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DEBOTTLENECKING OF THE AIRLIFT CULTIVATION PROCESS FOR *Chaetoceros calcitrans*

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ภุชงค์ ศรีอ่วม: การลดขีดจำกัดของกระบวนการเลี้ยงคีโตเซอรอส คีโตเซอรอส คาลซิแทรนซ์ แบบอากาศยก (DEBOTTLENECKING OF THE AIRLIFT CULTIVATION PROCESS FOR *Chaetoceros calcitrans*) อ.ที่ปรึกษาวิทยานิพนธ์:รศ.ดร. ประเสริฐ ภวสันต์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ดร. สรวิศ เผ่าทองศุข, 66 หน้า

การศึกษาปัจจัยที่มีผลต่อขีดจำกัดในการเพาะเลี้ยงไดอะตอม คีโตเซอรรอส คาลซิแทรนซ์ เช่น สารอาหาร การบังแสงจากฟองอากาศ และการบังแสงจากตัวไดอะตอมเอง พบว่า การปรับเปลี่ยนปริมาณของสารที่เป็นองค์ประกอบในอาหารชนิด Modified F/2 medium ได้แก่ ซิลิกอน (โซเดียมซิลิเกต) ไนโตรเจน (ไนเตรต) และ ฟอสฟอรัส (ฟอสเฟต) ไม่ส่งผลต่อค่าความเข้มข้นเซลล์สูงสุด แต่กลับมีผลต่อค่าอัตราการเจริญเติบโตจำเพาะ (μ) การบังแสงจากจำนวนฟองอากาศที่มากเกินที่เกิดในส่วนของไหลไหลลง (Downcomer) ในระบบอากาศยก เรียกว่า "การบังแสงจากฟองอากาศ" ในการเพาะเลี้ยงคีโตเซอรอส คาลซิแทรนซ์ สามารถลดปัญหาการบังแสงจากฟองอากาศได้ด้วยการใช้ถังปฏิกรณ์ชีวภาพที่มีการขยายขนาดของยอดหอ ซึ่งการแยกตัวของฟองอากาศจากของเหลวที่บริเวณยอดหอทำให้ความสามารถในการส่องผ่านของแสงเข้าสู่ระบบมีค่าสูงขึ้น แต่อย่างไรก็ตาม เมื่อเทียบกับการใช้ถังปฏิกรณ์เชิงแสงแบบเดิม จำนวนความเข้มข้นเซลล์สูงสุดที่ได้จากถังปฏิกรณ์แบบขยายขนาดยอดหอมิได้เพิ่มขึ้น นอกจากนี้ในการเพาะเลี้ยงไดอะตอม พบว่ายังมีผลของ "การบังแสงจากตัวไดอะตอมเอง" ซึ่งเกิดจากการที่ความเข้มข้นของไดอะตอมมีค่ามาก มากจนบังแสงที่จะส่องเข้าไปข้างในถังปฏิกรณ์ส่งผลให้ไดอะตอมที่อยู่ด้านในได้รับปริมาณความเข้มแสงไม่เพียงพอที่จะสังเคราะห์แสง ในที่สุดไดอะตอมก็จะไม่เจริญเติบโตและได้ความเข้มข้นน้อย การใช้ถังปฏิกรณ์ขนาดเล็กและต่อกันแบบอนุกรมโดยเลี้ยงในระบบต่อเนื่องสามารถลดปัญหาการบังแสงจากตัวไดอะตอมได้ เนื่องจากไดอะตอมจะกระจายไปไอยู่ในถังปฏิกรณ์แต่ละถังทำให้ความเข้มข้นในถังที่ 1 มีความเข้มข้นน้อยลง แสงส่องผ่านได้มากขึ้นส่งผลให้ไดอะตอมเจริญเติบโตได้มากขึ้น แต่อย่างไรก็ตาม จากการวิเคราะห์หาค่าความสามารถในการผลิตจำเพาะ (Specific productivity) พบว่าการเพาะเลี้ยงไดอะตอมในถังปฏิกรณ์ขนาดเล็กแบบถังเดียว ให้ค่าความสามารถในการผลิตจำเพาะสูงกว่าการเพาะเลี้ยงแบบ 3 ถังต่ออนุกรม

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PUCHONG SRIOUAM: DEBOTTLENECKING OF THE AIRLIFT CULTIVATION PROCESS FOR *Chaetoceros calcitrans*. THESIS ADVISOR: ASSOC. PROF. PRASERT PAVASANT, Ph.D., THESIS CO-ADVISOR: SORAWIT POWTONGSOOK, Ph.D., 66 pp

The growth limiting factors were investigated for the cultivation of *Chaetoceros calcitrans*, i.e. nutrient limitation, bubble shading, and self shading effects. The nutrient content in the modified F/2 medium such as silicon (as sodium silicate), nitrogen (as nitrate), plus phosphorus (as phosphate) did not have significant impacts on the cell culture in terms of final cell concentration, but did have some influence on the specific growth rate. Light obstruction due to the presence of excessive bubble in the airlift system was also found to limit the growth of the diatom, and this was called "bubble shading effect". The cultivation of the diatom in the expanded-top airlift photobioreactor facilitated the disengagement of the bubbles at the top section of the system and could help reduce the bubble shading effect. However, although the shading effect at high aeration rate was eliminated, the growth was not significantly enhanced the maximum cell concentration. At high cell concentration, the growth was limited by the self shading effect which the light penetration was obstructed by the dense cell culture. The use of reactors-in-series could decrease this effect because cells were allowed to grow in separate column which connected in series. In this configuration, the first compartments contained culture at low concentration and therefore was increased to high light intensity, and the last column would contain high cell density. The effect of self shading therefore could be limited to the last column in the series. With this configuration, it was possible to achieve an ultrahigh cell density. However, the final analysis indicated that, although the single reactor configuration could be subject to self shading effect, it could provide a better productivity than the three reactors-in-series configuration.

Department	Chemical Engineering	Student's signature	Puchong Sriouam
Field of study	Chemical Engineering	Advisor's signature	
Academic year	2007	Co-advisor's signature	

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CHAPTER I
INTRODUCTION

1.1 Motivations

The growth of aquaculture industries can be strongly illustrated by shrimp industries which began in early 1970s and continued to grow until 1990s (Liang et al., 1997 and Smith, 1998). Among the various types of common microorganisms, the diatom *Chaetoceros calcitrans* is one of the most popular strains used in feeding shrimp larvae.

Recently, the cultivation of diatom *Chaetoceros calcitrans* in airlift photobioreactor for feeding shrimp larvae achieved a satisfactory result, i.e. in Thailand a maximum cell density obtained from 2.5 L glass bubble column was approximately 5.8 x 10^6 cells mL^{-1} with a maximum specific growth rate of 3.80 x 10^{-2} h^{-1}. A high maximum cell concentration of 8.88 x 10^6 cells mL^{-1} could be achieved from the 17 L batch cultivation in airlift photobioreactor (maximum specific growth rate = 7.41 x 10^{-2} h^{-1} at superficial gas velocity, u_{sg} = 3 cm s^{-1}) (Krichnavaruk et al., 2005). Moreover, the cultivation of *Chaetoceros calcitrans* in the 17 L semi-continuous airlift system could provide an average maximum cell concentration of around 3.86 x 10^6 cells mL^{-1} with a specific growth rate of about 9.25 x 10^{-2} h^{-1} (Krichnavaruk et al., 2007). However, the cultivation of diatom *Chaetoceros calcitrans* in airlift photobioreactors often suffered drawbacks from “bubble shading effect” and “self shading effect” which obstructed the light path and decreased cell growth.

Bubble shading effect in airlift photobioreactor occurs due to the formation of a gas bubble swarm which is dragged down into the downcomer and obstructs the light penetration. Cells thus suffer from the inadequate light intensity and a retarding growth is observed. Self shading effect takes place due to the over growth of the diatom which often occurs at the stationary phase. In other words, the culture can be too dense for the light to penetrate and this decreases the cell growth.

This work aimed to study the growth limiting factors for the cultivation of *Chaetoceros calcitrans*, i.e. nutrient limitation, bubble and self shading effects in

airlift photobioreactors and proposed alternative options in terms of operation or configuration to improve for the performance regarding the growth of *Chaetoceros calcitrans*. The ultimate goal was to have systems running at very high cell density and high production capacity.

1.2 Objectives

The objectives of this work were to:

1. Study the effect of nutrient content in modified F/2 medium such as silica (as sodium silicate), nitrogen (as nitrate), and phosphorus (as phosphate),
2. Study bubble shading effects and self shading effects for the cultivation of *Chaetoceros calcitrans* in airlift photobioreactor, and
3. Propose alternative options in terms of design and operation of airlift photobioreactors for the cultivation of high cell density/productivity of *Chaetoceros calcitrans*.

1.3 Working Scopes

The conditions which were proposed by Loataweesup (2002) were employed for the cultivation in this work. For example, the culture medium and light intensity used in this work were the modified standard F/2 (Guillard's) medium (with a two-fold of silica and phosphorus concentrations) and 135 μmol photons $m^{-2}s^{-1}$, respectively.

Types of airlift photobioreactors employed in this work are:

- 3 L concentric airlift photobioreactor
- 3 L concentric airlift photobioreactors in series
- 17 L concentric airlift photobioreactor
- 25 L expanded top airlift photobioreactor

The configuration of the reactors will be described in Chapter 3.

CHAPTER II

BACKGROUNDS AND LITERATURE REVIEW

2.1 Microscopic algae

It is believed that the progenitors of microscopic algae may have originated from blue-green algae born about 3,600 million years ago, and may be divided into about 20,000 to 30,000 different species. The size of most microscopic algae ranges from about 3 to 100 micro meter. Some can live for about two to three months in a dry, dark place. Generally, microscopic algae can be found in water (including as saltwater, estuary, and fresh water), soil, and air.

Diatoms

Algae can be unicellular or multicellular organisms. The most numerous unicellular forms are the diatoms. Diatoms are in a division *Chromophyta (class Bacillariophyceae).* The characteristic feature of the class *Bacillariophyceae* is their ability to secrete an external wall composed of silica, so called the "frustule" (a rigid two part boxlike cell wall). Diatoms are immediately recognizable by their cases or frustules. The chloroplasts contain chlorophylls *a*, c_1 and c_2 with the major carotenoid being fucoxanthin, which gives the cells their characteristic colors as yellowish or brownish (Lewin *et al*., 1984).

Diatoms normally store oil or fatty acids as the end product of photosynthetic activity. That is the main cause for using diatoms as a food for aquatic larvae. Diatoms widely used in hatcheries are *Skeletonema, Chaetoceros, etc*. (Marasigan, 1989). *Chaetoceros* was among the first diatoms to be utilized effectively as a live, natural food for shrimp larvae (Berner, 1993).

Chaetoceros calcitrans

Chaetoceros sp. is one of the well known diatoms as it serves as nutritious food for marine hatcheries. It is widely found in warm and cold waters with 17-30 ppt salinity, temperature around 20–30^{o}C and light intensity of 500–10,000 luxes. Several species of *Chaetoceros* sp. can be identified, *e.g. Chaetoceros gracilis, Chaetoceros*

debilis, *Chaetoceros calcitrans*, etc. However, the most popular strain is *Chaetoceros calcitrans* (Phatarpekar, P.V. et al, 2000 and Rico-Villa, B et al, 2006).

Chaetoceros calcitrans is a unicellular floating diatom organism/ phytoplankton, a rectangular diatom with four long acantha (sharp spiny parts) on its corners. Generally, cell growth and reproduction is accomplished by dividing into two cells. The size of the cell is about 8-12 micro meter (0.008-0.012 mm) in length and about 4 to 10 micro meter (0.004 to 0.01 mm) in width. 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. The chaetoceros is comprised of highly-unsaturated fatty acids with a good balance of vitamins. The cell is found rich in protein (27%), nucleic acid (10%) and lipid (11%) (Zhukova and Aizdaicher, 1995).The percentages of biochemical composition and total fatty acids of *Chaetoceros calcitrans* are shown in Tables 2.1 and 2.2, respectively. *Chaetoceros* is also excellent in terms of its predatory qualities, and is therefore utilized as optimal bait for shellfish (such as clams, and oyster) in their larval form, echinoderms (such as sea cucumbers and sea urchins), and crustaceans (such as shrimps and crabs).

2.2 Factors controlling algal growth

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 another.

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. Silicate is specifically 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. Two enrichment media that have been used extensively and are suitable for the growth of most algae are the Walne medium (Table 2.3.) and the

standard Guillard's F/2 medium (Table 2.4). Various specific recipes for algal culture media are described by Vonshak (1986). Commercially available nutrient solutions may reduce preparation labor. The complexity and cost of the above culture media often excludes their use for large-scale culture operations. Alternative enrichment media that are suitable for mass production of micro-algae in large-scale extensive systems contain only the most essential nutrients and are composed of agriculture-grade rather than laboratory-grade fertilizers.

Light and Photoperiod

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 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 (Brown, J. S., 1980, Glover et al., 1987 and 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)

$$1\,\mu\text{mol}\,(\text{m}^{-2}\text{s}^{-1}) \;=\; 1\;\;\mu\text{E}\,\text{m}^{-2}\text{s}^{-1} = \frac{119.7}{\lambda}\;\;\text{W}\,\text{m}^{-2} \;=\; 50\;\;\text{luxes} \quad \ldots. (2.1)$$

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, as for *Chaetoceros calcitrans*, our previous work had shown that the loss rate of photon trap could will be replaced by the recovery rate at the light intensity of around 10,000 luxes (200 µmol photon $m^{-2}s^{-1}$).Therefore this diatom can be cultivated without the dark period, and will not cause danger to the cell. The duration of artificial illumination for the cultivation of *Chaetoceros calcitrans* should be 24 hours of light per day, and light intensity 10,000 luxes (200 µmol photon m^{-2} s^{-1}) (Laotaweesup, 2002).

Temperature

The temperature for *Chaetoceros calcitrans* cultures is generally between 20 and 40 °C. But the optimal temperature is generally between 20 and 30 °C. At 40 °C, cells are grown with no color but if temperature drops to 20 or 30 °C, an increase in growth and color is observed. 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.

Salinity

Chaetoceros calcitrans are extremely tolerant to changes in salinity. Range of salinity for the cultivation of *Chaetoceros calcitrans* are 6- 50 ppt (g/L). Salinity of 20-30 ppt has been found to be optimal.

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.

Carbon dioxide (CO_2)

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.

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 (containing approximate 0.03% CO_2) 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 jetpumps (ponds). However, it should be noted that not all algal species can tolerate vigorous mixing.

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.

2.3 Culture systems for diatom and single cell algae

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

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.

Open/Closed

- Open Systems

Open cultures such as uncovered ponds and tank (indoors or outdoors) are more readily contaminated than closed culture vessels such as tubes, flasks, carboys, etc.

All very large commercial systems used today are shallow open-air systems. The culture is agitated or circulated by some mechanical means such as paddle wheel or rotating scraper (Lee, 1997). The major types of open-air systems currently used are tanks, shallow ponds, circular ponds and raceway ponds. The success of this open-air system depends significantly on the climate factor. In most case, the ambient temperature in the vicinity of the culture pond was appropriate for the growth of algae.

- Closed Systems

The aims of these systems are to increase the volumetric yield and biomass quality under the controlled conditions by proficiency experiences. The systems were designed for cultivation of the microorganism in specified environment suitable for the growth of each species to prevent the growth of the contaminants. Growth conditions, such as temperature, could be easily regulated to achieve the maximum productivity (Lee, 2001; Lee and Richmond, 1998; Vonshak, 1997 and Richmond, 1996).

Axenic(sterile) / Xenic

Axenic cultures are free of any foreign organisms such as bacteria and require a strict sterilization of all glassware, culture media and vessels to avoid contamination.

Batch, Continuous, and Semi-Continuous

These are the three basic types of phytoplankton culture which will be described as follows:

- Batch culture

The batch culture consists of a single inoculation of cells into a container of fertilized seawater followed by a growing period of several days and finally harvesting when the algal population reaches its maximum or near-maximum density. In practice, algae are transferred to larger culture volumes prior to reaching the stationary phase and the larger culture volumes are then brought to a maximum density and harvested. The following consecutive stages might be utilized: test tubes, 2 L flasks, 5 and 20 L carboys, 160 L cylinders, 500 L indoor tanks, 5,000 L to 25,000 L outdoor tanks

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 labor to harvest, clean, sterilize, refill, and inoculate the containers.

- Continuous culture

The continuous culture method (*e.g.* a culture in which a supply of fertilized seawater is continuously pumped into a growth chamber and the excess culture is simultaneously washed out) 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. Adding 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.

- Semi-continuous culture

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, predators and/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.

2.4 Airlift photobioreactor

2.4.1 Classification

Airlift photobioreactors can be classified into two major types; the internal loop and external loop, as shown in Figure 2.1. The internal loop airlift photobioreactor is the cylindrical column with a concentric cylindrical tube that separates the column into four sections; riser, downcomer, gas separator, and bottom section (Figure 2.1a).

In external loop airlift photobioreactors, riser and downcomer are two separate columns (Figure 2.1b) which are connected by two connecting tubes. These connecting tubes support liquid flow between riser and downcomer.

2.4.2 Transport mechanism in airlift photobioreactor

An airlift photobioreactor can be divided into four main parts, riser, downcomer, gas separator and bottom section as shown in Figure 2.1. A concentric airlift photobioreactor (Figure 2.1 a) is used to describe the function of each of the section.

1. Riser is the section through which gas is supplied, and the upward flow of liquid is induced.
2. Gas separator is the section where the flow pattern is highly turbulent, similar to that in CSTRs. In this section, a large portion of gas disperses out at the liquid surface and creates heavier fluid.
3. Downcomer is the section that the heavier fluid from the gas separator section and some remaining gas bubbles flow downwards.
4. The final bottom section is provided to allow the liquid to re-enter the riser again together with the supplied gas.

Two factors govern the liquid flow pattern in the system: (i) density or the hydrostatic pressure difference between the riser and downcomer due to the difference in local gas holdups; and (ii) the energy transfer from the gas bubbles from the air compressor.

2.4.3 Cultivation of Chaetoceros calcitrans in airlift photobioreactor

Few attempts have been carried out for the cultivation of microalgae in airlift photobioreactors. Merchuk *et al.*, (1998) investigated the productivity of the red microalgae *Porphyridium* sp. for the cultivation in the airlift photobioreactor (ALPBR) when compared with the conventional bubble column. They found that a higher cell number of the red microalgae *Porphyridium* sp. in the ALBR could be obtained than that in the bubble column.

Recently, airlift photobioreactors have been employed for the cultivation of *Chaetoceros calcitrans* at the Biochemical Engineering Laboratory, Department of Chemical Engineering, Faculty of Enginering, Chulalongkorn University. The elaboration on this is offered here below.

Loataweesup (2002) cultivated the diatom *Chaetoceros calcitrans* in a small glass bubble column and airlift photobioreactor to investigate the optimal conditions for the growth of the diatom. The optimal light intensity which yielded the maximum cell concentration was reported to be 400 μ molphoton $m^{-2}s^{-1}$. It was claimed that the suitable nutrient concentration was a two fold of silica and phosphorus concentrations in a modified standard F/2 (Guillard's) medium, which resulted in a better specific growth rate of the diatom, whilst vitamin B_{12} did not significantly affect the growth of the diatom. The maximum cell concentration for the cultivation in a 2.5 L glass bubble was reported to be 5.8×10^{6} cells mL^{-1} with a maximum specific growth rate of 3.80×10^{-2} h^{-1}.

The investigation also discovered that the cultivation of *Chaetoceros calcitrans* in the 17 L airlift photobioreactor yielded higher specific growth rate and cell concentration when compared with the bubble column of the same size. The maximum cell concentration in a batch culture was obtained at the superficial gas velocity of 3 cm s^{-1}, which achieved the highest cell concentration of 8.88×10^{6} cells mL^{-1} (specific growth rate = 7.41×10^{-2} h^{-1}). It was concluded that the well defined flow pattern in airlift photobioreactor provided an effective light utilization for the diatom particularly at high cell concentration as the cells were regularly circulated to the reactor outer surface where light was supplied. This circulation was not found in other bioreactors such as bubble column which a liquid circulation was poor.

Krichnavaruk et al., (2007) examined the various modes of cultivation of *Chaetoceros calcitrans* in airlift photobioreactor. The cultivations in both semi-continuous and continuous systems resulted in a high cell productivity, although the final cell concentrations in both systems were lower than that obtained from the batch system. The behavior of the large-scale airlift system was not significantly different from the conventional bubble column where the diatom could only be produced at low cell density and low growth rate. Moreover, it was also reported that the cultivation of *Chaetoceros calcitrans* in airlift photobioreactor suffered two main problems; bubble and self shading effects (Krichnavaruk, 2005). However, they did not report alternatives to deal with such problems.

Table 2.1 Biochemical composition of *Chaetoceros calcitrans* expressed as a percentage of dry weight (Zhukova and Aizdaicher, 1995)

Biochemical Composition	% Dry weight
Protein	27.21
Nuecleic acid	10.00
Total lipids	11.80
Polyunsaturated fatty acids (PUFAs)	0.90

Table 2.2 Fatty acids composition of *Chaetoceros calcitrans* expressed as a percentage of the total fatty acids (Zhukova and Aizdaicher, 1995)

Fatty acids		% total fatty acids
Symbol	Scientific Name	
14:0	Myristic	13.0
16:0	Palmitic	18.0
16:1n7	Palmitoleic	28.8
18:1n9	Oleic	0.3
18:2n6	Linoleic	0.7
18:3n3	Linolenic	0.8
18:3n6	Calendic	0.3
18:4n3	Parinaric	0.2
20:4n6	Arachidonic	2.3
20:5n3	Eicosapentaenoic (EPA)	34.0
22:6n3	Decosahexaenoic (DHA)	1.2

Table 2.3 Composition and preparation of Walne medium (Lavens and Sorgeloos, 1996)

Constituents	Quantities
Solution A (at 1 ml per liter of culture)	
Ferric chloride ($FeCl_3$)	0.8 g[(a)]
Manganous chloride ($MnCl_2$, $4H_2O$)	0.4 g
Boric acid (H_3BO_3)	33.6 g
EDTA[(b)], di-sodium salt	45.0 g
Sodium di-hydrogen orthophosphate (NaH_2PO_4, $2H_2O$)	20.0 g
Sodium nitrate ($NaNO_3$)	100.0 g
Solution B	1.0 ml
Make up to 1 litre with fresh water[(c)]	Heat to dissolve
Solution B	
Zinc chloride ($ZnCl_2$)	2.1 g
Cobaltous chloride ($CoCl_2$,6 H_2O)	2.0 g
Ammonium molybdate ($(NH_4)_6Mo_7O_{24}$, $4H_2O$)	0.9 g
Cupric sulphate ($CuSO_4$, $5H_2O$)	2.0 g
Concentrated HCl	10.0 ml
Make up to 100 ml fresh water[(c)]	Heat to dissolve
Solution C (at 0.1 ml per liter of culture)	
Vitamin B_1	0.2 g
Solution E	25.0 ml
Make up to 200 ml with fresh water[(c)]	
Solution D (for culture of diatoms-used in addition to solutions A and C, at 2 ml per liter of culture)	
Sodium metasilicate (Na_2SiO_3, $5H_2O$)	40.0 g
Make up to 1 litre with fresh water[(c)]	Shake to dissolve
Solution E	
Vitamin B_{12}	0.1 g
Make up to 250 ml with fresh water[(c)]	
Solution F (for culture of *Chroomonas salina* - used in addition to solutions A and C, at 1 ml per liter of culture)	
Sodium nitrate ($NaNO_3$)	200.0 g
Make up to 1 litre with fresh water[(c)]	

(a) Use 2.0 g for culture of *Chaetoceros calcitrans* in filtered sea water;

(b) Ethylene diamine tetra acetic acid;

(c) Use distilled water if possible.

Table 2.4 Composition and preparation of standard Guillard's F/2 medium (Lavens and Sorgeloos, 1996)

Nutrients	Final concentration (mg L^{-1} sea water)	Stock solution preparations
$NaNO_3$	75	*Nitrate/Phosphate solution*
$NaH_2PO_4.H_2O$	5	*Working stock:*
		add 75 g $NaNO_3$ + 5 g NaH_2PO_4 to 1 L distilled water (DW)
$Na_2SiO_3.9H_2O$	30	*Silicate solution* : add 30 g $NaSiO_3$ to 1 L DW
$CoCl_2.6H_2O$	0.01	*Trace metal/EDTA solution*
$CuSO_4.5H_2O$	0.01	*Primary stocks*: make 5 separate
$MnCl_2.4H_2O$	0.18	1 L stocks of (g L^{-1} DW) 10.0 g $CoCl_2$, 9.8 g $CuSO_4$,
$Na_2MoO_4.2H_2O$	0.006	180 g $MnCl_2$, 6.3 g Na_2MoO_4 , 22.0 g $ZnSO_4$
$ZnSO_4.7H_2O$	0.022	*Working stock*:
Na_2EDTA	4.36	add 1 mL of each primary stock solution +
$FeCl_3.6H_2O$	3.15	4.35 g Na_2EDTA + 3.15 g $FeCl_3$ to 1 L DW
Thiamin HCl	0.1	*Vitamin solution*
Biotin	0.0005	*Primary stocks:*
B_{12}	0.0005	add 20 g thiamin HCl + 0.1 g biotin + 0.1 g B_{12} to 1 L DW
		Working stock: add 5 mL primary stock to 1 L DW

add 1 mL each of the four working stock solutions per liter of seawater

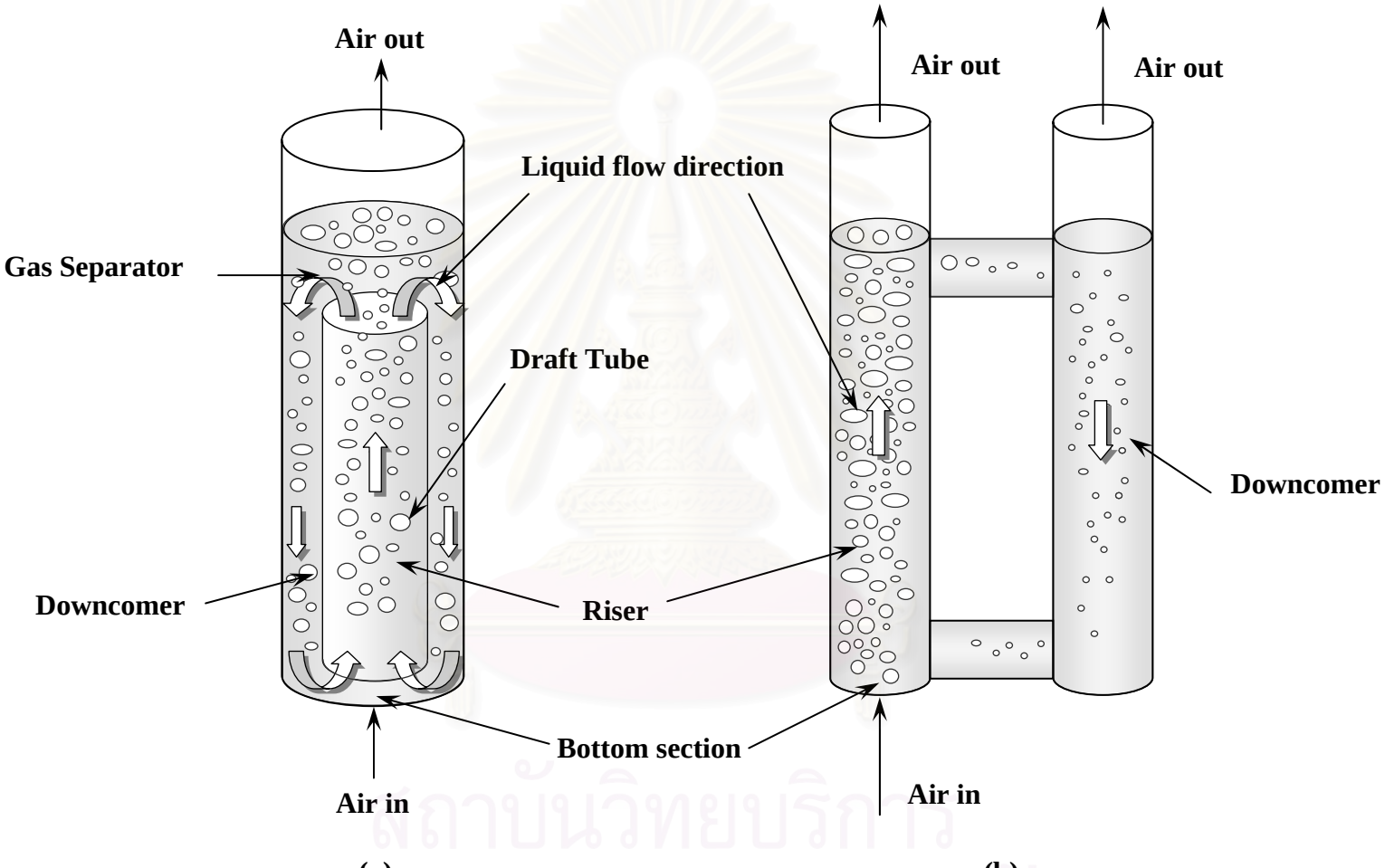


Figure 2.1 Classification of airlift photobioreactor: (a) internal loop (b) external loop

CHAPTER III

EXPERIMENTS

3.1 Experimental Apparatus

3.1.1 Conventional setup

Airlift photobioreactors employed in this investigation were made from clear acrylic plastic to allow the visual observation of the ongoing phenomena, and also to allow the light passage through the column. For the cultivation of *Chaetoceros calcitrans*, the internal loop ALPBR with the size of 17 L (ALPBR-1) was used where the draft tube was installed centrally in the outer column separating the downcomer from riser with the ratio between downcomer and riser cross sectional were of 2.62 and 3.27 (see Figure 3.1 for a schematic and Table 3.1 for dimensions). A calibrated rotameter was used to control the volume of gas volumetric flow rate which was supplied to the system through a porous gas sparger at the base of the column, with superficial velocity of 3.0 cm s^{-1}. This level of superficial velocity was found to be optimal for the cultivation of this diatom (Krichnavaruk et al., 2005). Fluorescent lighting bulbs (18 and 36 watts) were provided on both sides of the column as shown in Figure 3.1. These light bulbs were place 10 cm away from the column for photosynthesis to obtain the average light intensity at the draft tube surface of 135 μmol photon $m^{-2}s^{-1}$. This light intensity was measured by "Digicon LX-50 lux meter", and converted into μmol photon $m^{-2}s^{-1}$ using Equation 3.1. The investigation was carried out at room temperature which was around 27–35 °C.

3.1.2 Study of nutrient content in modified F/2 medium

ALPBR-3 was employed to study the concentration of nutrients in the modified F/2 medium, which was originally formulated by Laotaweesup (2002). This modified F/2 medium was examined again in this work to study the effect of each main nutrient component, i.e. silicon (as sodium silicate), nitrogen (as nitrate), and phosphorus (as phosphate) that could play an important role in the control of the growth of the *Chaetoceros calcitrans* culture. In this experiment, the culture was started with an initial cell concentration of 1.0 x 10^5 cells mL^{-1}. Compressed air was

supplied through a porous sparger located centrally at the base of the column. Fluorescent light bulbs were supplied on both sides of the column for photosynthesis. The temperature of the system was controlled around 27–35°C. Samples were taken for measurement at every 6 hours to determine the cell concentration and concentration of nutrient content.

3.1.3 Study of bubble shading effects

To study the effect of bubble shading, the expanded top airlift photobioreactor (ALPBR-2) were employed. The gas separating section of ALPBR-2 was enlarged to ensure more complete gas disengagement. Dimensions for this system are given in Figure 3.2. With the expanded top section, this airlift system had a volume of 25 L. This system was operated using the same conditions as ALPBR-1, however, the locations of light bulbs were slightly adjusted to obtain the same light intensity at the draft tube surface (see Figure 3.2 for the schematic of this system).

3.1.4 Study of self shading effects

The airlift photobioreactor (ALPBR-3) employed for this investigation was the 3 L concentric airlift photobioreactor as displayed in Figure 3.1 (all dimensions are detailed in Table 3.1). However, these reactors were connected in series using connecting tubes as illustrated in Figure 3.3. The operation of such system was performed in three configurations. The first configuration was the single column (System I) which was used as blank. System II was operated with two connecting columns whereas System III was three connecting columns. Medium feed rates were varied as indicated in Table 3.2. Compressed air was supplied through a porous sparger located centrally at the base of the column. Fluorescent light bulbs were supplied on both sides of the column for photosynthesis. The temperature of the system was controlled around 27–35°C. The sterilized modified standard F/2 medium was pumped into the column where the overflow stream was provided to control the total volume at 3 L. Samples were taken for measurement at every 6 hours to determine the cell concentration and specific growth rate.

3.2 Experimental Methods

3.2.1 Experimental preparation

3.2.1.1 Treatment of fresh seawater

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

1. Dilute the seawater to 30 ppt with tap water.
2. Disinfect the seawater with 50 ppm (parts per million) of chlorine (as sodium hypochloride).
3. Supply compressed air through all over the pond for about 3 days or until no remaining chlorine is detected.

3.2.1.2 Preparation of culture medium

The modified standard F/2 (Guillard's) medium is prepared according to the composition as shown in Table 3.3.

3.2.2 Study of nutrient content in modified F/2 medium

1. Sterilize ALPBR-3 with 50 ppm chlorine (as sodium hypochloride)
2. Supply compressed air through the porous sparger located centrally at the bottom of the column for 1 day
3. Drain all the water and rinse the column with tap water to eliminate the remaining chlorine
4. Fill in the column with fresh seawater, culture medium together with the pure culture and adjust the total volume to 3 L. Initial cell concentration were controlled at 1×10^5 cells mL^{-1}
5. Cover the column with a plastic funnel to minimize airborne contamination
6. Supply sterilized compressed air (treat with the air filter size 0.2 μm) through a porous sparger and adjust the superficial gas velocity to 3 cm s^{-1}
7. Supply both sides of column with fluorescent light bulbs which are placed along the column height (10,000 ± 1,000 luxes or 135 ± 14 μmol photon $m^{-2}s^{-1}$) as shown in Figure 3.1

8. 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 (see Appendix A for the detail of measurement) at every 6 hours until the stationary growth is observed
9. Calculate the specific growth rate using Equation 3.3, the productivity using Equation 3.4, and the specific productivity using Equation 3.6.

3.2.3 Batch culture system (Study of bubble shading effects)

1. Sterilize ALPBR-1 and ALPBR-2 with 50 ppm chlorine (as sodium hypochloride)
2. Supply compressed air through the porous sparger located centrally at the bottom of the column for 1 day
3. Drain all the water and rinse the column with tap water to eliminate the remaining chlorine
4. Fill in the column with fresh seawater, culture medium together with the pure culture and adjust the total volume to 17 L and 25 L. Initial cell's concentration of both columns are controlled at 1×10^5 cells mL^{-1}
5. Cover the column with a plastic funnel to minimize airborne contamination
6. Supply sterilized compressed air (treat with the air filter size 0.2 μm) through a porous sparger and adjust the superficial gas velocity to 3 cm s^{-1}
7. Supply both sides of column with fluorescent light bulbs, placed along the column height (10,000 ± 1,000 luxes or 135 ± 14 μmol photon $m^{-2}s^{-1}$) as shown in Figures 3.1 and 3.2
8. Take samples and count for the cell density using a Haemacytometer (mentioned in Section 3.3.2) and measure the light intensity in the draft tube at every 6 hours until the stationary growth is observed
9. Calculate the specific growth rate using Equation 3.3, the productivity using Equation 3.4, and the specific productivity using Equation 3.6
10. Repeat Steps 1–9 again but change the superficial gas velocity to 4 cm s^{-1} in Step 6

3.2.4 Semi-continuous culture system

1. Sterilize both columns (ALPBR-1 and ALPBR-2) with 50 ppm chlorine (as sodium hypochloride)
2. Supply air through the porous sparger at the bottom of the column for 1 day
3. Drain all the water and rinse the column with tap water to remove residual chlorine
4. Fill in the column with fresh seawater, culture medium together with the pure culture and adjust the total volume to 17 L and 25 L. Initial cell's concentration of both columns are controlled at 1×10^5 cells mL^{-1}
5. Cover the column with a plastic funnel to minimize airborne contamination
6. Supply sterilized compressed air (treat with the air filter size 0.2 μm) through a porous sparger and adjust the superficial gas velocity to 3 cm s^{-1}
7. Supply both sides of column with fluorescent light bulbs, placed along the column height (10,000 ± 1,000 luxes or 135 ± 14 μmol photon $m^{-2}s^{-1}$)
8. Take samples and count for the cell density using Haemacytometer every 6 hours until the stationary growth is observed
9. Calculate the specific growth rate using Equation 3.3, the productivity using Equation 3.4, and the specific productivity using Equation 3.6
10. Subculture the cells and adjust its concentration to 1×10^6 cells mL^{-1} and the culture medium as necessary for the quantity of the additional seawater
11. Take samples after 48 hours of the cultivation, determine the cell density and repeat Step 9 again every 48 hours or other appropriate time as will be estimated from the batch culture
12. Repeat Steps 1–11 again but change the superficial gas velocity to 4 cm s^{-1} in Step 6

3.2.5 Continuous culture system

1. Fill in the ALPBR-3 with 3 L of water and disinfect with 50 ppm chlorine (as sodium hypochloride)
2. Sparge air through the porous sparger at the bottom of the column for 1 day

3. Drain all the water and rinse the column with tap water to remove residual chlorine at the bottom of the column
4. Fill in the column with fresh seawater, culture medium together with the pure culture and adjust the total volume to 3 L. The initial cell's concentration is controlled at 1×10^5 cells mL^{-1}
5. Cover the column with a plastic funnel to prevent contaminants in the column
6. Supply sterilized compressed air (treat with the air filter size 0.2 μm) through porous sparger and adjust the gas velocity to 3 cm s^{-1}
7. Take samples every 6 hours and examine the cell density using Haemacytometer until the stationary growth is initially observed
8. Connect the overflow line to the column to control the volume of the medium to 3 L
9. Pump in the culture medium using a peristaltic pump with the volumetric flow rate of 1 mL min^{-1}
10. Take samples every 6 hours and count for the cell concentration using Haemacytometer
11. Calculate the specific growth rate using Equation 3.3, the productivity using Equation 3.5, and the specific productivity using Equation 3.6
12. Repeat Steps 1–11 again with medium feed rates of 2 and 3 mL min^{-1}

3.2.6 Reactor-in-series culture system (Study of self shading effects)

1. Connect two ALPBR-3s for the cultivation in series as shown in Figure 3.3 (System II)
2. Fill in each column with 3 L of water and disinfect with 50 ppm chlorine
3. Sparge air through the porous sparger at the bottom of the columns for 1 day
4. Drain all the water and rinse the columns with tap water to remove residual chlorine at the bottom of the columns
5. Fill in the first column with fresh seawater, culture medium together with the pure culture and adjust the total volume to 3 L. The initial cell's concentration is controlled at 1×10^5 cells mL^{-1}

6. Cover the column with a plastic funnel to prevent contaminants in the column
7. Supply sterilized compressed air (treat with the air filter size 0.2 μm) through porous sparger and adjust the gas velocity to 3 cm s^{-1}
8. Take samples every 6 hours and examine the cell density by using a Haemacytometer until the stationary growth is initially observed
9. Pump in the culture medium by using a peristaltic pump with the volumetric flow rate of 2 mL min^{-1}
10. Take samples both columns every 6 hours and count for the cell concentration using Haemacytometer
11. Calculate the specific growth rate using Equation 3.3, the productivity using Equation 3.5 and the specific productivity using Equation 3.6
12. Repeat Steps 2–11 again with medium feed rate of 4 and 6 mL min^{-1}
13. Connect three ALPBR-3s for the cultivation in series as shown in Figure 3.3 (System III)
14. Repeat Steps 2–11 again with medium feed rate of 3, 6 and 9 mL min^{-1}

3.3 Analyses

3.3.1 Determination of light intensity

The light intensity can be calculated from Equation 3.1 as follows:

$$I = \frac{E}{74} \qquad \text{....(3.1)}$$

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 0.04 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 5 medium squares on the grid (25 medium squares per 1 large square)
5. Calculate the cells number, using Equation 3.2:

$$N = \frac{1}{4} \times \frac{n}{5} \times 10^6 \qquad \text{..... (3.2)}$$

where

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

n = number of cells on 5 medium squares (cells)

3.3.3 Determination of specific growth rate

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

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

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:

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

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)

For continuous cultivation:

$$P = \frac{N \times F}{60} \qquad \text{.....(3.5)}$$

where

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

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

F = volumetric flow rate of fresh medium (mL min^{-1})

3.3.5 Determination of specific productivity

The specific productivity for the cultivation of the diatom in the ALPBR can be calculated from Equation 3.6:

$$SP = \frac{P}{V} \qquad \text{.....(3.6)}$$

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 is determined by using a “Refractometer”, and adjusted the salinity of the water to 30 ppt (parts per thousand).

Table 3.1 Dimensions of conventional concentric ALPBR (ALPBR-1 and ALPBR-3) employed in this work

Parameters	Dimensions (cm)	
	ALPBR-1	ALPBR-3
Column outside diameter (D)	15	10
Draft tube outside diameter (d)	8	5
Column and draft tube thickness	0.3	0.3
Column height (H)	120	60
Draft tube height (h)	100	40

Table 3.2 Condition of conventional concentric ALPBR (ALPBR-3)

Experiment	System	Medium feed rates			
		Stream A	Stream B	Stream C	Stream D
Set 1	I	1 mL min^{-1}	-	-	Overflow
Set 2	I	2 mL min^{-1}	-	-	Overflow
Set 3	I	3 mL min^{-1}	-	-	Overflow
Set 4	II	2 mL min^{-1}	2 mL min^{-1}	-	Overflow
Set 5	II	4 mL min^{-1}	4 mL min^{-1}	-	Over flow
Set 6	II	6 mL min^{-1}	6 mL min^{-1}	-	Over flow
Set 7	III	3 mL min^{-1}	3 mL min^{-1}	3 mL min^{-1}	Over flow
Set 8	III	6 mL min^{-1}	6 mL min^{-1}	6 mL min^{-1}	Over flow
Set 9	III	9 mL min^{-1}	9 mL min^{-1}	9 mL min^{-1}	Over flow

Table 3.3 Composition of the modified standard F/2 (Guillard's) stock solution

Nutrients	Final concentration (mg L^{-1} seawater)	Stock solution preparations
$NaNO_3$	75	*Nitrate/Phosphate solution*
$NaH_2PO_4.H_2O$	10	*Working stock:*
		add 75 g $NaNO_3$ + 10 g NaH_2PO_4 to 1 L distilled water (DW)
$Na_2SiO_3.9H_2O$	60	*Silicate solution* : add 60 g Na_2SiO_3 to 1 L DW
$CoCl_2.6H_2O$	0.01	*Trace metal/EDTA solution*
$CuSO_4.5H_2O$	0.01	*Primary stocks*: make 5 separate
$MnCl_2.4H_2O$	0.18	1 L stocks of (g L^{-1} DW) 10.0 g $CoCl_2$, 9.8 g $CuSO_4$,
$Na_2MoO_4.2H_2O$	0.006	180 g $MnCl_2$, 6.3 g Na_2MoO_4 , 22.0 g $ZnSO_4$
$ZnSO_4.7H_2O$	0.022	*Working stock*:
Na_2EDTA	4.36	add 1 mL of each primary stock solution +
$FeCl_3.6H_2O$	3.15	4.35 g Na_2EDTA + 3.15 g $FeCl_3$ to 1 L DW
Thiamin HCl	0.1	*Vitamin solution*
Biotin	0.0005	*Primary stocks:*
B_{12}	0.0005	add 20 g thiamin HCl + 0.1 g biotin + 0.1 g B_{12} to 1 L DW
		Working stock: add 5 mL primary stock to 1 L DW

add 1 mL each of the four working stock solutions per liter of seawater

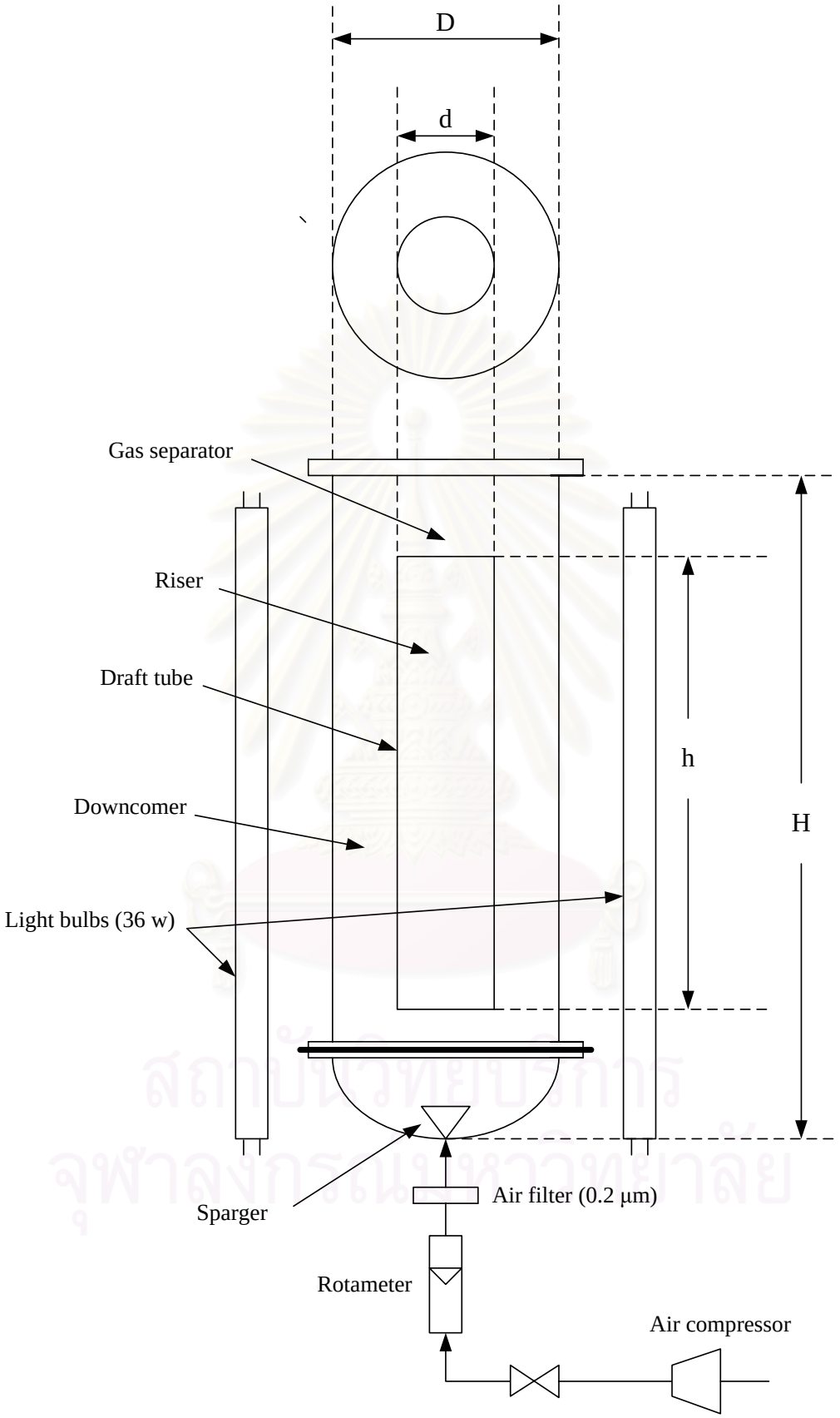


Figure 3.1 Experimental setup for the cultivation of *Chaetoceros calcitrans* in ALPBR-1 and ALPBR-3

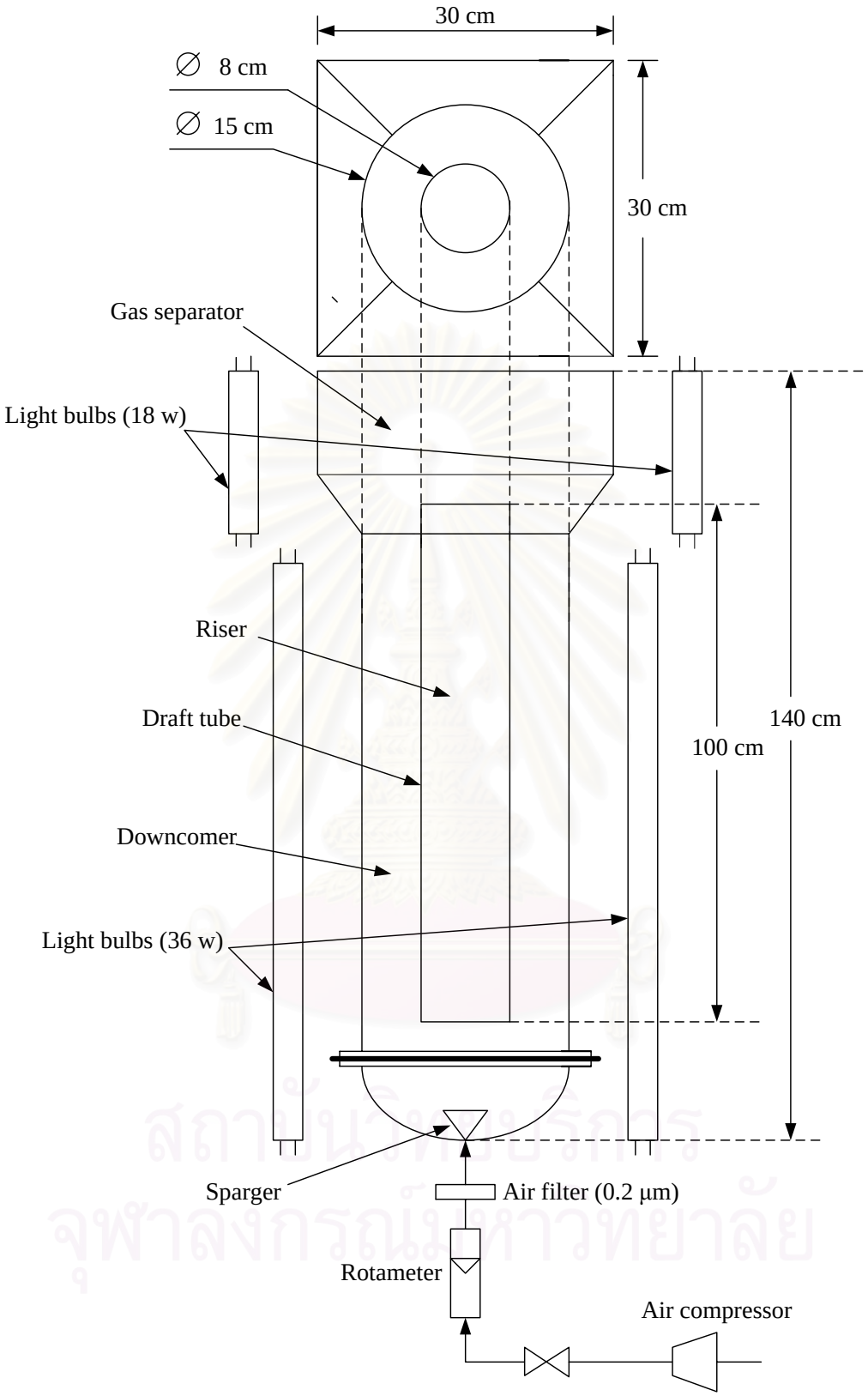


Figure 3.2 Experimental setup for the cultivation of *Chaetoceros calcitrans* in ALPBR-2

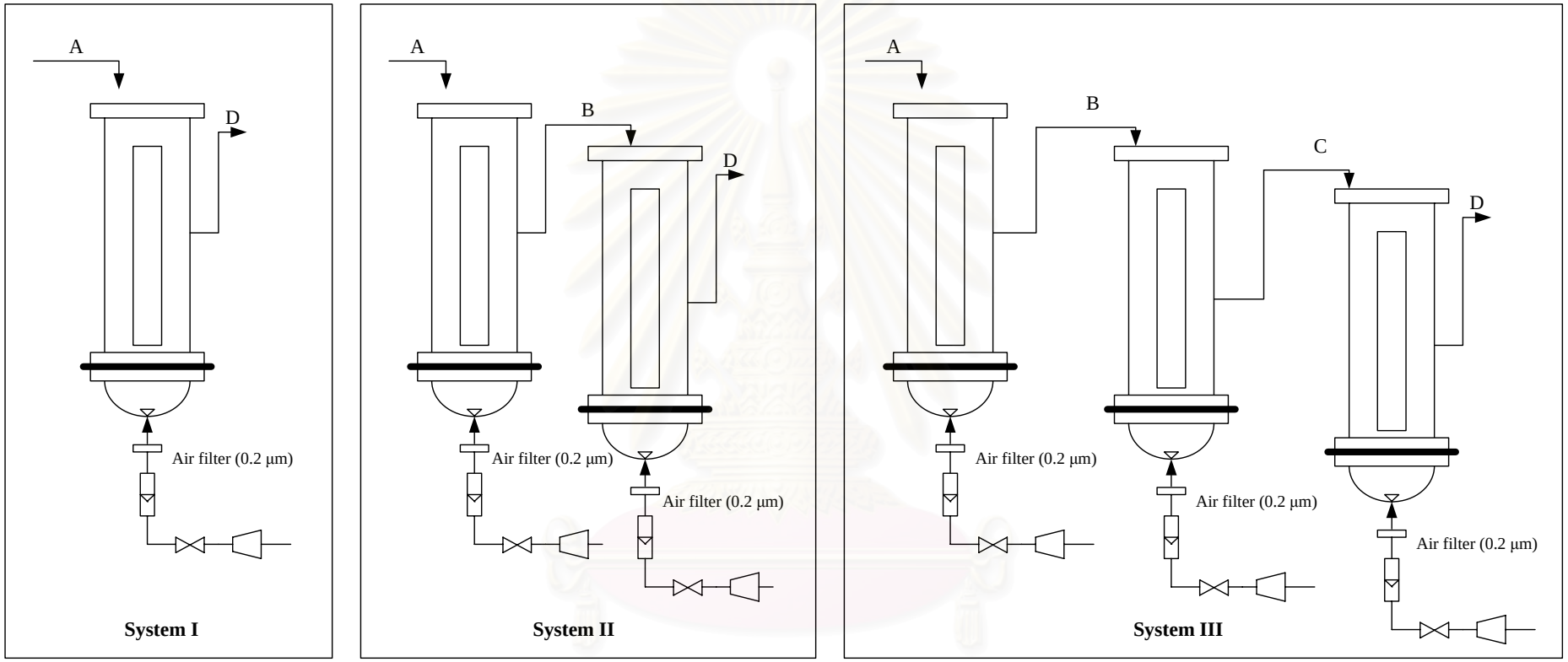


Figure 3.3 Experimental setup for the cultivation of *Chaetoceros calcitrans* in ALPBR-3 in series

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Effect of nutrient concentration

The nutrient used in this work was adopted from the work of Laotaweesup (2002) where the F/2 (Guillard's) medium was examined. In that work, the optimal composition of nutrient was found to be F/2 with two-fold of silicon (3.2 mg Si L^{-1}) and two-fold of phosphorus (2.4 mg PO_4^{2-} L^{-1}). However, that particular work has not provided in-depth analysis of how *Chaetoceros calcitrans* was fed on the medium. This modified F/2 (Guillard's) medium was examined again in this work to study the effect of each main nutrient component, i.e. silicon (as sodium silicate), nitrogen (as nitrate), and phosphorus (as phosphate) that could play an important role in the control of the growth of the *C.calcitrans* culture. In this experiment, the culture was started with an initial cell concentration of 1.0 x 10^5 cells mL^{-1}. Detail of the modified F/2 (Guillard's) medium is shown in Table 3.3.

4.1.1 Effect of silicon concentration

The growth curve and the time profile of silicon concentration in the culture with various initial silicon concentration are shown in Figure 4.1. The specific growth rate (μ) and maximum cell concentration (X_{max}) obtained from the cultivation with various initial silicon concentration are illustrated below:

For	1x silicon content	μ	=	7.3 x 10^{-2} h^{-1}
		X_{max}	=	6.7 x 10^6 cell mL^{-1}
	2x silicon content	μ	=	7.9 x 10^{-2} h^{-1}
		X_{max}	=	7.9 x 10^6 cell mL^{-1}
	3x silicon content	μ	=	8.8 x 10^{-2} h^{-1}
		X_{max}	=	7.9 x 10^6 cell mL^{-1}

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

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

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

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

The culture with the modified F/2 medium with various initial silicon concentration ended up with various $Y_{X/Si}$ as shown below:

For 1x silicon content $Y_{X/Si} = 1.97 \times 10^8$ cell/µg Si

2x silicon content $Y_{X/Si} = 1.29 \times 10^8$ cell/µg Si

3x silicon content $Y_{X/Si} = 0.95 \times 10^8$ cell/µg Si

From this calculation, it could be seen that silicon does help cell growth in that an increase in silicon content enhanced the specific growth rate of *C.calcitrans*. However, an increase in silicon from 2x to 3x did not seem to increase the final cell concentration. The analysis of the yield illustrated that cells assimilated different quantity of silicon depending on the availability of the silicon in the medium. In fact, diatoms cannot survive with insufficient supply of silicon as silicon is not only needed in the cell-wall formation, but it is also required for deoxyribonucleic acid (DNA) synthesis. In addition, it is generally known that the assimilation of silicon into cell is directly connected with the formation of new cell wall, therefore there was a definite low concentration of silicate below which a population could not survive. The accumulation of silicon in the cell structure seemed to provide strength to the cell and the culture with 3x silicon content could survive longer after the stationary phase than the culture with 2x and 1x silicon content (results not shown).

Nevertheless, an increase in the cell number with an increase in silicon content did not seem to provide promising results, i.e. a two fold or 100% increase in silicon content could only give approx. 18% increase in the final cell concentration. The level of enhancement is considered not useful when compared with the cost of silicon-compound required to prepare the nutrient. Hence, in this work, it was concluded at this point that further experiments would be conducted with the same silicon concentration as that stated in the modified F/2 medium.

4.1.2 Effect of nitrogen concentration

Nitrogen is essential for the microbial growth and the availability of nitrogen is often one of the major factor influencing the growth and chemical composition of the algae. In general, the major problem associated with nitrogen starvation was a rapid reduction in photosynthetic performance. Figure 4.2 illustrates the results from the experiment which could be summarized as:

For	1x nitrogen content	μ	=	3.9×10^{-2} h^{-1}
		X_{max}	=	7.1×10^{6} cell mL^{-1}
	2x nitrogen content	μ	=	6.5×10^{-2} h^{-1}
		X_{max}	=	6.7×10^{6} cell mL^{-1}
	3x nitrogen content	μ	=	8.5×10^{-2} h^{-1}
		X_{max}	=	7.6×10^{6} cell mL^{-1}

This indicated that the concentration of nitrogen could not be enhanced by increasing the availability of nitrogen in the nutrient. However, the specific growth rate seemed to be promoted significantly with an increase in nitrogen in the nutrient.

Analysis for the yield of cell with nitrogen (as nitrate) or $Y_{X/N}$ is as follows:

$$Y_{X/N} = \frac{\Delta X}{\Delta N} \qquad \text{.....(4.2)}$$

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

N nitrogen concentration (mg NO_3^- L^{-1})

The culture with the modified F/2 medium with various initial nitrogen concentration ended up with various $Y_{X/N}$ as shown below:

For	1x nitrogen content	$Y_{X/N}$	=	3.17×10^{5} cell/µg NO_3^-
	2x nitrogen content	$Y_{X/N}$	=	2.10×10^{5} cell/µg NO_3^-
	3x nitrogen content	$Y_{X/N}$	=	1.73×10^{5} cell/µg NO_3^-

This analysis shows that, at 1x nitrogen content, the specific uptake quantity of nitrogen was quite low, and this was enhanced when more nitrogen was supplied in

the nutrient. However, an increase in the nitrogen content from 2x to 3x did not further enhance the specific nitrogen uptake (and the yield was more or less constant). As stated above, an increase in nitrogen content could help increase the growth rate but did not have significant impacts on the final cell concentration. Therefore it could be concluded that nitrogen compounds did make the cells of *C.calcitrans* more active but the final cell concentration was controlled by other factors. In the following discussion, the nutrient used in the cultivation of this alga was still maintained at 1x nitrogen content.

4.1.3 Effect of phosphorus concentration

Phosphorus is needed for the microbial growth as a component of an energy precursor, and it was an important component of cell membrane. The most important role of phosphorus is in energy transfers through energy carrying agents e.g. adenosine triphosphate (ATP), NADPH, etc. Therefore the quantity required is usually not as much as other nutrient constituents such as silicon or nitrogen which are directly assimilated into the culture tissue. Inadequate or excess of phosphorus could have negative effect on the growth of the culture. When external phosphate concentrations were high, the ability of cells in the assimilation of phosphorus compound was repressed and the growth was inhibited. On the other hand, an inadequate level of external phosphorus reduces the cell capacity in authorizing ATP and other energy compounds which also led to a limited cell growth. According to Nelson *et al*. (1979), a phosphorus limited algae had an enzymatic activity of 25 times less than algae with sufficient available phosphorus.

Laotaweesap (2002) observed that the suitable level of phosphorus concentration *C.calcitrans* was 2.4 mg (PO_4^{2-}) L^{-1} which was exactly the amount reported in the modified F/2 medium. Hence, the study for the optimal quantity of phosphorus was not repeated here, rather, the cultivation of *C.calcitrans* with this phosphorus level was carried out in this work using the phosphorus level reported by Laotaweesup (2002). The analysis of the remaining phosphorus in the medium was also provided as shown in Figure 4.3. The results indicated that, although phosphorus was depleted, *C.calcitrans* 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.

4.2 Analysis of bubble shading effect

4.2.1 Existence of bubble shading effect

As described in previous works, Laotaweesup (2002) and Krichnavaruk (2005) reported that the suitable superficial gas velocity for the cultivation of *C.calcitrans* in ALPBR-1 was 3 cm s^{-1} because this condition allowed the light to pass through the column at the highest intensity. In this work, the waterproof probe was inserted into the riser to determine the light intensity in the riser at various superficial gas velocities in the airlift system ALPBR-1 without the cultivation of *C.calcitrans*. The results as given in Table 4.1 illustrate that the light intensity decreased from 10,950 Luxes (148 µmol photons $m^{-2}s^{-1}$) without aeration to 9,950 Luxes (134 µmol photons m^{-2} s^{-1}) with aeration at u_{sg} of 3 cm s^{-1}. This clearly demonstrates the light obstruction/shading effect from the bubbles resided in the airlift. This shading effect was even more obvious at the aeration with u_{sg} of 4 cm s^{-1} where more gas bubbles were retained in both riser and particularly in the downcomer section of the airlift and the light intensity became even lower. In the normal cylindrical airlift configuration (like ALPBR-1), when the system was aerated at high gas throughput, the liquid circulated at a faster speed which therefore contained with it a higher drag force. This could drag more bubbles to go down in the downcomer of the airlift which could then obstruct more light penetration ability. In this case, the light intensity decreased from 10,950 Luxes (148 µmol photons $m^{-2}s^{-1}$) as in the case of without aeration to 9,020 Luxes (122 µmol photons $m^{-2}s^{-1}$). This phenomenon was, in this work, called “bubble shading effect”. The mechanism of bubble shading effect is shown in Figure 4.4.

4.2.2 Bubble shading and cell growth rate

The cultivation of *C.calcitrans* in ALPBR-1 was set for the studying of bubble shading effect. Figure 4.6 demonstrates that the maximum cell concentration of *C.calcitrans* in ALPBR-1 at u_{sg} of 3 cm s^{-1} was about 7.92 x 10^6 cell mL^{-1} and this was reduced to about 7.27 x 10^6 cell mL^{-1} at u_{sg} of 4 cm s^{-1}. This was because, at

superficial gas velocity higher than 3 cm s^{-1}, a large number of gas bubbles were dragged down into the downcomer and obstructed the light penetration. This resulted in a slower growth rate and the maximum cell concentration decreased.

4.2.3 Alternative design for alleviation of bubble shading

In this work, the expanded-top ALPBR (ALPBR-2) was used for the investigation for alleviation of bubble shading effect on the growth of *C.calcitrans*. The expanded-top ALPBR was the conventional internal loop of airlift column which the gas separating section was enlarged to support the disengagement of gas bubbles from liquid surface (detailed in Figure 3.2). The waterproof probe was inserted into the riser to determine the light intensity in the riser at various gas velocities in the system without cell cultivation. Table 4.1 illustrates the light penetration ability in ALPBR-2 and it was clear that, with this design, the obstruction of light seemed to disappear, i.e. the light intensities in the system without and with aeration were similar, and even with the aeration at u_{sg} of 4 cm s^{-1}, the light still could penetrate into the system at the same intensity as that at u_{sg} of 3 cm s^{-1}. The mechanism of bubble shading effect in ALPBR-2 is shown in Figure 4.5.

This finding suggested that the light penetration ability in the system could be improved by allowing bubbles to leave the system, and for this case, this was achieved by enlarging the gas separator section of ALPBR.

Figure 4.6 illustrates the result from the cultivation of the diatom in ALPBR-1 and ALPBR-2 with u_{sg} = 3 and 4 cm s^{-1} in the batch-wise mode. It was found that, at u_{sg} = 3 cm s^{-1}, both the maximum cell concentration and specific growth rate of ALPBR-1 was similar to ALPBR-2 (detailed in Figure 4.6 (a) and Table 4.2). In the ALPBR-1, more gas bubbles existed in the downcomer, however, this did not seem to have significant effect on the cell growth. Hence, the maximum cell concentration and specific growth rate in both columns were similar. On the other hand, as the aeration was enhanced to u_{sg} = 4 cm s^{-1}, there were a larger number of gas bubbles being dragged down into the downcomer, and the light obstruction occurred in ALPBR-1 seemed to place considerable impact on the growth, i.e. the maximum cell concentration of ALPBR-1 was lower than ALPBR-2 (6.5×10^6 and 8.1×10^6 cells mL^{-1}, respectively), but the specific growth rate of ALPBR-1 was higher than ALPBR-2 (5.2×10^{-2} and 4.7×10^{-2} h^{-1}, respectively). Note that the growth rate of *C.calcitrans* in ALPBR-2 at both u_{sg} were quite similar which indicated that the

bubble shading effect in this system was not significant. However, the cultivation in ALPBR-2 demonstrated that an increase in the aeration from u_{sg} = 3 to 4 cm s^{-1} did not have substantially positive effect on the cell growth. This means that, even though the bubble shading effect could be avoided, the growth of *C.calcitrans* seemed to reach its maximum at u_{sg} = 3 cm s^{-1}.

4.3 Self shading effect

4.3.1 Existence of self shading effect

Krichnavaruk et al., (2007) reported the self shading phenomena when they tried to enhance the productivity of *C.calcitrans*. This self shading effect occurred due to the over growth of the diatom and this prevented the light penetration into the dense culture and resulted in the eventual cease of the growth.

4.3.2 Self shading and cell growth rate

Figure 4.7 illustrates the effect of cell density on the penetration capacity of the light through the cell culture. The left y-axis is the deduction of light intensity as it passed through the cell culture at any specific time period whereas the right y-axis is the cell concentration time profile. It was obvious that as cells grew, the culture became more dense and the light could not pass through as effectively as that when the culture was not dense. Krichnavaruk (2005) tried to prove this self shading effect by adding fresh concentrated nutrients into the dense cell culture where the results showed that cells could not grow effectively in that particular circumstance as the growth was limited by the low light intensity.

4.3.3 Alternative design for alleviation of self shading

The use of reactors-in-series was proposed to lessen the self shading problem. In this system, cells were allowed to grow in separate compartments connected in series. The first compartments would then contain the culture at low concentration and therefore was exposed to high light intensity, and only the last compartment would be exposed to high cell density. The effect of self shading therefore could be limited to the last column in the series.

The cultivation of the diatom *C.calcitrans* in ALPBRs-in-series was carried out to investigate the self shading effect due to the dense cell culture. In this system, the diatom was firstly cultivated in the ALPBR-3 (3L) with the ratio between downcomer and riser cross sectional of 3.27 in a similar fashion to the batch cultivation. Compressed air was supplied with u_{sg} = 3 cm s^{-1} and light intensity was controlled at approx. 10,000 Luxes (measured at the outer wall). When the growth of the diatom reached the mid exponential region, the columns were connected in series and fresh medium was fed into the first column (at dot line in the figure). The schematic diagram of this setup is illustrated in Figure 3.3. The volumetric feed rates (F) were manipulated using peristaltic pumps. The reactor-in-series setup separates the growth of the diatom into several stages, each with different level of cell concentration. In the first column, the cell concentration was remained at low level to ensure effective light penetration and a high level of photosynthesis. On the other hand, the last column contained culture at high cell density, as required for the harvest. Although this high density would block the light penetration, the low light intensity zone was limited only to the last column instead of having only one column which would cause the whole culture to be subject to low light condition.

Figure 4.8 illustrates the result from the cultivation of the diatom in System I (F = 1, 2 and 3 mL min^{-1}). It was observed that, when medium was fed to the ALPBR (dot line) the cell concentration decreased and could be stable. The highest harvest cell concentration was observed about 6.3 x 10^5 cell mL^{-1} with F = 3 mL min^{-1}. The maximum cell concentration was increased by the two and three connected ALPBR-3 as shown in Figure 4.9 and Figure 4.10, respectively. Figure 4.9 and Figure 4.10 show that, cell concentration in the first column was decreased (at F = 4 and 6 mL min^{-1} in system II, and at F = 6 and 9 mL min^{-1} in system III) by the dilution rate. More details were shown in Table 4.4.

4.4 Harvesting modes for the cultivation of *C. calcitrans*

4.4.1 Batch cultivation

The batch cultivation is perhaps one of the most conventional methods for the cultivation of microbial population. It is the method that, more often than not, gives the highest cell concentration. However, this system often requires high labor

cost and some time high operating cost due to the batch change-over and other unnecessary steps regarding the unsteady state operation such as washing tanks, etc. In this work, the batch cultivation in the 17 L ALPBR was performed and yielded the final cell concentration of 7.9 x 10^6 cell mL^{-1} with specific growth rate of 7.1 x 10^{-2} h^{-1}. This was converted to the specific productivity of 2.85 x 10^4 cell L^{-1} s^{-1}.

4.4.2 Semi-continuous cultivation

The batch cultivation, although could provide a relatively high final cell concentration, but often suffered from an initially slow growth regime and loss of time during the final system cleansing. The cultivation of *C.calcitrans* in the semi-continuous system was therefore investigated to examine the probability of enhancing the productivity of the diatom. Figure 4.11 illustrates the result for the semi-continuous cultivation of *C.calcitrans* in the ALPBR-1 in which the diatom was cultivated with an initial cell concentration of about 1.0 x 10^5 cells mL^{-1} and u_{sg} = 3 cm s^{-1}. The diatom was initially cultured as a batch system for 72 hours to ensure an active cell condition before harvesting. Approximately 15 L of culture was harvested where a fresh modified standard F/2 solution was then added to ALPBR-1 to replace the harvested volume. The new initial cell concentration was approximately 1.0 x 10^6 cells mL^{-1}. The culture was then continued for 20 hours before the next harvest cycle to allow the diatom to reach the exponential phase where the diatom was most active. An average maximum cell concentration achieved from each cycle was around 6.32 x 10^6 cells mL^{-1} with a specific growth rate of about 8.97 x 10^{-2} h^{-1}. The summary of the performance in each cycle of the semi-continuous system is provided in Table 4.3. The cultivation of *C.calcitrans* in the semi-continuous system achieved an average specific productivity of 7.32 x 10^4 cell $L^{-1}s^{-1}$.

4.4.3 Continuous cultivation

The continuous cultivation in this work was carried out in three small airlift systems, i.e. one 3L airlift photobioreactor, two 3L airlift photobioreactors-in-series, and three 3L airlift reactors-in-series. At the most suitable operating conditions, these systems could provide the culture different specific productivity and growth rate as summarized below:

For one 3L airlift system:

Final cell concentration	6.3×10^6 cell mL^{-1}
Specific productivity	10.5×10^4 cell L^{-1} s^{-1}
Specific growth rate	0.06 h^{-1}

For two 3L airlifts-in-series system:

Final cell concentration	8×10^6 cell mL^{-1}
Specific productivity	4.44×10^4 cell L^{-1} s^{-1}
Specific growth rate	0.02 h^{-1}

For three 3L airlifts-in-series system:

Final cell concentration	12×10^6 cell mL^{-1}
Specific productivity	6.67×10^4 cell L^{-1} s^{-1}
Specific growth rate	0.02 h^{-1}

It can be seen that, although the three reactors-in-series could provide the culture with ultrahigh cell density (12 million cells per one milliliter), the final specific productivity was not quite attractive. On the other hand, the one tank mode could provide a better specific productivity at 10.5×10^4 cell L^{-1} s^{-1} which was considered the highest among all the options considered in this work.

4.5 Economics of cultivation systems for *C. calcitrans*

The analytical economics for the cultivation of *C. calcitrans* in various modes was carried out where the results are shown in Table 4.5. The systems involved in this analysis were the batch cultivation (ALPBR-1), semi-continuous cultivation (ALPBR-1), continuous cultivation (ALPBR-3) and the continuous in series cultivation (3 ALPBR-3). This analysis was based on the production of 1×10^{12} cells. It was observed that the most attractive system for the cultivation of the diatom *C. calcitrans* was the continuous system with the medium feed rate of 3 mL min^{-1}. The specific operating cost, approx. 6.31×10^{-3} THB $L^{-1}h^{-1}$ (1.31×10^{-3} THB mg^{-1} h^{-1}), which was the lowest when compared to any other systems. The benefits of this system were derived from a number of advantages when compared to the larger systems, i.e. low overhead charge due to the labor cost, minimal lost of time during the start up and shut down period or for system maintenance. The maintenance and equipment costs in this

system, i.e. reactor, air compressor and light source, were also lower than the set up for larger systems.

Table 4.1 Light intensity in ALPBR before the cultivation of *C.calcitrans*

Superficial gas velocity, u_{sg} (cm s^{-1})	Light intensity (Luxes)			
	ALPBR-1		ALPBR-2	
	Without aeration	With aeration	Without aeration	With aeration
3	10,950	9,950	11,010	10,960
4	10,950	9,020	11,010	10,950

Table 4.2 Maximum cell concentration and specific growth rate in ALPBR for the cultivation of *C.calcitrans*

Superficial gas velocity, u_{sg} (cm s^{-1})	ALPBR-1		ALPBR-2	
	Maximum cell concentration (cell mL^{-1})	Specific growth rate (h^{-1})	Maximum cell concentration (cell mL^{-1})	Specific growth rate (h^{-1})
3	$(7.9 \pm 0.02) \times 10^6$	7.1×10^{-2}	$(7.3 \pm 0.02) \times 10^6$	7.0×10^{-2}
4	$(6.5 \pm 0.07) \times 10^6$	5.2×10^{-2}	$(8.1 \pm 0.02) \times 10^6$	4.7×10^{-2}

Table 4.3 Initial and harvest cell concentrations, productivity and specific growth rate of *C.calcitrans* with semi-continuous culturing system in ALPBR-1 (harvested at every 20 h)

Cycle	Initial cell concentration (cell mL^{-1})	Harvested cell concentration (cellmL^{-1})	Specific growth rate (h^{-1})	Specific productivity (cell $L^{-1}.s^{-1}$)	Specific productivity (cell $L^{-1}.s^{-1}$) (Krichnavaruk 2005)
1	1.00×10^6	6.00×10^6	8.96×10^{-2}	6.94×10^4	6.35×10^4
2	1.05×10^6	6.25×10^6	8.92×10^{-2}	7.22×10^4	6.64×10^4
3	1.06×10^6	6.62×10^6	9.16×10^{-2}	7.72×10^4	6.01×10^4
4	1.10×10^6	6.42×10^6	8.82×10^{-2}	7.39×10^4	6.38×10^4
Average	$\mathbf{1.05 \times 10^6}$	$\mathbf{6.32 \times 10^6}$	$\mathbf{8.97 \times 10^{-2}}$	$\mathbf{7.32 \times 10^4}$	$\mathbf{6.35 \times 10^4}$

Table 4.4 Productivity and specific productivity for the cultivation of *C.calcitrans* in ALPBRs-in-series systems

Experiment	System	Medium feed rate (mL min^{-1})	Dilution rate (h^{-1})	Productivity (cell s^{-1})	Specific productivity (cell L^{-1} s^{-1})	Specific productivity (cell L^{-1} s^{-1}) (Krichnavaruk et al., 2005)
set 1	I	1	0.02	0.82×10^5	2.72×10^4	-
set 2	I	2	0.04	1.77×10^5	5.89×10^4	2.32×10^4
set 3	I	3	0.06	3.15×10^5	10.5×10^4	2.89×10^4
set 4	II	2	0.02	2.7×10^5	9×10^4	-
set 5	II	4	0.04	washout	washout	washout
set 6	II	6	0.06	washout	washout	-
set 7	III	3	0.02	6.06×10^5	6.74×10^4	-
set 8	III	6	0.04	washout	washout	-
set 9	III	9	0.06	washout	washout	-

Table 4.5 Economical analysis for the cultivation of *C.calcitrans*

	Working volume			
	Batch (ALPBR-1)	**Semi-continuous (ALPBR-1)**	**Continuous (ALPBR-3)**	**Continuous in series (3 ALPBR-3)**
Effective volume (L) [A]	**17**	**14**	**-**	**-**
Cycle time (h) [B]	**84**	**20**	**-**	**-**
Productivity (cells s^{-1}) [C]	**4.93×10^5**	**1.02×10^6**	**3.15×10^5**	**6.03×10^5**
Specific productivity (cells $L^{-1}s^{-1}$) [D]	**2.9×10^4**	**7.32×10^4**	**10.5×10^4**	**6.7×10^4**
Cell mass concentration (mg L^{-1}) [E]*	**4.90**	**4.79**	**4.79**	**5.18**
Cultivation time (h) [F=(1x10^{12}÷3,600)÷C]	**564**	**271**	**882**	**461**
Number of cycle (-) [G=F÷B]	**6.7**	**14**	**-**	**-**
Total volume of seawater used (L) [H=AxG]	**114**	**196**	**159**	**83**
Cost of water, 0.06 THB L^{-1} (THB) [I=0.06xH]	**6.84**	**11.76**	**9.54**	**4.98**
Cost of nutrient, 1 THB L^{-1} (THB) [J=Hx1]	**114**	**196**	**159**	**83**
Power of air compressor (W) [K]	**100**	**100**	**30**	**100**
Power of light source (W) [L]	**480**	**480**	**240**	**720**
Total electrical unit (units) [M=(K+L)xF÷1,000]	**327**	**157**	**239**	**378**
Electrical cost, 3 THB per unit (THB) [N=3xM]	**981**	**471**	**715**	**1,134**
Total operating cost (THB) [O=I+J+N]	**1,102**	**679**	**884**	**1,222**
Operating cost per hour (THB h^{-1}) [P=O÷F]	**1.95**	**2.51**	**1**	**2.65**
Specific operating cost (volume) (THB $L^{-1}h^{-1}$) [Q=P÷H]	**1.71×10^{-2}**	**1.28×10^{-2}**	**6.31×10^{-3}**	**3.19×10^{-2}**
Specific operating cost (mass) (THB $mg^{-1}h^{-1}$) [R=Q÷E]	**3.49×10^{-3}**	**2.67×10^{-3}**	**1.31×10^{-3}**	**6.16×10^{-3}**

* calculated from correlation reported by Laotaweesup (2002)

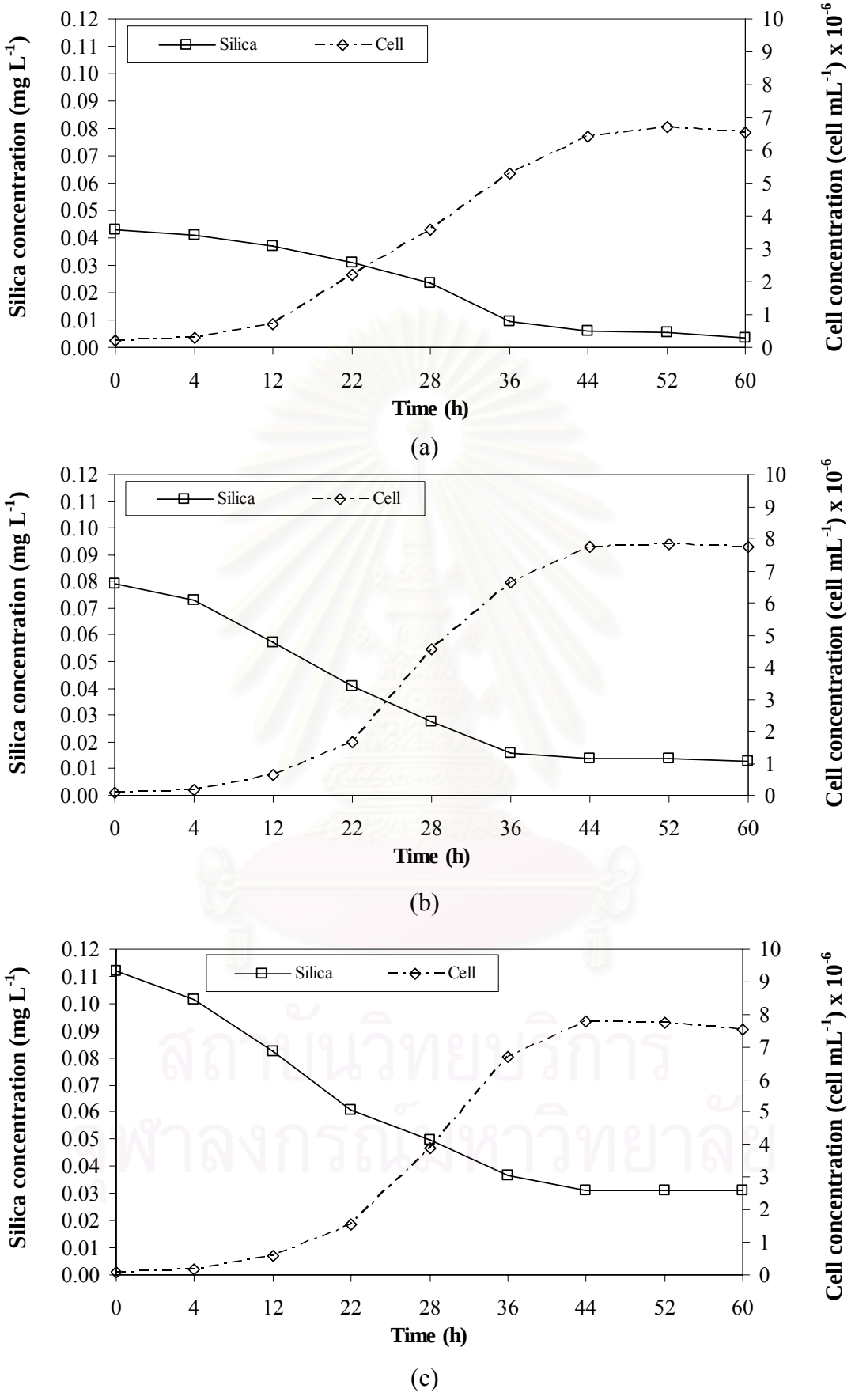
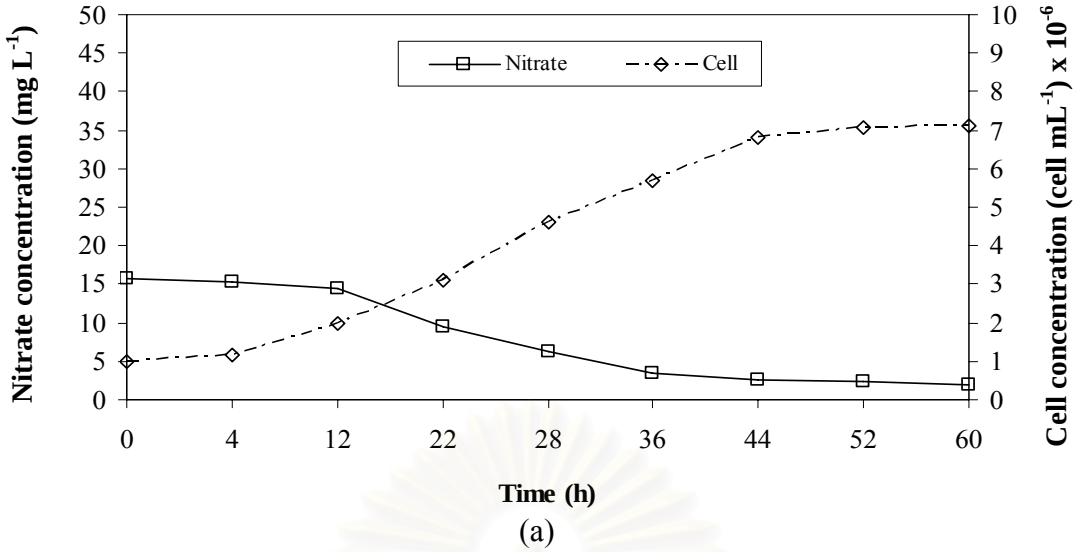
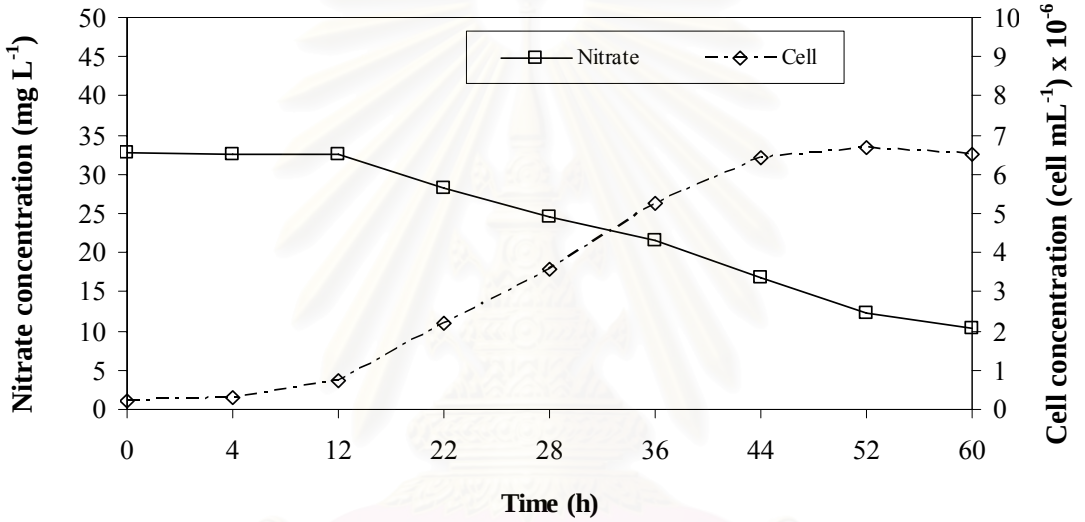


Figure 4.1 Growth behavior and silica concentration from the cultivation of *C.calcitrans* in the modified F/2 medium: (a) 1x silica content, (b) 2x silica content and (c) 3x silica content



(a)



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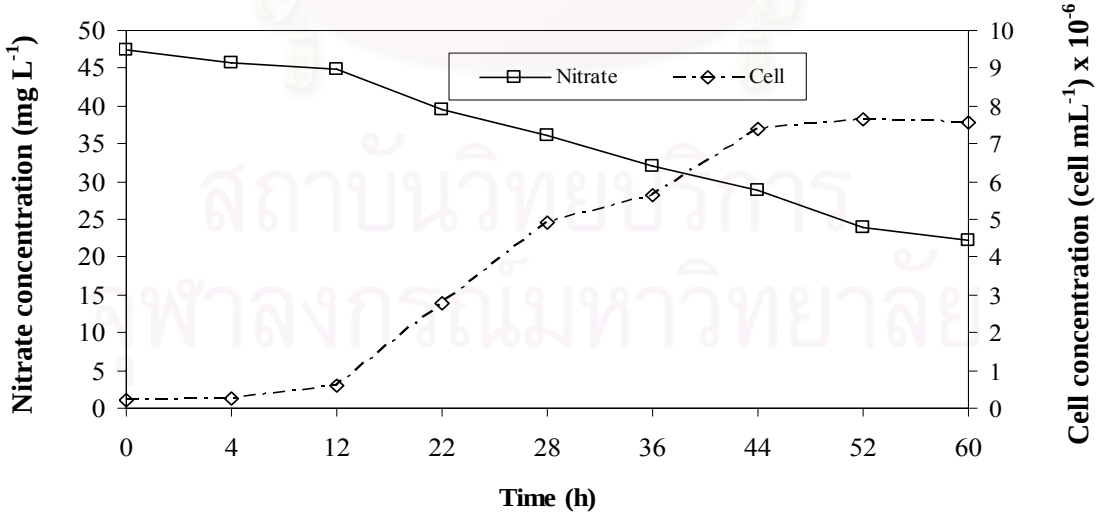


(c)

Figure 4.2 Growth behavior and nitrate concentration from the cultivation of *C.calcitrans* in the modified F/2 medium: (a) 1x nitrate content, (b) 2x nitrate content and (c) 3x nitrate content

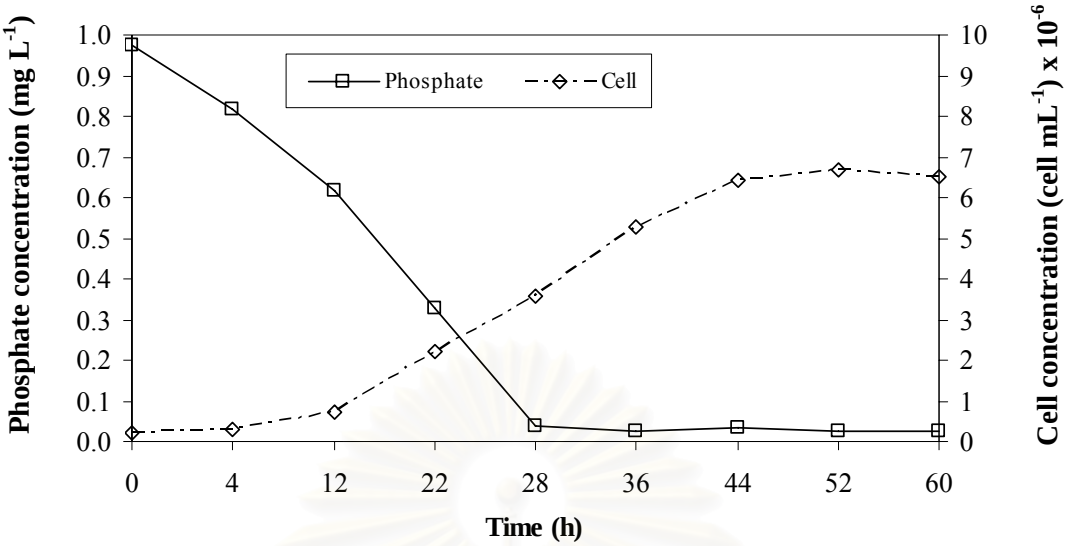


Figure 4.3 Growth behavior and phosphate concentration from the cultivation of *C. calcitrans* in the modified F/2 medium

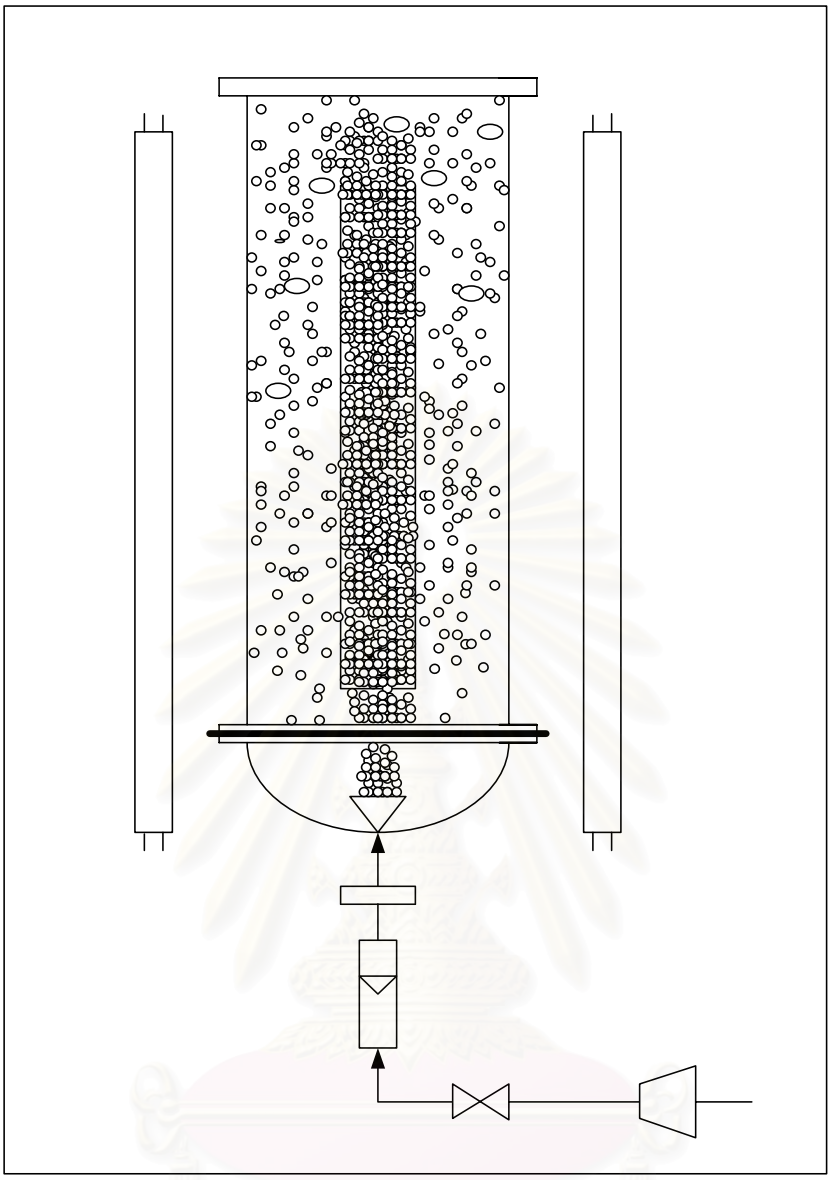


Figure 4.4 Mechanism of bubble shading effect in ALPBR-1

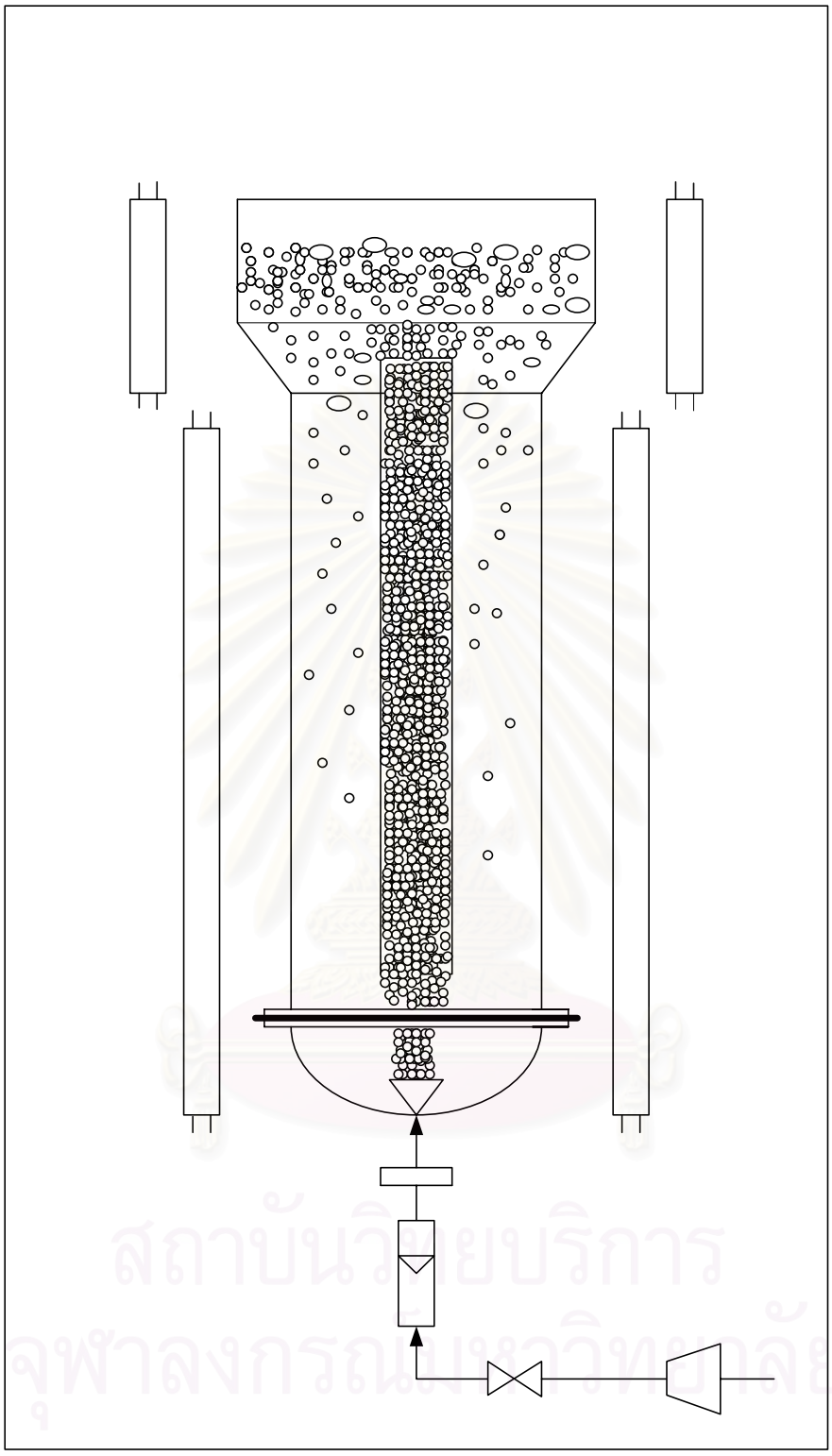
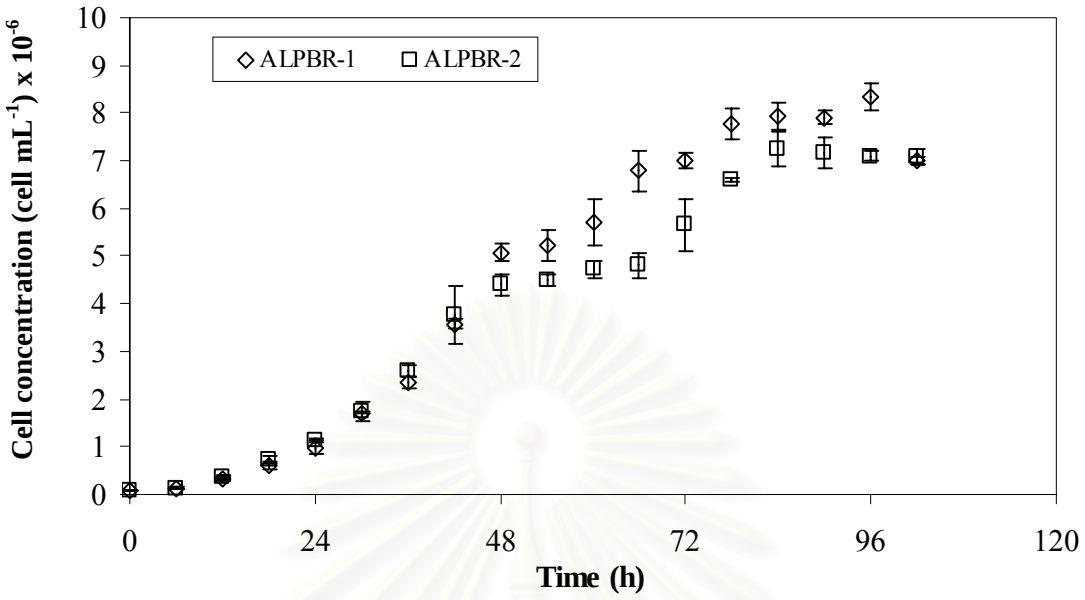
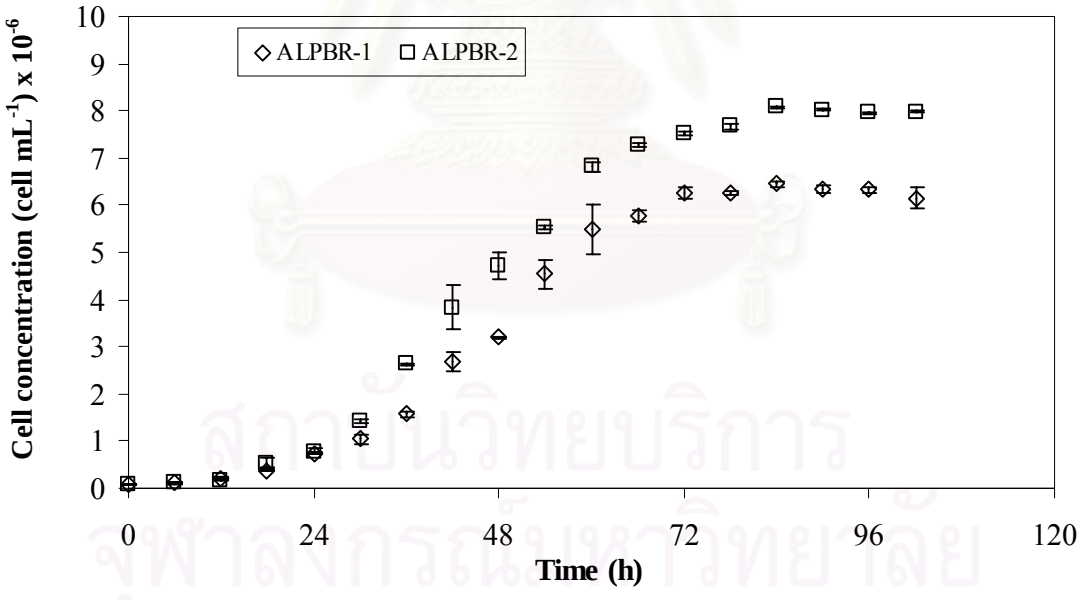


Figure 4.5 Mechanism of bubble shading effect in ALPBR-2



(a)



(b)

Figure 4.6 Comparison between growth behavior of *C. calcitrans* in ALPBR-1 and ALPBR-2: (a) u_{sg} = 3 cm s^{-1} and (b) u_{sg} = 4 cm s^{-1}

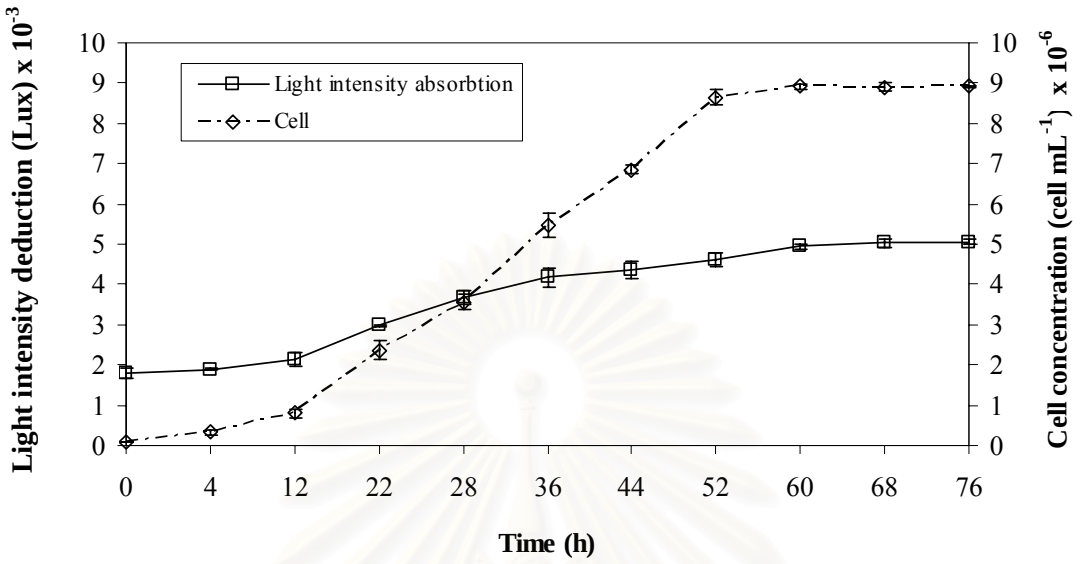


Figure 4.7 Growth behavior of *C.calcitrans* in ALPBR-3 with u_{sg} of 3 cm s^{-1}

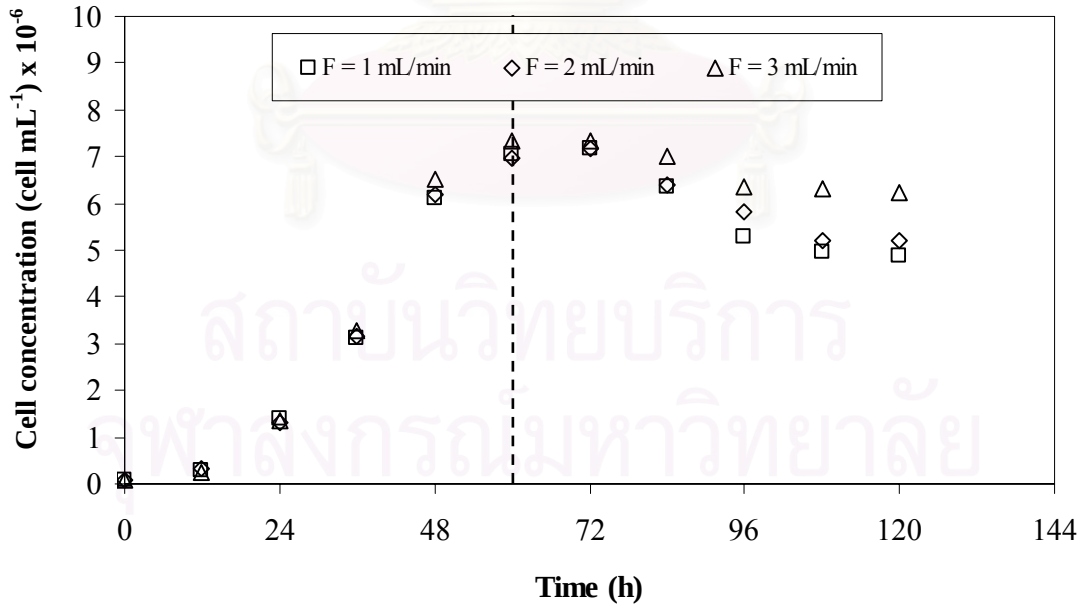


Figure 4.8 Growth behavior of *C.calcitrans* in ALPBRs-in- series (System I) with medium feed rate, F of 1, 2 and 3 mL min^{-1}

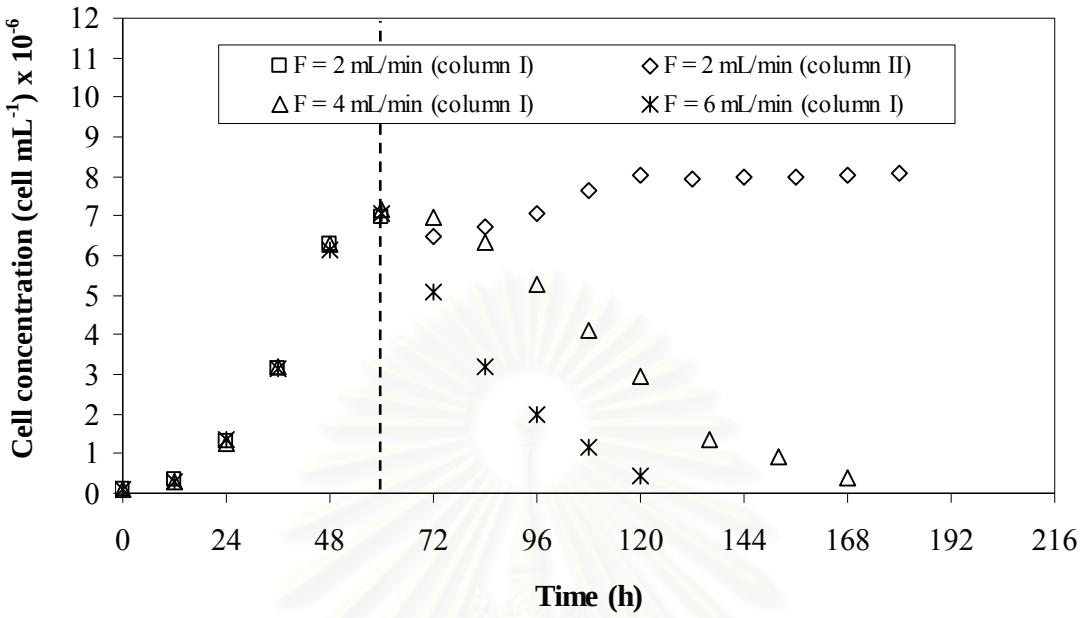


Figure 4.9 Growth behavior of *C.calcitrans* in ALPBRs-in-series (System II) with medium feed rate, F of 2, 4 and 6 mL min^{-1}

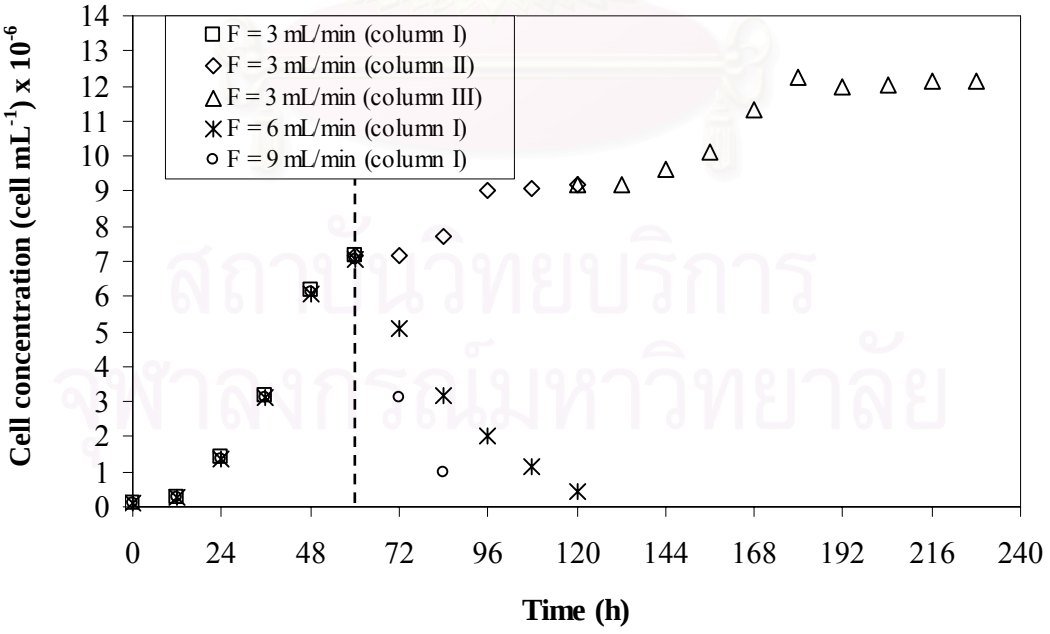


Figure 4.10 Growth behavior of *C.calcitrans* in ALPBRs-in-series (System III) with medium feed rate, F of 3, 6 and 9 mL min^{-1}

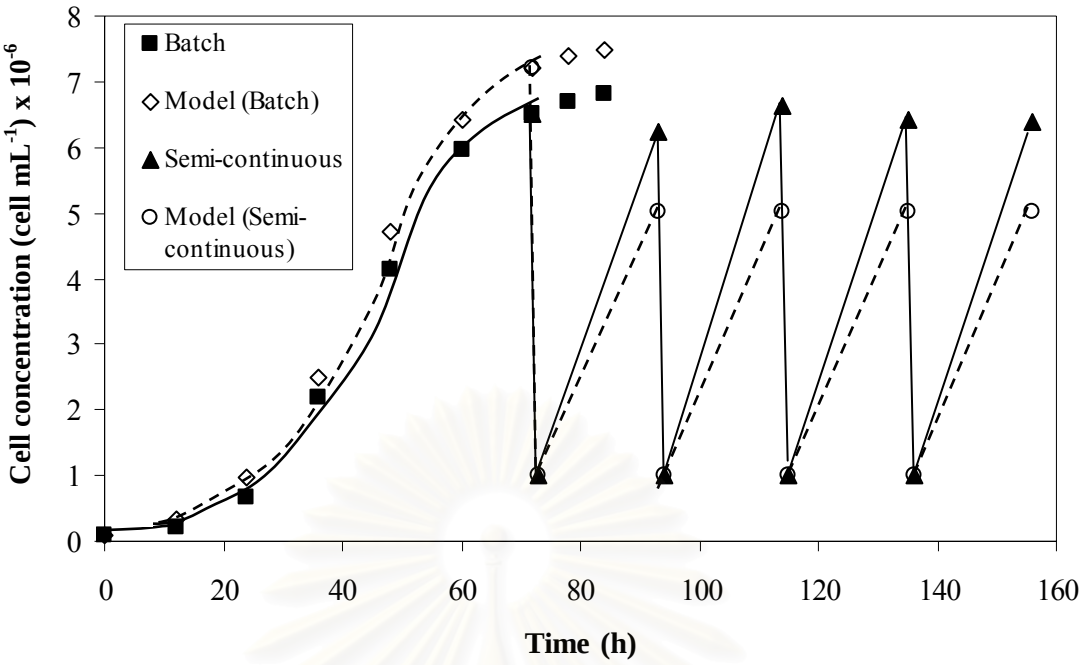
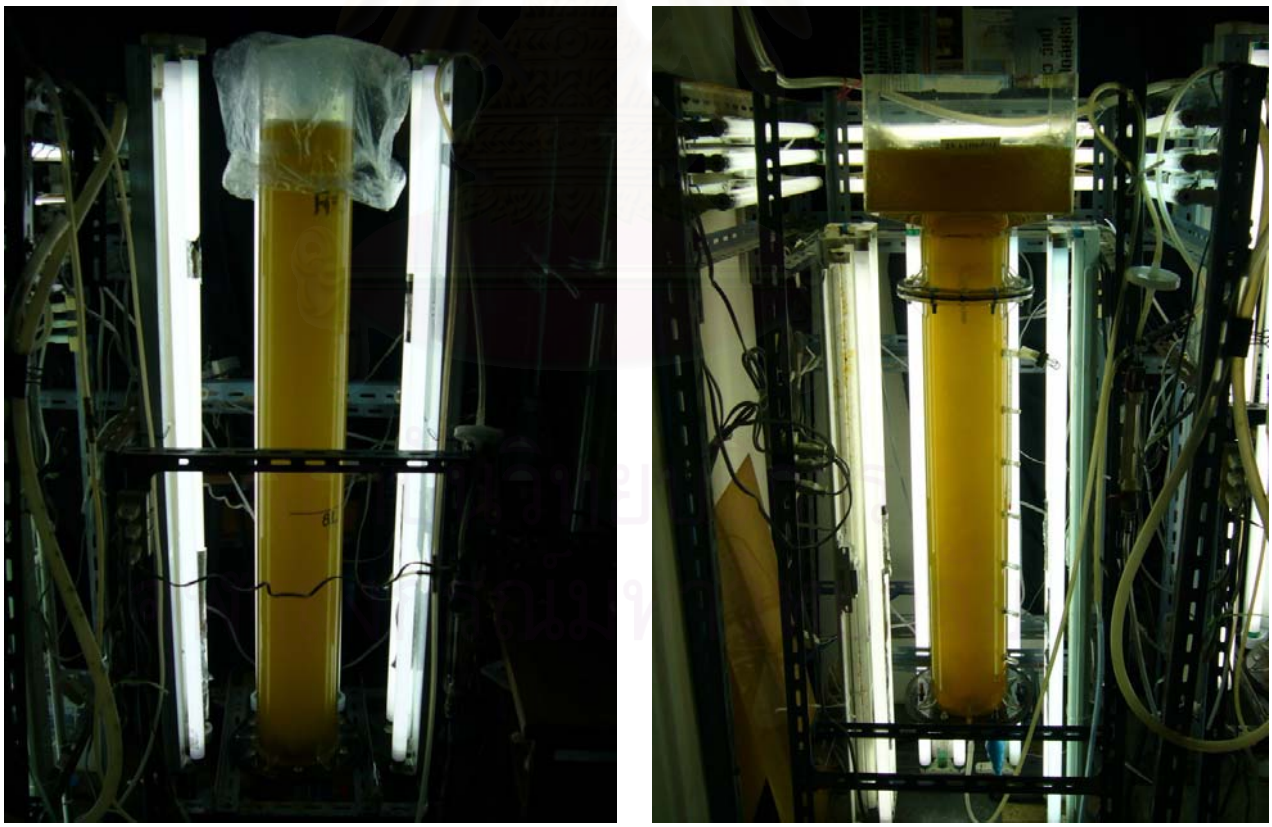


Figure 4.11 Growth behavior of *C.calcitrans* in batch and semi-continuous cultures (ALPBR-1 with u_{sg} = 3 cm s^{-1}) and growth behavior from model prediction



(a) (b)

Figure 4.12 Cultivation of *C.calcitrans* in airlift photobioreactor: (a) ALPBR-1 and (b) ALPBR-2

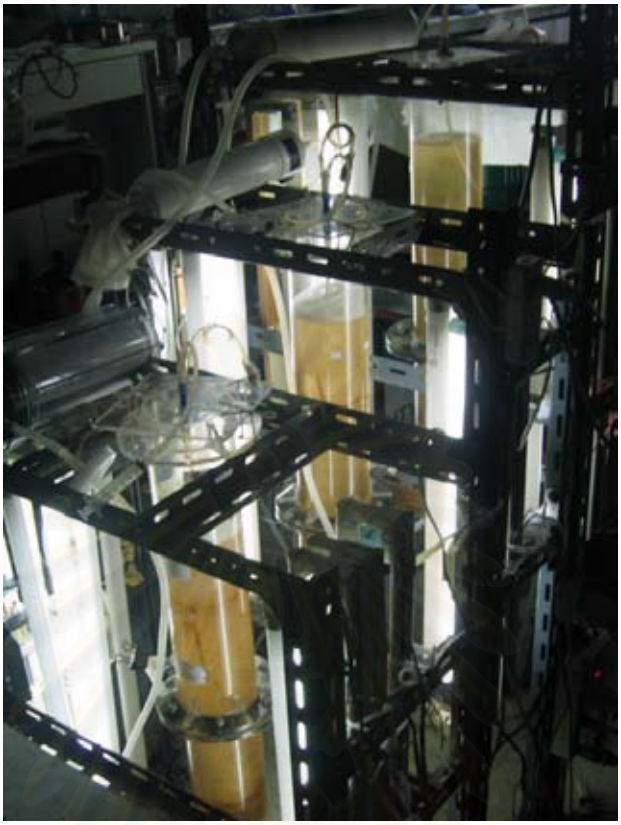


Figure 4.13 Cultivation of *C.calcitrans* in ALPBR-3

CHAPTER V

CONCLUSIONS AND CONTRIBUTION

5.1 Conclusions

The growth limiting factors were investigated for the cultivation of *Chaetoceros calcitrans*. These factors included nutrients, light obstruction with bubbles and with dense cells. In addition, the cultivations of *Chaetoceros calcitrans* in several types of photobioreactors were examined for the most suitable reactor configuration that gave the highest productivity. Major findings from this work can be summarized as follows:

5.1.1 Effect of nutrient content

The nutrient content in the modified F/2 medium did not have significant impacts on the cell culture in terms of final cell concentration, but did have some influence on the specific growth rate.

5.1.2 Bubble shading effect

Light obstruction due to the presence of excessive bubble in the airlift system was also found to limit the growth of the diatom, and this was called "bubble shading effect". The cultivation of the diatom in the expanded-top airlift photobioreactor made easy the disengagement of the bubbles at the top section of the system and reduced the bubble shading effect. However, although the shading effect at high aeration rate was eliminated with this modified configuration of airlift system, the growth was not significantly enhanced the maximum cell concentration.

5.1.3 Self shading effect

At high cell concentration, the growth was limited by the self shading effect in which the light penetration was obstructed by the dense cell culture. The use of reactors-in-series could decrease this effect because cells were allowed to grow in separate columns connected in series. In this configuration, the first column contained

culture at low concentration and therefore was subject to high light intensity, and the last column would contain high cell density where the light availability was limited. The effect of self shading therefore was confined to the last column in the series. With this configuration, it was possible to achieve an ultrahigh cell density. However, the final analysis indicated that, although the single reactor configuration could be subject to self shading effect, it could provide a better productivity than the three reactors-in-series configuration.

5.2 Contribution

This work provides the more in-depth analysis of the requirement of several types of nutrients in the cultivation of *Chaetoceros calcitrans*. It was found that some of the nutrients should be enhanced to augment the growth rate of the diatom, but this did not seem to be able to improve the maximum cell density. Several modes of cultivation of *Chaetoceros calcitrans* were proposed in this work including batch, semi-continuous and continuous cultivations. The most suitable operating conditions for each type of cultivation were investigated, and it was the first time that the cultivation of *Chaetoceros calcitrans* could reach the level of more than 10×10^6 cell mL^{-1}, i.e. 12.13×10^6 cell mL^{-1} could be obtained from the cultivation in the three reactors-in-series in a continuous mode as the limiting factors both in terms of bubble shading and cell shading effects were dealt with effectively. Among all the systems examined in this work, the most suitable system was the 3 L continuous system with a medium feed rate of 3 mL min^{-1}. This was because this system could provide the highest specific productivity when compared with all other cultivation configurations. In addition, the small system also benefits from low investment and operating costs, ease of maintenance, e.g. the startup and shut down periods was easy and does not require intensive labor. Although the addition of CO_2 might further enhance the growth of the diatom, this was not examined in this work. This was due to economical reasons as the supply of CO_2 might not justify the cost of the culture product. Hence, the outcome from this work is ready to be reported and to be applied directly in the actual aquaculture industry.

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APPENDIX

Appendix A : Measurement of nutrient concentrations in the modified F/2 medium

The concentration of nutrient in the medium can be determined using Spectrophotometer as follows:

Silicon (Resuced Molybdosilicate Spectrophotometric Method)

- Take the sample for m ALPBR and separate diatom from the culture medium by centrifuge at 3,000 rpm with 30 min
- Take the clear solution about 1 ml for the measurement of silicon
- Add 1 mL of sample solution to 0.4 mL of Molybdate in the polyethelene bottle which cleaned by sample solution, mix and wait about 10 min
- Add 0.6 mL of reducing reagent (ISO 3361-1975) into the mix solution
- After 2-3 h, measure the solution by Spectrophotometer with wavelength 810 nm
- Calculate the concentration of silicon (mg Si L^{-1}) with standard curve as shown in Figure A-1

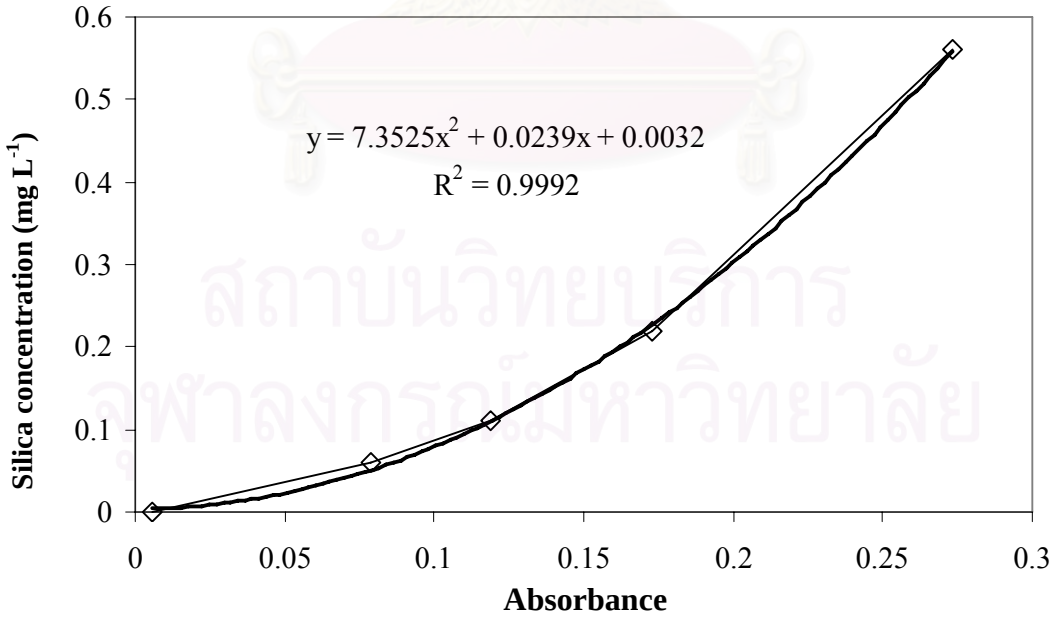

Figure A-1: Standard curve for the calculation of silica concentration in modified F/2 medium

Nitrogen (as nitrate)

(Ultraviolet Spectrophotometric Screening Method)

- Take the sam ple for m ALPBR and separate diatom from the culture medium by centrifuge at 3000 rpm with 30 min
- Take the clear solution about 3 ml for the measurement of nitrate
- Measure the sam ple solution by Spect rophotometer with nitrate m ode (Clesceri et al, 1998), a nd calculate the concentr ation of nitrate (m g NO_3 L^{-1}) with standard curve as shown in Figure A-2

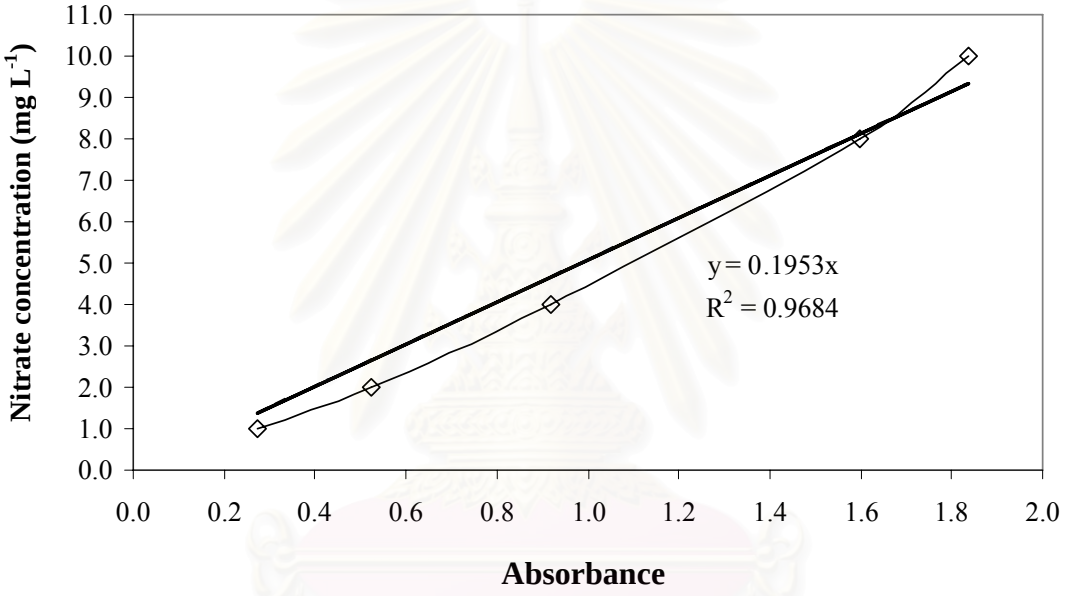

Figure A-2: Standard curve for the calculation of nitrate concentration in modified F/2 medium

Phosphorus (as phosphate)

(Ascorbic Acid Methods)

- Take the sam ple for m ALPBR and separate diatom from the culture medium by centrifuge at 3000 rpm with 30 min
- Take the clear solution about 1 ml for the measurement of phosphate
- Add 0.1 mL of m ix reagent (Clesceri et al, 1998) into the sample solution and shake

- Measure the sample solution by Spectrophotometer with phosphate mode, and calculate the concentration of phosphate (mg PO_4 L^{-1}) with standard curve as shown in Figure A-3

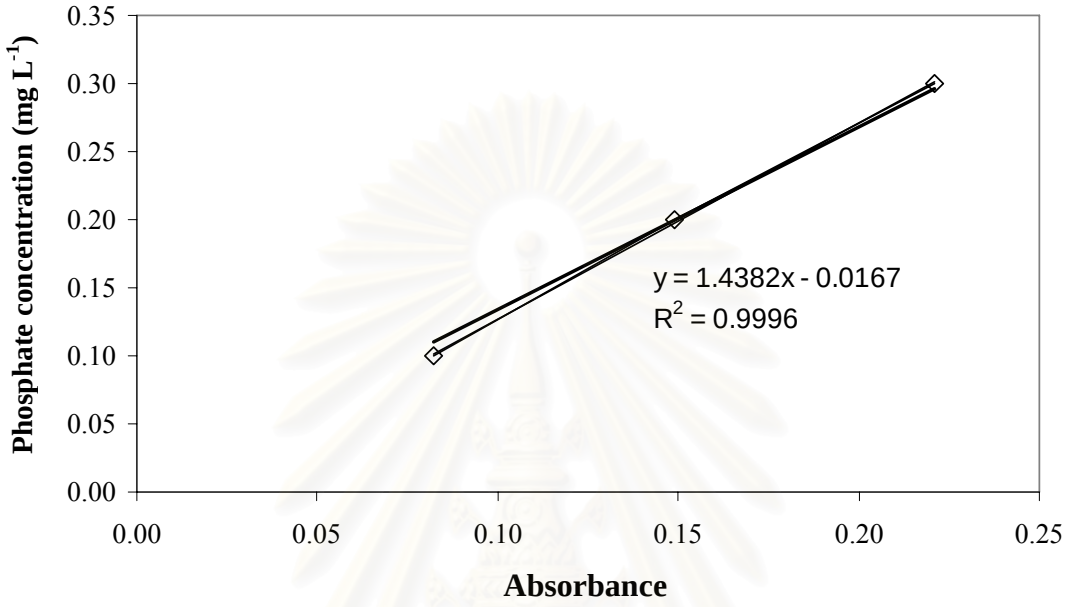

Figure A-3: Standard curve for the calculation of phosphate concentration in modified F/2 medium

BIOGRAPHY

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