การเพาะเลี้ยงไดอะตอม *Skeletonema costatum* ในถังปฏิกรณ์ชีวภาพเชิงแสงแบบอากาศยก

นางสาวศรัญญา มงคลสิทธิ์

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

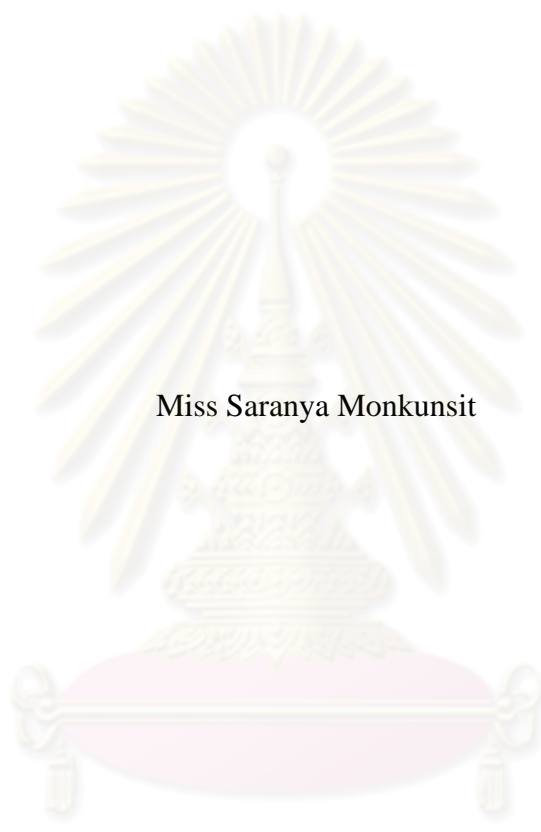CULTIVATION OF A DIATOM *Skeletonema costatum*
IN AIRLIFT PHOTOBIOREACTOR

Miss Saranya Monkunsit

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Engineering Program in Chemical Engineering
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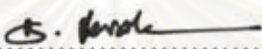Faculty of Engineering
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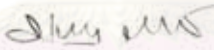
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ศรัญญา มงคลสิทธิ์ : การเพาะเลี้ยงไดอะตอม *Skeletonema costatum* ในถังปฏิกรณ์ชีวภาพเชิงแสง
แบบอากาศยก (CULTIVATION OF A DIATOM *Skeletonema costatum* IN AIRLIFT
PHOTOBIOREACTOR) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : รศ.คร. ประเสริฐ ภวสันต์, อ.ที่ปรึกษา
วิทยานิพนธ์ร่วม: คร. สรวิศ เผ่าทองศุข, 63 หน้า.

งานวิจัยนี้ได้ศึกษาการเพาะเลี้ยงไดอะตอม *Skeletonema costatum* ในถังปฏิกรณ์ชีวภาพเชิงแสงแบบ
อากาศยกเปรียบเทียบกับถังสัมผัสแบบธรรมดาปริมาตร 3 ลิตร การทดลองเริ่มต้นโดยการหาสูตรอาหาร
ที่เหมาะสมต่อการเจริญเติบโตของ *Skeletonema costatum* พบว่าเซลล์เจริญเติบโตได้ดียิ่งขึ้นเมื่อเพาะเลี้ยงในสูตร
อาหารที่เพิ่มความเข้มข้นของซิลิกา 4 เท่าของปริมาณที่ใช้ในสูตรอาหารมาตรฐาน F/2 (กิลลาร์ค) การเพาะเลี้ยง
เซลล์ในถังปฏิกรณ์ชีวภาพเชิงแสงแบบอากาศยกพบว่า ให้ผลดีกว่าการเพาะเลี้ยงในถังสัมผัสแบบธรรมดา
เล็กน้อย เมื่อเลี้ยงในถังปริมาตร 3 ลิตรเท่ากันและให้อากาศที่ 1.5 เซนติเมตรต่อวินาที ความเข้มแสง 34 ไมโคร
โมลโฟตอนต่อตารางเมตรต่อวินาที จากสภาวะการเพาะเลี้ยงดังกล่าวทำให้ได้ความเข้มข้นของเซลล์สูงสุด,
ค่าอัตราการเจริญเติบโตจำเพาะ และผลผลิตของเซลล์สูงสุด ที่ได้จากการเพาะเลี้ยงเซลล์ในถังปฏิกรณ์ชีวภาพ
เชิงแสงแบบอากาศยก คือ 4.6 x 10^6 เซลล์ต่อมิลลิลิตร, 0.07 ต่อชั่วโมงและ 6.6 x 10^4 เซลล์ต่อวินาที
เมื่อเปรียบเทียบกับถังสัมผัสแบบธรรมดา คือ 3.8 x 10^6 เซลล์ต่อมิลลิลิตร, 0.06 ต่อชั่วโมงและ 4.1 x 10^4 เซลล์ต่อ
วินาที ทั้งนี้เนื่องจากการไหลของของไหลในถังปฏิกรณ์ชีวภาพแบบอากาศยกเป็นไปอย่างมีรูปแบบ ส่งผลให้
เซลล์ได้รับและสัมผัสกับแสง อาหาร และอากาศได้อย่างทั่วถึงส่งผลดีแก่การเจริญเติบโตของเซลล์ สภาวะ
ที่เหมาะสมในการเพาะเลี้ยงเซลล์สำหรับการดำเนินการแบบกะในถังปฏิกรณ์ชีวภาพเชิงแสงแบบอากาศยกคือ
มีพื้นที่ของส่วนให้อากาศต่อส่วนที่ไม่ให้อากาศเท่ากับ 3.27 ทำการให้อากาศที่ 1.5 เซนติเมตรต่อวินาที ความเข้ม
แสง 34 ไมโครโมลโฟตอนต่อตารางเมตรต่อวินาที

ภาควิชา วิศวกรรมเคมี	ลายมือชื่อนิสิต
สาขาวิชา วิศวกรรมเคมี	ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก
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SARANYA MONKUNSIT: CULTIVATION OF A DIATOM *Skeletonema costatum* IN AIRLIFT PHOTOBIOREACTOR. THESIS ADVISOR: ASSOC. PROF. PRASERT PAVASANT, Ph.D., THESIS CO-ADVISOR: SORAWIT POWTONGSOOK, Ph.D., 63 pp

This work aimed to examine the performance of 3 L airlift photobioreactor system on the cultivation of *Skeletonema costatum* compared to that in bubble column. Firstly, the experiment was set out to determine appropriate medium for *Skeletonema costatum* where it became apparent that the standard F/2 medium (Guillard's medium) could only yield the best growth character when the silicon concentration increased 4 times than the normal value. The cultivation of cell in airlift photobioreactor was found to provide a slightly better performance than the bubble column where the maximum cell concentration, specific growth rate, and productivity in the airlift were 4.6×10^6 cell mL^{-1}, 0.07 h^{-1}, and 6.6×10^4 cell s^{-1} compared with 3.81×10^6 cell mL^{-1}, 0.06 h^{-1}, and 4.1×10^4 cell s^{-1} in the bubble column of the same size (3L) and operated at the same aeration rate ($u_{sg} = 1.5$ cm s^{-1}) and light intensity (34 µmol photons m^{-2} s^{-1}). This was because the airlift photobioreactor allowed circulatory flow in the system, minimizing cell precipitation and enhancing light utilization efficiency, and this positively affected the cell growth. In batch culture, the optimal operation was found to occur when the ratio between downcomer and riser cross sectional area (A_d:A_r) was 3.27, the superficial gas velocity (u_{sg}) was 1.5 cm s^{-1} and the light intensity was 34 µmol photons m^{-2} s^{-1}.

ศูนย์วิทยทรัพยากร

จุฬาลงกรณ์มหาวิทยาลัย

Department : __Chemical Engineering__	Student's Signature _Saranya Monkunsit_
Field of Study : __Chemical Engineering__	Advisor's Signature _____
Academic Year : __2008__	Co-Advisor's Signature _____

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CHAPTER I

INTRODUCTION

1.1 Rationale

One important factor of effective shrimp cultivation is the feed which must have the following characteristics: easily digesting, proper size and shape, containing completed nutritions. The food for shrimp larvae in the first protozoea stage (zoea stage) in Thailand is generally diatoms. The use of diatom as shrimp larvae's food was found to be better than artificial feed (Pechmanee, 1997). The other advantage of diatom as shrimp's larvae food is the size of diatom, which must be small enough and suitable for the shrimp larvae. Moreover, diatom food will induce less water pollution compared to artificial feed. The small diatom such as *Chaetoceros calcitrans* and *Skeletonema costatum* already contains essential nutrients required for shrimp larvae growth (Enright et al., 1986). *Chaetoceros calcitrans* is usually used in the eastern part of Thailand whereas *Skeletonema costatum* is usually in the south, both being equally popular, the choice of which depends on the experience of the farmers.

The cultivation of both *Chaetoceros calcitrans* and *Skeletonema costatum* in Thailand is generally performed in 4 steps. Step 1; stock culture of the diatom is cultivated as dense cell culture in a 1 L Erlenmeyer flask for 1−2 days: Step 2; the culture inoculated from Step 1 is expanded to a bigger container such as glass bottle or flask containing fresh sea water (30 ppt). Step 3; essential nutrients are added. Step 4; the cells from Step 3 are being scaled up in a larger scale tank such as cement tank or plastic container. After this stage, the diatom is then moved to larger ponds, 2−3 tons in volume, and left growing for a few more days before it is ready to be used in feeding the shrimp larvae. However, the drawback of this method is that the cultivation in the last steps is done in an open pond which is more susceptible to contamination. These contaminants may be harmful for the aquatic cultures as they cause many subsequent destructive diseases, and hence, the old fashioned cultivation

yields a relatively low specific growth rate, providing enough time for the blooming of the unwanted species in the systems. Also the climate conditions directly influence the growth of the diatom. The design of closed system bioreactors is therefore treated as a solution to these problems.

Airlift bioreactor (ALBR) is one of the potential alternatives for the cell cultivation (Merchuk et al., 1998). Airlift bioreactors achieve mixing of the growth medium by injecting air into the bottom of a bioreactor vessel. As a result, well defined fluid flow pattern and relatively high gas-liquid mass transfer rate are obtained. The mixing in the airlift bioreactor could be obtained without causing too much shear force in the liquid phase which could inhibit the growth of the diatom. Our recent work at the Department of Chemical Engineering, Faculty of Engineering, Chulalongkorn University has proven that, by giving it a good design and operating conditions, the use of airlift system as a photobioreactor could drastically improve the quality of the cultivation of *Chaetoceros calcitrans* (Krichnavaruk et al., 2005; Krichnavaruk et al., 2007; Sriouam, 2007)

This work aimed to extend the applicability of the airlift system onto the cultivation of other types of diatom and *Skeletonema costatum* is among the good choices as it is also used as a shrimp larvae feed. The main target was to determine the optimal design configurations and operating conditions for the growth of *Skeletonema costatum* which yielded high cell density.

1.2 Objective

The objective of this work was to investigate the cultivation of *Skeletonema costatum* in an airlift photobioreactor. This included the determination of the optimal medium, design parameters and operating conditions of such airlift photobioreactor.

1.3 Working scopes

1.3.1 The determination of suitable nutrient condition was based on the standard F/2 medium (Guillard and Ryther, 1962) and was tested in 2000 mL glass bottles.

1.3.2 The photobioreactor employed in this work was a small lab-scale 3 L contactor with the dimension as stated in Table 3.3.

1.3.3 The light intensity was controlled in a range of 20-47 μmol photons $m^{-2}s^{-1}$.

1.3.4 The superficial gas velocity was approximately controlled in a range of 1-2 $cm\ s^{-1}$.

1.3.5 The ratio between the downcomer and riser cross section area (A_d/A_r) was fixed at 0.98, 1.87, 3.27 and 14.87. These were set according to the standard tube sizes available commercially.

Chapter II

Backgrounds and Literature Review

2.1 *Skeletonema costatum*

The diatoms genus *Skeletonema* (Greville) are important components of the marine and estuarine phytoplankton. Although there are few truly freshwater species (e.g. *Skeletonema potamos*), but the best known species, *S. costatum*, is a marine and occasionally brackish, commonly distribution in coastal waters (Cupp, 1997). *Skeletonema costatum* (Fig. 2.1) are joined in long chains of 6-24 cells (one chain could have up to 30 cells), with varying spaces between sibling cells. Each cell has 1 or 2 chloroplasts. The valves are flat or slightly convex (Fig. 2.1 (a) and (b)). The areolae are rectangular and elongated, arranged radially; on the mantle they are parallel to the pervalvar axis (Fig. 2.1 (c) and (d)). The external parts of the fultoportulae are long, tubular and split throughout from the base (Fig. 2.1 (c) - (e)). There are 3 satellite pores and the processes are separated by 3 areolae. There are 12-24 processes per valve, depending on the diameter. The rimoportula is a tubular structure, usually longer on the terminal valves than in the intercalary ones (Fig. 2.1 (d)) (Castillo et al., 1995).

2.2 General growth factors for diatoms

The biochemical composition of algae is generally affected by cultivation conditions and many other factors such as nutrient composition, temperature, light intensities, photoperiod, carbon dioxide, salinity, pH, aeration, etc. These are described below:

2.2.1 Culture medium (nutrients)

Diatoms do not require equal amounts of all nutrients, therefore they are added in different quantities. Natural seawater can only supply enough nutrients for limited growth of some marine species. Most media recipes supply nutrients vastly in excess of the concentrations in order to support high biomass cultures. Conventionally, standard F/2 medium was reported to be suitable for the cultivation of diatom. However, different diatoms might require the various nutrients differently and it is important that the F/2 medium be modified to suit each individual diatom species. The modified standard F/2 medium was commonly employed as standard medium for diatoms. For example, maximum specific growth rates of *Phaeodactylum tricornutum* and *Chaetoceros muelleri* by Liang et al. (2006) in F/2 medium supplied with more nitrogen at 0.88 mM were 1.38 ± 0.01 day^{-1} and 0.80 ± 0.01 day^{-1}, respectively. Guerrini et al. (2007) investigated the influences of N and P in the medium on the growth of *Protoceratium reticulatum* and reported that the optimal concentration of nitrogen and phosphate were 17.7 and 7.3 µM, respectively, which were different from the concentrations stated in the general standard F/2 medium (75 µM and 5 µM). The result also showed that the maximum cell density and specific growth rate were 5.5×10^3 cell ml^{-1} and 0.5 day^{-1}, respectively.

Our recent works (Krichnavaruk et al., 2005) on the examination of optimal growth conditions of the diatom *Chetoceros calcitrans,* the standard F/2 medium was modified with a two fold of silica and phosphorus concentrations as this was illustrated to result in a better growth of this diatom. On the other hand, Vitamin B$_{12}$ in the range from 1 to 3 µg L^{-1} did not significantly affect the growth.

In the following text, general information on the nutrient requirement is described.

Nitrogen

Nitrogen is most commonly added as nitrate. However, some algal species grow better in ammonium (Thompson et al, 1989). Unfortunately, ammonium is often found to be toxic at >100 mM and a much lower concentration is generally used.

Guillard suggested the level of 500 mM for cell cultivation (Guillard, 1983) but this concentration should be tested prior to use. In some situations it is desirable to have ammonium available.

Phosphate

Phosphate is another key contributor for the growth of algae in the aquarium. Phosphorus is a part of every living animal and is necessary, but phosphorus in elevated amounts could activate undesirable algae growth and inhibit calcification. Phosphate is added to the sea water in several ways, but more often through food and water sources.

Silica

Silica is only required by diatoms (excepting the rarely cultured silicoflagellates) and is therefore often left out of media which are selective for other organisms. Diatoms only need trace quantities of silica because they are extremely efficient at scouring silica from the environment. Some diatoms may grow for many generations in seawater media which has no added silica and some may continue to divide for several weeks, albeit with poor frustule development.

Carbon

Generally diatoms will need roughly 6 times more carbon than nitrogen. For marine species the carbon requirement in small batch cultures is met by the 2 mM contained within seawater, and by allowing atmospheric exchange, the carbon can be supplied over time. Large batch cultures, generally greater than 500 mL to 1 L may need to be aerated with either air or an air/CO_2 mix to prevent carbon limitation. Typical CO_2 concentrations are in the range 0.5 - 5.0 % v/v

Vitamins

The three most widely used vitamins in order of significance to algae are vitamin B_{12} (cobalamin or cyanocobalamin), thiamine and biotin. Effects of vitamin

are several, for example, an essential ingredient for growing algae that are critical, manufacture essential protein, again bacteria, etc.

2.2.2 Culture environments

Temperature

Batch cultures have been used for many years to study relationships between specific growth rate of the diatom and temperature. The upper and lower temperature limits and the optimum temperature i.e. that resulting in the maximum growth rate, are reproducible parameters and can be used to characterize species. In general, growth increases exponentially with temperature up to an optimum temperature, after which it declines rapidly.

Salinity

Salinity for growth of algal is important parameter control. For example, continuous cultures (12 h light:dark cycle) of *S. costatum* were adapted to constant salinity in natural (16.1ppt) (Rijstenbil and Sinke, 1989). In continuous light, photosynthesis and cell growth of ammonium-limited *Skeletonema costatum* was inhibited after a salinity decrease (22.4 to 8.6 ppt) (Rijstenbil and Sinke, 1989).

2.3 Photobioreactors

After the identification of a strain and product of interest, the step to follow is the development of bioprocess to establish the link between discovery and commercialization. The cultivation system can vary depending on the product and strain. **Open systems** can be used for very fast growing strains or for strains that grow at extreme conditions, such as high pH or high salinities. However, for the majority of the strains and products with application in the pharmaceutical industry, monoalgal cultures are required. In order to meet these standards and aiming at attaining a cost-

effective process, several **closed systems** have been developed in the last twenty years (Pulz and Scheibenbogen, 1998). Typically, high volumetric productivities are required to reduce the size of cultivation system and consequently reduce productivity and downstream processing costs. This entails a high efficiency of light utilization besides high biomass concentrations because light energy is the growth limiting substrate. The basic idea of using sunlight to produce high-value compounds brings along several limitation, which are related to the light regime inside the cultivation system and have to be considered in the design and scale-up of photobioreactors.

2.3.1 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.

2.3.2 Air-lift photobioreactor

Airlift photobioreactors are generally classified as pneumatic reactors without any mechanical stirring arrangements for mixing. The turbulence caused by the fluid flow ensures adequate mixing of the liquid. The draft tube is provided in the central section of the reactor. The introduction of the fluid (air/liquid) causes upward motion and results in circulatory flow in the entire reactor. Airlift photobioreactors can be used for both free and immobilized cells, however, there are relatively few reports on using such system for metabolite production when compared with other types of bioreactors. 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

Transport mechanism in air-lift photobioreactor

An air-lift photobioreactor can be divided into four main parts, riser, gas separator, downcomer, and bottom section (Fig. 2.2)

A. Riser the section through which gas is supplied, and the upward flow of liquid is induced.

B. 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.

C. Downcomer is the section that the heavier fluid from the gas separator section and some remaining gas bubbles flow downwards.

D. The final bottom section is provided to allow the liquid to re-enter the riser again together with the supplied gas.

2.4 Applications of airlift photobioreactor for the cultivation of algae/diatom

2.4.1 Cultivation in airlift photobioreactors

The development of photobioreactor for mass cultivation of single cell algae or diatom always enables the adjustment of the light intensity, which leads to a successful production of high cell density culture. Examples of the novel designed photobioreactors include a tubular reactor (Scragg et al., 2002) and the flat plate bioreactor (Richmond and Cheng-Wu, 2001). However, these existing closed systems suffer serious drawbacks from poor mixing and gas–liquid mass transfer. Airlift bioreactors (ALBR) have recently become an attractive alternative for cell cultivation and Merchuk et al., 1998 indicated that the growth of the red microalga *Porphyridium* sp. in airlift photobioreactor was much better than that obtained from the cultivation in the bubble column.

Recently, Krichnavaruk et al. (2005) proposed the use of airlift photobioreactor and bubble column as an alternative cultivation system for *Chaetoceros calcitrans*. The performance of the airlift photobioreactor was clearly

superior to that of the bubble column both in terms of maximum cell density and growth rate, i.e. maximum growth rate and maximum cell concentration were 7.41×10^{-2} h^{-1} and 8.88×10^{6} cells mL^{-1} in the airlift photobioreactor, respectively, and 6.3×10^{-2} h^{-1} and 7.68×10^{6} cells mL^{-1} in the bubble column.

Similar airlift systems were also proposed for the cultivation of vegetative cells of *Haematococcus pluvialis* (Kaewpintong et al., 2007). The comparison between the performances both airlift photobioreactor and bubble column at $u_{sg} = 0.4$ cm s^{-1} at the same operating condition showed that the maximum cell density and of specific growth rate *H. pluvialis* grown in airlift bioreactor were 79.5 x 10^{4} cells mL^{-1} and 0.45 d^{-1}, which were higher than those in bubble column which were 42 x 10^{4} cells mL^{-1} and 0.36 d^{-1}, respectively.

2.4.2 Cultivation of *Skeletonema costatum*

Table 2.1 provides an extensive summary of the recent advancement in the cultivation of different types of algae and diatoms. Details of the cultivation such as environmental conditions, nutrients, and growth data are provided. Most of the works, however, are still at the enfant stage where no bioreactors have been developed to suit each species needs. The bioreactors often used in the cultivation are bubble columns, airlift bioreactors, tubular reactors, each with their unique pros and cons. For the cultivation of *S. costatum*, most of the work was only performed in a small scale system with the highest growth rate of 1.51 d^{-1} (Varum and Myklestad, 1984). The only large scale was done in 5L erlenmeyer flask and achieved a low level of growth rate at 0.63 d^{-1} (Urbani et al., 2005).

Table 2.1 Summary of the operation for cultivation of microalgae

Microalgae	Photobioreactor			Modes of operation				Medium	Biomass productivity $(g.L^{-1}h^{-1})$	Cell density (**)	μ (d^{-1})	Remarks	Reference
	Type	Volume	Culture mode	Temp (^{o}C)	Light (μE^*)	Photoperiod (light:dark)	Light source						
Skeletonema custatum	Conical flasks	250 ml	Batch	13	200	14:10	Lamps	F/2 medium (Guillard & Ryther., 1962)			1.51	Effect of light, salinity and nutrient limitation on the production of β-1,3-D-glucan and exo-D-glucan (Both glucan increasing nutrient deficiency)	Varum and Myklestad, 1984
	Poly-carbonate flasks	200 ml	Batch	22	100	12:12	Lamps	F/2 nutrients (Harrison et al., 1980)		3.8	0.52	Growth and photosynthesis limitation by low concentra-tions of Zn^{2+} (Zn^{2+} 66 pM)	Hul et al., 2003
	Water-jacketed culture glasses	500 ml	Batch	20-25	250		Lamps	Walne medium	2.2×10^{-3}		0.96	A good nutritive value were achieved with Walnemedium at 1 % stock solution and an aeration rate of 1.5 vv^{-1} min^{-1}	Sebastián et al., 2004
	Poly-carbonate flasks	200 ml	Batch	22	100	12:12	Lamps	Modified F/2 nutrients (Harrison et al., 1980)		2.6 - 5.2		Effect of Zn^{2+} on utilization of dissolved organic phos-phorate compounds (Cell concentration increased when Zn^{2+} was increased from 0 to 24 pM, in growth medium)	Shi et al., 2004
	Flasks	5 L	Batch	18	112	16:8	Cool-white lamps.	F/2 medium		6.42 ± 0.05	0.63	Effect of P-depletion and growth status(6 μM Phosphorus)	Urbani et al., 2005

(μE^*) = μmol photons $m^{-2} s^{-1}$, (**) = x 10^5 cells mL^{-1}

Table 2.1 (cont.) Summary of the operation for cultivation of microalgae

Microalgae	Photobioreactor			Modes of operation			Medium	Biomass productivity $(\text{g m}^{-2}\text{ h}^{-1})$	Cell density (**)	μ (d^{-1})	Remarks	Reference
	Type	Volume	Culture mode	Temp. (^oC)	Light $(\mu\text{E*})$	Light source						
Chaetoceros muelleri	Bench-top	0.6 L	Batch	23 ± 1	190	fluorescent lamps	F/2 medium	0.09			Effects of light path lengths and initial culture density on the cultivation (1 cm. light path)	Göksan et al., 2003
	Bench-top	1.8 L	Batch	23 ± 1	190	fluorescent lamps	F/2 medium	0.14			Effects of light path lengths and initial culture density on the cultivation (3 cm. light path)	Göksan et al., 2003
Chaetoceros calcitrans	Bubble column	17 L	Batch	30 ± 2	400	36 W lamps	Modified f/2 medium		58.0	0.91	Two fold of silica and phosphorus concentrations in growth medium	Krichnavaruk et al., 2005
	Airlift	17 L							88.8	1.78		
	Bubble column	17 L	Batch						49.6	0.69		
	Airlift	17 L	Batch	30 ± 2	350	36 W fluorescent light bulbs	F/2 medium		88.8	1.78	Enhanced productivity in airlift photobioreactors	Krichnavaruk et al., 2007
	Airlift	12 L	Semi-continuous						38.6	2.22		
	Airlift	2.8 L	Conti-nuous						16.2	1.54		

$(\mu\text{E*}) = \mu\text{mol photons m}^{-2}\text{ s}^{-1}$, (**) $= \text{x } 10^5 \text{ cells mL}^{-1}$

Table 2.1 (cont.) Summary of the operation for cultivation of microalgae

Microalgae	Photobioreactor			Modes of operation			Medium	Cell density (**)	Biomass productivity (g.L⁻¹h⁻¹)	μ (d⁻¹)	Remarks	Reference
	Type	Volume	Culture mode	Temp. (^{o}C)	Light (μE*)	Light source						
Chaetoceros calcitrans	Bubble column Airlift	170 L	Batch	30 ± 2	350	36 W fluorescent light bulbs	F/2 medium	25.1 29.6		0.59 0.62	Enhanced productivity in airlift photobioreactors	Krichnavaruk et al., 2007
Chlorella vulgaris	Flat panel airlift	3 L	Batch	22	980	HQI-vapor lamp	DS-medium (Pohl et al., 1987)		0.11		A novel airlift photobioreactor with baffles for improved light utilization through the flashing light effect	Degen et al., 2001
	Tubular	250 L	Batch	25	130	Gelvwess white daylight	Watanabe's medium (Watanabe ,1960)	570	0.40	0.40	Growth of microalgae with increased calorific values	Scragg et al., 2002
	Tubular	250 L	Batch	25	130	Gelvwess white daylight	Watanabe's medium	70	0.24	0.69	Growth of microalgae with increased calorific values (low nitrogen medium (203 mg l⁻¹ (NH₄)₂HPO₄)	Scragg et al., 2002
Chlorella emersonii								33	0.41	0.38	Growth of microalgae with increased calorific values	Scragg et al., 2002
	Tubular	250 L	Batch	25	130	Gelvwess white daylight	Watanabe's medium	0.36	40	0.38	Growth of microalgae with increased calorific values (low nitrogen medium (203 mg l⁻¹ (NH₄)₂HPO₄))	

(μE*) = μmo lphotons m⁻² s⁻¹, (**) = x 10⁵ cells mL⁻¹

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Table 2.1 (cont.) Summary of the operation for cultivation of microalgae

Microalgae	Photobioreactor			Modes of operation				Medium	Biomass productivity $(g.l^{-1}h^{-1})$	Cell density (**)	μ (d^{-1})	Remarks	Reference
	Type	Volume	Culture mode	Temp. (^{o}C)	Light (μE*)	Photoperiod (light:dark)	Light source						
Dunaliella tertiolecta	Bubble column	65 L	Conti-nuous	20	50-70	16:8		Artificial seawater medium			0.11	Cultivation method called acceleration-stat	Maria et al., 2003
Haematococcus pluvialis	Tubular	2 L	Conti-nuous	20	130			Inorganic medium free of acetate (Garcia et al., 2005)	9.84			Comparative analysis of the outdoor culture in tubular and bubble column photobioreactors	Garcia et al., 2005
	Novel double-layered		Batch	25	40±3		Fluorescent lamps	Bold's Basal medium (Park et al., 2001)		4.0.		Cell growth and astaxanthin accumulation	Suh et al., 2006
	Airlift	3 L	Batch	21±1	20	Continuous	Fluorescent lamps	F1 (Fábregas et al., 1998)		4.2	0.36	Photoautotrophic high-density cultivation of vegetative cells in airlift bioreactor	Kaewpintong et al., 2007
	Bubble column	3 L	Batch	21±1	20	Continuous	Fluorescent lamps	F1		7.95	0.45		Kaewpintong et al., 2007

(μE*) = μmol photons m^{-2} s^{-1}, (**) = x 10^5 cells mL^{-1}

Table 2.1 (cont.) Summary of the operation for cultivation of microalgae

Microalgae	Photobioreactor			Modes of operation				Medium	Biomass productivity $(g.l^{-1}h^{-1})$	μ (d^{-1})	Remarks	Reference
	Type	Volume	Culture mode	Temp. (^oC)	Light (μE^*)	Photoperiod (light:dark)	Light source					
Haematoco ccus pluvialis	Bubble column	2 L	Conti-nuous	20	70			Inorganic medium free of acetate (Garcia et al., 2005)	1.44		Comparative analysis of the outdoor culture in tubular and bubble column photobioreactors	Garcia et al., 2005
Isochrysis galbana	Water-jacketed culture glasses	500 ml	Batch	15	40-43	Continuous	Lamps	Ukeles (Buffered with a concentration of 4.13mM Tris-HCl.)	2.25	0.37	Biomass production in relation to culture medium	Sánchez et al., 2000
Phaeodacty lum tricornutum	Airlift tubular	20 L	Batch	20	114.67		Halogen lamp	Mann and Myers ,1968 medium	0.08	0.06	Tubular photobioreactor design for algal cultures	Molina et al., 2001

$(\mu E^*) = \mu mol$ photons $m^{-2} s^{-1}$, $(**) = x\ 10^5$ cells mL^{-1}
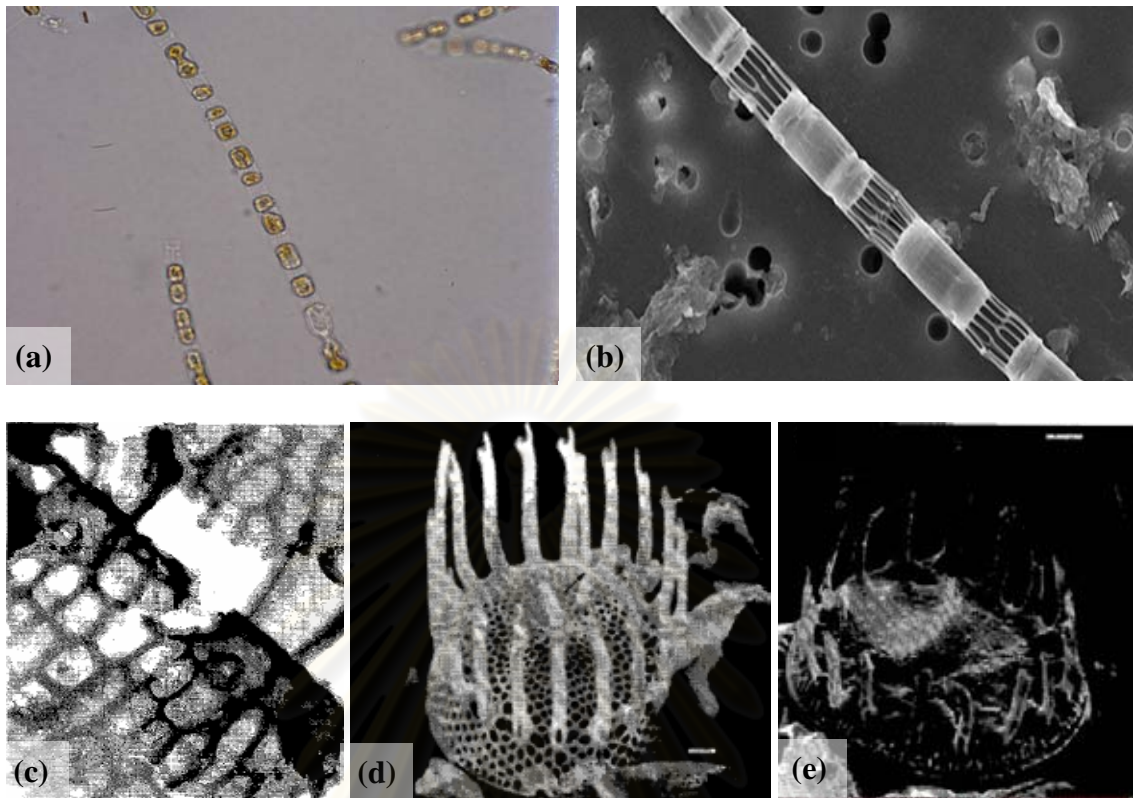
Figure 2.1 Morphology of *S.costatum* : (a) *S.costatum*; (b)scanning
electronmicroscope (SEM) of *S.costatum* (http://www.chbr.noaa.gov/pmn/
resources.htm); (c)valve mantle with some fultoportulae, Transmission electron
microscope; (TEM) (Castillo et al., 1995); (d)terminal valve with several external
parts of fultoportulae and one slightly excentric tube of therimoportula (arrow)
(Castillo et al., 1995); (e)An intercalary valve with various tubes of fultoportulae and
one short process (arrow) of the rimoportula, (Castillo et al., 1995)

Air out

Liquid flow direction

Gas separator
section

Draft tube

Downcomer

Riser

Bottom section

Air in
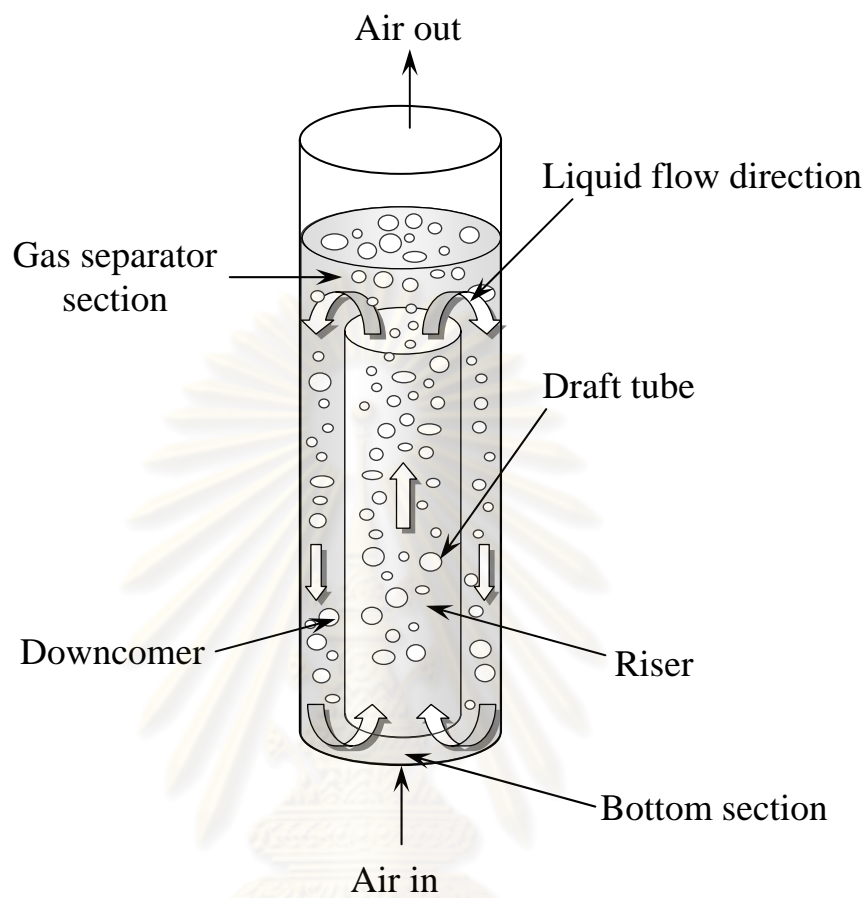
Figure 2.2 Airlift photobioreactor

CHAPTER III

MATERIALS AND METHODS

3.1 Source of diatom

Skeletonema costatum BIMS-PP0115 is obtained from Bangsaen institute of marine science, Burapha university, Thailand.

3.2 Culture medium

F/2 medium as recommended by Guillard and Ryther (1962) is employed as the standard medium for the cultivation of *Skeletonema costatum*. The composition of this standard medium is given in Table 3.1.

3.3 Set up of photobioreactor

The growth of *Skeletonema costatum* is investigated in clear acrylic plastic bubble column and airlift photobioreactor (see Fig. 3.1A and 3.1B for schematics of bubble column and airlift photobioreactor and Table 3.2 for their dimensions). The airlift bioreactor is equipped with a draft tube installed centrally in the column. Air from an air compressor is metered through a flowmeter, sterilized with a 0.45 μm Gelman filter and passed into the culture at the bottom of both reactors. This allowed liquid circulation in the airlift system. Light is supplied through fluorescent lamp (18 watts) at the side along the length of the column, and light intensity is measured by "Digicon LX-50 lux meter" around the column. This is then converted into μ mol photon $m^{-2}s^{-1}$ using Equation 3.4. The temperature is controlled at 25-30°C.

3.4 Experimental methods

3.4.1 Preparation of pure culture

1) Prepare culturing medium in a 1 L Erlenmeyer flask (500 mL of 30 ppt fresh seawater with standard F/2 medium)

2) Add to the medium 4 g of agar powder, this yields 0.8%wt agar in the medium

3) Add Stock A, B and C nutrients (see Table 3.1) before autoclaving

4) Cover the flask with aluminum foil

5) Autoclave the agar medium and the dried Petri dishes at $120^{o}C$ for 20 min

6) Sterilize test tube by incubation for 30 minutes at $150^{o}C$

7) Prepare agar plates aseptically by pouring the warm autoclaving agar into the sterile test tube (3-5 mL for a test tube) and cool for about 2 h

8) When agar plates cool down, streak the algal sample onto the agar surface with a sterile loop (previously heated to red-hot and cooled)

9) Place the test tube upside-down on an illuminated rack

10) Observe cell colonies which should grow on the surface after 5-21 days

3.4.2 Initial preparation of stock culture

1) Select the colony and transfer them with a sterile loop into a test tube filled with 5-10 mL of sterilized culture liquid medium in sterilized test tube and shake it regularly during incubation

2) When a color of the culture becomes brownish, check the isolated algal strain under the microscope

3) Inoculate cells from test tube into 50mL sterilized culture medium

4) Scale up the inoculums to 250 mL and 500 mL sterilized culturing mediums and shake well during this period

3.4.3 Study of nutrient content in modified F/2 medium

1) Sterilize airlift photobioreactor 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 as shown in Figure 3.1B

8) Take samples and count for the cell density using Haemacytometer (mentioned in Section 3.5.1) and measure the concentration of each nutrient content in the medium (see Appendix B for the detail of measurement) at every 12 hours until the stationary growth is observed

9) Calculate the specific growth rate using Equation 3.2, the productivity using Equation 3.3, and the specific productivity using Equation 3.5.

3.4.4 Bubble column and airlift photobioreactor culture systems

1) Sterilize Bubble column and Airlift photobioreactor 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 sterilized water to remove residual chlorine at the bottom of the column

4) Fill in the column with sterilized sea water, culture medium together with the pure culture, adjust the total volume to 3 L

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

6) Supply sterilized compressed air (sterilized with a 0.045 μm Gelman autoclave filter) through a porous sparger and adjust the superficial gas velocity to 2 cms^{-1}

7) Supply both sides of column with five 18 W fluorescent light bulbs, placed along the column height

8) Take samples and count for the cell density using Haemacytometer (mentioned in Section 3.5.1)

9) Calculate the specific growth rate using Equation 3.2, the productivity using Equation 3.3 and the specific productivity using Equation 3.5

10) Repeat Steps 1–9 again with superficial gas velocity changed from 2 to 3, 4 and 5 cm s^{-1}

11) Repeat Steps 1–9 with the draft tube diameter changed to 3, 5, 6 and 6.95 cm (indicated in Table 3.3)

3.5 Analyses

3.5.1 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 all cells in the chain of *Skeletonema costatum* (to obtain the total number of cells in the large square in the Haemacytometer, the number of cells in each of the 5 medium squares are counted)

5) Calculate the cells number, using Equation 3.1:

$$N = \frac{n}{5} \times 10^4 \qquad\qquad \text{..... (3.1)}$$

where

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

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

3.5.2 Determination of specific growth rate

The specific growth rate can be calculated from Equation 3.2 as follows:

$$\mu = \frac{\ln(N_2) - \ln(N_1)}{t_2 - t_1} \qquad\qquad (3.2)$$

where

μ =   specific growth rate (h^{-1})

N_1 =   cell concentration at t_1 (cell L^{-1})

N_2 =   cell concentration at t_2 (cell L^{-1})

t_1 =   first sampling time (h)

t_2 =   second sampling time (h)

3.5.3 Determination of productivity

The productivity is calculated by the following equation:

$$P = \frac{C_2 - C_2}{t_2 - t_1} \qquad\qquad (3.3)$$

where P = productivity (mg chlorophyll a L^{-1})

C_1 = cell concentration at t_1 (cell L^{-1})

C_1 = cell concentration at t_2 (cell L^{-1})

t_1 = first sampling time (h)

t_2 = second sampling time (h)

3.5.4 Determination of light intensity

The light intensity can be calculated from Equation:

$$I \approx \frac{E}{74} \qquad\qquad (3.4)$$

where I = light intensity (μ molphoton m^{-2}s^{-1})

E = light intensity (lux)

3.5.5 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.

3.5.6 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).

3.5.7 Determination of total protein (Kjeldahl's Method)

(References: AOAC, "Official methods of analysis," Method 945.18-B)

1) Sample: grind the sample to a homogeneous, fine powder. 1 g of sample is accurately weighed and quantitatively transferred into a digestion tube.

2) Reagents for digestion to each sample in the digestion tube add:
   – 2 Kjeltabs ST (selenium catalyst)
   – 20 ml sulfuric acid (H_2SO_4), concentrated 96%

After addition of reagents, shake gently to wet the sample.

3) Digestion: heat for 30 min at 150 °C and then 60 min at 420 °C.

4) Cooling: let the digestion tubes cool to 50 °C–60 °C.

5) Distillation: position the Erlenmeyer flask containing 25 ml of boric acid solution (4%) and a digestion tube with a digested sample in the steam distillation unit. Add reagents: H2O = 50 ml, NaOH = 50 ml (35%).

6) Titration: titrate for determination.

3.5.8 Determination of total lipid

(References: AOAC, "Official methods of analysis," Method 920.39)

1) Extract 2 g test portion on small paper in funnel with five 20 mL portion H_2O prior to drying for ether extraction.

2) Use thimble with porosity permitting rapid passage of ether.

3) Extraction period may vary from 4 hr at condensation rate of 5-6 drop s^{-1} to 16 hr at 2-3 drop s^{-1}.

4) Dry extract 30 min at $100^{o}C$

5) Cool and weigh.

3.5.9 Determination of total carbohydrate

This method is applicable to the determination of total carbohydrate in food by subtraction the sum of protein, fat, moisture and ash from total weight of food (The carbohydrate concentration can be calculated from Equation 3.5).

% Total carbohydrate = 100-(%protein + %fat + %moisture + %ash)   (3.5)

Table 3.1 Chemical composition of standard F/2 (Guillard's) stock solution

Solution A: Nitrate and Phosphate stock solution (1 L)

Nutrient	Amount
$NaNO_3$	84.15 g
$Na_2HPO_4 \cdot H_2O$	6.0 g
$FeCl_3 \cdot 6H_2O$	2.90 g
$Na_2EDTA \cdot 2H_2O$	10.0 g

Solution B: Silicate stock solution (1 L)

Nutrient	Amount
$Na_2SiO_3 \cdot 9H_2O$	33.0 g

Solution C: Trace metal stock solution (1 L)

Nutrient	Amount
$CuSO_4 \cdot 5H_2O$	1.96 g
$ZnSO_4 \cdot 7H_2O$	4.40 g
$Na_2MoO_4 \cdot 2H_2O$	1.26 g
$MnCl_2 \cdot 4H_2O$	36.0 g
$CoCl_2 \cdot 6H_2O$	2.0 g

Solution D: Vitamin stock solution (1 L)

Nutrients	Amount
Vitamin B1	0.4 g
Vitamin B12	0.002 mg
Biotin	0.10 mg

Note: To prepare the culture medium for *Skeletonema costatum*, simply add 1 mL of solutions A, B, C and D in 1 L of fresh sea water.

Table 3.2 Main dimension of the bioreactor

	Bubble column	Airlift photobioreactor
Column inner diameter (D)	9.4	9.4
Column height (H)	60	60
Draft tube height (h)	40	40

Table 3.3 Design configurations of airlift bioreactor for the investigation on optimal configuration for the growth

Reactor	Draft tube		$A_d:A_r$ (-)
Inner diameter (cm)	Inner diameter (cm)	Outer diameter (cm)	
9.4	2.31	3	14.87
	4.40	5	3.27
	5.37	6	1.87
	6.33	7	0.98

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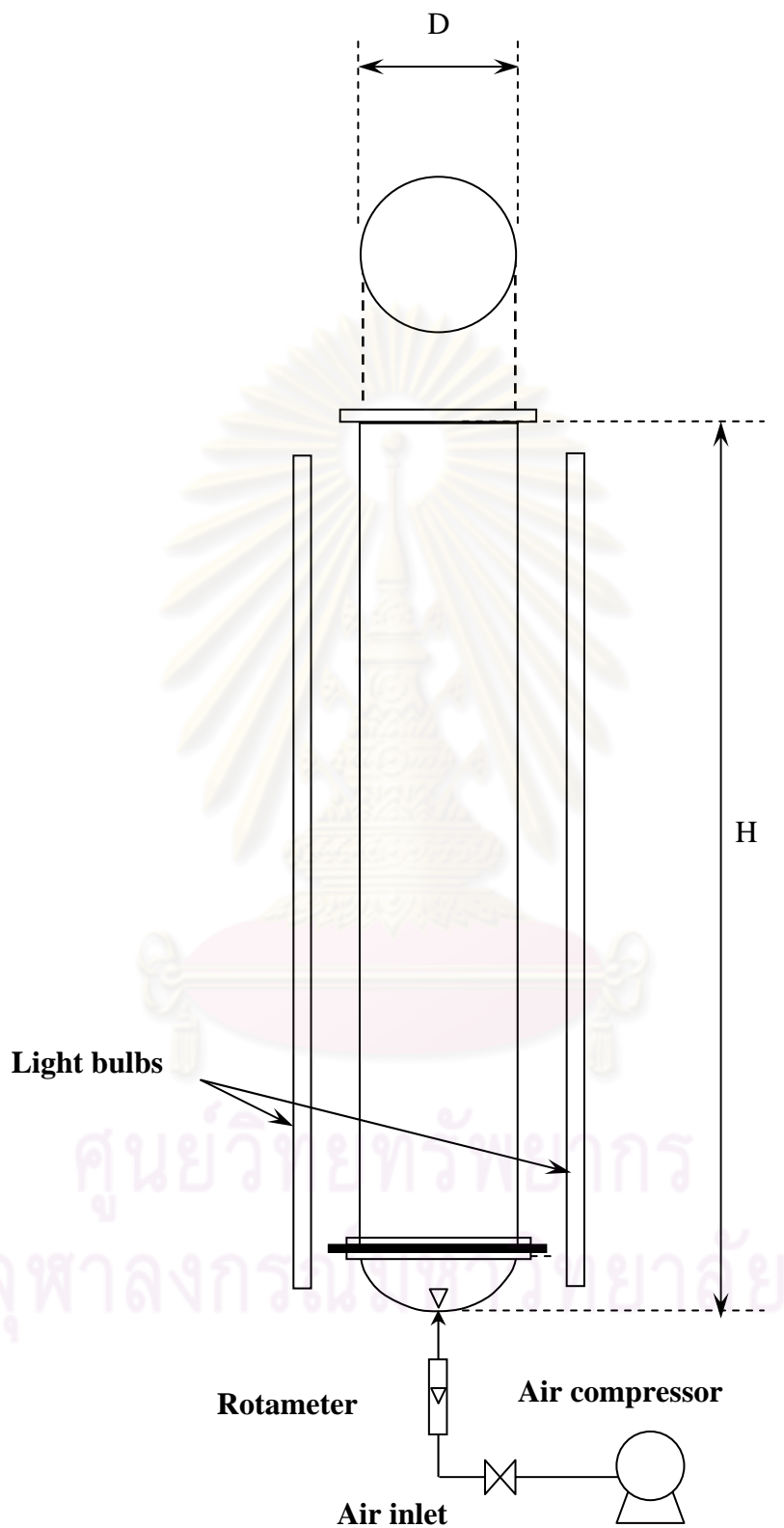
Figure 3.1 Experimental setup for the cultivation of *Skeletonema costatum* in bubble column

Figure 3.2 Experimental setup for the cultivation of *Skeletonema costatum* in airlift photobioreactor

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Determination of appropriate nutrient concentrations

4.1.1 Limiting nutrients for growth of *S. costatum* with standard F/2 medium

Laboratory batch experiments were carried out to determine growth limiting nutrients in 2 L glass bottle (superficial gas velocity of 1.5 cm s^{-1} and light intensity of 33.78 μmol photons m^{-2} s^{-1}). Time profiles of the nutrient concentrations during the growth of the diatom were illustrated in Fig. 4.1. During the initial lag phase where very slow growth was observed, nutrients concentration remained at the concentration level in F/2 medium. Once cells entered the exponential phase, all nutrients were being consumed quite quickly. Phosphate concentration seemed to be consumed significantly and the level of phosphate in the medium went down to almost zero. The reason for the significant uptake of phosphorus could be that cells accumulated energy during the growth in the form of adenosine triphosphate (ATP) which required the uptake of phosphorus. Silicon was also being consumed quite significantly and the level of silicon was also close to zero. This is because 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. Nitrogen is one important element for protein synthesis by photosynthesis and is conventionally supplied to the culture in nitrate form. Nitrate, on the other hand, despite being consumed in a much higher quantity when compared with Si and P, was still left in a reasonably high content.

As a preliminary assessment, the results in this section led to the conclusion that phosphate and silicon could be rate limiting factor for the growth of *S. costatum*. This issue is discussed further in the next section.

Based on the test results in growth limiting nutrients, the concentration of nutrient in F/2 standard medium was not especially designed for the growth of *S.*

costatum. This section discusses the investigation on the optimal nutrient concentration which should be modified to the standard F/2 medium. Therefore it is important to identify the main nutritional factors that controlled the growth of *S. costatum*, i.e. silicon (as sodium silicate), nitrogen (as nitrate), and phosphorus (as phosphate). In this experiments, the cultures were started with an initial cell concentration of 0.4×10^6 cells mL^{-1}.

4.1.2 Silicon

Fig. 4.2 illustrates clearly that *S. costatum* grew better when extra quantity of silicon was added into the standard F/2 medium. Increasing silicon from 1 to 4 fold of the standard concentration clearly induced higher growth rate and productivity. The highest maximum cell concentration of almost 4.6×10^6 cells mL^{-1} occurred at 4 fold silica content. However, the maximum cell concentration tended to decrease when the silicon concentration increased more than 4 folds.

To describe this finding, it is better to recall that algae/diatoms contain a number of minerals in cell wall components, and silicon is one of the main constituents of the diatom shell. Silicon is not only needed in the cell-wall formation, but it is required for the synthesis of DNA (Lesley et al., 1997). As a result, there was a definite low concentration of silicate below which a population could not survive. This explanation could explain the finding in this work that increasing silica concentration in the medium resulted in the increase in the growth rate of the diatom *S. costatum*. Nevertheless, it was found here also that increasing silica by five-fold silicon content could lead to a decrease in the growth rate. This could be due to an inhibition effect caused by the overdose of silica.

4.1.3 Phosphorus

Phosphorus plays an important role in almost all cell metabolic activities, and it is an important component of cell membrane. Phosphate is instrumental in the storage of energy as adenosine triphosphate (ATP) molecules, Nicotinamide adenine dinucleotide phosphate (NADPH), etc. Insufficient supply of phosphorus can, hence, reduce the cell growth (Berg et al., 2002). For this work, the effects of phosphorus

concentration on the growth of *S. costatum* are demonstrated in Fig. 4.3. Note that this experiments were performed with silicon content of 4-fold the standard F/2 medium. This analysis showed that the addition of phosphorus concentration from 1x to 3x did not have significant impacts on the maximum cell concentration and productivity.

4.1.4 Nitrogen

The concentration of nitrogen can cause important changes in their growth character and biochemical composition of the algae as inadequate nitrogen could lead to a rapid reduction in photosynthetic performance. The following description is based on the cultivation in the modified F/2 medium with 4x silicon content.

As shown in Fig. 4.4, nitrogen contents at 1x and 2x the standard F/2 medium did not have significant impacts on the maximum cell concentration. An increase in the nitrogen content did, in fact, enhance the specific growth rate, but only slightly. A further increase more than 2x did not have appreciating results on the growth parameters of *S. costatum*. Therefore it was concluded that nitrogen compounds did make the cells *S. costatum* more active but only marginally and the final cell concentration should be controlled by other factors. For this reason, the nutrient used in the cultivation of this alga was still maintained at 1x nitrogen content.

4.1.4 Analysis of nutrient requirements

The cellular yield based on the nutrient component or $Y_{Nu/X}$ can be evaluated using the following equation:

$$Y_{Nu/X} = \frac{\Delta Nu / \Delta t}{\Delta X / \Delta t} \qquad (4.1)$$

when $\Delta Nu /\Delta t$ = specific nutrient uptake (mg L^{-1} s^{-1})

$\Delta X /\Delta t$ = cell production (cell mL^{-1} s^{-1})

In this work, the cellular yield for silicon, phosphorus and nitrogen can be summarized as follows (Table 4.1):

silicon content $\quad\quad Y_{Si/X} = 0.4$ x 10^{-3} pg $cell^{-1}$

phosphorus content $\quad Y_{P/X} = 2.9$ x 10^{-3} pg $cell^{-1}$

nitrogen content $\quad\quad Y_{N/X} = 5.5$ x 10^{-3} pg $cell^{-1}$

The ratio of the three major cellular chemical compositions, N: Si: P assimilated into the cell calculated from the ratio between these yields became 1.9 : 0.1 : 1.

Literature revealed that the ratio of N: Si: P of *S. costatum* cultivated in chemostats under non-limiting nutrient (Harrison et al., 1976) is 10:5.4:1 which means that the ratios between N and Si to P were much higher in the literature than those found in this work. This implied that, although P was totally consumed in the cultivation of *S. costatum*, P was already present in relatively high content when compared with other nutrients such as Si and N. On the other hand, Si seemed to be inadequately provided in the standard F/2 medium (almost 50 times lower than the reported Si:P ratio of 5.4:1). Nitrogen was also not being supplied sufficiently, i.e. N:P ratio of only 1.9:1 when compared with the reported requirement of 10:1, but this was considered not as significant as the silicon case. This could answer the finding in Section 4.1.2 where an increase in Si clearly augmented the growth of the diatom.

4.2 Growth of *S. costatum* under different light intensities

These experiments were conducted in the 3L airlift photobioreactor with A_d/A_r = 3.27 and 3L bubble column with an inner diameter of 9.4 cm operating at different levels of light intensities and at superficial velocity of 1.5 cm s^{-1}. The comparison between the effects of light intensity on the growth in airlift photobioreactor with standard F/2 and modified F/2 mediums (from Section 4.2) are illustrated in Fig. 4.5. It becomes quite clear that the modified F/2 medium outperformed the standard F/2 medium at all light intensities. The highest cell concentration, specific growth rate and productivity obtained from the standard F/2 medium at the light intensity around 34 μmol photons m^{-2} s^{-1} were about 1.8 x 10^6 cell mL^{-1}, 0.04 h^{-1} and 2.2 x 10^4 cell s^{-1}, respectively, which were less than those obtained from the culture with the modified F/2 medium were maximum also at the same light intensity of 34 μmol photons m^{-2} s^{-1} at about 4.6 x 10^6 cell mL^{-1}, 0.07 h^{-1} and 6.4 x10^4 cell s^{-1}, respectively.

At low range of light intensity (< 34 μmol photons m^{-2} s^{-1}), increasing the light intensity (from 20 to 34 μmol photons m^{-2} s^{-1}) helped induce photosynthesis and therefore an increase in maximum cell concentration, specific growth rate and productivity were observed. However, increasing light intensity above 34 μmol

photons m^{-2} s^{-1} was found to have negative impacts on the growth. This could be explained using the damage mechanism of the photosystem II (PS II) D1 protein (Sukenik et al., 1987; Davison., 1991; Vymazal., 1994; Grima et al., 1996), where the over-saturation of light caused damages to the PS II D1 protein that carried the binding sites for the electron carrier. In fact, a simultaneous repair-mechanism existed which produced new D1 molecules to replace damaged ones. At adequately low light intensity, all damaged D1 protein molecules were replaced almost immediately, and the net damage to the photosynthetic was negligible. At high light intensity, on the other hand, the repair occurred more slowly than the damage and this led to an apparently lower photosynthetic rate.

Fig. 4.6 illustrates the effects of light intensity on the performance of airlift photobioreactor compared with that of bubble column. Similar correlation between light intensity and growth in both types of reactors was observed. The best results were obtained from the airlift system at the light intensity of 34 μmol photons m^{-2} s^{-1}. The performance of bubble column was, unexpectedly, better than the airlift at high light intensity. This could be due to the effect of circulatory flow in the airlift which allowed all the cells to be exposed to high light intensity zone, and therefore the effect of high light intensity was distributed evenly throughout the culture. Since the flow in the bubble column was not homogeneous and there existed some precipitation at the bottom, only some fractions of cells were exposed to high light intensity whereas the cells under shading were not. Hence, the effect of light intensity in the bubble column was not as severe as that in the airlift.

4.3 Effect of aeration rate on growth of *S. costatum*

These experiments were conducted in the 3L airlift photobioreactor with A_d/A_r = 3.27 and 3L bubble column with an inner diameter of 9.4 cm operating at different levels of light intensities and at the light intensity of 34 μmol photons m^{-2} s^{-1}.

In airlift system, an increase in the aeration rate from 1 to 1.5 cm s^{-1} led to higher cell concentration, specific growth rate and productivity in both types of mediums (standard and modified F/2 medium). However, increasing aeration rate

above 1.5 cm s^{-1} was found to have negative impacts on the growth. This effect is illustrated in Fig. 4.7.

An increase in the aeration rate from 1 to 1.5 cm s^{-1} induced liquid circulation rate and mass transfer between gas and liquid phase in the system (Gavrilescu and Tudose, 1998) which facilitated the removal of metabolic gases such as oxygen, preventing the accumulation of such gases, avoiding potential adverse effects on the growth rate (Tung et al., 1998). The mixing of liquid caused a more homogeneity between the medium and cell which resulted in a more sufficient utilization of nutrients. Therefore, this lead to a higher maximum cell concentration and productivity. On the other hand, a much too high aeration rate ($u_{sg} > 1.5$ cm s^{-1}) led to a system with more gas bubbles along the downcomer. These gas bubbles were somewhat undesirable as they could prevent the passage of light to the center of the bioreactor as the light penetration ability was obstructed and dissipated by the swarm of gas bubbles. Also the mass transfer rate between phases decreased as a result of decreasing specific mass transfer area (of large bubbles). A low growth rate was apparent at this aeration rate. (Sriouam, 2007)

Fig. 4.8 compares the results from the cultivation in bubble column and airlift photobioreactor and reveals that the airlift system still provided a better performance, but only at u_{sg} of 1.5 cm s^{-1}. At other u_{sg}, no significant differences between the two types of reactors were observed.

4.4 Growth in airlift with different ratios between downcomer and riser cross section area

This section investigates the effect of design configuration of the airlift photobioreactor system, i.e. the ratio between downcomer and riser cross sectional area (A_d:A_r) on the growth of *S. costatum*. Experiment in this section was performed with the light intensity of 34 μmol photons m^{-2} s^{-1}. This parameter could be simply altered by changing the draft tube size. The four sizes of commercially available clear column were employed, i.e. at 3, 5, 6 and 7 cm, which gave A_d/A_r of approximately 14.87, 3.27, 1.87 and 0.98, respectively.

Fig. 4.9 describes the behavior of these airlift systems. The range of A_d/A_r examined in this work spanned from 0.98 to 14.87. At all range of u_{sg}, there seemed to be the optimal A_d/A_r that provided the highest maximum cell concentration, and for this work, this occurred at A_d/A_r of 3.27. At high aeration rate (u_{sg} = 2 cm s^{-1}) however, the system at A_d/A_r of 3.27 was found to be operated with low growth rate, but otherwise, the growth seemed to be better when A_d/A_r became larger. The selection of the most appropriate design of airlift was based on the productivity level and the airlift with A_d/A_r of 3.27 provided the best result at u_{sg} of 1.5 cm s^{-1}. At this condition, the maximum cell concentration, specific growth rate and productivity were 4.6 x 10^6 cell mL^{-1}, 0.07 h^{-1} and 6.4 x10^4 cell s^{-1}, respectively.

The light was only supplied on the outer wall of the airlift photobioreactor therefore cell in downcomer were better disclosed to light than those in riser. Therefore in general, if the riser is large, bubbles will play an important role in controlling the growth as bubbles could disperse or blockage of light pathway. On the other hand, if the riser is small and this enhanced the riser liquid velocity whilst considerably decreased the downcomer liquid velocity. At the same time, a high aeration rate could lead to low light intensity in the column as the blockage of light pathway from the swarm of bubbles was considerable. Therefore, the optimal configuration for the growth of *S. costatum* in the airlift photobioreactor was concluded to be at A_d:A_r of 3.2 with aeration rate at 1.5 cm s^{-1}.

4.5 Preliminary economic evaluation

The preliminary economic consideration for the production of 1 kg of *S. costatum* was based on the cultivation in the airlift photobioreactor and bubble column operated in batch mode, and the results are presented in Table 4.2 The differences in the performance of the bubble column and airlift required that the total volume of sea water for the cultivation in airlift photobioreactor (1450 L) was less than that for bubble column (2223 L). Besides, some other advantages of airlift photobioreactor, such as the reducing number of time cycle for the cultivation of *S. costatum* allowed the airlift to be operated more effectively, and this was reflected in the analysis as the airlift seemed to be more attractive than the bubble column The

specific operating cost for the cultivation in the airlift was approx. US$ 0.09 g^{-1} (3.41 THB g^{-1}), which was 27 % lower when compared with the cultivation in the bubble column (0.13 US$ g^{-1} or 4.66 THB g^{-1}) (see Appendix A for the example of calculation). Note that the investment cost was not included in this preliminary evaluation.

4.6 Nutritional value of *S. costatum* obtained from this work

This section analyses the nutritional value on the cultivation of *S. costatum*. The experiment was operated at the most suitable conditions determined from previous experiments as reported above, i.e. superficial gas velocity of 1.5 cm s^{-1}, light intensity of 34 μmol photons m^{-2} s^{-1} and with the modified F/2 medium (from Section 4.1. Carbohydrate, protein and lipid of the diatom were analyzed using the standard methods as follows:

Protein: Kjeldahl technique (AOAC, 1990)

Lipid: AOAC method (AOAC, 1995)

Carbohydrate: Calculation from humidity, ash and lipid

Expressed as percentage of dry weight, the range for the level of carbohydrate, protein and lipid are 21.5%, 31.2%, 1.26%, respectively.

The nutritional value of any algal species for a particular organism depends on its cell size, digestibility, production of toxic compounds, and biochemical composition. Generally, the gross composition of micro algae is compared in Table 4.2 where the general ranges of protein, lipid, and carbohydrate are 12-35%, 7.2-23%, and 4.6-23%, respectively (Brown, 1991). Although there are marked differences in the compositions of the microalgal classes and species, protein is always the major organic constituent, followed usually by lipid and then by carbohydrate. *Skeletonema* cultivated in this work provided reasonable amount of protein and carbohydrate but a slightly low level of lipid. When compared to *S. costatum* reported by Brown (1991) in Table 4.3, a much higher level of lipid was found than the level achieved in this work. Exact reasons for this were not evaluated in this work.

**Table 4.1** Cellular yield based on nutrient component

	Intitial Conc. (mg L^{-1})	Final Conc. (mg L^{-1})	Specific nutrient Uptake (x 10^{-10}mg L^{-1})	Cellular yield (x 10^{-3} pg Nutrient cell^{-1})
Silicon	3.7	0.6	3.1	0.4
phosphorus	24.3	1.9	22.4	2.9
Nitrogen	75.5	32.8	42.7	5.5

**Table 4.2** Economical analysis of the cultivation of 1 kg (dry weight) of *S. costatum*

Variable		Value	
		ALPBR	BC
Effective volume (L)	[A]	3	3
Cycle time (h)	[B]	45	36
Maximum cell concentration (cell mL^{-1})	[C]	4.6 x 10^6	2.9 x 10^6
Cell mass concentration (kg L^{-1})	[D]	0.69 x 10^{-3}	0.45 x 10^{-3}
Total volume of sea water used (L) (based on 1 kg)	[E=1÷D]	1450	2223
Number of reactor (reactor)	[F=E÷A]	484	741
Cost of water, 0.06 THB L^{-1} (THB)	[G=0.06 x E]	87	134
Cost of nutrient, 1 THB L^{-1} (THB)	[H=1 x E]	1450	2223
Power of air compressor (W)	[I]	900	1350
Power of light source (W)	[J]	13044	20000
Total electrical unit (units)	[K=(I+J) x B÷1,000]	628	769
Electrical cost, 3 THB per unit (THB)	[L=3xK]	1883	2306
Total operating cost (THB)	[M=G+H+L]	3419	4662
Total operating cost (US$) (36 THB per 1 US$) (Update 13/3/2009)	[N]	94	130
Total operating cost per gram (THB g^{-1})	[R=P÷1000]	3.41	4.66
Total operating cost per gram (US$ g^{-1})	[P=Q÷1000]	0.09	0.13

**Table 4.3** Concentration of protein, carbohydrate and lipid of microalgae commonly used in aquaculture industry

| Algal class	Percentage of dry weight			Source
Species	Protein	Carbohydrate	Total fat	
Bacillariophyceae				
Nitzchia closterium	26	9.8	13	Brown (1991)
Chaetoceros calcitrans	31.3	16.4	22.9	Nakseedee (2008)
Chaetoceros gracilis (Chaetoceros muelleri)	48.2	18.9	28.9	Coutteau (1996)
Skeletonema costatum	25	4.6	10	Brown (1991)
Skeletonema costatum	31	21.5	1.3	this work
Cyanophyceae				
Spirulina platensis	54.2	-	26.6	Li et al. (2003)
Spirulina sp.	67.0	25.5	30.1	Hernández and Olguín (2002)
Chlorophyceae				
Dunaliella tertiolecta	20	12.2	15	Brown (1991)
Nannochloris atomus	30	23.0	21	Brown (1991)
Cryptophyceae				
Chroomonas salina	29	9.1	12	Brown (1991)
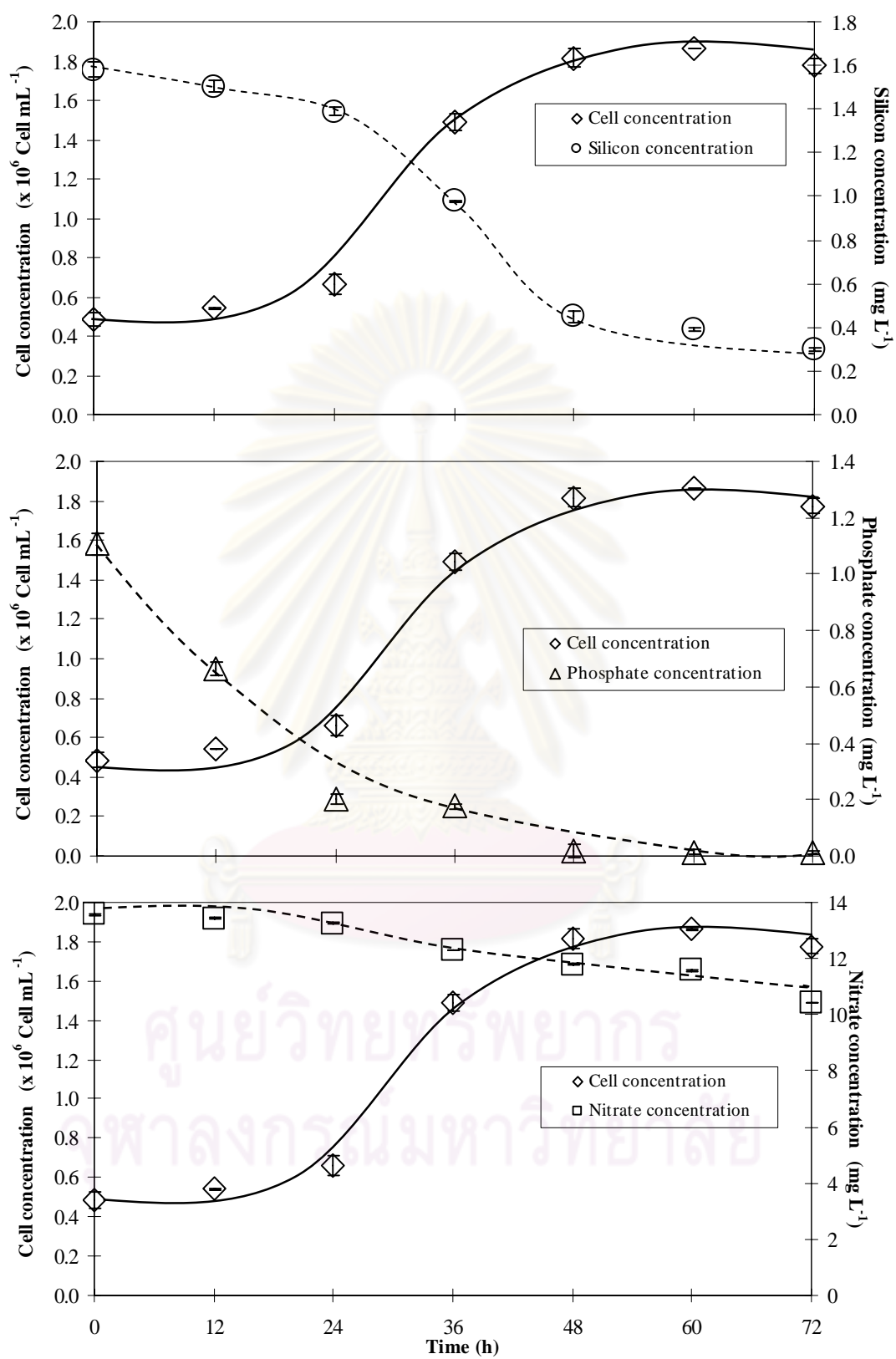
Figure 4.1 Growth behavior and nutrient concentration from cultivation
of *S. costatum*
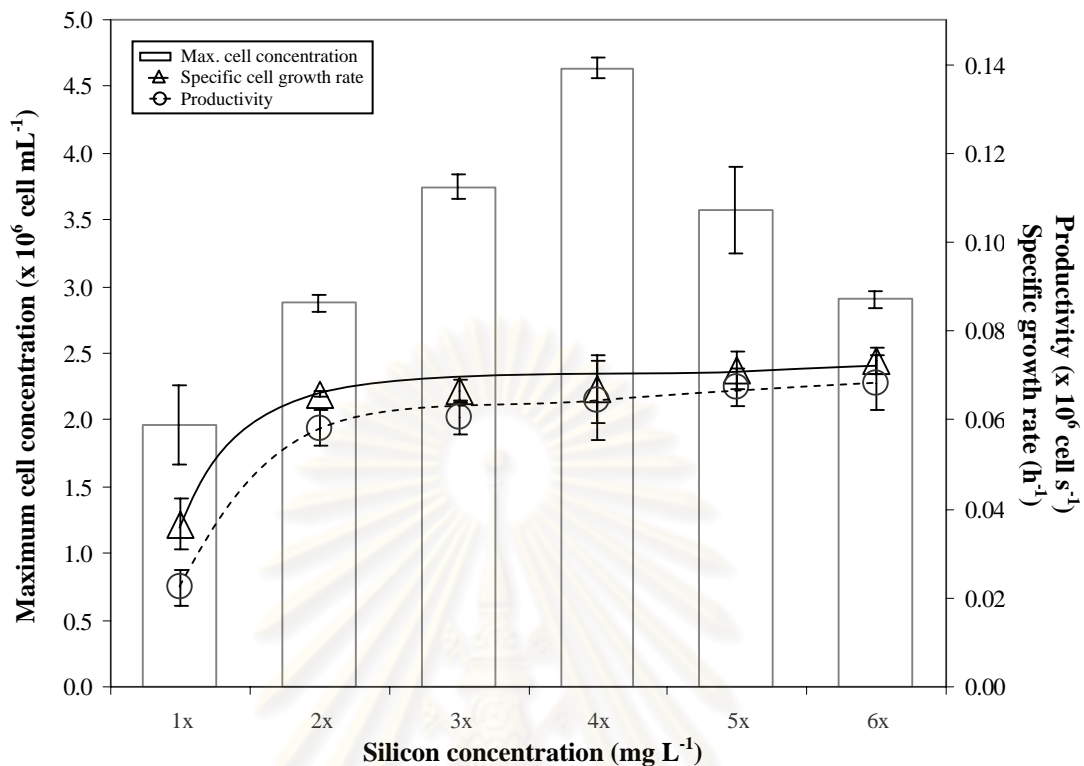
Figure 4.2 Effect of silica concentration on cell growth
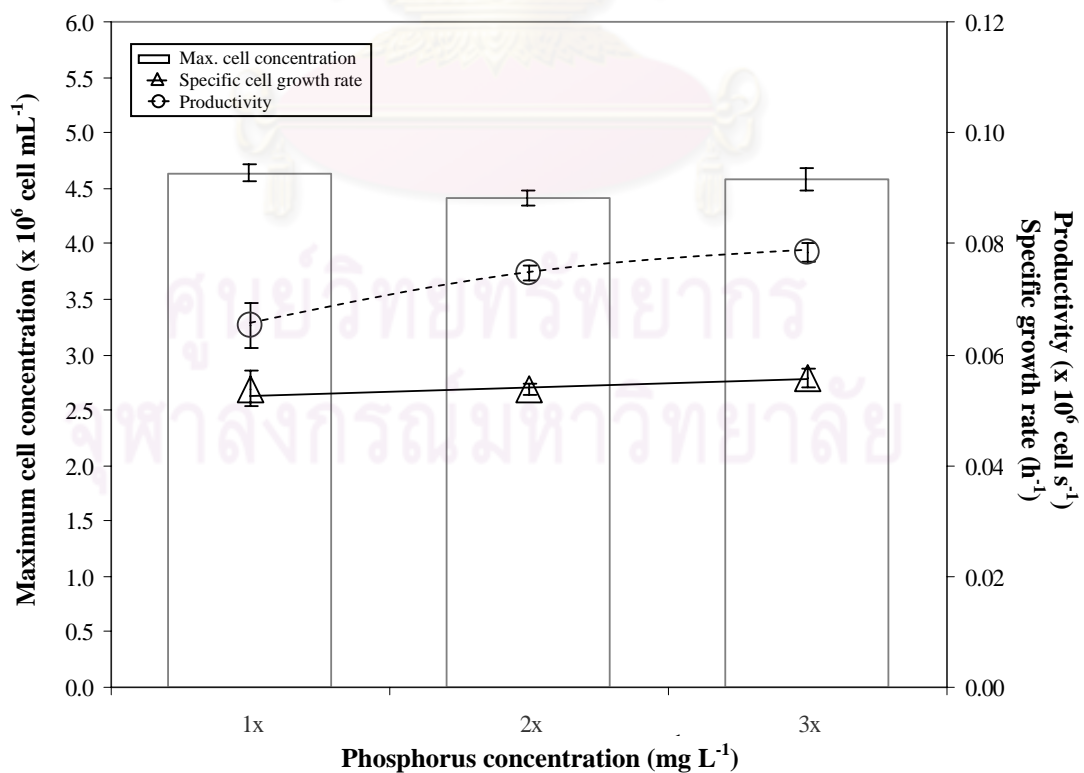
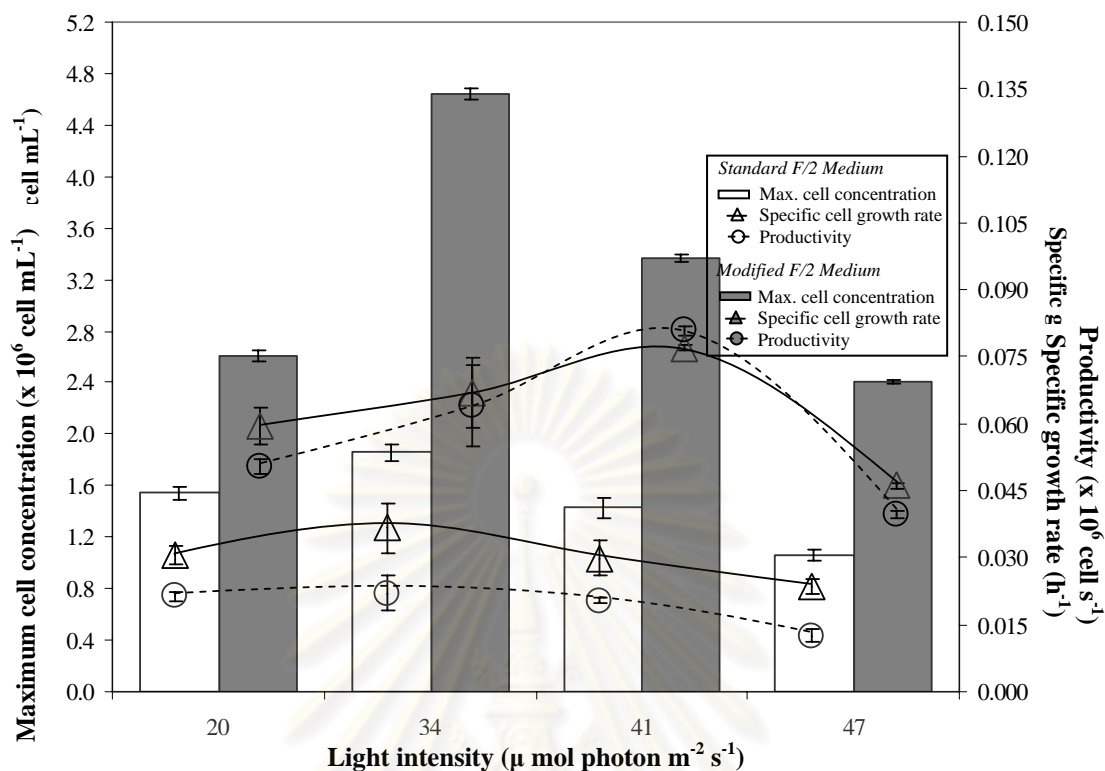**Figure 4.3** Effect of phosphorus concentration on cell growth
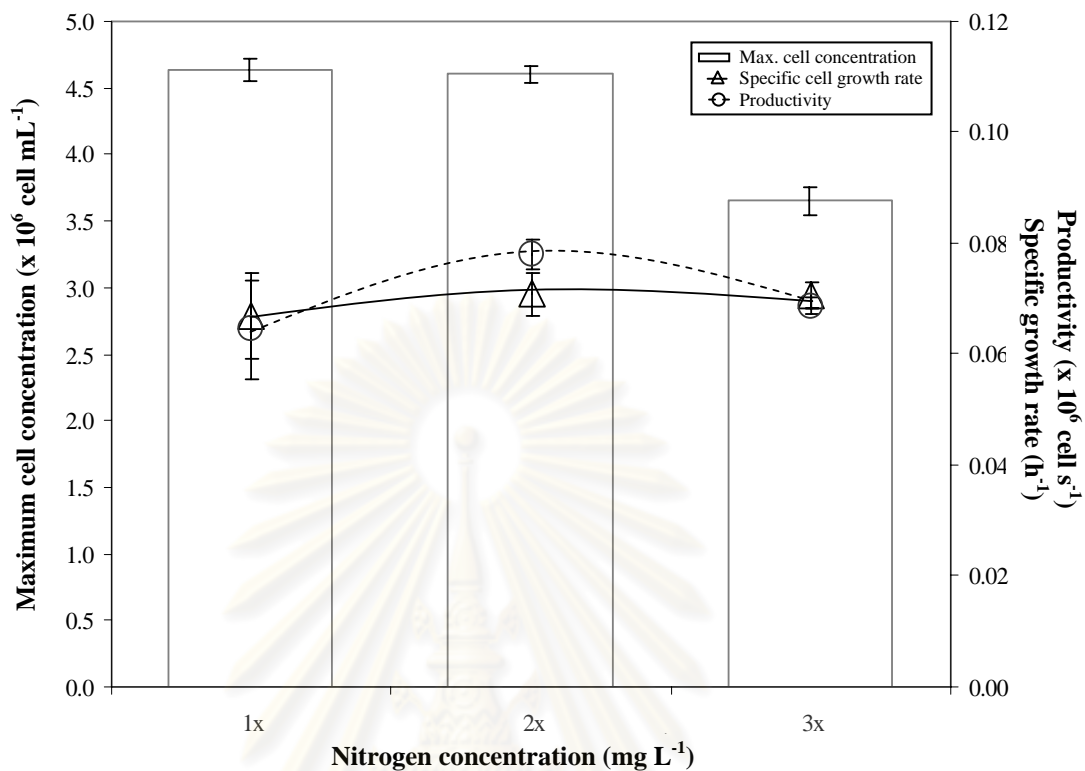
Figure 4.4 Effect of nitrogen concentration on cell growth

Figure 4.5 Effect of light intensities in airlift photobioreactor with standard F/2 and modified F/2 mediums
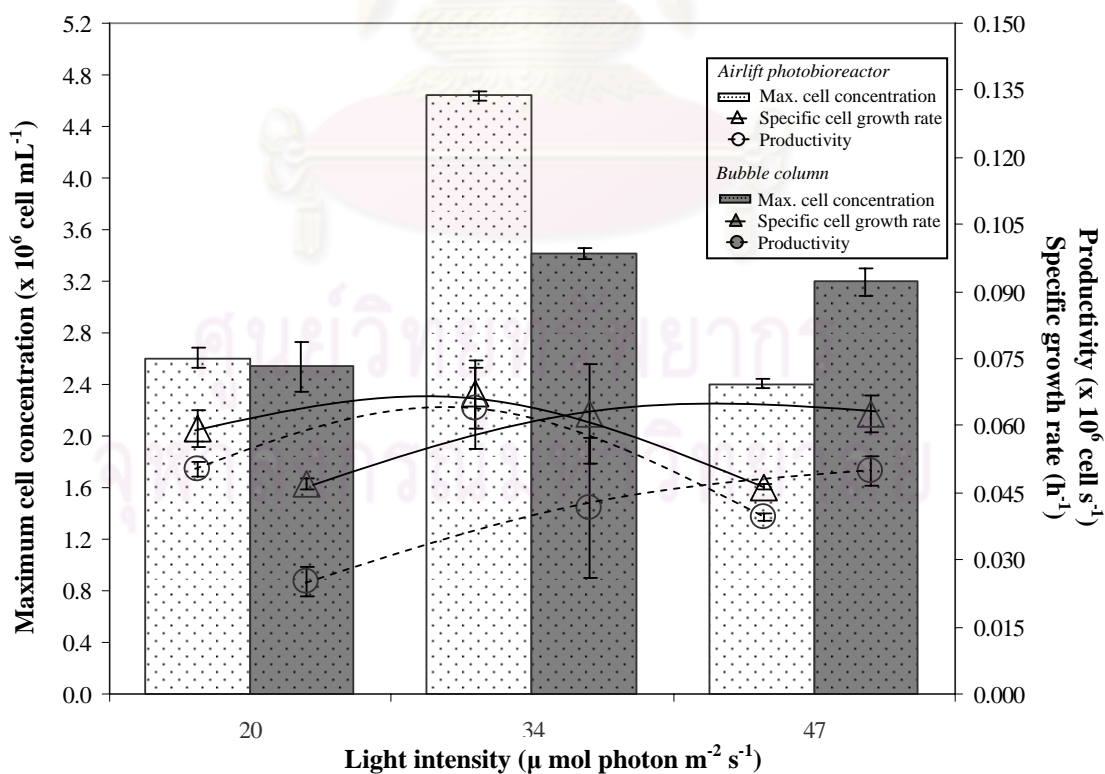
Figure 4.6 Effect of light intensities in airlift photobioreactor and bubble column operated with modified F/2 medium
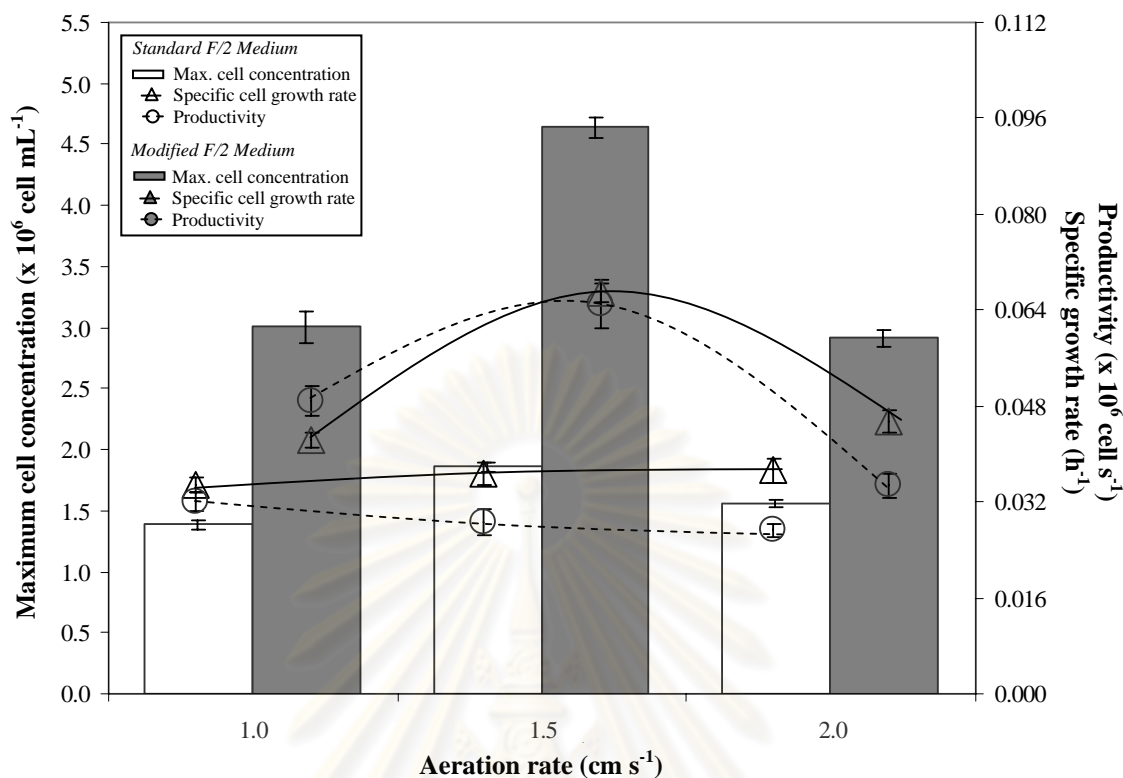
Figure 4.7 Effect of aeration rate on cell growth in airlift photobioreactor operated with standard F/2 and modified F/2 mediums
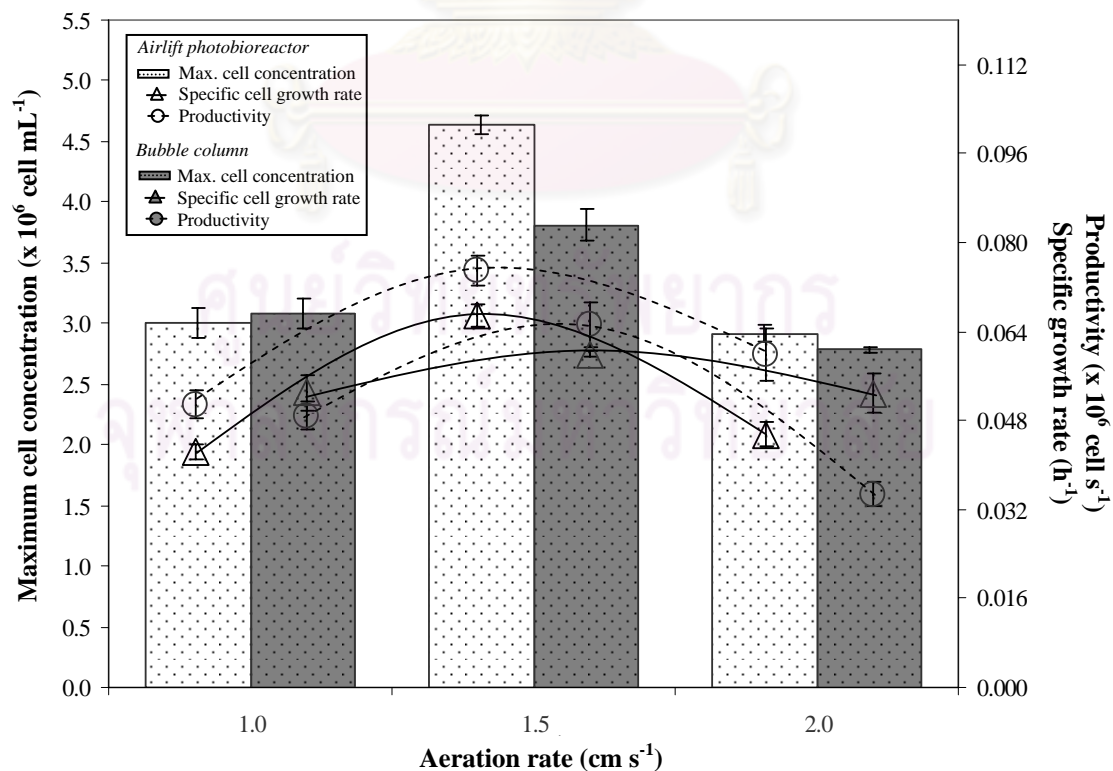
Figure 4.8 Effect of aeration rate on cell growth in airlift photobioreactor and bubble column operated with modified F/2 mediums

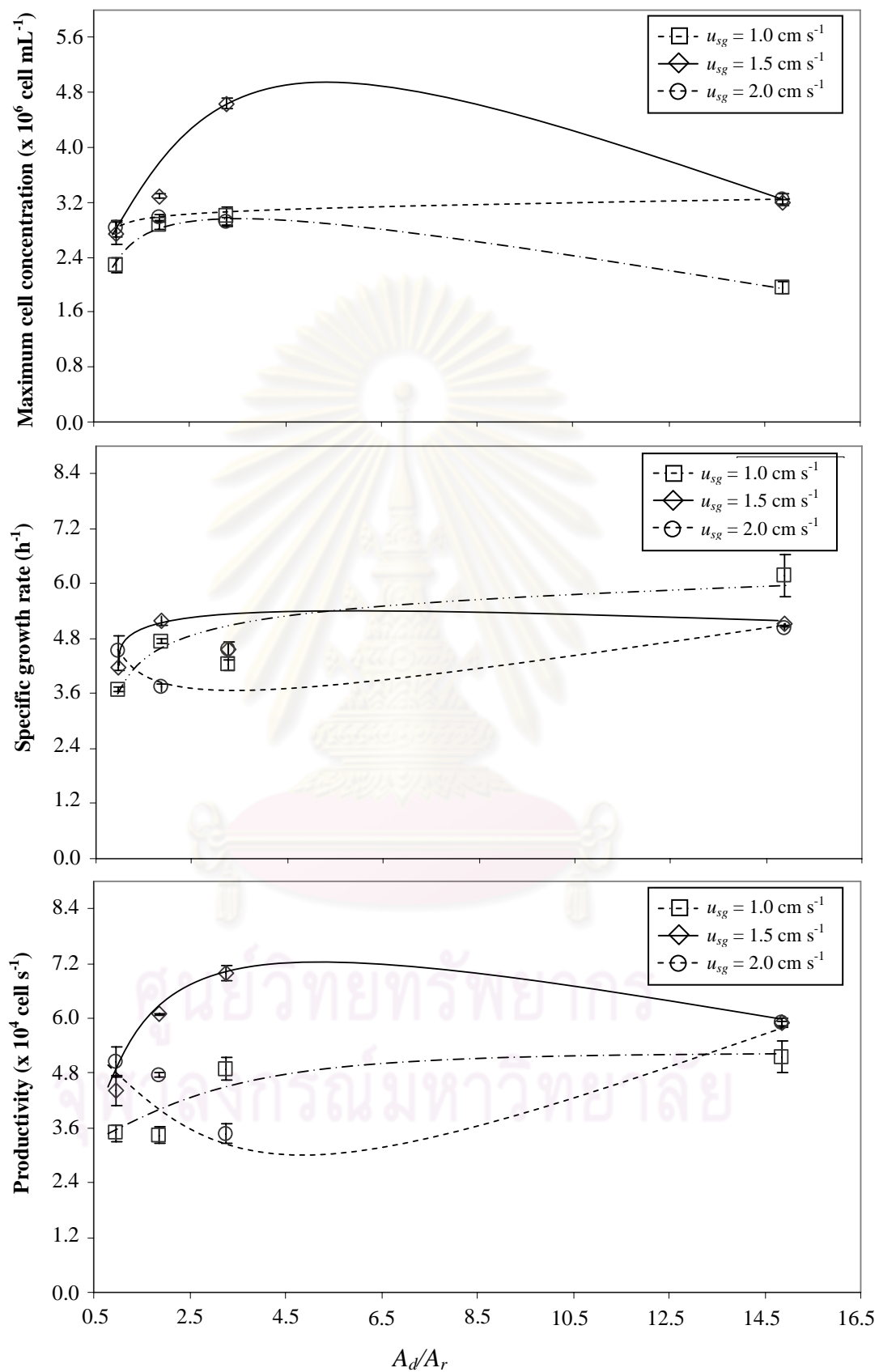**Figure 4.9** Growth performance in airlift systems at various A_d/A_r operated with

modified F/2 medium

CHAPTER V

CONCLUSIONS, CONTRIBUTIONS, AND RECOMMENDATIONS

5.1 Conclusions

This work demonstrates that an airlift system was suitable for the cultivation of *S. costatum*. Appropriate nutrient concentrations, light intensities, aeration rate and ratio between downcomer and riser cross sectional area were identified and these are summarized below:

Parameter	Optimum
Nutrient concentration	Standard F/2 medium with 4 fold silica concentrations
Light intensity	34 μmol photon m^{-2}s^{-1}
Aeration rate	1.5 cm s^{-1}
A_d/A_r	3.27

Furthermore, this work revealed that the cultivation of *S. costatum* in the airlift photobioreactor was superior to that in the bubble column. This was due primarily to the well defined flow pattern in the airlift photobioreactor which led to a more effective light utilization of the algae. The optimal culture yielded the specific growth rate of 0.046 h^{-1} with the maximum cell concentration of 4.6x10^6 cells mL^{-1} and the productivity of 6.5x10^4 cell s^{-1}.

5.2 Contributions

S. costatum is widely used as feed in aquaculture industry particularly prawn larvae cultivation and therefore the optimization of the growth condition of this diatom is important for the effective mass production. The cultivation of *S. costatum*

is generally performed in bubble columns. However, bubble columns suffer drawbacks from its noncirculatory flow causing problems with mixing of air and medium. In addition, precipitation of cells could be significant and all these result in an inhibited growth. This research offers an alternative airlift photobioreactor which is developed to achieve circulatory flow in the reactor, minimizing cell precipitation, enhancing the light utilization efficiency, and hence, high cell growth is obtained. This work aims to examine the performance of airlift system on the cultivation of *Skeletonema costatum* compared to that in bubble columns. The experiment reported here is also one of the first experiences in the culture of *S. costatum* in the airlift bioreactor and constitutes the initial step in testing the potential of this strain for mass production.

5.3 Recommendations

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

1. the influence of CO_2 on the growth of this diatom;
2. the cultivation in the semi-continuous culture to ensure the highest biomass productivity.
3. the upscale of the airlift photobioreactor; and
4. the usage of airlift photobioreactor as an outdoor culture to make the most advantage of the natural light and minimizing the electricity cost.

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http://www.chbr.noaa.gov/pmn/resources.htm

APPENDIX

Appendix A : Example for the calculation of Economic for cultivation of *S. costatum*

Parameters for the cultivation of *S. costatum* in photobioreactor are shown below:

Effective volume	=	3 L
Cycle time	=	45 h
Maximum cell concentration	=	4.6×10^6 cell mL^{-1} (4.6×10^9 cell L^{-1})
Cell mass concentration	=	0.69 g L^{-1} (0.69×10^{-3} gL^{-1})
Power of light source	=	13044 W
Power of air compressor	=	900 W

$$\text{Total volume of seawater used} = \frac{1\,kg}{\text{cell mass concentration}}$$

$$= \frac{1\,kg}{0.69 \times 10^{-3}\,kg\,L^{-1}}$$

$$\approx \underline{1450\,L}$$

$$\text{Number of reactor} = \frac{\text{Total volumn of seawater used}}{\text{Effective volumn}}$$

$$= \frac{1450\,L}{3\,L}$$

$$\approx \underline{484}$$

Cost of water (0.06THB L^{-1})   = 0.06 THB L^{-1} x Total volume of seawater used

$$= 0.06\text{ THB L}^{-1}\text{x }1450\text{ L}$$

$$\approx \underline{87\text{ THB}}$$

Cost of nutrient (1 THB L^{-1})   = 1 THB L^{-1} x Total volume of seawater used

$$= 1 \text{ THB L}^{-1} \text{ x } 1450 \text{ L}$$

$$= \underline{1450 \text{ THB}}$$

Total electrical unit (unit or kW h) $\quad$ = Power of light source and air compressor

$\quad$ x Cultivation time

$$= (13044 \text{ W} + 900 \text{ W}) \text{ x } 45 \text{ h}$$

$$\approx \underline{628 \text{ unit}}$$

Electric cost (3 THB unit^{-1}) $\quad$ = 3 THB unit^{-1} x 628 unit

$$\approx \underline{1883 \text{ THB}}$$

Total operating cost $\quad$ = Electric cost + Cost of water + Cost of nutrient

$$= 1883 \text{ THB} + 87 \text{ THB} + 1450 \text{ THB}$$

$$= 3420 \text{ THB} = \frac{3420 \text{ THB}}{36 \text{ THB / US \$}}$$

$$\approx \underline{95 \text{ US \$}}$$

Total operating cost per gram $\quad = \dfrac{\text{Total operating cos t}}{1000 \text{ g}}$

$$= \frac{3420 \text{ THB}}{1000 \text{ g}}$$

$$\approx 3.41 \text{ THB g}^{-1} \approx \frac{3.41 \text{ THB / g}}{36 \text{ THB / US \$}}$$

$$\approx \underline{0.09 \text{ US \$ g}^{-1}}$$

Appendix B: Measurement of silicon concentration

(Molybdosilicate Spectrophotometric Method

(Fanning and Pilson, 1973))

Reagents

- Sodium bicarbonate ($NaHCO_3$)

- Sulfuric acid (H_2SO_4) 1 N

- Hydrochloric acid (HCl)

- Ammonium molybdate reagent

Dissolve 10 g $(NH_4)_6Mo_7O_{24}.4H_2O$ in distilled water, with stirring and gentle warming, and dilute to 100 mL. Filter if necessary. Adjust to pH 7 to 8 with silica-free NH_4OH or NaOH and store in a polyethylene bottle to stabilize.

- Oxalic acid solution

Dissolve 7.5 g $H_2C_2O_4.2H_2O$ in distilled water and dilute to 100 mL.

- Stock silica solution

Dissolve 4.73 g sodium metasilicate nonadrate, $Na_2SiO_3.9H_2O$, in freshly boiled and cooled distilled water and dilute to approximately 900 mL and store in a plastic bottle.

Procedure

- Preparation of sample

Take the sample (50 mL) and separate diatom from the culture medium by centrifuge at 3,000 rpm with 30 min. Add in rapid succession 1 mL HCl and 2 mL ammonium molybdate reagent in. Mix by inverting at least six time and let stand for 5 to 10 min. Add 2 mL oxalic acid solution and mix throughtly. Read color after 2 min but before 15 min, measuring time from addition of oxalic acid.

- Preparation of standards

Dilute 10 mL stock solution to 1000 mL with freshly boiled and cooled distilled water; 1 mL = 10 µg SiO_2 and store in a plastic bottle.

- Photometric measurement

Measure the solution by Spectrophotometer with wavelength 810 nm

- Calculation

Calculate the concentration of silicon (mg SiO_2 L^{-1}) with standard curve as shown in Figure A-1

$$y = 7.3525x^2 + 0.0239x + 0.0032$$
$$R^2 = 0.9992$$

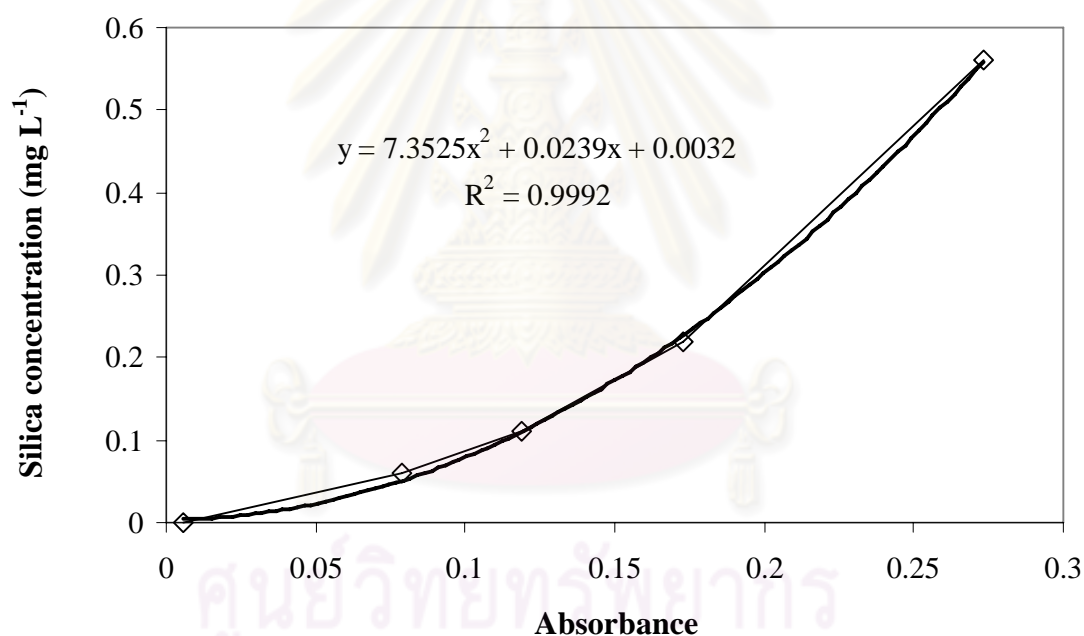Figure A-1: Standard curve for the calculation of silica concentration in modified F/2 medium

Appendix C: Measurement of nitrogen concentration

(Ultraviolet spectrophotometric screening method
(Armstrong, 1963))

Reagents

- Nitrate free water

Use redistilled or distilled, deionized water of highest purity to prepare all solution and dilutions.

- Stock nitrate solution

Dry potassium nitrate (KNO_3), in an oven at 105 ^{o}C for 24 hr. Dissolve 0.7218 g in water and dilute to 1000 mL.

- Standard nitrate solution

Dilute 50 mL stock nitrate solution to 500mL with water; 1 mL = 10 µg NO_3^-.

- Hydrochloric acid (HCl) 1 N

Procedure

- Preparation of sample

Take the sample (50 mL) and separate diatom from the culture medium by centrifuge at 3,000 rpm with 30 min. Add in rapid succession 1 mL HCl solution and mix thoroughly.

- Preparation of standards curve

Prepare NO_3^- calibration standards in the range 0 to 11 mg NO_3^- L^{-1} by diluting to 50 mL the following volumes of standard nitrate solution. Treat NO_3^- standard in same manner as samples.

- Spectrophotometric measurement

Read absorbance or transmittance against redistilled water set at zero absorbance or 100% transmittance. Use a wavelength of 220 nm to obtain

NO$_3^-$ reading and a wavelength of 275 nm to determine interference due to dissolve organic matter.

- Calculation

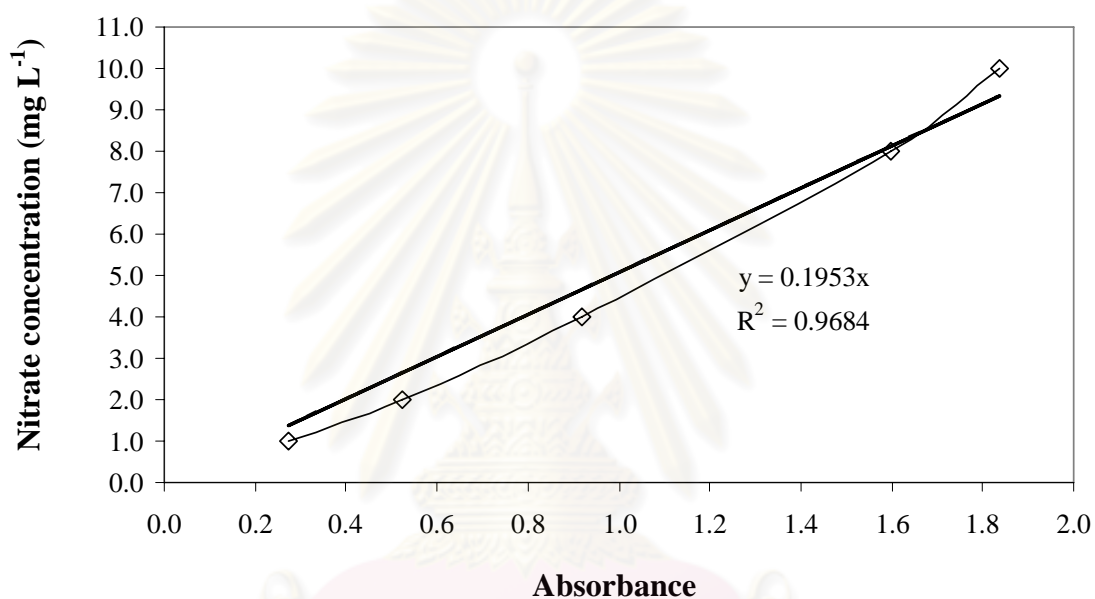Calculate the concentration of silicon (mg NO$_3$ L^{-1}) with standard curve as shown in Figure A-2

$y = 0.1953x$
$R^2 = 0.9684$

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

Appendix D: Measurement of phosphorus concentration

(Fontaine, 1942)

Reagents

- Phenolphthalein indicator solution

Dissolve 5 g phenolphthalein in 500 ml ethyl alcohol and add 500 mL distilled water. Then add 0.02 N NaOH until a faint pink color appears.

- Standard phosphate solution

Dissolve in distilled water 0.7164 g of potassium dihydrogen phosphate KH_2PO_4, which has been dried in an oven at 105 ^{o}C. Dilute the solution to 1 L. The dilute 100 mL of this to 1 L. Of this second solution, 1000 mL = 0.05 mg of PO_4.

- Sulfuric acid solution

Slowly add 120 ml concentration H_2SO_4 to about 750 mL distilled water. Cool the solution and dilute to 1 L.

- Amino-naphthol-sulfonic acid solution

Grind 0.5 g 1-amino-2-naph-thol-4-sulfonic acid with 5 mL sodium sulfite solution containing 1 g anhydrous Na_2SO_3 per 5 mL; and dissole in 200 mL sodium metabisulfite solution containing 30 g $Na_2S_2O_5$ per 200 mL. Filter. Keep in brown, tightly stoppered bottleat room temperature and prepare fresh every 2 weeks. The reagent become hightly colored as deterioration proceeds.

- Hydrochloric acid (HCl) 1 N

Procedure

- Take the sample (50 mL)and separate diatom from the culture medium by centrifuge at 3,000 rpm with 30 min. Add 1 drop phenolphthalein indicator. If the sample turns pink, add sufficient dilute H_2SO_4 dropwise, to discharge the color.

- Dilute to approximately 70 ml with distilled water. Add, mixing thoroughtly after each reagent addition, 10 mL amino-naphthol-sulfonic acid solution. Dilute to 100 mL with distilled water. The rate of color developed depend on the temperature of the final solution; hence sample, standard, and

reagents should be within $3^{o}C$ of one another and at a temperature between $20^{o}C$ and $30^{o}C$. After exacty 10 min, measure the color photometrically and calibration curve (wavelength of 650 nm), using a distilled water blank. Calculate the concentration of silicon (mg PO_4 L^{-1}) with standard curve as shown in Figure A-3.

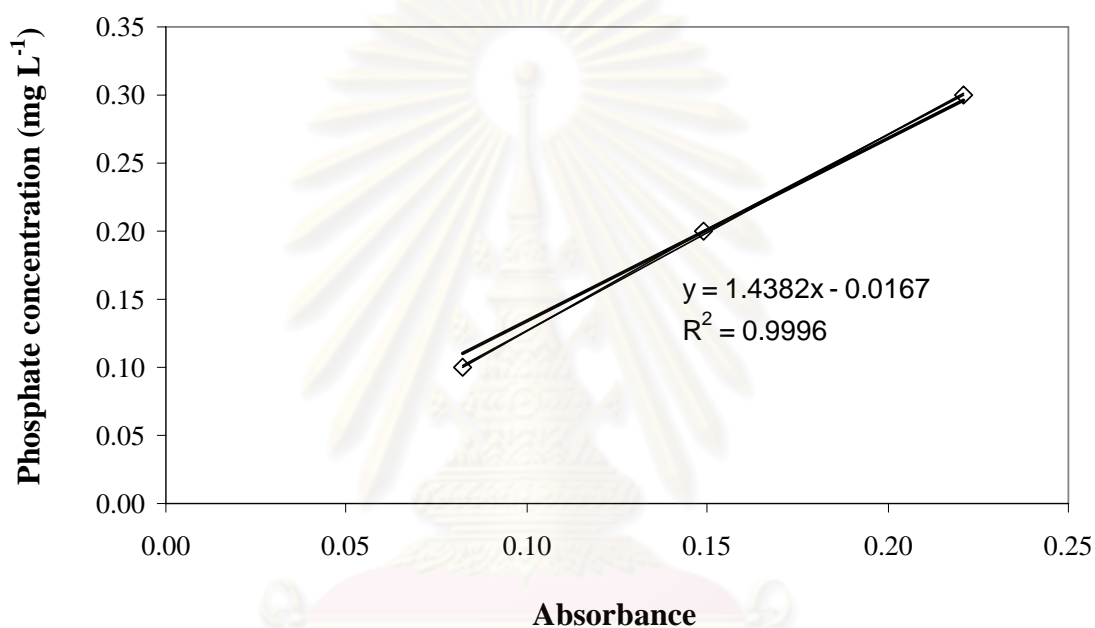Figure A-3: Standard curve for the calculation of phosphate concentration in modified F/2 medium

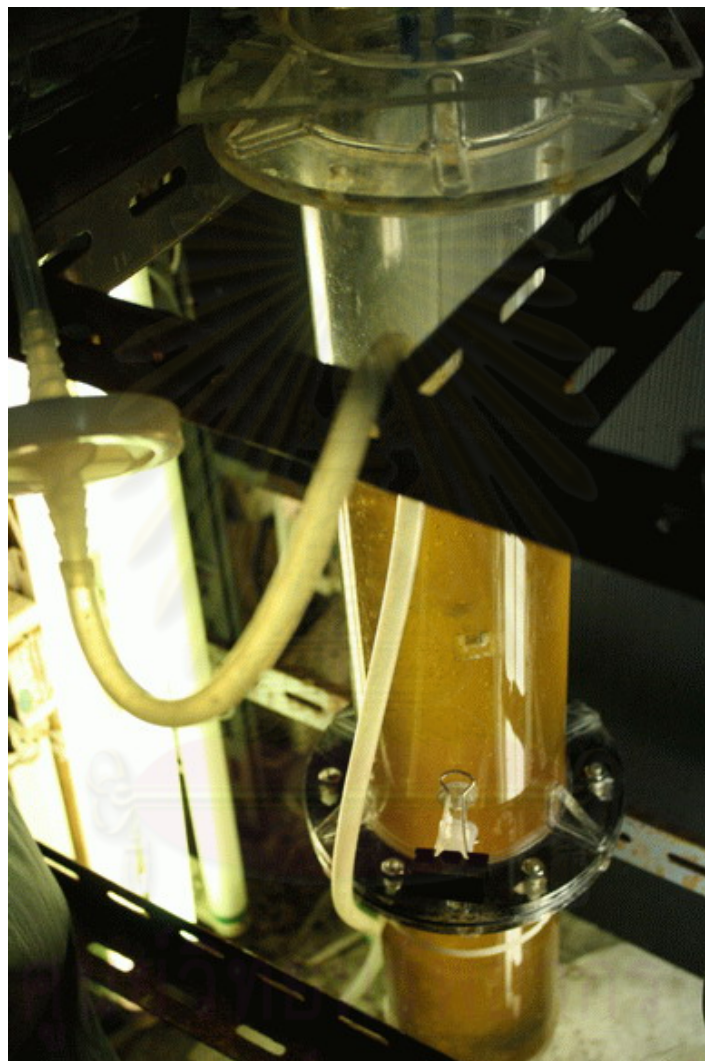# Appendix E: Cultivation of *S. costatum* in airlift photobioreactor

Figure A-4: Experimental setup for the cultivation of *S. costatum* in airlift

photobioreactor

BIOGRAPHY

Miss. Saranya Monkunsit was born on 1st June, 1983 in Chiang Mai. She finished the higher secondary course from Suanboon Yopatham Lamphun School in March, 2001. After that, she studied in the major of Biotechnology in Faculty of Science at King Mongkut's Institute of Technology Ladkrabang. She continued her further study for Master's degree in Chemical Engineering (Biochemical engineering research group) at Chulalongkorn University in 2005.