneis* sp. under sunlight

Section 4.4.1 illustrates clearly that *Entomoneis sp.* grew better at high light intensity (270 μmol photons m^{-2} s^{-1}), and therefore it is interesting to see if the culture could withstand the outdoor, extremely high light intensity (e.g. more than 1,000 μmol photons m^{-2} s^{-1} at noon). The effect of light intensities on the growth of *Entomoneis* sp. under sunlight was investigated using 2.5 L bubble column

photobioreactor at the aeration rate of 0.5 cm s^{-1}. The resulting growth performances are compared in Figure 4.9.

Experimental results demonstrate that the cultivation under sunlight decreased the growth. The maximum cell concentration was small, equal to $27.5x10^4$ cell mL^{-1} on Day 8. This could be explained as the cultivation under Sunlight means that the culture was only exposed to light during the day time, and the light inadequacy during the night time could lower the cell growth. Indeed, in nature, the light regime is discontinuous and the intensity varies daily. Literature indicates that, depending on the species of microalgae, growth can be controlled by the photoperiod (Paasche, 1967, 1968; Foy et al., 1976; Loogman et al., 1980). However, the use continuous light was still reported to allow for an increase in the final concentration (Janssen et al., 2000; Meseck et al., 2005). Another possible reason is that the cultivation under sunlight could result in the culture being exposed to an extremely high light intensity (500-1,000 μmol photon m^{-2} s^{-1}) when compared to the condition in the indoor system (270 μmol photon m^{-2} s^{-1}). This adverse influence to the algal growth at high light intensity was called photo-inhibition which occurs due to the damage mechanism of the photosystem II (PS II) D1 protein where the over-saturation of light caused damages to the PS II D1 protein that carried the binding sites for the electron carrier (Sukenik et al., 1987; Davison., 1991; Vymazal., 1994; Grima et al., 1996). The temperature could also be an important parameter affecting the growth. The optimal temperature for the growth of cell microalga was also reported to be in the range of 25-30^oC (Converti et al., 2009), however, in this experiment, the cultivation under sunlight raised the temperature upto about 39-40^oC at noon, and could adversely affect the growth. Exact reasons on the effect of sunlight on *Entomoneis* sp. still need further verification. Nevertheless, the cultivation under sunlight could save energy and therefore the cooling system might be required to prevent the overshoot of temperature.

4.5 Cultivation in flat-plate airlift photobioreactor

4.5.1 Batch cultivation mode

In this section, the cultivation in the large scale flat-plate airlift photobioreactor was investigated using 17 L flat-plate airlift photobioreactor with A_d/A_r = 0.4 with an initial cell density of $1x10^4$ cells mL^{-1}. The cultivation was exposed to sunlight during the day time and fluorescent light during the night. The light intensity from fluorescent light was about 43 μmol photon m^{-2} s^{-1}. The aeration was supplied at u_{sg} of 0.5 cm s^{-1} and the temperature was controlled in an evaporative room at 27±2°C. The use of evaporative room was selected to save the energy during the cultivation and to ensure that the cultivation was performed in the condition close to the actual one in industrial scale. The resulting best growth performances in 17 flat-plate airlift and 2.5 L bubble column photobioreactor at the same batch mode condition are compared in Figure 4.10.

The cell concentration of *Entomoneis* sp. in 2.5 L bubble column was $28.8x10^4$ cell mL^{-1} on Day 7 with specific growth rate of 0.45 d^{-1}, whereas the cultivation in 17L flat plate airlift provided the cell concentration of $31.05x10^4$ cell mL^{-1} on Day 6 and the growth rate of 0.51 d^{-1}. Although airlift employed in this work had a larger size than the bubble column, the final cell concentration was higher and cell growth is higher, than the bubble column at smaller scale. This was because airlift system contains a draft tube or flat-plate which improves circulation and oxygen transfer and equalizes shear forces in the reactor. The liquid circulation facilitates the uplift of the cells which could form aggregation at the bottom of the column. This results in cells being well circulated in the system, and being better exposed to light and nutrient. It can then be concluded that the airlift outperformed bubble column for the cultivation of *Entomoneis* sp.

4.5.2 Semi-continuous cultivation mode

Semi-continuous cultivation was operated at best conditions as examined in Section 4.5.1, i.e. A_d/A_r = 0.4 under sunlight during the day time and fluorescent light during the night. The light intensity from fluorescent light was about 43 μmol photon

m^{-2} s^{-1}. The temperature was controlled in an evaporative room at 27±2^{o}C and the aeration was supplied at u_{sg} of 0.5 cm s^{-1}. The initial cell density was set at 10x10^{4} cells mL^{-1} until it reached the desired maximum cell concentration typically in 2-3 days. Once the desire concentration was reached, the culture was harvested by replacing the old culture with fresh culture. The harvesting volume was calculated such that the initial cell concentration became 10x10^{4} cells mL^{-1}. This harvesting cycle was repeated five times. Semi-continuous cultivation helped maintain the Si concentration and there was no need to periodically add Si as suggested for the batch culture. The harvest re-occurred at an approximately 3-day interval. The culture was then left to grow, and the next harvest was performed when the target cell concentration was reached again.

This section demonstrates that the cultivation of *Entomoneis* sp. could be achieved successfully in a semi-batch mode with the maximum cell concentration was 37.49x10^{4} cell mL^{-1} (average from five times of repeated harvesting cycle). The results on the growth from each cycle are shown in Figure 4.11.

4.6 Effect of CO_2 on growth of *Entomoneis* sp.

4.6.1 Cultivation in 2.5 L bubble column photobioreactor

The effect of CO_2 concentration on the growth of *Entomoneis* sp. was preliminary investigated in the 2.5 L bubble column photobioreactor as batch cultivation with the initial cell density of 1x10^{4} cells mL^{-1} under continuous fluorescent lighting at 270 μmol photon m^{-2} s^{-1}. The culture was aerated at u_{sg} of 0.5 cm s^{-1} with a mixture of air and CO_2 at 2% by volume. The CO_2 fixation was related directly to cell density as cells need carbon for their growth and the only source of carbon in this system was from CO_2. Hence, it was expected that the culture aerated with CO_2 which was enriched with more carbon source should exert positive effect to cell growth rate. Figure 4.12 indicates clearly that *Entomoneis sp.* grew better in the system with 2% CO_2. The maximum cell concentration of 52.27x10^{4} cells mL^{-1} and 0.50 d^{-1} in specific growth rate were relatively high when compared with the results from the cultivation without CO_2 (max. cell = 41.29x10^{4} cells mL^{-1}, sp. growth rate =

0.48 d^{-1}). This finding agrees well with literature where the addition of CO_2 at 2% could increase the maximum cell density on the growth (Chiu et al., 2008 and 2009).

4.6.2 Cultivation in 17 L flat-plate airlift photobioreactor

In this section, the effect of CO_2 concentration on the growth of *Entomoneis* sp. was further investigated in the large scale 17 L flat-plate airlift photobioreactor with A_d/A_r = 0.4, operating in a semi-continuous mode with an initial cell density of $10x10^4$ cells mL^{-1} under sunlight during the day time and fluorescent light during the night. The light intensity from fluorescent light was about 43 μmol photon m^{-2} s^{-1}. The temperature was controlled at 27±2°C, and the aeration was supplied at u_{sg} of 0.5 cm s^{-1}. The aeration was mixed with CO_2 at 1, 3, 5, and 7% by volume. The cultures were harvested at a 3-day interval and this was repeated 2 times. The results are illustrated in Figure 4.13 where the best growth occurred at 5% CO_2 with the maximum cell concentration and specific growth rate of $53.89x10^4$ cell mL^{-1} and 0.68 d^{-1}, respectively. The next best growth with cell concentration of $52x10^4$, $42.5x10^4$ and $39.92x10^4$ cell mL^{-1} occurred at CO_2 of 3%, 1%, and 7%, respectively.

The evaluation of CO_2 utilization by *Entomoneis* sp. is illustrated in Table 4.5. The results indicated that, for the 17 L flat-plate airlift photobioreactor, cells consumed about 8.3, 11.2, 11.5 and 8.3 g CO_2 in the culture aerated with 1, 3, 5 and 7% CO_2, respectively. These were equivalent to the CO_2 conversion of 95, 43.1, 26.6, and 13.6% (Note : the calculation of total CO_2 inlet, CO_2 ultilization and CO_2 conversion are provided in Appendix B). This reveals that, with the conditions used in this work, only very limited quantity of CO_2 could be utilized, and more CO_2 conversion was obtained at low CO_2 content in the air feed (at 1% CO_2, CO_2 conversion equals 95%). Based on the result from Table 4.8 could be concluded as follows,

1) at low CO_2 throughput, high conversion could be achieved but still not enough CO_2 was supplied,
2) at higher CO_2 throughput, higher CO_2 was being converted to cell but the CO_2 conversion was low,

3) at very high CO_2 throughput, pH could play a significant role in limiting the growth.

4.7 Economic evaluation on the cultivation of *Entomoneis* sp.

This section describes the economical analysis of the various systems for the cultivation of *Entomoneis* sp., i.e. 17 L flat plate airlift system in batch and semi-continuous modes and 2.5 L bubble column in batch mode. Table 4.9 indicates that the operation of the 17L flat plate airlift as semi-continuous mode was the most attractive for the cultivation of *Entomoneis* sp. The operating cost for the production of 1 kg (dry cell) was approximately 1,278 THB which was the lowest when compared to any other investigated systems. It should be noted that the cultivation of *Entomoneis* sp. in the 17 L flat plate airlift photobioreactor was conducted with the most suitable operating conditions obtained from Sections 4.5 whereas the 2.5L bubble column was performed with conditions stated in Sections 4.4.1, and this analysis did not include the installation cost for the equipments, and neither the reuse of the medium was considered.

Table 4.1 Influences of different culture media on the growth of *Entomoneis* sp.

Media	**Specific growth rate (d^{-1})**	**Maximum cell concentration x10^4 (cell mL^{-1})**	**Specific productivity x10^{-2} (g L^{-1} d^{-1})**
F/2	0.28	5.7	4.3
F/2M	0.30	11	5.9
WC	0.32	10.5	5.7
Chu's	0.25	3.9	4.2
Walne	0.20	6.6	8.3

Table 4.2 Cost comparison of F/2M and WC media

	F/2M		**WC**	
Nutrient	**Final conc. (mg/L)**	**Cost (Bath/ L)**	**Final Conc. (mg/ L)**	**Cost (Bath/ L)**
Na_2SiO_3. $9H_2O$	60	0.072	28.42	0.034
$NaNO_3$	75	0.05625	85.01	0.064
$NaH_2PO_4.H_2O$	10	0.0101	-	-
K_2HPO_4	-	-	8.71	0.007316
$CaCl_2.2\ H_2O$	-	-	36.76	0.022
MgSO4.$7H_2O$	-	-	36.97	0.024
H_3BO_3	-	-	1.0	0.001
Sum	-	0.1385	-	0.1523

Table 4.3 Silicon concentration and feeding schedule

Batch	**Si conc. (mg L^{-1})**	**Day (Si addition)**	**Amount of added Si* (mg L^{-1})**	**Total concentration of Si (mg L^{-1})**
B-0	Typical	0	13	13
B-1	4.8	0,3,4,5,6	11.02, 2.54, 2.6, 2.10, 1.50	22.36
B-2	6.7	0, 3, 4, 5	9.50, 4.47, 4.24, 3.80	22.01
B-3	13.7	0,3,5	11.52, 10.77, 10.60	32.89
B-4	30.60	0	30.60	30.60

*** This amount of Si is needed to replenish the consumed Si. By adding this Si, the concentration of Si will be back to the original level as indicated in the second column.**

Table 4.4 Cellular yield based on Si-component

Experiment (see in Table 4.2)	**Cell conc. (g L^{-1})**		**Si conc. (g Si L^{-1})**		$Y_{X/Si} = \frac{\Delta X}{\Delta Si}$
	Initial	Final	Initial	Final	
B-0	0.06	0.22	13	0.2	11.7
B-1	0.06	0.55	32.89	6.7	26.8
B-2	0.06	0.57	22.36	4	27
B-3	0.06	0.47	22.01	3.3	15.5
B-4	0.06	0.32	30.57	5	9.9

Table 4.5 Cellular yield based on P-component

phosphorus content	Cell conc. (g L^{-1})		Phosphorus conc. (g P L^{-1})		$Y_{X/P} = \frac{\Delta X}{\Delta P}$
	Initial	Final	Initial	Final	
1x	0.06	0.53	1.85	0.166	299.8
2x	0.06	0.57	3.85	0.644	160
3x	0.06	0.59	6.15	1.750	120.2

Table 4.6 Effect of aeration on the growth

u_{sg} (cm s^{-1})	Specific growth rate (d^{-1})	Max. cell conc. x10^4 (cell mL^{-1})	Max. cell mass (g L^{-1})	Specific Productivity x10^{-2} (g L^{-1} d^{-1})
0.3	0.4	18.47	0.37	5.6
0.5	0.43	32.75	0.57	7.8
1.0	0.42	30.87	0.55	7.5
2.0	0.42	26.94	0.49	7.6

Table 4.7 Effect of light intensity on the growth

Light intensity (µmol photons m^{-2} s^{-1})	Specific growth rate (d^{-1})	Max. cell conc. x10^4 (cell mL^{-1})	Max. cell mass (g L^{-1})	Specific Productivity x10^{-2} (g L^{-1} d^{-1})
81	0.43	32.75	0.57	7.8
135	0.46	33.33	0.58	8.6
270	0.48	41.29	0.70	10.7
Sunlight	0.40	27.5	0.50	7.1

Table 4.8 Analysis of CO_2 utilization by *Entomoneis* sp. in 17 L flat-plate airlift photobioreactor

CO_2 concentration (%)	**cell mass (g L^{-1})**	**pH**	**Total CO_2 inlet (g)**	**CO_2 utilization (g)**	**CO_2 conversion (%)**	**Max. cell concentration x10^4 (cell mL^{-1})**	**Specific growth rate (d^{-1})**
1	0.71	8.0	8.66	6.17	95	42.5	0.59
3	0.85	7.6	25.97	7.77	43.1	52	0.65
5	0.87	7.1	43.28	8.20	26.6	53.89	0.68
7	0.68	6.5	60.59	5.79	13.5	39.92	0.56

Table 4.9 Economical analysis for the cultivation of *Entomoneis* sp.

Feasibility	**17 L FP Airlift Semi- continuous**	**17 L FP Airlift Batch**	**2.5 L Bubble column**
Area (m^2)	0.12	0.12	0.015
Reactor Area (m^2)	0.04	0.04	0.005
Height (m)	0.5	0.5	0.6
Volume (m^3)	0.02	0.02	0.003
growth rate (d^{-1})	0.544	0.591	0.474
Max cell density (cell/mL^{-1})	$37.5x10^4$	$31.1x10^4$	$41.3x10^4$
Dry weight (g L^{-1})	0.645	0.548	0.7
Initial cell density (cell mL^{-1})	$10x10^5$	$1x10^4$	$1x10^4$
Harvesting period (d)	2.43	5.81	7.85
Harvesting volume (m^3)	$1.47x10^{-2}$	$1.94x10^{-2}$	$2.94x10^{-3}$
Productivity (g dry cell d^{-1})	9.45	10.6	2.06
Productivity (g dry cell $year^{-1}$)	$2.84x10^3$	$3.18 x10^3$	$6.18 x10^2$
Nutrient requirements for the cultivation at the target productivity			
Nutrient charge (B/kg)	**88.7**	**99.5**	**90**
Electricity requirements			
1. Lighting (kWh)	129.6	129.6	648
2. Fan (kWh)	333	333	0
3. Controlling system (kWh)	54	54	0
4. Air pump (kWh)	720	720	720
Total electricity requirements (kWh)	1236.6	1236.6	1701
Electricity charge (B/kWh)	3	3	3
Total Electricity Charge (B/kg)	**1170**	**1310**	**5265**
Water requirements			
1. Nutrient solution (L)	$1.55 x10^3$	$1.83x10^3$	$1.43x10^3$
2. Washing activity (L)	$4.56x10^2$	$5.7x10^2$	$9.52x10^2$
Total water consumption (L)	$2.01x10^3$	$2.36x10^3$	2.3810^3
Water charge (B/cum.)	10	10	10
Total Water Charge (B/kg)	**20**	**23**	**23.8**
Sea water charge	neglect	neglect	neglect
Harvesting method	neglect	neglect	neglect
Cost to yield cell 1 kg (B/kg)	**1278.7**	**1432.5**	**5378.8**

Note: This analysis focused only on the operating cost for the production of 1 kg of *Entomoneis* sp.

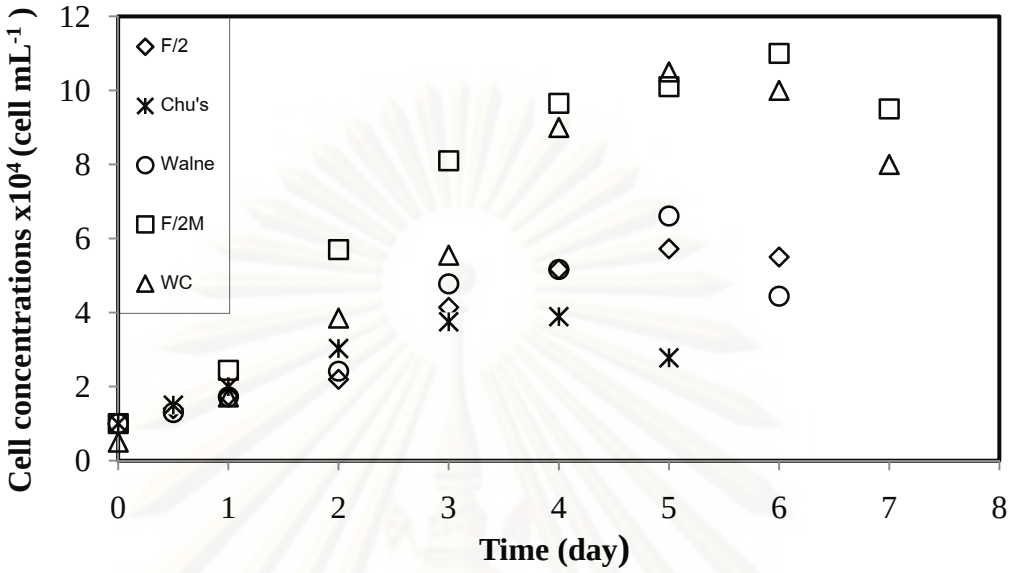


Figure 4.1 Influence of culture media on the growth of *Entomoneis* sp.: Culture conditions: Light intensity = 81 µmol photons m^{-2} s; Superficial aeration velocity (u_{sg}) = 0.5 cm s^{-1}

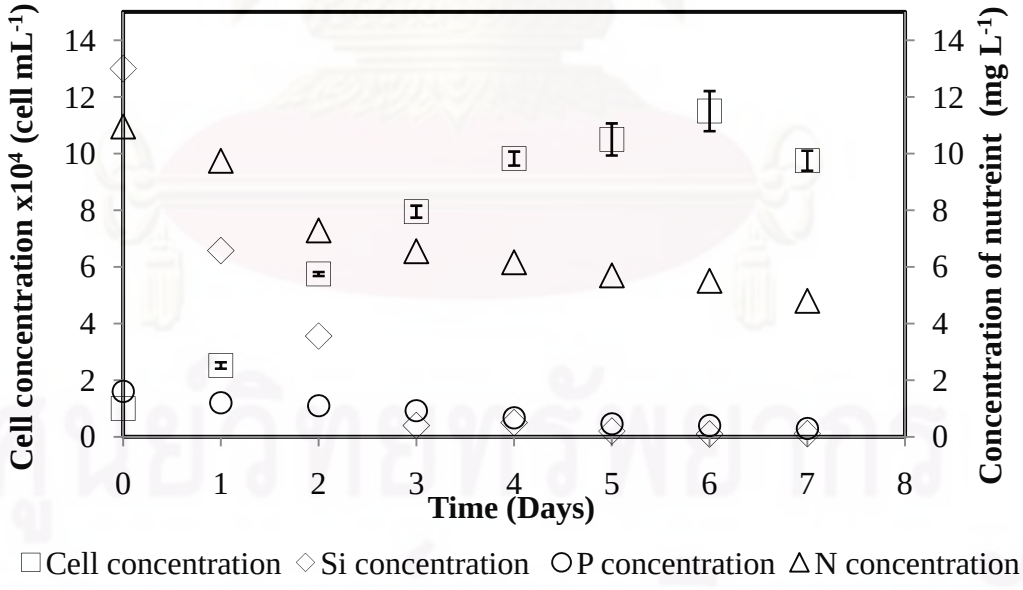


Figure 4.2 Growth behavior and nutrient concentration from cultivation of *Entomoneis* sp.

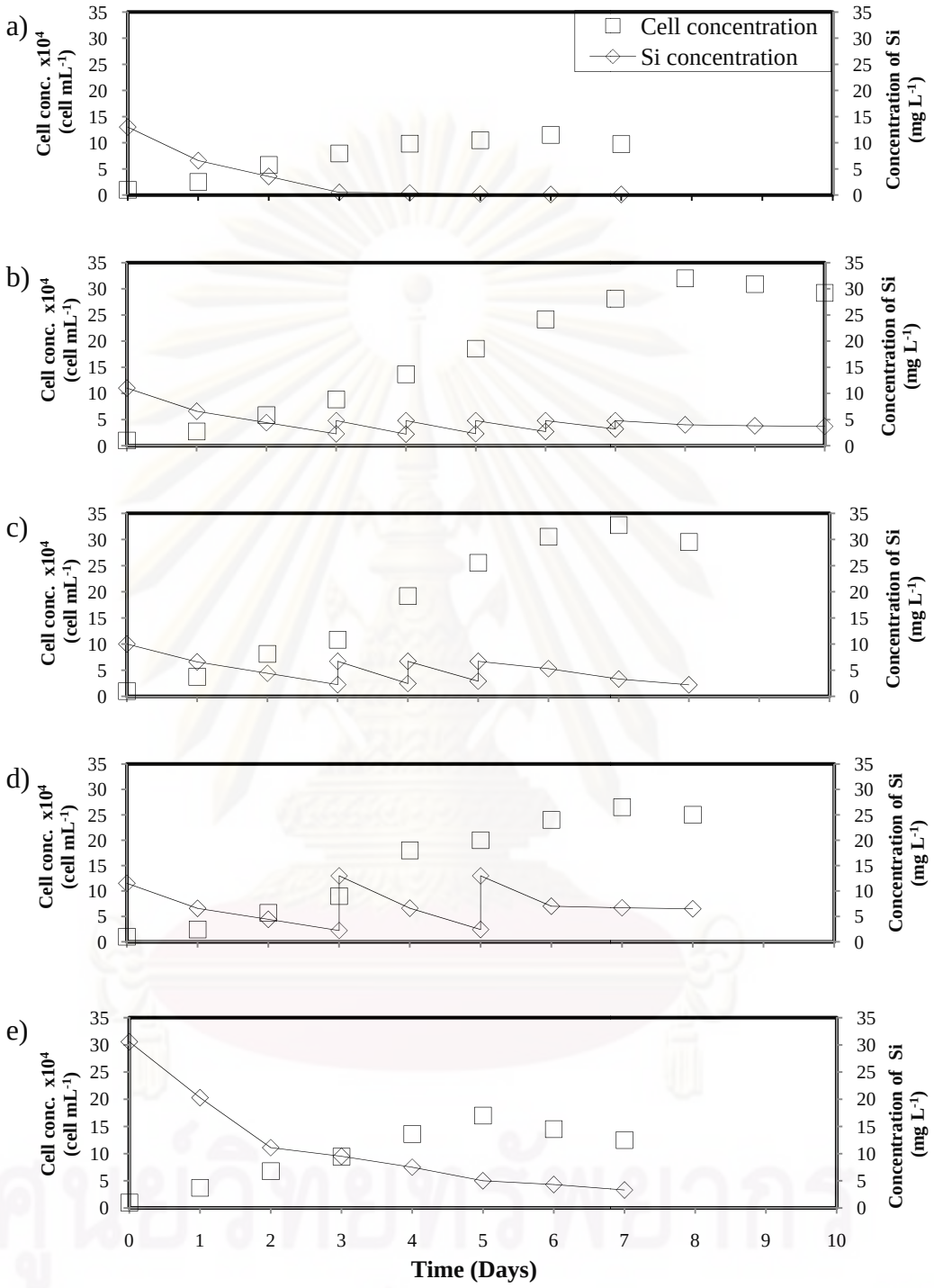


Figure 4.3 Effect of feeding Si into the medium at Day 3 with Si concentrations of: (a) typical value; (b) 4.8 mg L^{-1}; (c) 6.7 mg L^{-1}; and (d) 13.7 mg L^{-1} (e) Excess

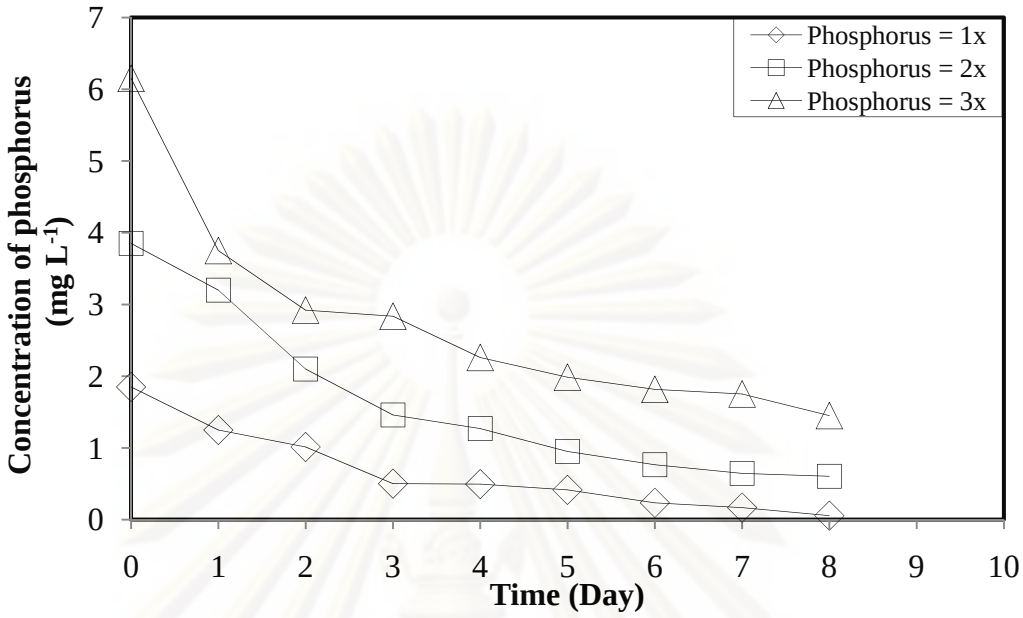


Figure 4.4 Effect of phosphorus concentration on the growth of *Entomoneis* sp.

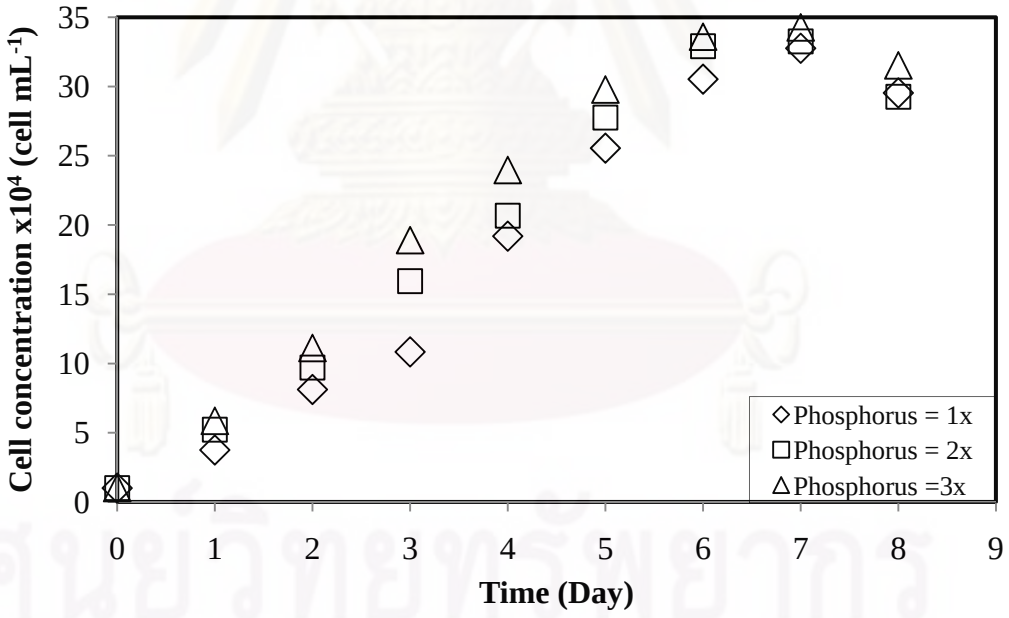


Figure 4.5 Effect of concentration of phosphorus on microalgal cell growth

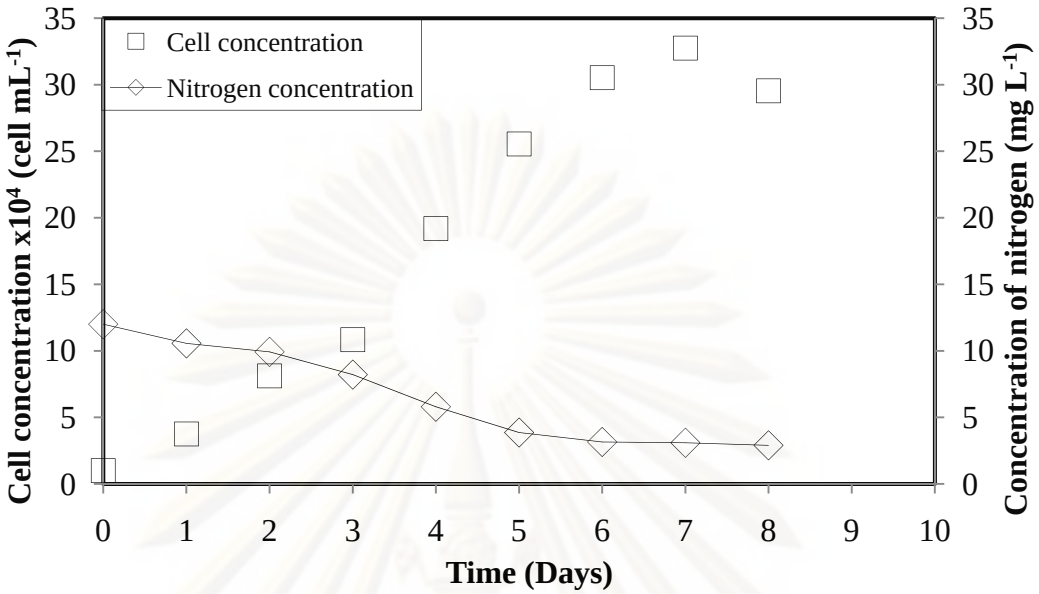


Figure 4.6 Effect of level of concentration of nitrogen on microalgal cell growth

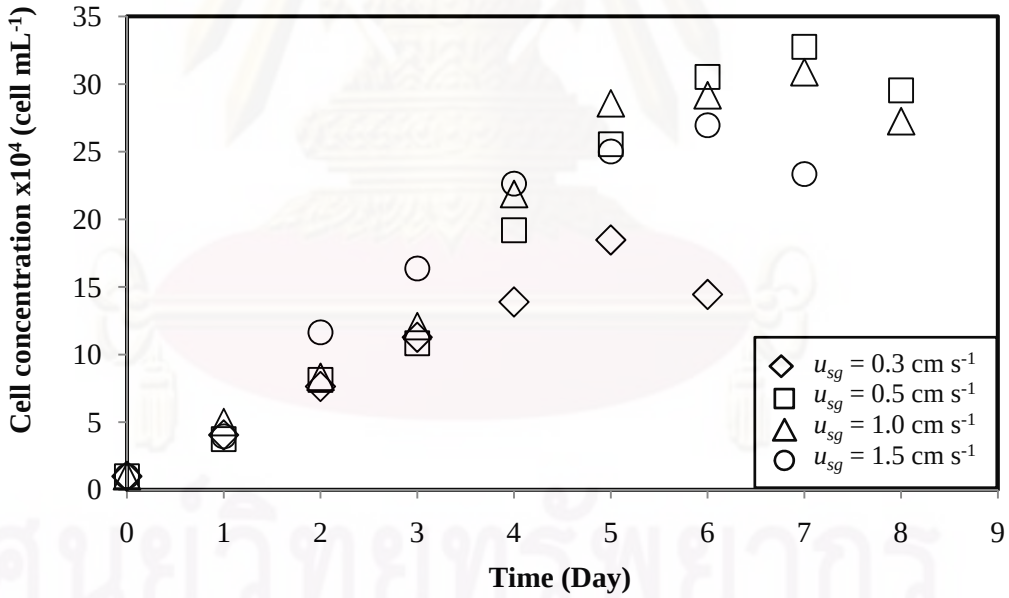


Figure 4.7 Effect of aeration rate on growth of *Entomoneis* sp.

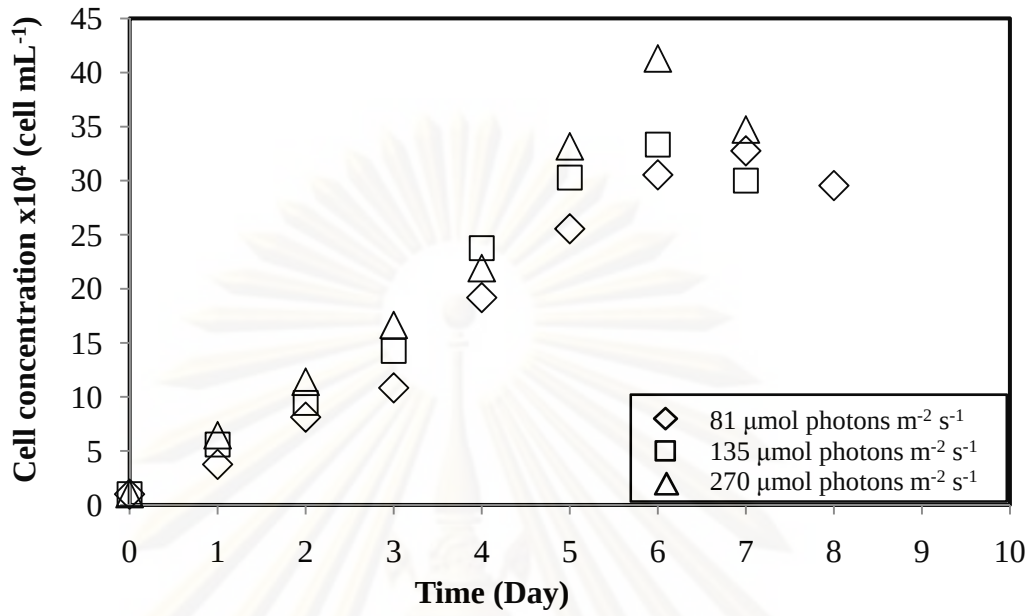


Figure 4.8 Effect of light intensities on growth of *Entomoneis* sp.

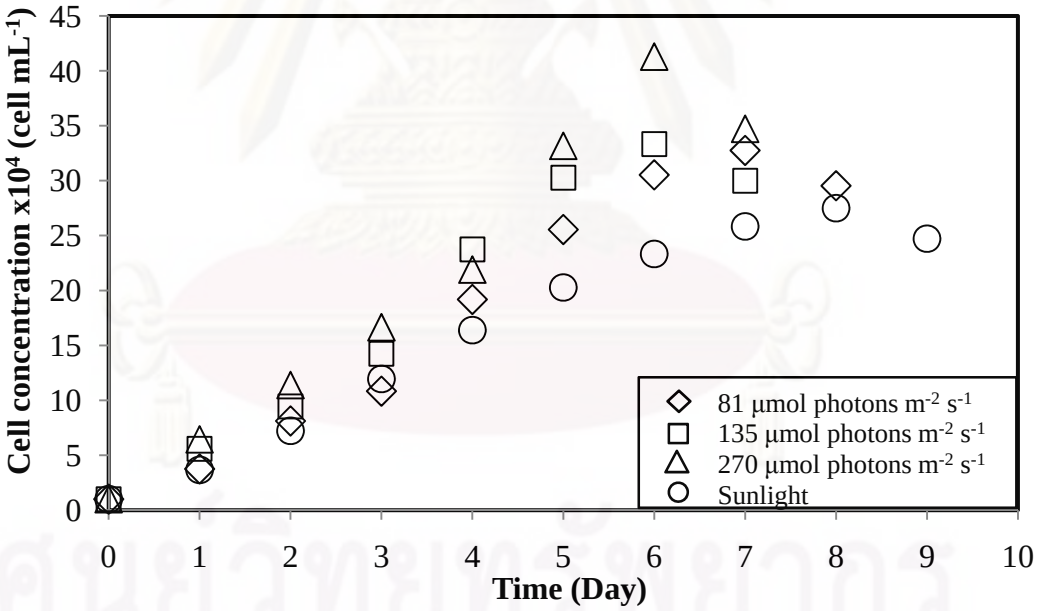


Figure 4.9 Comparisons on the growth of *Entomoneis* sp. under fluorescent lamp and sunlight

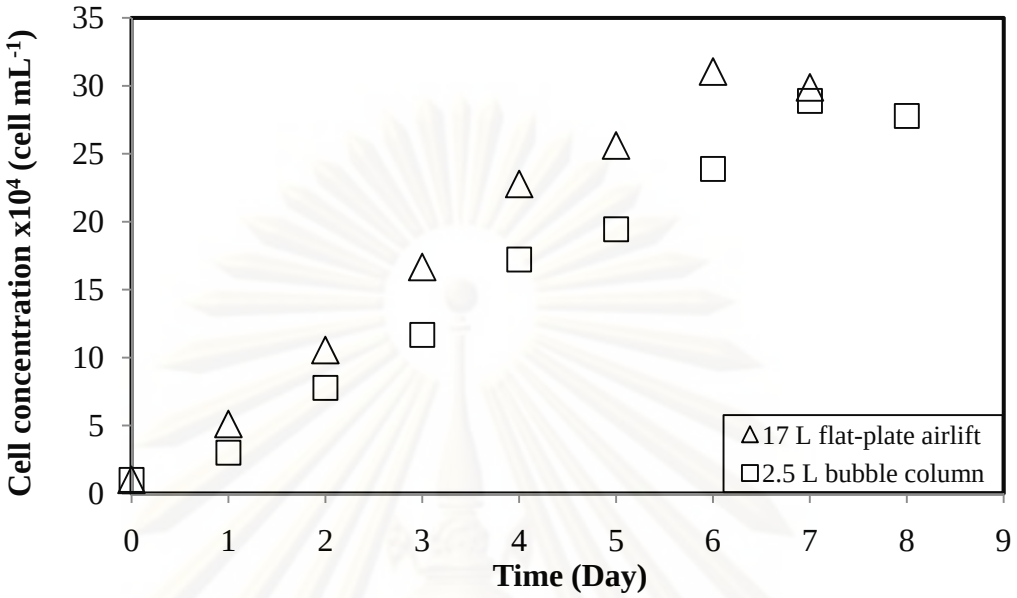


Figure 4.10 Growth of *Entomoneis* sp. in 17 L flat-plate airlift and 2.5 L bubble column photobioreactors

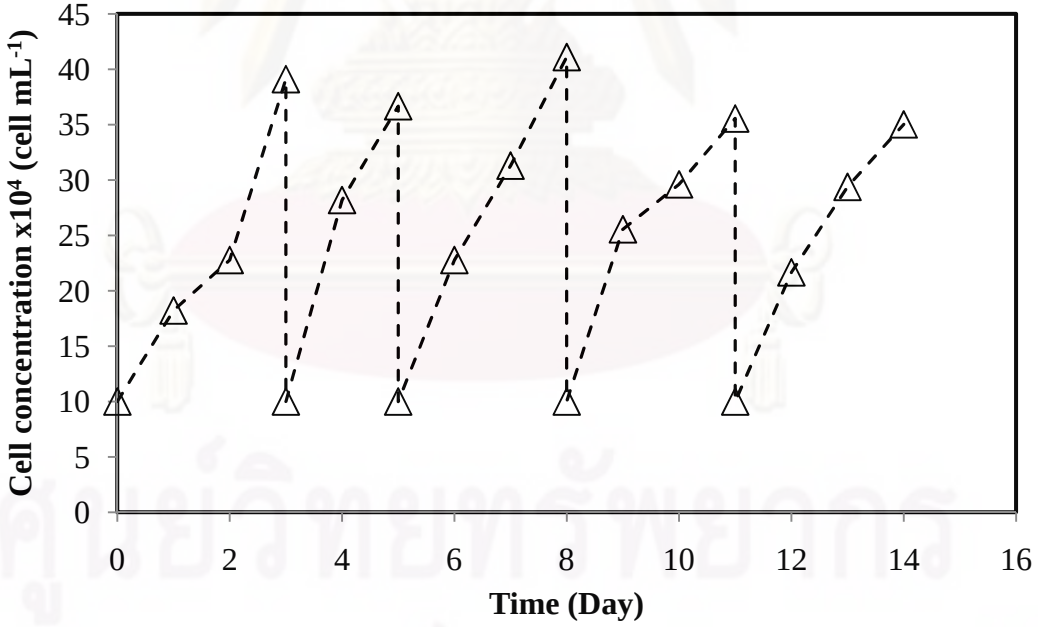


Figure 4.11 Growth of *Entomoneis* sp. in 17 L flat-plate airlift in semi-continuous mode

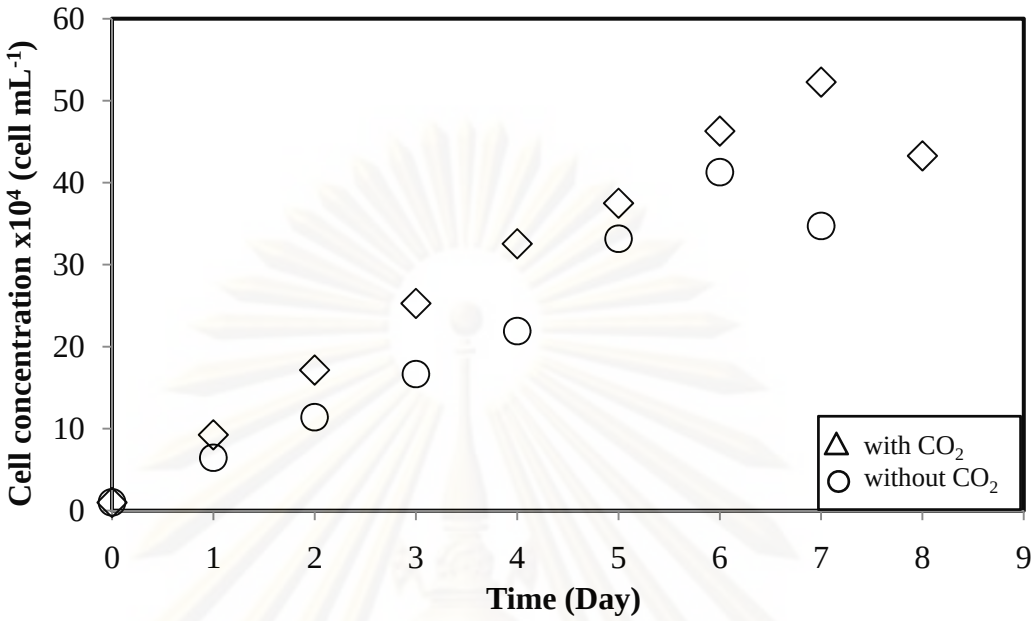


Figure 4.12 Effect of CO_2 on growth of *Entomoneis* sp. in 2.5 L bubble column photobioreactor

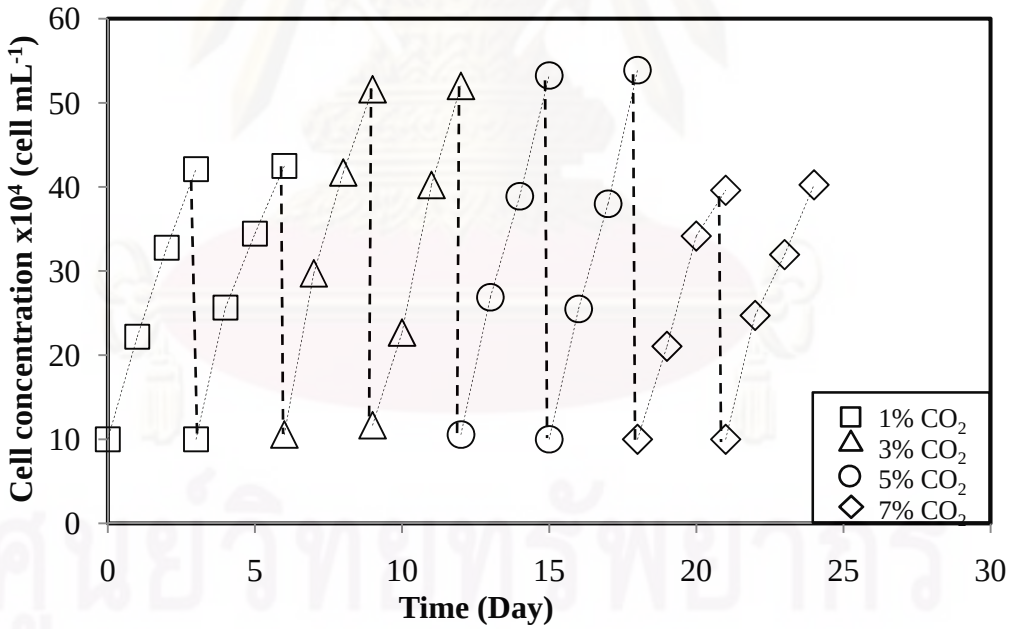


Figure 4.13 Effect of CO_2 on growth of *Entomoneis* sp. in 17 L flat-plate airlift photobioreactor

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Major findings from this work can be summarized as follows:

1. The optimal condition for cultivation of *Entomoneis* sp. was investigated in 2.5 L bubble column photobioreactor as batch mode. The results revealed the maximum cell concentration of 32.75×10^4 cell mL^{-1} and the specific growth rate of 0.429 d^{-1}. The appropriate nutrient concentrations, light intensities, aeration rate were identified and summarized below:

Parameter	Optimal condition
Nutrient concentration	Standard F/2 medium with adding silicon concentrations (fixed 6.7 mg L^{-1}) at Day 3,4 and 5
Light intensity	270 μmol photon $m^{-2}s^{-1}$
Aeration rate (in terms of superficial velocity)	0.5 cm s^{-1}

2. *Entomoneis* sp. cultured in a large scale 17 L flat plate airlift photobioreactor as batch mode with A_d/A_r = 0.4 was achieved with the maximum cell concentration of 31.05×10^4 cell mL^{-1} and the specific growth rate of 0.51 d^{-1}.
3. The semi-continuous culture in large scale 17 L flat plate airlift photobioreactor was successfully implemented which helped maintain the silicon concentration in the medium. In this system, there was no need to periodically add silicon as needed for the batch culture. The harvest at 3-day interval provided the maximum cell concentration of 37.49×10^4 cell mL^{-1} and the specific growth rate of 0.54 d^{-1}.
4. Preliminary investigation of the effect of CO_2 on the growth of *Entomoneis* sp. in bubble column demonstrated clearly that *Entomoneis sp.* grew better in the

system with 2% CO_2. The attainable maximum cell concentration of $52.27x10^4$ cells mL^{-1} and the specific growth rate of 0.502 d^{-1} were relatively high when compared with the results from the cultivation without CO_2 where the maximum cell density of $32.75x10^4$ cells mL^{-1} and specific growth rate of 0.429 d^{-1} could only be obtained.

5. The highest cell concentration was maintained with 5% CO_2 in 17 L flat plate airlift system where the maximum cell concentration of $53.89x10^4$ cell mL^{-1} and the specific growth rate of 0.68 d^{-1} could be achieved.
6. *Entomoneis* sp. could be produced at the cost of approx. $1.07x10^3$ THB/kg dry biomass

5.2 Contributions

The cultivation of *Entomoneis* sp. in this work is generally performed in bubble columns in batch mode. The result demonstrates that silicon was limiting factor of the growth of this alga, and a periodic addition of Si could, to some extent, overcome this limitation.

The result demonstrates also that the airlift system could be use effectively as a cultivation method for *Entomoneis*. This is because airlift systems help improve medium circulation and oxygen transfer resulting in cells being well circulated in the system, and being better exposed to light. As a result, the cell growth in flat-plate airlift grew better than that in the bubble column photobioreactor. Furthermore, the cultivation of *Entomoneis* sp. could be achieved successfully in a semi-continuous mode and this culture help maintain the silicon concentration and there was no need to periodically add silicon as suggested for the batch culture.

The cultivation of *Entomoneis* sp. with addition of CO_2 might further enhance the growth of the diatom. This indicates that microalgae can uptake CO_2 from the atmosphere and help cool down the global warming problem.

5.3 Recommendation

There are still other aspects of research that should be conducted to enhance the basic knowledge in the cultivation of *Entomoneis* sp. and also to complete the fundamentals on the photobioreactor design. These are:

1. The reuse of medium is strongly suggested to reduce the operating cost (nutrient cost)
2. The upscale of the flat plate airlift photobioreactor should be more closely looked at.
3. Utilization of natural sunlight should be given priority to minimize the electricity cost.
4. The use of CO_2 from the flue gas for the culture of *Entomoneis* should be considered. This will not only improve the biomass productivity, but will help lessen the climate change dilemma.

REFERENCES

Akulut, A. 2003. Planktonic Diatom (Bacillariophyceae) Flora of Sultan SazlÝÛÝ Marshes (Kayseri). Turk J Bot 27 : 285-301.

APHA. 1992. American Public Health Association. Standard methods for the examination of water and wastewater. Maryland : Victor Graphics.

Alvarez, S., Diaz, P., Lopez-Archillac, A.I., Guerrero, M.C. 2006. Phytoplankton composition and dynamics in three shallow temporary salt lakes (Monegros, Spain). Journal of Arid Environments 65 : 553–571.

Amin S. 2009. Review on biofuel oil and gas production processes from microalgae. Energy Conversion and Management.

Barasanti, L. and Gualtieri, P. 2006. Algae: Anatomy, Biochemistry, and Biotechnology. Tarlor & Francis Group, Boca Ration : FL.

Berg, J.M., Tymoczko, J.L., and Stryer, L. Biochemistry. New York : W.H. Freeman, 2002.

Bold, Harold C., Constantine J. Alexopoulos, and Theodore Delevoryas. 1987. Morphology of Plants and Fungi. Harper and Row Publishers : New York.

Brown, J. S. 1980. Absorption and fluorescence spectra of chlorophyll-proteins isolated from *Euglena gracilis*. Biochimica et Biophysica Acta. BBA - Bioenergetics 591 : 9-21.

Camacho Rubio, F., Acien Fernandez, F.G., Sanchez Perez, J.A., Garcia Camacho, F., Molina Grima, E. 1999. Prediction of dissolved oxygen and carbon dioxide concentration profiles in tubular photobioreactors for microalgal culture. Biotechnology and Bioengineering 62 : 71–86.

Chisti, Y. 2007. Biodeisel from microalgae. Biotechnology Advances 25 : 294-306.

Chiu S.Y., Kao C.Y., Tsai M.T., Ong S.C., Chen C.H., Lin C.S. 2009. Lipid accumulation and CO_2 utilization of *Nannochloropsis oculata* in response to CO_2 aeration. Bioresource Technology 100 : 833–838.

Converti, A., Lodi, A., Borghi, A. D., and Solisio, C. 2006. Cultivation of *Spirulina platensis* in a combined airlift-tubular reactor system. Biochemical Engineering Journal 32 : 13-18.

Davison, I.R. 1991. Minireview. In: Environmental effects on algal photosynthesis. Journal of Phycology 27 : 2–8.

Degen, J., Uebele, A., Retze, A., Schmidt-Staigar, U., Trosch, W.A. 2001. A novel airlift photobioreactor with baffles fro improved light utilization through flashing light effect. Journal of Biotechnology 92 : 89–94.

Douskova, I., Doucha, J., Livansky, K., Machat, J., Novak, P., Umysova, D., Zachleder, V., Vitova, M. 2009. Simultaneous flue gas bioremediation and reduction of microalgal biomass production cost. Appl. Microbiol Biotechnol 82 : 179-185.

Fernando, R., Silva-Aciares, Riquelme C. E. 2008. Comparisons of the growth of six diatom species between two configurations of photobioreactors. Aquacultural Engineering 38 : 26–35.

Gudin, C., Chaumont, D. 1991. Cell fragility-The key problem of microalgae mass production in closed photobioreactor. Bioresource Technology 38:145-151.

Glover, H. E., Keller, M. D. and Spinrad, R. W. 1987. The effects of light quality and intensity on photosynthesis and growth of marine eukaryotic and prokaryotic phytoplankton clones. Journal of Experimental Marine Biology and Ecology 105 : 137-159.

Grima, E. M., Sanchez Perez A. S., Camacho, F. G., Garcia Sanchez, J. L., Acien Fernandez F. G., and Alonso, D. L. 1996. Productivity analysis of outdoor chemostat culture in tubular air-lift photobioreactors. Journal of Applied Phycology 8 : 369-380.

Guillard, R. L. 1975. Culture of Phytoplankton for Feeding Marine Invertebrates. In W. L. Smith, M. H. Chanley (eds.), Culture of Marine Invertebrates Animal, pp. 15-41. New York : Plenum Press (For general algae culture techniques).

Harker, M., Tsavalos, A.J., Young, A.J., 1996. Autotrophic growth and carotenoid production of *Haematococcus pluvialis* in a 30 liter airlift photobioreactor. Journal of Fermentation and Bioengineering 82 : 113–118.

Hase, R., Oikawa, H., Sasao, C., Morita, M. and Watanabe, Y. 2000. Photosynthetic production of microalgal biomass in a raceway system under greenhouse conditions in Sendai city. Journal of Bioscience and Bioengineering 89 : 157-163.

Hata, N., Ogbonna, J.C., Hasegawa, Y., Taroda, H., Tanaka. 2001. Production of astaxanthin by *Haematococcus pluvialis* in a sequential heterotrophic photoautotrophic culture. Journal of Applied Phycology 13 : 395-402.

Hong, H. C., Mazumder, A., Wong, M. H., Liang, Y.2008. Yield of trihalomethanes and haloacetic acids upon chlorinating algal cells, and its prediction via algal cellular biochemical composition. Water research : 1 – 8.

Hsieh, C. H., Wu, W. T. 2009. Cultivation of microalgae for oil production with a cultivation strategy of urea limitation. Bioresource Technology.

Hu, Q., Guterman, H., Richmond, A., 1996. A flat inclined modular photobioreactor for outdoor mass cultivation of phototrophs. Biotechnology and Bioengineering : 51, 51–60.

Jeffrey, S. W. and Vesk, M. 1977. Effect of blue-green light on photosynthetic pigments and chloroplast structure in the marine diatom *Stephanopyxis turris*. Journal of Phycology 13 : 271-279.

Kaewpintong, K., Shotipruk, A., Powtongsook, S. and Pavasant, P. 2007. Photoautotrophic high-density cultivation of vegetative cells of *Haematococcus pluvialis* in airlift bioreactor. Bioresource Technology 98 : 288-295.

Knuckey, R. M., Brown, M. R., Barrett, S. M., Hallegraeff, G. M. 2002. Isolation of new nanoplanktonic diatom strains and their evaluation as diets for juvenile Pacific oysters (Crassostrea gigas). Aquaculture 211 : 253–274.

Krichnavaruk, S., Loataweesup, W., Powtongsook, S. and Pavasant, P. 2005. Optimal Growth Conditions and the Cultivation of *Chaetoceros calcitrans* in Airlift Photobioreactor. Chemical Engineering Journal 105 : 91-98.

Krichnavaruk, S., Powtongsook, S. and Pavasant, P. 2007. Enhanced Productivity of *Chaetoceros calcitrans* in Airlift Photobioreactors. Bioresource Technology 98 : 2123-2130.

Laing, I. 1991. Cultivation of marine unicellular algae. MAFF Laboratory Leaflet Number, 67. Directorate of Fisheries Research Lowestoft : UK.

Lavens, P., and Sorgeloos, P. 1996. Manual on the production and use of live food for aquaculture: Micro-algae. Rome.

Lesley, J., and Benjamin E. 1997. Role of silicon in diatom metabolism. Journal of Hives of Microbiology 112 : 147-152.

Lunning, K. Light. In J. Vymazal (ed.). 1981. Algae and Element Cycling in Wetlands, pp. 81-95. Boca Raton, Florida : CRC Press.

Mann, J.E. and Myers, J. 1986. On pigments, growth and photosynthesis of *Phaeodactylum tricornutum*. Journal of Phycology 4 : 349–355.

Matsudo M. C., Bezerra, R. P., Sato, S., Perego, P., Converti, A., Carvalho, J. C. M. 2008. Repeated fed-batch cultivation of *Arthrospira* (*Spirulina*) *platensis* using urea as nitrogen source. Biochemical Engineering Journal.

Mirón, A. S., Garc´ıa, M. C. C., Camacho, F. G., Grima, E. M. and Chisti, Y. 2002. Growth and biochemical characterization of microalgal biomass produced in bubble column and airlift photobioreactors: studies in fed-batch culture. Enzyme and Microbial Technology 31 : 1015-1023.

Nelson, D. M. and Brand, L. E. Bacillariophyta.1979. Chap. 12. In T. Berner (Ed.), Ultrastructure of microalgae, Boca Raton, Florida: CRC Press.

Ogbonna, J.C., Ichige, E., Tanaka, H. 2002. Interactions between photoautotrophic and heterotrophic metabolism in photoheterotrophic cultures of Euglena gracilis. Appl. Microbiol Biotechnol 58 : 532–538.

Oncel, S., Sukan F. V. 2008. Comparison of two different pneumatically mixed column photobioreactors for the cultivation of *Artrospira platensi*s (*Spirulina platensis*). Bioresource Technology 99 : 4755–4760.

Powell, E. E., Mapiour, M. L., Evitts, R. W., Hill, G. A. 2009. Growth kinetics of *Chlorella vulgaris* and its use as a cathodic half cell. Bioresource Technology 100 : 269–274.

Ranjbar, R., Inoue, R., Shiraishi, H., Katsuda, T., Katoh, S. 2008. High efficiency production of astaxanthin by autotrophic cultivation of *Haematococcus pluvialis* in a bubble column photobioreactor. Biochemical Engineering Journal 39 : 575–580.

Rebolloso Fuentes, M. M., Garcia Sanchez, J. L., Fernandez Sevilla, J. M., Acien Fernandez, F. G., Sanchez Perez, J. A., and Grima, E. M. 1999. Outdoor continuous culture of *Porphyridium cruentum* in a tubular photobioreactor: quantitative analysis of the daily cyclic variation of culture parameters. Journal of Biotechnology 70 : 271-288.

Richmond, A., and Cheng-Wu, Z. 2001.Optimization of a flat plate glass reactor for mass production of *Nannochloropsis* sp. outdoors. Journal of Biotechnology 85 : 259–269.

Cohen, Z. 1986. Products from Microalgae. In Richmond, A. (Ed.), CRC Handbook of Microalgal Mass Culture, pp. 421-454, Boca Raton, Fla. (USA) : CRC Press.

Sa´nchez, J.F., Ferna´ndez, J.M., Acie´n F.G., Rueda A., Pe´rez-Parra J, Molina E. 2008. Influence of culture conditions on the productivity and lutein content of the new strain *Scenedesmus almeriensis*. Process Biochemistry 43: 398–405.

Smith, L.L., Fox, J.M., and Granvil, D.R. 1993. Intensive algae culture techniques. In McVey J.P. (Ed.), CRC Handbook of mariculture: Crustacean Aquaculture, 2nd ed, pp. 3-13. Boca Raton, Florida: CRC Press.

Strickland, J. D. and Parson, T. R. 1972. A Practical Handbook of Water Analysis, 2nd edition. Otawa: Fishery Research Board of Canada.

Sriouam, P. 2007. Debottlenecking of the Airlift Cultivation Process for *Chaetoceros calcitrans*. Thesis, Chulalongkorn University, Thailand,

Sukenik, A., Wyman, KD., Bennett, J., Falkowski, P.G. A novel mechanism for regulating the excitation of photosystem II in a green alga. Nature Journal 327 (1987) : 704-707.

Ugwu, C.U., Ogbonna, J.C., Tanaka, H., 2002. Improvement of mass transfer characteristics and productivities of inclined tubular photobioreactors by installation of internal static mixers. Appl. Microbiol Biotechnol 58 : 600–607.

Vymazal, J. 1994. Algae and Element Cycling in Wetlands. Lewis Press : Chelsea.

Xu, Z., Dapeng, L., Yiping, Z., Xiaoyan, Z., Zhaoling, C., Wei, C. and Fan, O. 2002. Comparison of photobioreactors for cultivation of *Undaria pinnatifida* gametophytes. Biotechnology Letters 24 : 1499-1503.

Yim, J. H., Kim, S. J., Ahn, S. H. and Lee, H. K. 2003. Optimal conditions for the production of sulfated polysaccharide by marine microalga *Gyrodinium impudicum* strain KG03. Biomolecular Engineering 20 : 273-28.

APPENDICES

Appendix A

Nutrient Analysis

Appendix A-1: Determination of reactive silicate

(modified from Strickland and Parson, 1972)

Method

A. Calibration

Standard silicate from Concentration of Na_2SiF_6 stock = 14.935 mg Si/ L

(Na_2SiF_6 stock: Dissolved 100 mg of KNO_3 in 1000 ml of sea water)

A: Dilute 500 fold of Na_2SiF_6 stock, Concentration = 0.02987 mg Si/ L

B: Dilute 250 fold of Na_2SiF_6 stock, Concentration = 0.05974 mg Si/ L

C: Dilute 100 fold of Na_2SiF_6 stock, Concentration = 0.14935 mg Si/ L

D: Dilute 50 fold of Na_2SiF_6 stock, Concentration = 0.2987 mg Si/ L

E: Dilute 10 fold of Na_2SiF_6 stock, Concentration = 1.4935 mg Si/ L

F: Dilute 5 fold of Na_2SiF_6 stock, Concentration = 2.987 mg Si/ L

B. Reagents

1. Stock molybdate solution: Dissolve 4 g of $(NH_4)_6Mo_7O_{24}.4H_2O$ and add 12 ml of HCl 10% in 300 ml of DI water. Store the solution in a plastic bottle where it is stable indefinitely.
2. Metol-Sulphite solution: Dissolve 6 g of Na_2SO_3 and add 10 g of Metol (P-methylaminophenol Sulphate) in 500 ml of DI water. Filter the solution with filter paper No.1. Store in a glass bottle tightly stopped by a rubber bung. The solution is stable for many months if no air is allowed to enter the bottle unnecessarily.
3. Oxalic acid solution: Dissolve 50 g of Oxalic acid dihydrate $(COOH)_2.2H_2O$ in 500 ml of DI water. Store in a glass bottle tightly stopped by a rubber bung.
4. Sulphuric acid solution: Dissolve 250 ml of 50 % v/v of Sulphuric acid in 500 ml of DI water.

5. Reducing agent: Mixed 10 ml of Metol with 60 ml of Oxalic acid and slowly add 60 ml of 50 % v/v of Sulphuric acid in the 300 ml of DI water. Prepare afresh each day.

C. Blank

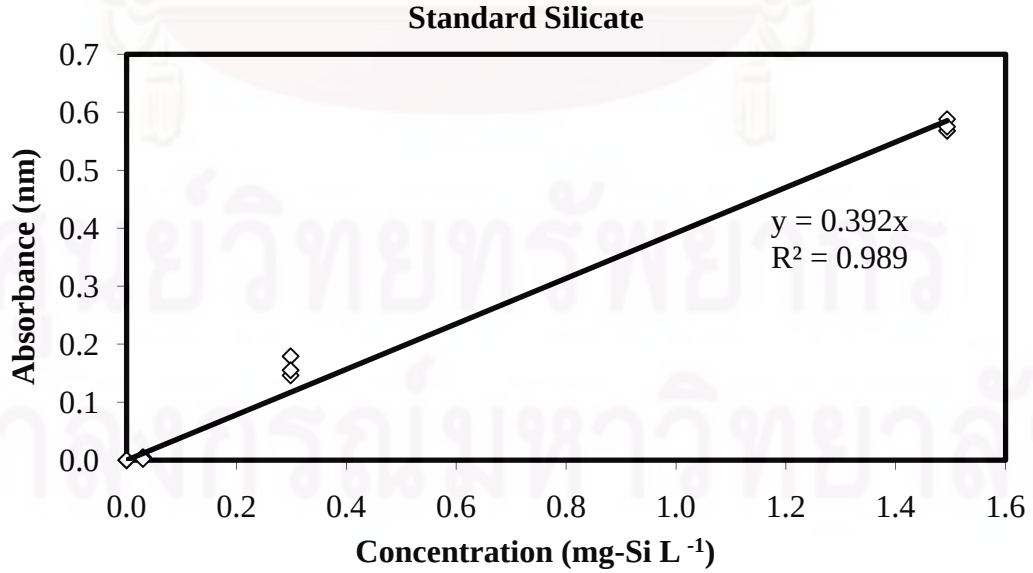
1. Add 0.5 ml of DI water to 0.2 ml of molybdate solution keep for 10 min.
2. Add 0.3 ml of reducing agent, allow 2- 3 hr for reaction time.
3. Measure the solution by Spectrophotometer at wavelength of 810 nm (set blank equal zero).

D. Procedure

1. Add 0.5 ml of Sample to 0.2 ml of molybdate solution mix for 10 min
2. Add 0.3 ml of reducing agent, allow 2- 3 hr for reaction time.
3. Measure the solution by Spectrophotometer at wavelength of 810 nm

E. Calculation

Calculate the concentration of silicon (mg Si L^{-1}) with standard curve as shown in Figure A-1



Appendix A-2: Determination of nitrate

(APHA, 1998)

Screening method

A. Calibration

Standard Nitrate from Concentration of KNO_3 stock = 100 mg NO_3-N/ L (KNO_3 stock: Dissolved 0.7128 g of KNO_3 in distilled water, Diluted the solution to 1000 ml with distilled water. Store the stock in a dark glass bottle and keep with 1 ml of Chloroform)

A: Dilute 200 fold of KNO_3 stock, Concentration = 0.5 mg NO_3-N/ L
B: Dilute 125 fold of KNO_3 stock, Concentration = 0.8 mg NO_3-N/ L
C: Dilute 100 fold of KNO_3 stock, Concentration = 1.0 mg NO_3-N/ L
D: Dilute 50 fold of KNO_3 stock, Concentration = 2.0 mg NO_3-N/ L
E: Dilute 40 fold of KNO_3 stock, Concentration = 2.5 mg NO_3-N/ L
F: Dilute 30 fold of KNO_3 stock, Concentration = 3.0 mg NO_3-N/ L
G: Dilute 25 fold of KNO_3 stock, Concentration = 4.0 mg NO_3-N/ L
H: Dilute 20 fold of KNO_3 stock, Concentration = 5.0 mg NO_3-N/ L
I: Dilute 15 fold of KNO_3 stock, Concentration = 8.0 mg NO_3-N/ L
J: Dilute 10 fold of KNO_3 stock, Concentration = 10 mg NO_3-N/ L

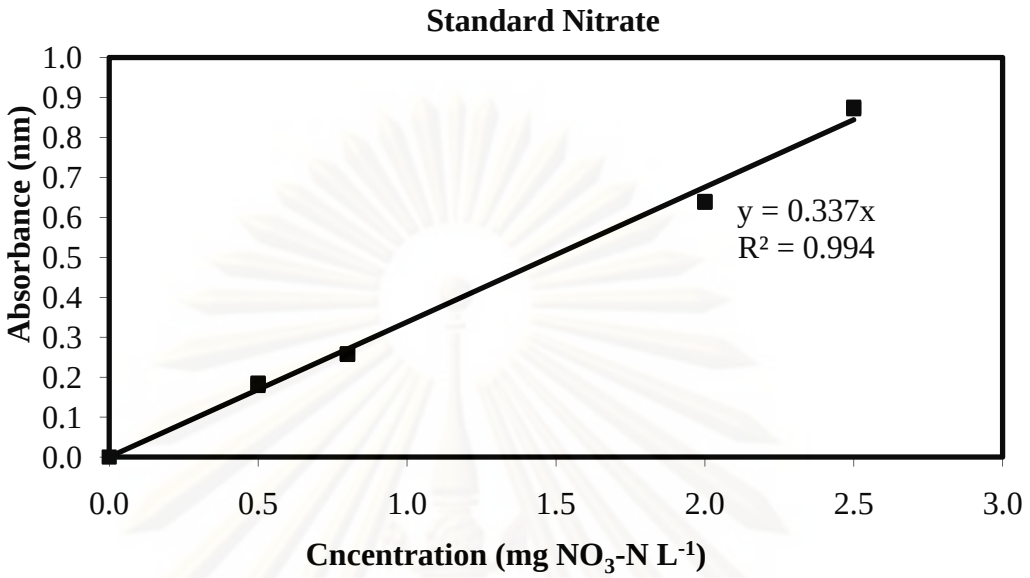
B. Blank: 1 ml of distilled water, Measure by Spectrophotometer at wavelength of 220 and 275 nm. Set blank equal zero

C. Procedure

Measure the sample by Spectrophotometer at a wavelength of 220 nm to obtain NO_3^- reading and a wavelength of 275 nm to determine interference due to dissolved organic matter.

D. Calculation

Calculate the concentration of nitrate (mg NO_3-N L^{-1}) with standard curve as shown in Figure A-2



Appendix A-3: Determination of reactive phosphate

(modified from Strickland and Parson, 1972)

Method

A. Calibration

Standard Nitrate from Concentration of KH_2PO_4 stock = 186 mg PO_4-P/ L (KH_2PO_4 stock: Dissolved 0.816 g of KH_2PO_4 in 1000 ml of distilled water. Store the stock in a dark glass bottle and keep with 1 ml of Chloroform)

A: Concentration = 0.0186 mg PO_4-P/ L

B: Concentration = 0.093 mg PO_4-P/ L

C: Concentration = 0.186 mg PO_4-P/ L

D: Concentration = 0.372 mg PO_4-P/ L

B. Reagents

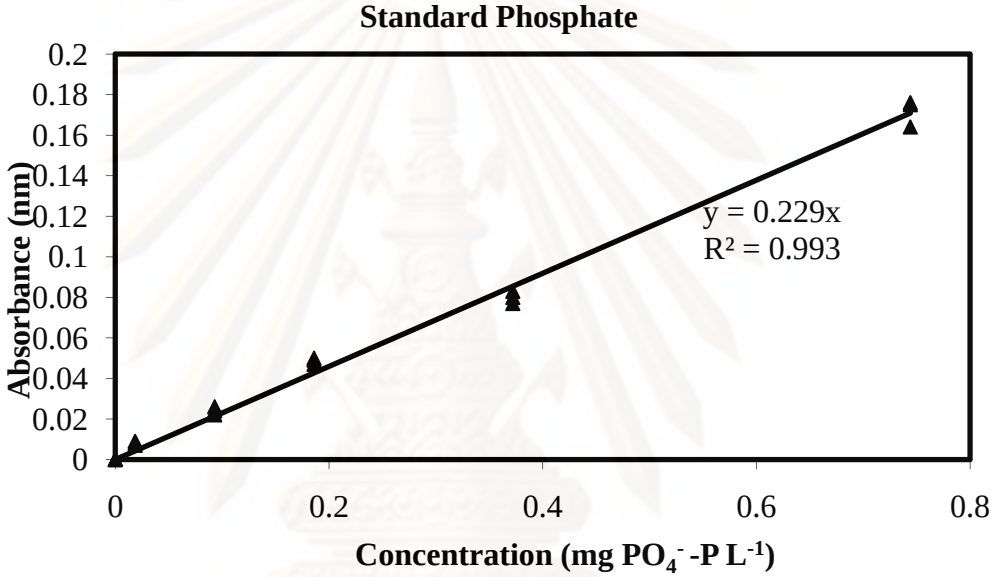
1. Stock Ammonium molybdate solution: Dissolve 15 g of $(NH_4)_6Mo_7O_{24}.4H_2O$ in 500 ml of distilled water. Store the solution in a plastic bottle where it is stable indefinitely.
2. Sulfuric acid solution: Dissolve 15 ml of Sulfuric acid in 500 ml of distilled water. Store the solution in a glass bottle and keep in refrigerator.
3. Ascorbic acid solution: Dissolved 27 g of ascorbic acid in 900 ml of distilled water. Store in a glass or plastic bottle and keep in refrigerator.
4. Potassium antimonyle tartrate solution: Dissolved 0.34 g of $KNaC_4H_4C_6.4H_2O$ in 250 ml of distilled water. Store in a glass or plastic bottle and keep in refrigerator.
6. Mixed Reagent: Mix 2 ml of Ammonium molybdate solution, 5 ml of Sulfuric acid solution, 2 ml of Ascorbic acid solution and 1ml of Potassium antimonyle tartrate solution. Prepare afresh each day.

C. Blank: Add 0.1 ml of mixed reagent in 1 ml distilled water, allow 10 min for reaction time. Measure by Spectrophotometer at wavelength of 885 nm. Set blank equal zero

D. Procedure: Add 0.1 ml of mixed reagent in 1 ml sample, allow 10 min for reaction time. Measure by Spectrophotometer at wavelength of 885 nm.

E. Calculation

Calculate the concentration of phosphorus (mg PO^{-}_{4}-P L^{-1}) with standard curve as shown in Figure A-3



Appendix B

The calculation of conversion of CO_2 by *Entomoneis* sp.

Example case: Cultivation of *Entomoneis* sp. with air (3% CO_2)

Mass of CO_2 feed into reactor

Conditions: Aeration rate (Q) = 0.005 m s^{-1}

Area of riser (A_r) = 0.00034 m^2

Time to input air to reactor (t) = 259,200 s

Composition of CO_2in air (CO_2 conc.) = 3% = 0. 03 %v/v

Molecular weight of CO_2 (MW CO_2) = 44 g mol^{-1} CO_2

The amount of CO_2 in the input air can be calculated from:

$$CO_2 = Q \times area\ riser \times CO_2\ conc. \times t \times MW\ CO_2 \times \frac{1\ mol}{22.4\ L}$$

$$CO_2 = 0.005\ \frac{m}{s} \times 0.00034\ m^2 \times 0.03 \times 259{,}200\ s \times 44\ g\ CO_2 \times \frac{1\ mol}{22.4\ L} \times 1000\ \frac{L}{m^3}$$

$$= 25.96\ gCO_2$$

CO_2 uptake to cell mass

Conditions: Cell initial (X_0) = 100,000 cell mL^{-1}

Cell maximum (X_m) = 520,000 cell mL^{-1}

Cell density ($X_{density}$) = 1.64x10^{-9} g $cell^{-1}$

Volume of reactor (V) = 17 L

Ratio of molecule of cell ($CH_4N_{0.14}P_{0.15}Si_{0.3}O_{0.9}$) per carbon (C) (ratio) = 0.26

Molecular weight of CO_2 (MW CO_2) = 44 g mol^{-1} CO_2

Molecular weight of C (MW C) = 12 g mol^{-1} C

The amount of CO_2 uptake can be calculated from:

$$CO_2 = \frac{(X_m - X_o) \times X_{density} \times V \times ratio \times MWCO_2}{MWC}$$

$$CO_2 = \frac{(520{,}000 - 100{,}000) \times 1.64 \times 10^{-9} \times 17\ L \times 1000 \times 0.26 \times 44}{12} = 11.2g\ CO_2$$

% conversion of CO_2

$$Conversion = \frac{CO_2\ uptake\ to\ cell\ mass}{mass\ of\ CO_2 feed\ into\ reactor} \times 100$$

$$Conversion = \frac{11.2}{25.96} \times 100 = 43.1\%$$

BIOGRAPHY

Miss. Konkanok Sahasthat was born on 17th February, 1986 in Udonthani. She finished the higher secondary course from Nonsungpittayakarn School in March, 2004. After that, she studied in the major of Chemical Engineering in Faculty of Engineering and Industrial Technology at Silpakorn University. She continued her further study for Master's degree in Chemical Engineering (Environmental and Safety research group) at Chulalongkorn University in 2010.