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ปีการศึกษา 2556

REDUCTION OF PRODUCTION COST CHAETOCEROS GRACILIS CULTIVATION IN AIRLIFT PHOTOBIOREACTORS WITH REUSE CULTURE MEDIUM

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Engineering Program in Chemical Engineering
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ปัทมา ซัง : การลดต้นทุนการเพาะเลี้ยงสาหร่ายคีโตเซอรอส ในถังปฏิกรณ์ชีวภาพอากาศยก โดยการนำสารอาหารกลับมาใช้ใหม่. (REDUCTION OF PRODUCTION COST CHAETOCEROS GRACILIS CULTIVATION IN AIRLIFT PHOTOBIOREACTORS WITH REUSE CULTURE MEDIUM) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร. ประเสริฐ ภวสันต์, หน้า.

การเพาะเลี้ยงสาหร่าย คีโตเซอรอส กราซิลิส ในถังปฏิกรณ์ชีวภาพอากาศยกขนาด 5 ลิตร ที่อัตราความเข้มข้นเซลล์เริ่มต้น 5 x 10^5 เซลล์ต่อมิลลิลิตร ใช้ความเข้มแสง 135 ไมโครโฟตรอน ต่อตารางเมตร ต่อวินาที ในงานวิจัยนี้แบ่งการทดลองออกเป็นสี่ส่วน คือ การเลี้ยงคีโตเซอรอส กราซิลิสโดยการนำสารอาหารกลับมาใช้ใหม่ตามสูตร modified F/2 medium พบว่า การเลี้ยงในรอบสารอาหารใหม่ให้การเจริญเติบโตที่ดีที่สุด โดยให้ความหนาแน่นเซลล์สูงสุดอยู่ที่ 10.73 ± 0.35 x 10^6 เซลล์ต่อมิลลิลิตร ขณะที่การเลี้ยงโดยการนำสารอาหารกลับมาใช้ใหม่ความหนาแน่นเซลล์สูงสุดลดลง50% ทั้งนี้ในส่วนขององค์ประกอบทางชีวเคมีคือ ในการเลี้ยงโดยนำสารอาหารกลับมาใช้ใหม่ปริมาณไขมันเพิ่มขึ้นจากการเลี้ยงแบบสารอาหารใหม่ แต่ในขณะเดียวกันปริมาณคาร์โบไฮเดรตลดลงและโปรตีนมีค่าคงที่ สำหรับส่วนที่สองเป็นการทดลองหาความเข้มข้นของสารอาหารหลักคือ ซิลิเกต ไนเตรต และ ฟอสเฟต ที่เหมาะสมต่อการเจริญเติบโต โดยการค่อยๆเติมสารอาหารที่แต่ละความเข้มข้นในแต่ละวันเป็นระยะเวลา 4 วัน พบว่าที่ความเข้มข้น 50%ของสารอาหารหลักแต่ละชนิด ให้ความหนาแน่นเซลล์ใกล้เคียงกับการเลี้ยงโดยใช้สารอาหารตามสูตรตั้งต้น(ถังควบคุม) ในขณะที่องค์ประกอบทางชีวเคมี พบว่า ปริมาณคาร์โบไฮเดรตจะเพิ่มขึ้น ในขณะที่ปริมาณไขมันลดลง และปริมาณโปรตีนใกล้เคียงกันเมื่อเทียบกับค่าที่ได้จากถังควบคุม ในการทดลองส่วนนี้พบว่าที่50%ของสารอาหารหลักแต่ละชนิดให้ผลที่ดีที่สุดจึงนำไปทดลองต่อในส่วนที่สาม คือการนำสารอาหารกลับมาใช้ใหม่ จากการทดลองพบว่าการเลี้ยงรอบสารอาหารใหม่ที่ 50%ซิลิเกต 50%ไนเตรต และ 50%ฟอสเฟต ให้ความหนาแน่นเซลล์สูงสุด คือ 11.07 ± 0.78 x10^6, 11.64 ± 0.11 x10^6 และ 9.83 ± 0.12 x10^6 เซลล์ต่อมิลลิลิตร ตามลำดับ ซึ่งได้ผลเทียบเท่าการเลี้ยงในถังควบคุมคือ 11.61 ± 0.82 x10^6เซลล์ต่อมิลลิลิตร ในขณะที่การทดลองโดยการนำสารอาหารกลับมาใช้ใหม่ความหนาแน่นเซลล์สูงสุดลดลงจากรอบสารอาหารใหม่50% สำหรับองค์ประกอบทางชีวเคมีของรอบการนำสารอาหารกลับมาใช้ใหม่นั้น ปริมาณคาร์โบไฮเดรตลดลง ขณะที่ปริมาณไขมันเพิ่มขึ้นและปริมาณโปรตีนค่อนข้างคงที่ เมื่อเทียบกับการเลี้ยงรอบสารอาหารใหม่และการเลี้ยงในถังควบคุม ในส่วนสุดท้ายของงานวิจัยเป็นการศึกษาเปรียบเทียบผลทางเศรษฐศาสตร์ พบว่าในการเลี้ยงโดยการนำสารอาหารกลับมาใช้ใหม่ มีต้นทุนที่สูงกว่าการเลี้ยงในรอบสารอาหารใหม่ เนื่องจากต้องใช้ปัจจัยในการผลิตมากขึ้นเพื่อให้ได้ผลการผลิตเทียบเท่าการเลี้ยงในรอบสารอาหารใหม่

ภาควิชา วิศวกรรมเคมี ลายมือชื่อนิสิต .....

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PATTHAMA SUNG: REDUCTION OF PRODUCTION COST CHAETOCEROS GRACILIS CULTIVATION IN AIRLIFT PHOTOBIOREACTORS WITH REUSE CULTURE MEDIUM. ADVISOR: ASSOC. PROF. PRASERT PAVASANT, Ph.D., pp.

Chaetoceros gracilis was cultivated in the batch cultivation system using 5 L airlift photobioreactor. The initial cell concentration was 5 x 10^5 cells mL^{-1} and Light was supplied at 135 μmol photon m^{-2} s^{-1}. This research is divided into four sections. Firstly, the cultivation of *C. gracilis* with fresh sea water which was cultivated using modified F/2 medium and the reused medium which was cultivated again with the medium after cell separation. A maximum cell density obtained from the fresh medium was approximately 10.73 ± 0.35 x 10^6 cell mL^{-1}. The maximum cell density of reused medium was 50% lower than that from the fresh medium. Regarding the biochemical composition, total lipid obtained from the reused medium experiment was higher than that from the fresh medium, and the opposite was found for carbohydrate, whereas the level of protein remained unaltered regardless of the management of the medium. Secondly, the culture with varying macronutrient was examined, i.e. silicate, nitrate and phosphate concentration. For the case of 50% nutrient (daily added equally for 4 days) the system exhibited similar performance with that of the control, fresh medium. Carbohydrate was higher with the 50% nutrient experiment whereas lipid seemed to be a major component in the control experiment. Thirdly, the cultivation of C. gracilis with reused 50% macronutrients. The maximum cell densities from the cultivation with 50%silicate, 50% nitrate and 50%phosphate were approximately at the same level, i.e. 11.07 ± 0.78 x10^6, 11.64 ± 0.11 x$10^6$and 9.83 ± 0.12 x10^6 cell mL^{-1}, respectively. The maximum cell density of reused medium was 50% lower than that from the control experiments. Finally, economics demonstrates that the reuse of medium did not contribute to the cost effectiveness as it affected the growth rate and therefore the system with reused medium requires more inputs to give a comparative performance with the control experiment.

Department: Chemical Engineering Student's Signature .....

Field of Study: Chemical Engineering Advisor's Signature .....

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CHAPTER 1

INTRODUCTION

1.1 Motivations

The production of biofuels from microalgae has stimulated numerous attentions recently. Although the oil product from the microalgae still is quite costly when compared with oil derived from vegetation, the growth of algae is relatively faster leading to a better yield and there is room to improve on process efficiency. In addition, microalgae can be cultivated all year long and can be harvested on a daily basis which allows a better process management (rather than harvesting once a year and the raw material will have to be stored in silo for months). Moreover the use of microalgae to produce biofuels does not lead to the problem of food security, unlike the other land-crops where the energy plants are blamed for the invasion and reduction of food-crop lands (Gouveia & Oliveira, 2009). Marine microalgae have been applied in aquaculture industry, especially in nursery stages of many aquatic lives, e.g. shrimp and fish (Liang *et al*., 1997). For shrimp larvae, *Chaetoceros gracilis* is one of the most popular species in Thailand as it can be cultivated quite easily. Preliminary examination reveals that this alga contains a reasonable amount of triglyceride and might be a good source of biofuels.

Recently, there has been a study on the optimal growth and reactor design for the cultivation of *Chaetoceros calcitrans* where the maximum cell density of approximately 8.88×10^{6} cell mL^{-1} with a maximum specific growth rate of 7.41×10^{-2} h^{-1} were achieved (Krichnavaruk *et al*., 2005). Moreover, the culture could be grown in various modes of operation both in semi-continuous and continuous, and both indoor and outdoor. One of the most important economic factors for the cultivation of *Chaetoceros* is the cost of nutrients, as the high cost silica which is one of the main nutritional components constitutes more than 80% of the overall cost and this makes the nutrient cost as high as more than 1 THB per liter.

This work investigates the effectiveness in the use of culture medium and to examine preliminary economic analysis for such *Chaetoceros gracilis* cultivation from the management of nutrient. The work will start by examining the requirement of major nutrients for the growth of *Chaetoceros gracilis* and design the most efficient nutrient formula for such alga. Finally the economic analysis of such nutrient management will be performed and compared with the use of conventional F/1

medium. The reuse of nutrients will also be examined in cases where excess nutrients have to be used for a better growth.

1.2 Objectives

The objectives of work were to

- Examine the needs of major nutrients from *Chaetoceros gracilis*
- Design the nutrients which best suits the growth of *Chaetoceros gracilis*
- Investigate the reuse of nutrients if necessary
- Conduct preliminary economic analysis from the use of new composed nutrient compared with typical F/2 nutrients

1.3 Working Scopes

- The cultivation of *Chaetoceros* gracilis in batch cultivation system and varied in the initial cell concentration range of 1-10 (x105 cells mL-1)
- Employed in this work is 5 L acrylic airlift photobioreactor. The configuration of reactor will be dimensions in Table 3.1
- The ratio between the downcomer and riser cross section areas (AD/Ad) of 2.17
- The superficial gas velocity of 3 cm s^{-1}
- The light intensity is controlled at 135 μmol photon m^{-2} s^{-1}
- Only nutrients that constitute the major cost of the nutrient will be examined

CHAPTER 2

BACKGROUND AND LITERATURE REVIEW

2.1 Microscopic algae

2.1.1 Diatoms

Diatoms are unicellular algae, can be found in freshwater and seawater. Diatoms are in the division of *Chromophyta* (class *Bacillariophyceae*). Diatoms generally range in size from ca. 2-200 microns (Hasle and Syvertsen, 1997). Their major identify lie in the cell wall that is composed primarily of silica (Horner, 2002). Major pigments of diatoms are chlorophylls a and c, beta-carotene, fucoxanthin, diatoxanthin and diadinoxanthin (Hasle and Syvertsen, 1997). There are about 10,000 different species of diatoms with different shapes such as a sphere or egg dishes, etc. The propagation is by cell division. Depending on environmental conditions, diatoms can divide as much as $1x10^{9}$ cells within one month. Diatoms have many implications to the environment, such as food fish, shellfish and food for aquatic larvae. Diatom widely used in hatcheries are *Skeletonema, Navicula, Asteroplonus, Chaetoceros, etc*.(Marasigan, 1989). Diatom *Chaetoceros* was a popular culture especially as the food for shrimp larvae (Berner, 1993).

2.1.2 *Chaetoceros gracilis*

Chaetoceros gracilis is one of the well-known diatoms as it serves as nutritious food for marine hatcheries and has been shown to be an adequate exclusive feed source for larvae and postlarvae of the shrimp *Metapenaeus ensis* (Chu, 1989) and is commonly used as a food source for larval molluscs (Parrish and Wangersky, 1990). *Chaetoceros gracilis* is unicellular floating diatom organism with a rectangular shape. The size of the cell without the setae is 8 – 12 microns in length and about 7 – 10 microns in width. It is widely found in warm and cold waters with 17 – 30 ppt salinity, temperature around 20 – 30°C and light intensity of 500 – 10,000 lux.

Chaetoceros gracilis is found to contain total fatty acids at 4.6 – 11% (2.2 – 2.4 pg cell^{-1}). The percentage of total fatty acid composition of *Chaetoceros gracilis* is shown in Table 2.1. Lipids, consisting of glycolipids, phospholipids, chlorophylls and other lipids, were the major constituents of the lipid extracts with values > 65 % (Volkman *et al*., 1989). The percentage composition of lipid is shown in Table 2.2 whereas Brown (1991) reported the dry weight of 78.4 pg cell^{-1} (shown in Table 2.3).

Table 2.1 Fatty acid composition of *Chaetoceros gracilis* expressed as a percentage of the total fatty acid (Volkman *et al*., 1989)

Fatty acid		% total fatty acid
Symbol	Scientific Name (Johnson & Saikai, 2009)	
14:0	Myristic	8.8
15:0	Pentadecanoic	1.0
16:0	Palmitic	23.3
17:0	Margaric	0.3
18:0	Stearic	4.1
20:0	Arachidic	0.3
22:0	Behenic	0.6
24:0	Lignoceric	0.3
16:1n7	Palmitoleic	33.4
16:1n5	Uncommon monounsaturated	0.1
16:1n13t	Hexadecenoic	1.2
18:1ln9	Oleic	3.6
18:1n7	Vaccenic	1.7
16:2n7	Unusual fatty acid	2.9
16:2n4	Hexadecadienoic	1.7
16:3n4	Unusual fatty acid	2.3
18:2n9	Elaidic	2.0
18:2n6	Linoleic	0.5
18:3n6	Calendic	0.8
18:4n3	Parinaric	0.2
20:4n6	Arachidonic	4.5
20:5n3	Eicosapentaenoic (EPA)	4.6
22:6n3	Decosahexaenoic (DHA)	0.3

Table 2.2 Percentage composition of lipid classes in *Chaetoceros gracilis* (Volkman *et al*., 1989)

Lipid classes	% Composition
Hydrocarbons and wax esters	1.3
Triacylglycerol	34.0
Free fatty acid	14.4
Sterols and alcohols	6.0
Polar lipid	44.2

Table 2.3 Concentrations of chlorophyll a, protein, carbohydrate and lipid in 16 species of micro-algae commonly used in aquaculture (modified from Brown, 1991)

Composition	Weight of constituent (pg.cell^{-1})
Chlorophyll a	0.78
Protein	9.0
Carbohydrate	2.0
Lipid	5.2

2.2 Factors controlling algae growth

2.2.1 Culture medium/Nutrients

Generally, the standard F/2 (Guillard's) medium was used for the cultivation of the diatom. Nutrient elements are usually divided into macronutrients and micronutrients. Macronutrients include nitrate (N), phosphate (P), silicate (Si), potassium (K), carbon (C), and manganese (Mg), etc. Micronutrient include metal, thiamin (B_1), cyanocobalamin (B_{12}), copper (Cu), iron (Fe), molybdenum (Mo), and zinc (Zn), etc. Nutrient of the standard F/2 (Guillard's) medium (Guillard, 1975) is shown in Table 2.4. The growth of cells concentration, mass product in the diatom culture depends on the amount of nutrients that the cells receive.

2.2.2 Light intensity

The growth of cell cultivation depends on light intensity, if light intensity are lower and higher will cause a decrease of cell growth. Light may be natural or

supplied by fluorescent tube. Too high light intensity may result in photo-inhibition. Effect of cell growth, cell density, yield of biomass, protein content depends on the types of light (the blue and white light). Photo-inhibition occurred earlier in white light than in blue light (>498 and 565 μmol photon m^{-2} s^{-1}, respectively) (Saavedra and Voltolina, 1994).

2.2.3 Aeration/Mixing

Aeration is typically a mechanism for the transfer of O_2 from the gas phase to the liquid in order to increase the amount of O_2 dissolved in the liquid. For algae, this mechanism could be reversed as aeration will help remove O_2 from photosynthesis to the atmosphere and prevent the build-up of the oxygen in the medium which could be harmful to the cell. High oxygen concentration could be necessary during the night time where there is not enough light for photosynthesis.

However, the better mixing could potentially lead to a more efficient utilization of nutrients by the diatom. A higher mass transfer might also facilitate the removal of metabolic gases such as O_2, preventing the accumulation of these gases, which might adversely affect the growth rate (Lavens & Sorgeloos, 1996). Nevertheless extreme mixing will cause shear (Shearing Force) that might be harmful to microorganisms.

2.2.4 Salinity

The optimal salinity for *Chaetoceros gracilis* is between 20 – 30 ppt (Lavens & Sorgeloos, 1996).

2.2.5 pH

The pH range of the cultured *Chaetoceros gracilis* is between 7 and 9. The pH optimums range for the cultured being 8.2-8.7 (Lavens and Sorgeloos, 1996).

2.2.6 Temperature

Samonte et al. (1993) reported that *C. calcitrans* grows well in the temperature range 18 – 30 $^{\circ}C$. In addition, Raghavan et al. (2008) was cultured *C. calcitrans* at the temperature of 20, 25 and 30$^{\circ}C$. The growth rate increased when the temperature was in range 25 -30$^{\circ}C$ and the other composition: lipid, protein, carbohydrates, chlorophylls and etc. increased with temperature range 25 -30$^{\circ}C$. Moreover, Hemalatha et al. (2012) reported the experimental cultivation of *C. simplex* at 20, 25 and 29$^{\circ}C$. Maximum cell concentration 2.0 x 10^6 cell mL^{-1} at 25$^{\circ}C$

and the other composition (protein, lipid and carbohydrates) increased with temperature increase. In this work, temperature 24 - 35 ^{o}C were in the proper range for the growth of *C. gracilis*.

Table 2.4 Composition of the standard F/2 (Guillard's) stock solution (Guillard, 1975)

Nutrients	Final concentration [mg L^{-1} seawater]	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 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 $CuSO_4$, 180 g $MnCl_2$, 6.3 g Na_2MoO_4,
$Na_2MoO_4.2H_2O$	0.006	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

2.3 Culture systems

2.3.1 Open/Closed systems

Open systems are the oldest and simplest form of culture systems for algae cultivation. Open systems cultures such as uncover ponds and tanks are more easily contaminated than closed systems, but closed systems will have trouble in the scale up. In most open systems, the culture is agitated by some mechanical means such as paddle wheel (Large commercial system) (Lavens and Sorgeloos, 1996).

Closed systems can be glass bottle or bioreactors such as stirred tank, tubular and airlift reactor, etc. The simplest type is glass bottle and the easiest to control the environmental condition, which often shown higher biomass productivity than other types of bioreactors (Lavens and Sorgeloos, 1996). It is usually easier to control the growth parameters at optimal in closed systems and therefore the productivity could be controlled at high level (Lee (2001), Lee and Richmond (1998), Vonshak (1997)).

2.3.2 Batch culture

The most common culture system is the batch culture, due to its simplicity and low cost. In this method algal cells are allowed to grow and reproduce in a closed container (i.e. closed system) in which there is no input or output of materials. The algal population cell density increases constantly until the exhaustion of some limiting factor, while other nutrient components of the culture medium decrease over time. When that nutrient is exhausted, their growth stops and eventually they die. These types of cultures typically last for about one week.

2.3.3 Continuous culture

This method of culturing algae differs from the batch culture method in that fresh medium is added to the culture at a constant rate and old media (and some of the algae cells) is removed at the same rate. The cultures therefore never run out of nutrients.

2.3.4 Semi-continuous culture

In the semi-continuous culture, periodic harvesting is followed immediately by topping up to the original volume and supplement with nutrients to achieve the original level of enrichment. It may be indoors or outdoors, but usually their duration is unpredictable depends by predators and/or contaminants and metabolites eventually build up, rendering the culture unsuitable for further use. The semi-

continuous method yields more algae than the batch method for a given tank size (Lavens and Sorgeloos, 1996)

The advantages and disadvantages of each culture systems are summarized in Table 2.5

Table 2.5 Advantages and disadvantages of various algal culture techniques (modified from Anonymous, 1991)

Culture types	Advantage	Disadvantage
Open	Cheaper	Contamination more likely
Close	Contamination less likely	Expensive
Batch	Easiest, most reliable	Least efficient, quality may be inconsistent
Continuous	Efficient, provides a consistent supply of high-quality cells, automation, highest rate of production over extended periods	Difficult, usually only possible to culture small quantities, complex, equipment expenses may be high
Semi-continuous	Easier, somewhat efficient	Sporadic quality, less reliable

2.4 Bioreactor

2.4.1 Bubble column photobioreactor

Bubble column reactors are cylindrical vessel with height greater than twice the diameter (Figure 2.1). The bubble column was high surface area to volume ratio, lack of moving parts, satisfactory heat and mass transfer, relatively homogenous culture environment and residual gas mixture. Mixing and CO_2 mass transfer is done through bubbling the gas mixture from sparger (Doran, 1995). Light is provided externally.

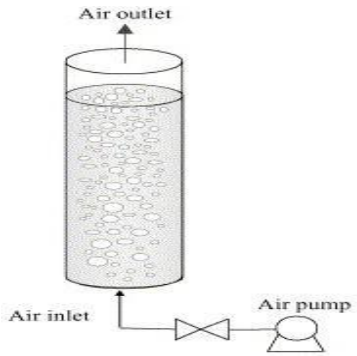

Figure 2.1 Schematic representation of Horizontal tubular photobioreactor (Krichnavaruk *et al*. 2005)

2.4.2 Flat panel photobioreactor

The flat panel reactor (shown in Figure2.2) can be made from transparent materials like glass, plexiglass, polycarbonate etc. It is characterized by high surface area to volume ratio and open gas disengagement systems. Agitation is provided either by bubbling gas from its one side through perforated tubes.

The flat panel by Barbosa et al. (Barbosa et al., 2005) was made from lexan (polycarbonate) held together in stainless steel frame having surface area to volume ratio of 0.34 cm^{-1}. The mixture of CO_2 and air was sparged through 17 needles with a diameter of 0.8 mm pinched through a piece of silicon placed at the bottom of the reactor. The reactor was illuminated at one surface with 10 fluorescent tubes having total light intensity of approximately 1,000 μmol photons m^{-2} s^{-1} (Barbosa et al., 2005). In addition, in a continuous culture of *Chlorella sorokiniana* using flat panel having short path length under high irradiance condition volumetric productivity obtained was 12.2 g L^{-1} d^{-1} (Cuareama *et al*., 2009).

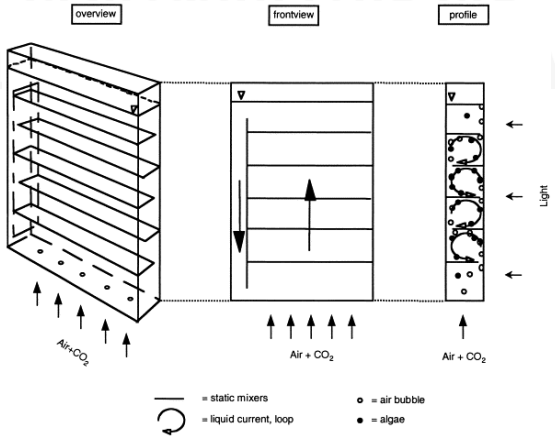

Figure 2.2 Schematic representation of Flat panel photobioreactor

(Degen *et al*., 2001)

2.4.3 Horizontal tubular photobioreactor

Horizontal tubular photobioreactor placed horizontally to the design of a series of parallel tubes configured at different shapes in horizontal plane (Figure2.3). The shape of its useful cultural outdoor there can be orientated towards sunlight resulting in high light conversion efficiency (Singh & Sharma, 2012**).**

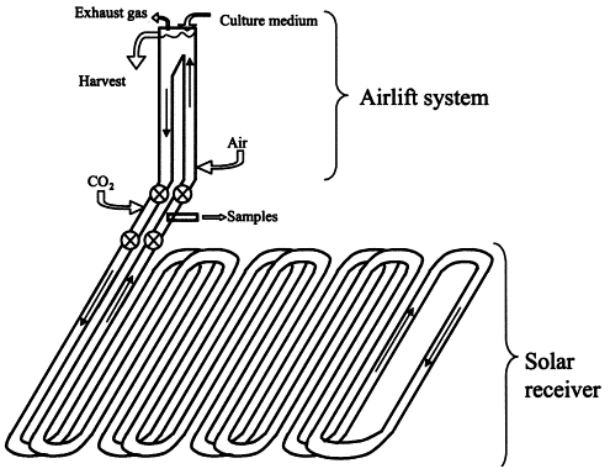

Figure 2.3 Schematic representation of Horizontal tubular photobioreactor (Singh & Sharma, 2012)

2.4.4 Stirred tank photobioreactor

Stirred tank reactor (shown in Figure 2.4) is most conventional where agitation is provided mechanically with the help of impeller of different sizes and shapes. Baffles are used in order to reduce vortex. This type of bioreactor has been turned into photobioreactor by illuminating it externally by fluorescent lamps or optical fibers but the main disadvantage of this system is low surface area to volume ratio which in turn decreases light harvesting efficiency (Singh and Sharma, 2012).

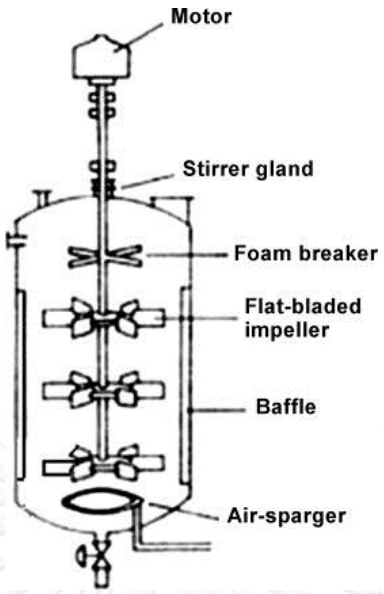

Figure 2.4 Schematic representation of the Stirred tank photobioreactor (Singh and Sharma, 2012)

2.4.5 Airlift photobioreactor

Airlift reactors (shown in Figure 2.5) are vessel with two interconnecting zones. One of the tubes is called riser where gas mixture is sparged whereas the other region is called downcomer which does not receive the gas .Generally it exists in two forms – internal loop and external loop. In the internal loop reactor, regions are separated either by a draft tube or a split-cylinder. Internal loop reactor has been modified into internal loop split airlift reactor and internal loop concentric tube reactor. In the external loop, riser and downcomer is separated physically by two different tubes.

Airlift reactor has characteristic advantage of creating circular mixing pattern where liquid culture passes continuously through dark and light phase giving flashing light effect to algal cells (Barbosa *et al*., 2003). As a result, the airlift is popularly applied in the cultivation of algae.

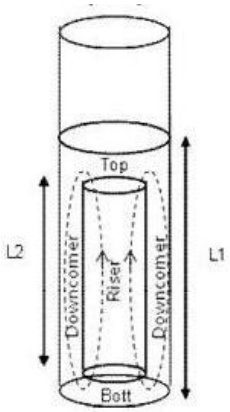

Figure 2.5 Schematic representation of airlift bioreactor (Jiménez & Rojas, 2011)

2.5 Airlift photobioreactor

2.5.1 Classification

Airlift photobioreactor has no agitation to assist in blending (Mixing), but the circulation of the fluid within the reactor is caused by the air in the bottom transported by air to float up to the top of the liquid. Airlift photobioreactor can be classified into two types; the internal loop and external loop. Figure 2.6 illustrates the 4 sections of the airlift, i.e. riser, downcomer, gas separator, and bottom section.

For external loop airlift photobioreactors, riser and downcomer are separated physically as separate columns (Figure 2.6). Mixing is done by bubbling the gas through sparger in the riser tube. (Singh & Sharma, 2012)

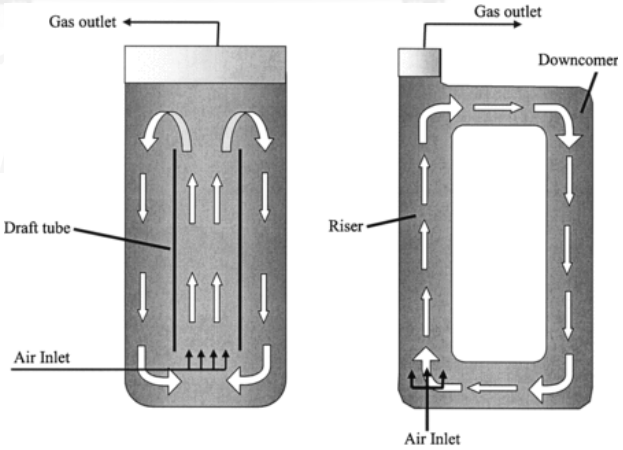

Figure 2.6 Schematic representation of an airlift bioreactor with internal recirculation (left panel) and external recirculation (right panel) (Warnock and Rubeait, 2006)

2.5.2 Transport mechanism in airlift photobioreactor

Airlift photobioreactor can be divided into three regions based on the flow and mixing within each area is as follows:

- Riser

The area is distributed with gas, and results in a lower fluid density than liquid in other sections of airlift. The fluid moves up the length of the reactor.

- Gas liquid separator

This is the area at the top of the reactor above the riser and downcomer. It is the location where gas separates out of the system. The liquid and some of the gas bubbles that cannot be separated from the system will continue to flow into the downcomer.

- Downcomer

Liquid freed of gas bubbles or containing lesser quantity of gas bubbles flows down into the unaerated downcomer.

2.5.3 Cultivation of *Chaetoceros calcitrans* in airlift photo bioreactor

Loataweesup (2002) and Krichnavaruk *et al*. (2005) investigated the culture to find optimum conditions for the growth of a diatom *Chaetoceros calcitrans* in a small glass 2.5L bubble column and 17L airlift photobioreactor. The modified standard F/2(Guillard's) medium with a twofold of silica and phosphorus concentrations was illustrated to result in a better growth of this diatom. Vitamin B12 in the range from 1 to 3 g L^{-1} did not significantly affect the growth. The optimum light intensity which yielded the maximum cell concentration was reported at 400 µmol photon m^{-2} s^{-1} where the maximum cell concentration for the cultivation in 2.5 L of glass bubble column was 5.8 x 10^{6} cells mL^{-1} with specific growth rate of 3.8x10^{-2} h^{-1}. The maximum cell concentration for the cultivation in 17 L of airlift photobioreactor was 8.88 x 10^{6} cells mL^{-1} with specific growth rate of 7.41x10^{-2} h^{-1}, but the maximum cell concentration from batch culture system was obtained at the superficial gas velocity of 3 cm s^{-1}. A long term semi-continuous operation could be achieved successfully with maximum specific growth rate of 9.65x10^{-2} h^{-1} and the maximum cell concentration reported to be 4.08 x 10^{6} cells mL^{-1}.

Krichnavaruk *et al*. (2007) examined the various modes of cultivation of *Chaetoceros calcitrans* in airlift photobioreactor. The cultivations in both semi-continuous and continuous culture systems resulted in a high cell productivity,

although the steady state 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. Despite this, among all of the systems investigated in this work, the large-scale system gave the highest productivity. The main limiting factor for the large-scale airlift culture was the availability of light. Based on economic analysis, the continuous cultivation in the 2.8L of airlift photobioreactor with a medium feed rate of 3 mL min^{-1} was most attractive where the operation cost could be maintained at a minimum of approximately 7.95 x 10^{-4} $L^{-1}h^{-1}$.

Table 2.6 Reviews of *Chaetoceros* cultivation

Author	Strains	Reactor	Medium	Volume (L)	T (°C)	Light intensity (μ mol photon m^{-2} s^{-1})	Aeration rate	Time (d)	Cell concentration (cell mL^{-1})	μ (d^{-1})	Chemical composition	
Corzo et al. (2000)	*C.calcitrans*	Erlenmeyer flask	f/4 supplied $NaNO_3$:	2	17	150		7			chl *a* *(μg L^{-1}):	
			25 μmol L^{-1}						4.5×10^5			50
			75 μmol L^{-1}						8×10^5			100
			150 μmol L^{-1}						13×10^5			240
			250 μmol L^{-1}						18×10^5			480
			450 μmol L^{-1}						22×10^5			680
Ying et al. (2001)	*C. gracilis*	flask	f/2 (Guillard's)	3	21 ± 1	20					total lipid	10.78%
				3	21 ± 1	68					total lipid	6.97%
Renard et al. (2002)	*Chaetoceros* sp.	Erlenmeyer flask	f/2 (Guillard's)	2	varied :	80	add CO_2	1				
					25		10 ml/min		3.98×10^6	0.74	lipid	16.8%
											carbohydrate	13.1%
											protein	57.3%
					27				2.90×10^6	0.84	lipid	14.8%
											carbohydrate	12.2%
											protein	57.1%
					30				3.28×10^6	0.87	lipid	12.2%
											carbohydrate	12.5%
											protein	64.1%
					33				2.95×10^6	0.76	lipid	12.4%
											carbohydrate	11.3%
											protein	62.5%
					35				2.22×10^6	0.78	lipid	12.1%
											carbohydrate	11.9%
											protein	47.3%

Table 2.7 Reviews of *Chaetoceros* cultivation (Continued)

Author	Strains	Reactor	Medium	Volume (L)	T (°C)	Light intensity (μ mol photon m^{-2} s^{-1})	Aeration rate	Time (d)	Cell concentration (cell mL^{-1})	μ (d^{-1})	Chemical composition
Goksan et al (2003).	*C. muelleri*	flat plate	f/2 (Guillard's)	0.6	23 ± 1	190	add 2% CO_2	8	22.39×10^6		
		flat plate	f/2 (Guillard's)	1.8	23 ± 1	190	add 2% CO_2	8	49.08×10^6		
Leonardos & Geider (2004)	*C. muelleri*		f/2(Guillard's) supplies ratio :		25			1			
			N:P 5			50			285×10^6	0.20	protein (pmol/cell) 7.43 cellular C (pmol/cell) 1.10 Chl *a* (pg/cell) 0.29
						700			$1,020 \times 10^6$	0.10	Protein 3.15 cellular C 0.90 Chl *a* 0.08
			N:P 15			50			358×10^6	0.31	Protein 8.90 cellular C 1.08 Chl *a* 0.52
						700			$1,035 \times 10^6$		Protein 3.51 cellular C 0.75 Chl *a* 0.08
			N:P 30			50			178×10^6	0.36	Protein 14.2 cellular C 1.27 Chl *a* 0.25
						700			977×10^6	0.10	protein 3.57 cellular C 0.59 Chl *a* 0.05

Table 2.8 Reviews of *Chaetoceros* cultivation (Continued)

Author	Strains	Reactor	Medium	Volume (L)	T (°C)	Light intensity (μ mol photon m^{-2} s^{-1})	Aeration rate	Time (d)	Cell concentration (cell mL^{-1})	μ (d^{-1})	Chemical composition
Leonardos & Geider (2004)	*C. muelleri*		f/2(Guillard's) supplies nitrate and phosphate ratio :		25						
			N:P 45			50			248 x10^6	0.15	protein (pmol/cell) 8.62 cellular C (pmol/cell) 1.12 Chl *a* (pg/cell) 0.30
						700			650 x10^6	0.21	protein 3.42 cellular C 0.73 Chl *a* 0.05
			N:P 90			50			249 x10^6	0.10	protein 9.27 cellular C 0.89 Chl *a*.46
						700			411 x10^6	0.18	protein 3.10 cellular C l0.86 Chl *a* 0.05
chnavaruk et al. (2005)	*C. calcitrans*	bubble airlift	modified f/2 (Guillard's)	2.5	30 ± 2	400	3 (cm s^{-1})		5.8 x 10^6	3.80 x 10^{-2}	
			modified f/2 (Guillard's)	17	30 ± 2	400	3 (cm s^{-1})		8.88 x 10^6	7.41 x 10^{-2}	
Liang et al (2006).	*C. muelleri*	glass flask	f/2 (Guillard's) supplies:	3	18 ± 1	60 (PAR)	1 (mL s^{-1})	2			Saturated Fatty acid :
			nitrate (N-Nt)							0.80 ± 0.01	35.40%
			ammonium (N-Am)							0.75 ± 0.01	38.29%
			urea (N-Ur)							0.76 ± 0.03	32.56%

Table 2.9 Reviews of *Chaetoceros* cultivation (Continued)

Author	Strains	Reactor	Medium	Volume (L)	T (°C)	Light intensity (μ mol photon m^{-2} s^{-1})	Aeration rate	Time (d)	Cell concentration (cell mL^{-1})	μ (d^{-1})	Chemical composition
chnavaruk et al. (2007)	*C. calcitrans*	bubble	f/2 (Guillard's)	17	30 ± 2	350	3 (cm s^{-1})	2.5	4.96×10^{6}	2.88×10^{-2}	
		airlift	f/2 (Guillard's)	17	30 ± 2	350	3 (cm s^{-1})	1.8	8.88×10^{6}	7.41×10^{-2}	
		bubble	f/2 (Guillard's)	170		48-52	250 (mL s^{-1})	3.4	2.51×10^{6}	2.46×10^{-2}	
		airlift	f/2 (Guillard's)	170		48-52	250 (mL s^{-1})	3.4	2.96×10^{6}	2.48×10^{-2}	
Pratiwi et al. (2009)	*C. gracilis*		f/2 (Guillard's)		16 – 19	54 – 68		3			SAFA** 59.57%
											MUFA*** 36.05%
											PUFA**** 4.38%
								7			SAFA 57.08%
											MUFA 33.83%
											PUFA 7.09%
								10			SAFA 43.61%
											MUFA 30.82%
											PUFA 25.56%
								14			SAFA 54.76%
											MUFA 27.49%
											PUFA 17.75%
								17			SAFA 64.28%
											MUFA 24.21%
											PUFA 11.51%
arthikeyan et al. (2012)	*C.curvisetus*	Erlenmeyer	f/2 (Guillard's) modifiled	5	24 ± 2	60		7	7.16×10^{4}		Chl *a* 4.7 pg.
		flask	12.35 mL^{-1} total N, 1.12 mL^{-1} total P								protein 19.7 pg $cell^{-1}$
	C. simplex	Erlenmeyer flask	f/2 (Guillard's)modifiled 12.35 mL^{-1} total N, 1.12 mL^{-1} total P	5	24 ± 2	60		7	3.88×10^{5}		Chl *a* 1.7 pg. protein 4.7 pg $cell^{-1}$

Table 2.10 Reviews of *Chaetoceros* cultivation (Continued)

Author	Strains	Reactor	Medium	Volume (L)	T (°C)	Light intensity (μ mol photon m^{-2} s^{-1})	Aeration rate	Time (d)	Cell concentration (cell mL^{-1})	μ (h^{-1})	Chemical composition	
molrat et al. (2010)	*C. gracilis*	Erlenmeyer flask	modified f/2 (Guillard's)	1	25 ± 1	68		2.5	8.59×10^6	9.81×10^{-2}	carbohydrate	7.47%
											protein	53.96%
											total Lipid	12.40 %
			add 0.05 g L^{-1} $NaHCO_3$	1	25 ± 1	68		2.5	8.49×10^6	10.73×10^{-2}	carbohydrate	13.79%
											protein	50.81%
											total Lipid	17.81%
			add 0.50 g L^{-1} $NaHCO_3$	1	25 ± 1	68		2.5	8.44×10^6	10.82×10^{-2}	carbohydrate	9.08%
											protein	46.08%
											total Lipid	11.47%
			add 5.00 g L^{-1} $NaHCO_3$	1	25 ± 1	68		2.5	5.05×10^6	6.91×10^{-2}	carbohydrate	5.21%
											protein	39.37%
											total Lipid	8.83%
ostard et al. (2012)	*Chaetoceros* sp.	flask		2	21 ± 2	180 (12:12; light:dark)	2.0 (L mim^{-1})	3			lipid	17.50%
											carbohydrate	1.61%
											protein	18.00%
								5			lipid	6.49%
											carbohydrate	0.93%
											protein	12.90%
								14	6.2×10^6		lipid	3.73%
											carbohydrate	1.50%
											protein	10.50%
ordova et al. (2012)	*C. muelleri*	fiber glass	f/2 (Guillard's)	80	21-23	140		4	2.94×10^6	0.90 (d^{-1})	lipid	27.34%
											carbohydrate	18.39%
											protein	18.06%
											ash	34.90%
		column	monoammonium phosphate	80	21-23	140		4	3.75×10^6	0.97 (d^{-1})	lipid	26.35%
											carbohydrate	22.75%

CHAPTER 3

EXPERIMENTS

3.1 Experiment setup

Airlift photobioreactor used in this investigation was made of clear acrylic plastic column in which light can shine through. For the cultivation of *Chaetoceros gracilis*, airlift photobioreactors with the size of 5 L (and diameter 12 cm) and a height of 58 cm (ALPBR) is employed where the draft tube is installed centrally inside the outer column separating the downcomer from riser with the ratio between the downcomer and riser cross section areas (A_D/A_d) of 2.17 (see Figure 3.1 for the schematic of the airlift photobioreactors and Table 3.1 for dimensions of the airlift photobioreactors). Air was supplied through a porous sparger at a superficial gas velocity of 3 cm s^{-1}. The temperature was controlled at 24 – 35°C. Light was supplied through 4 compact fluorescent 20W Lamps to reactor all day. The light intensity was around the height columns (shown in Figure 3.2) of 10,000 lux or 135 μmol photon m^{-2} s^{-1}. The light intensity was measured by "Digicon LX-50 lux meter".

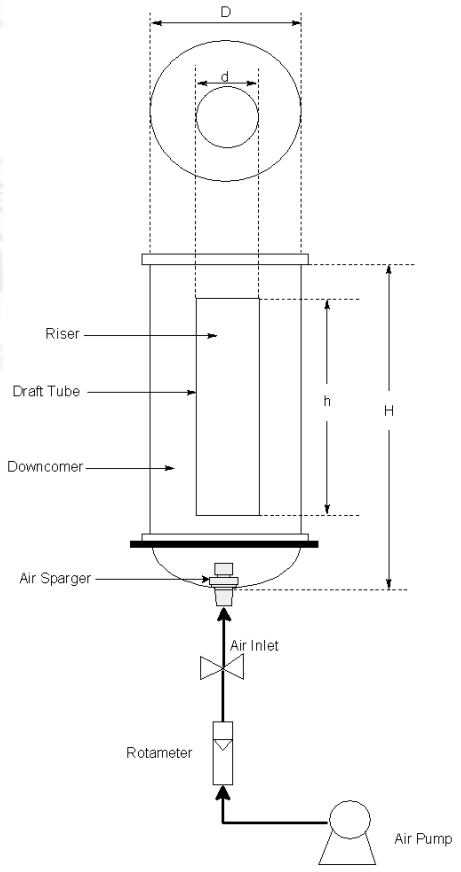

Figure 3.1 Experimental set up for an airlift photobioreactor

Table 3.1 Dimensions of conventional concentric airlift photobioreactor employed in this work

Parameters	Dimensions(cm)
Column outside diameter (D)	12
Draft tube outside diameter (d)	7
Column and draft tube thickness	0.3
Column height (H)	58
Draft tube height (h)	40

3.2 Experiment preparation

3.2.1 Treatment fresh seawater

Fresh seawater used in this study was from crystalline sea salt. The prepared step by step as follows:

1. Dissolve salt in tap water until the concentration 30 ppt (part per thousand).

2. Seawater disinfection with 50 ppm (part per million) of chlorine (as sodium hypo chloride)

3. Supply air through a porous sparger and adjust the superficial gas velocity to 3 cm s^{-1} for 2-3 days to remove the chlorine

4. Add sodium thiosulfate to test chlorine if the chlorine residual remains, sodium thiosulfate become yellow

3.2.2 Culture medium preparation

Chaetoceros gracilis was cultivated with modified standard F/2 (Guillard's) medium (shown in Table 3.2). The incubation was cultured in 2L bottles and scaled up to 5l of airlift photobioreactor.

Table 3.2 Composition of the modified standard F/2 (Guillard's) stock solution (Krichnavaruk *et al*., 2005)

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
$CoCl_2.6H_2O$	0.01	*Trace metal/EDTA solution*
$CuSO_4.5H_2O$	0.01	*Primary stocks*: make 5 separate 1 L stocks of [g L^{-1} DW] 10.0 g $CoCl_2$, 9.8 g $CuSO_4$, 180 g $MnCl_2$, 6.3 g Na_2MoO_4, 22.0 g $ZnSO_4$
$MnCl_2.4H_2O$	0.18	
$Na_2MoO_4.2H_2O$	0.006	
$ZnSO_4.7H_2O$	0.022	*Working stock*: add 1 mL of each primary stock solution
Na_2EDTA	4.36	4.35 g Na_2EDTA + 3.15 g $FeCl_3$ to 1 L DW
$FeCl_3.6H_2O$	3.15	
Thiamin HCl	0.1	*Vitamin solution*
Biotin	0.0005	*Primary stocks:* add 20 g thiamin HCl + 0.1 g biotin + 0.1 g B_{12} to 1 L DW
B_{12}	0.0005	*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

3.2.3 Study the cultivation of *Chaetoceros gracilis* in ALPBR

1. Fill 5L in the column with fresh seawater and sterilize fresh seawater in ALPBR with 50 ppm of chlorine (As sodium hypochlorite)
2. Supply air through the porous sparger centrally at the bottom of the column for 1-2 days
3. Test chlorine in the seawater by potassium iodide, if there was chlorine residual sodium thiosulfate become yellow
4. Determine the initial cell concentration of *Chaetoceros gracilis*, adjusted to of 1-10 (x10^5 cells mL^{-1})

5. Mix the algal inoculum with culture medium, adjusted to the total volume of 5 L
6. Cover the column with clear acrylic plastic
7. Supply air through a porous sparger and adjust the superficial gas velocity to 3 cm s^{-1}
8. Supply light around the height columns with fluorescent lamp. (10,000 lux or 135 µmol photon m^{-2} s^{-1})
9. Take the sample and measured cell growth rate using Haemacytometer and measured the medium concentration at 2 sample for 1 day until the stationary growth is observed
10. Measured temperature both outside and inside temperature by thermometer and measured pH using pH meter

3.3 Cultivation system

3.3.1 Batch cultivation system

Chaetoceros gracilis was cultivated in the batch cultivation system using 5 L acrylic airlift photo bioreactor (ALPBR). The initial cell concentration was 5 x 10^5 cells mL^{-1}. The experiment set up was shown in Figure 3.2.

The experiment was divided into 4 sets using follow conditions:

- Control (Fresh medium): 100% initial Fresh medium based on modified F/2 Guillard's medium
- 1st reused medium: The nutrient remaining after harvesting cells in the control experiment was adjusted to provide the amount of nutrients equivalent to what available in the fresh medium.
- 2nd reused medium: The used nutrient from the 1st reused experiment was adjusted to provide the amount of nutrients equivalent to what available in the fresh medium.
- 3rd reused medium: The used nutrient from the 2nd reused experiment was adjusted to provide the amount of nutrients equivalent to what available in the fresh medium.

3.3.1.1 Cultivation with Fresh medium

1. Fill in the column with fresh seawater and sterilize fresh seawater in 5L ALPBR
2. Add the initial cell from inoculums at cell concentration 5 x10^{5} cells mL^{-1} for *Chaetoceros gracilis*
3. Add nutrients with modified standard F/2 Guillard's medium
4. Supply air through a porous sparger and adjust the superficial gas velocity to 3 cm s^{-1} and measuring light intensity, pH and temperature
5. Take the sample and measured cell growth rate using Haemacytometer and measured the medium concentration twice a day until the stationary growth is observed

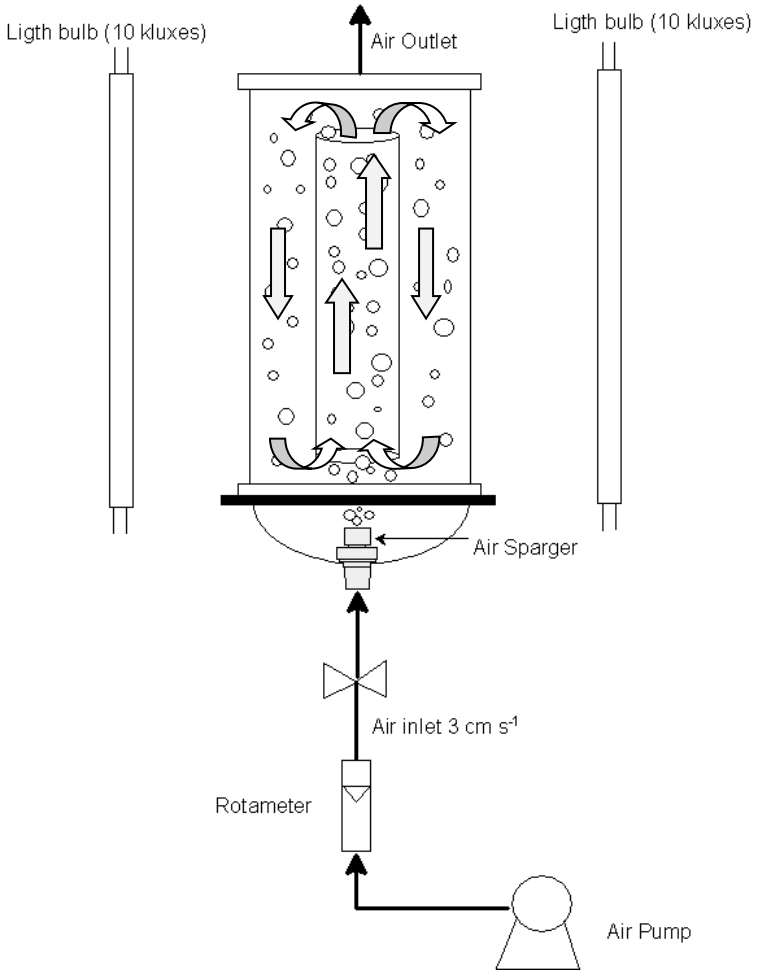

Figure 3.2 Experimental setup for the cultivation of *Chaetoceros gracilis* in ALPBR

3.3.1.2 Cultivation with reused medium

1. Repeat Steps 1-5 in section 3.3.1.1
2. Separate biomass in the fresh culture medium by centrifugation at 4500 rpm, 15 min and 10°C.
3. Measure the nutrient remaining after harvest cells in the fresh medium
4. Fill the remaining seawater with residual nutrient medium into 5L ALPBR and adjust nutrient content following 100% medium from modified standard F/2 (Guillard's)
1. Supply air through a porous sparger and adjust the superficial gas velocity to 3 cm s^{-1} and measuring light intensity, pH and temperature
2. Take the sample and measured cell growth rate using Haemacytometer and measured the medium concentration twice a day until the stationary growth is observed
3. Repeat steps 2-7 (include 3 times)

3.3.2 Fed-Batch cultivation system

A fed-batch culture with 3 levels of nutrient concentrations was set out as shown below:

- Control (Fresh medium): 100% initial fresh medium
- 50% macronutrient: The total amount of target nutrient (i.e. silicate, nitrate, and phosphate) was 50% of amount used in the fresh medium. The total feeding amount was split equally into 5 days. This was equivalent to 0.5 mL of the stock nutrient per day per 5 L of seawater.
- 100% macronutrient: The total amount of target nutrient (i.e. silicate, nitrate, and phosphate) was 100% of amount used in the fresh medium (same total amount). The total feeding amount was split equally into 5 days. This was equivalent to 0.5 mL of the stock nutrient per day per 5 L of seawater.
- 500% macronutrient: The total amount of target nutrient (i.e. silicate, nitrate, and phosphate) was 500% of amount used in the fresh medium. The total feeding amount was split equally into 5 days. This was

equivalent to 0.5 mL of the stock nutrient per day per 5 L of seawater.

1. Fill in the column with fresh seawater and sterilize fresh seawater (see section 3.2.1)
2. Add the initial cell concentration of *Chaetoceros gracilis* were controlled at 5 x10^5 cells mL^{-1}
3. Adjust macronutrient (i.e. silicate, nitrate, and phosphate) content following these conditions: control medium, 50%macronutrient, 100%macronutrient and 500%macronutrient from percentage of original concentration in modified standard F/2 (Guillard's) stock solution
4. Supply air through a porous sparger and adjust the superficial gas velocity to 3 cm s^{-1} and measuring light intensity, pH and temperature
5. Take the sample and measured cell growth rate using Haemacytometer and measured the medium concentration twice a day until the stationary growth is observed

3.3.3 Reused medium with Fed-batch cultivation system

Chaetoceros gracilis was cultivated in the fed-batch cultivation system using 5 L acrylic airlift photo bioreactor (ALPBR). The initial cell concentration was 5 x 10^5 cells mL^{-1}. For section 3.3.2, 50%macronutrient was best condition, therefore, this section use 50%macronutrient in reused medium

The experiment was divided into 4 sets using follow conditions:

- 50% medium: 50% of each macronutrient was being equally separated into 4 portions and each portion was added daily to the reactor (for a total of 4 days). This was equivalent to 0.5 mL of the studies nutrient per day per 5 L of seawater.
- 1^{st} reused medium: The nutrient remaining after the first harvest was adjusted to provide the amount of nutrients equivalent to what available in the fresh medium.
- 2^{nd} reused medium: The used nutrient from the 1^{st} reused experiment was adjusted to provide the amount of nutrients equivalent to what available in the fresh medium.
- 3^{rd} reused medium: The used nutrient from the 2^{nd} reused experiment was adjusted to provide the amount of nutrients equivalent to what available in the fresh medium.

1. Fill in the column with fresh seawater and sterilize fresh seawater (see section 3.2.1)
2. Add the initial cell concentration of *Chaetoceros gracilis* were controlled at 5 x10^{5} cells mL^{-1}
3. Adjust 50%macronutrient (i.e. silicate, nitrate, and phosphate) content from 100% of original concentration in modified standard F/2 (Guillard's) stock solution
4. Supply air through a porous sparger and adjust the superficial gas velocity to 3 cm s^{-1} and measuring light intensity, pH and temperature
6. Take the sample and measured cell growth rate using Haemacytometer and measured the medium concentration twice a day until the stationary growth is observed
7. Separate biomass in the fresh culture medium by centrifugation at 4500 rpm, 15 min and 10°C.
8. Fill the remaining seawater with residual nutrient medium into 5L ALPBR and measure the nutrient remaining after harvest cells in the fresh medium
9. Adjust nutrient content following 50%macronutreint from modified standard F/2 (Guillard's) stock solution
10. Supply air through a porous sparger and adjust the superficial gas velocity to 3 cm s^{-1} and measuring light intensity, pH and temperature
11. Take the sample and measured cell growth rate using Haemacytometer and measured the medium concentration twice a day until the stationary growth is observed
12. Repeat steps 1-11 (include 3 times)

3.4 Analyses

3.4.1 Determination of light intensity

Light intensity can be calculated from Equation 3.1:

$$I = \frac{E}{74} \quad (3.1)$$

where

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

E = light intensity (lux).

3.4.2 Determination of cell concentration

Cell concentration estimated from cell count with Haemacytometer (0.1 mm grid depth, 25 channel, 0.04 mm^2 channel area, see in Figure 3.4). The cell concentration can be determined as follows :

1. Clean counting chamber and cover glass
2. Take the 25 μL of sample into counting chamber and cover the counting chamber with cover glass
3. Count cell in the counting grid under microscope (x 40 objective) (see Figure 3.5)
4. Calculated the number of cells in the counting grid using Equation 3.2

$$N = n \times 10^4 \quad (3.2)$$

where

N = Cell number (cells mL^{-1})

n = Number of cells in the counting grid (cells).

3.4.3 Dry weight of algae

Dry weight was estimated from the dry weight of the cell when water was removed, the weight of the sample does not include the weight of the water in the optical density can be related to biomass production the next step.

1. Collect the sample approximate of 40 mL
2. Dry the Whatman GF/C filter paper with 1.6 μm pore size membrane in an

oven at 80°C until weight is constant

3. Filter the sample on glass fiber filter (1μm pore size) using Bunchner setup connected to vacuum pump
4. Wash filter by ammonium formate solution (0.5 M) to remove the deposited salts
5. Follow the procedure with control filters on which an equal volume of seawater is filtered. The strength of applied vacuum will determine amount of salts retained on the control filters.
6. Dry filter at 100°C for 4 hours and calculate dry weight from Equation 3.3

$$DW(g\ cell^{-1}) = \frac{(W_A - W_C)}{(N \times V)} \quad (3.3)$$

where

W_A = Average dry weight retained on algae filter (g)

W_C = Average dry weight retained on control filter (g)

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

V = Volume of alga culture (mL).

3.4.4 Determination of specific growth rate

Specific growth rate can be calculated from Equation 3.4

$$\mu = \frac{\ln(N_2) - \ln(N_1)}{t_2 - t_1} \quad (3.4)$$

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).

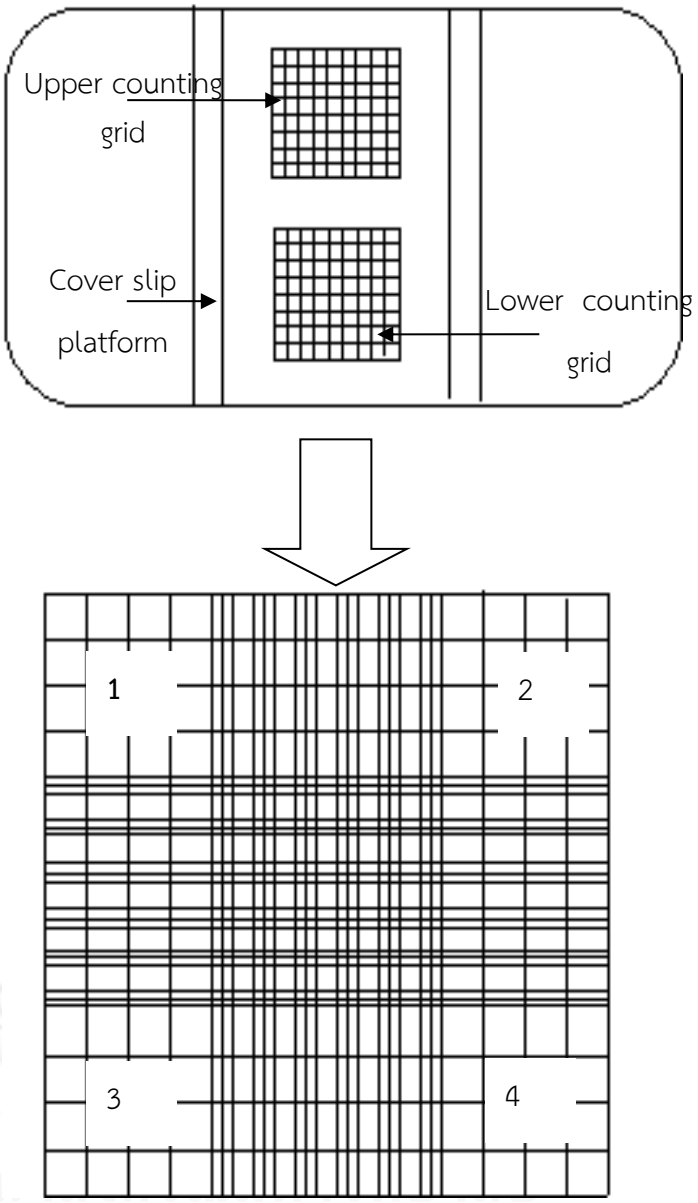

Figure 3.3 Schematic diagram of Haemacytometer (Fox, 1983)
The microalgae in the squares 1, 2, 3 and 4 are used for cell count

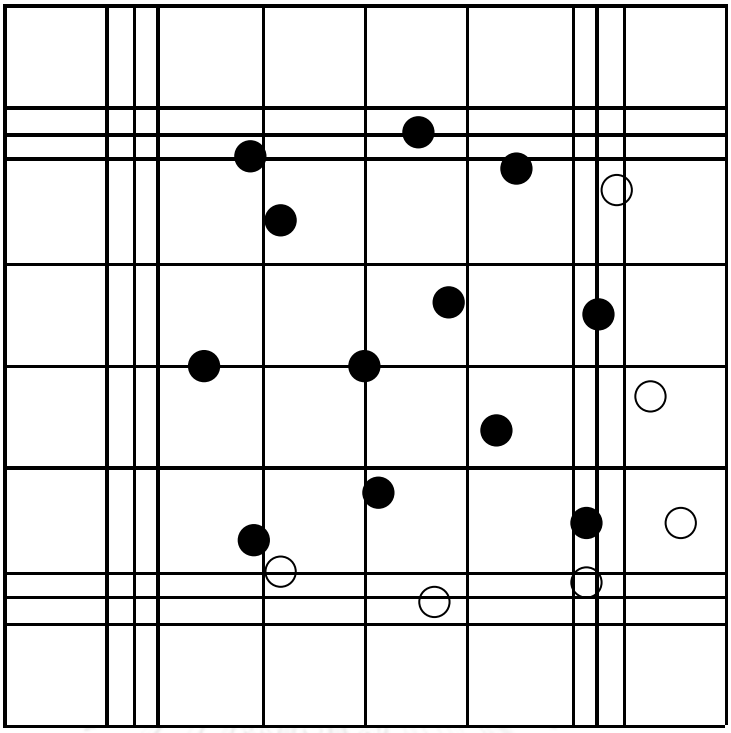

Figure 3.4 Counting cell density (Fox, 1983)

Count the cells in the square and those that touch the top and left borders (●)

Do not count the ones touching the right and lower borders (○)

3.4.5 Determination of productivity

Productivity of *Chaetoceros gracilis* can be calculated from Equation 3.5:

$$P = \frac{N_2 - N_1}{t_1 - t_1} \times \frac{V \times 1000}{3600} \qquad (3.5)$$

where

P	=	Productivity (cells s^{-1})
N_1	=	Cells concentration at t_1 (cells mL^{-1})
N_2	=	Cells concentration at t_2 (cells mL^{-1})
t_1	=	First sampling time (h)
t_2	=	Second sampling time (h)
V	=	Harvest volume (L).

3.4.6 Determination of salinity

The salinity of seawater was determined with “Refractometor”. Adjust the salinity of seawater was 30 ppt (part per thousand).

3.5 Determination of nutrient concentration

3.5.1 Determination of silicate

1. Silicate measure by UV-Visible spectrophotometer at wavelength of 810 nm (shown the measurement of silicate concentration in Appendix A-1)
2. Calculate the concentration of silicate (mg-Si L^{-1}) with standard curve as shown in Figure A-1.

3.5.2 Determination of nitrogen

1. Nitrogen measure by UV-Visible spectrophotometer at wavelength of 220 and 275 nm (shown the measurement of nitrate concentration in Appendix A-2)
2. Calculate the concentration of silicate (mg-N L^{-1}) with standard curve as shown in Figure A-2.

3.5.3 Determination of phosphorus

1. Phosphorus measure by UV-Visible spectrophotometer at wavelength of 885 nm (shown the measurement of phosphorus concentration in Appendix A-3)
2. Calculate the concentration of silicate (mg-P L^{-1}) with standard curve as shown in Figure A-3.

3.5.4 Determination of other element

The amount of other elements of modified standard F/2 (Guillard’s) medium such as Na, P, K, Co, Cl, Cu, Mn, Mo, Fe, B and Zn were measured by ICP-OES (700 series Inductively Couple Plasma-Optical Emission Spectrometer, Agilent technologies) with the steps as follows:

1. Collect approximate 5 mL –of medium sample
2. Filter the medium sample through the Whatman GF/C filter paper diameter 25 mm and 1.6 μm pore size membrane

3. Prepare 10 mL of the sample with dilute medium sample ratio 1:9 (1 mL of medium sample: 9 mL of DI water)
4. Prepare the standard solutions from standard mixture
5. Measure the concentration of elements by using ICP-OES

3.6 Determination of composition of cells

3.6.1 Determination of total lipid

1. Weigh 1 gram of the dried algae in thimble to the soxhlet extractor
2. Fill Chloroform and methanol as mixed solvent (120: 60 mL), up heat over until colorless
3. Weigh the flask and record them.
4. Take the flask of the evaporator until without solvent after that put it to desiccators for 2 hours
5. Record the weight of the flask

Total lipid can be calculated from:

$$T_L = W_1 - W_2 \tag{3.6}$$

where

TL = Total Lipid (g)

W1 = Flask weight before evaporator

W2 = Flask weight after evaporator.

3.6.2 Determination of moisture

1. Dry crucible dried in an oven at the 100°C for 2 hours, leave to cool in desiccators for 2 hours and record weight of crucible
2. Weigh 1 gram of dried algae into the crucible and record the weight of dried algae and crucible
3. Calcine algae and crucible at 100°C for 2 hours, leave to cool in desiccators for 2 hours
4. Record the weight of algae and crucible

3.6.3 Determination of ash

1. Dry crucible in an oven at 650°C for 2 hours, leave to cool in desiccators for 2 hours and record weight of crucible
2. Weigh 1 gram of dried algae into the crucible and record the weight of dried algae and crucible
3. Calcine algae and crucible at 650°C for 6 hours, leave to cool in desiccators for 2 hours
4. Record the weight of algae and crucible
5. Percent ash can be calculated from:

$$\%Ash=W_2-W_1-moisture \quad (3.7)$$

where

W_1 = weight initial

W_2 = weight finally.

CHAPTER 4

Results and Discussion

4.1 Effects of reusing nutrient in batch culture system

4.1.1 Growth of *C. gracilis* in reused nutrient

Chaetoceros gracilis was cultivated in the batch cultivation system using 5 L acrylic airlift photo bioreactor (ALPBR). The initial cell concentration was 5 x 10^5 cells mL^{-1}. Air was supplied through a porous sparger at a superficial velocity of 3 cm s^{-1}. The temperature was controlled at 24 – 30^{o}C. Light was supplied with fluorescent lamps where the intensity at the draft tube surface was maintained at 10,000 lux or 135 µmol photon m^{-2} s^{-1}. The cultivation was grown in fresh seawater enriched with modified standard F/2 (Guillard's) stock solution (Krichnavaruk *et al*., 2005).

The experiment was divided into 4 sets using follow conditions:

- Control (Fresh medium): 100% initial Fresh medium
- 1st reused medium: The nutrient remaining after harvesting cells in the control experiment was adjusted to provide the amount of nutrients equivalent to what available in the fresh medium.
- 2nd reused medium: The used nutrient from the 1st reused experiment was adjusted to provide the amount of nutrients equivalent to what available in the fresh medium.
- 3rd reused medium: The used nutrient from the 2nd reused experiment was adjusted to provide the amount of nutrients equivalent to what available in the fresh medium.

Figure 4.1 illustrates the result from the cultivation of *C. gracilis*. It can be seen from the figure that the cultures under the 4 different medium conditions exhibited similar growth pattern, i.e. lag phase of 1 day, 2-3 days exponential phase, very short stationary phase followed by cell decay at Day 4. Table 4.1 reports the cell concentration, specific growth rate, productivity and specific productivity from the cultivation with fresh medium, 1st reused, 2nd reused, and 3rd reused mediums. A maximum cell density obtained from the fresh medium was approximately 10.73 ± 0.35 x 10^6 cell mL^{-1} with a maximum specific growth rate of 0.79 ± 0.02 d^{-1}. This growth characteristic was similar to the reported value, e.g. a report by Lalanan *et al*. (2013) who reported that the maximum cell density of *C. gracilis* cultivated in

Erlenmeyer flask using fresh seawater enriched with F/2 (Guillard's) stock solution was 11.98 x 10^6 ± 0.52 cell mL^{-1}. Krichnavaruk *et al*. (2005) ; Krichnavaruk *et al*. (2007) found that the cultivation with modified standard F/2 (Guillard's) in airlift photo bioreactor was with the maximum cell density of 8.88 x 10^6 cell mL^{-1}.

C. gracilis was then separated from the fresh culture medium by centrifugation at 4500 rpm, 15 min and 10°C. The resulting clear solution was then reused in the following cultivation. In the 1st reused, 2nd reused, and 3rd reused mediums, the attainable maximum cell densities were approximately at the same level which were lower than that obtained from the cultivation with fresh medium, i.e. 5.28 ± 0.33 x 10^6, 6.09 ± 0.17 x 10^6 and 6.02 ± 0.13 x 10^6 cells mL^{-1}, respectively. The specific growth rate of the cultures with 1st, 2nd and 3rd reused medium took the same value of 0.74d^{-1}(see Table 4.1). Table 4.1 shows that the fresh medium condition gave the culture with the highest productivity at 13.03± 0.41 cell d^{-1} whereas the 1st reused, 2nd reused, and 3rd reused mediums provided similar productivities of 7.69 ± 0.41, 8.29 ± 1.10, 8.94 ± 0.61 cell d^{-1}, respectively.

4.1.2 Cultivation with reused medium

Figure 4.2 illustrates how cell density changed with silicate concentration when cells were cultivated with fresh, 1st reused, 2nd reused and 3rd reused mediums. The uptake of nutrients and yield of cell are reported in Table 4.2. It can be seen that as cell density increased silicate concentration decreased. Silicate concentration reduced quite quickly and reached steady level at a below 1 mg-Si L^{-1}. Silicate uptake was relatively high in the culture with fresh medium but this became lower in the reused rounds. Incidentally the growth of the culture with reused medium was also low. However, it is shown in Figure 4.5 that the uptake of silicate did not relate directly with the growth of *Chaetoceros gracilis*. In other words, there were cases where silicate was only slightly consumed, but the yield of the cell was high, and vice versa.

Krichnavaruk et al. (2005) demonstrated that silicate should be doubled to enhance the growth of *Chaetoceros gracilis* which indicated the significance of silicate. However, this was not the case in this work. Reasons for this cannot be

derived from this work, but it was possible that the cultures were cultivated with different light intensity and perhaps temperature (not reported in her work).

In a similar fashion, Figure 4.3 demonstrates the relationship between cell density and nitrate concentration. As expected, nitrate was consumed and cell grew. Nitrate was mostly uptake within the first day and the concentration remained low and constant in the following days. When plotted the uptake of nitrate and biomass yield as shown in Figure 4.5, it can be seen that the uptake of nitrate did not correspond well with the growth of the cell.

Similar findings were revealed for phosphate consumption as illustrated in Figures 4.4 and 4.5. It can then be concluded from this finding that the uptakes of the major nutrients like Si, N and P occurred quickly within the first day. After that, cell still grew but tended to reach its stationary phase. The uptakes of these nutrients did not seem to have direct effect on the growth of the alga, and it was shown that alga could grow high density when only limited amount of nutrients was consumed. However, cell might not grow so well if the concentration of nutrients was too low.

Figure 4.6 displays biochemical composition of the cell and this shows that %total lipid always moved in the opposite direction to %carbohydrate, whereas %protein remained relatively constant. Figure 4.7 suggests that %lipid and lipid productivity were high when $\Delta X/\Delta Si$ was high. This suggests some relationship between the uptake of silicate and lipid production/accumulation within the cell. This corresponds well with the report from Laing (2012) who stated that low silica cell contained a higher level of lipid than carbohydrate and protein.

%Lipid and lipid productivity were low when $\Delta X/\Delta N$ was high, and the opposite was found for carbohydrate. This supports the finding of Gao *et al*. (2013) who reported that nitrogen depriving medium could induce carbohydrate accumulation. However, it was unexpected to observe a constant level of protein when $\Delta X/\Delta N$ changed as N constitutes protein and a high consumption of N was anticipated to raise the protein productivity. There might be mechanism where nitrogen was transformed into some unusable nitrogen compounds such as nitrogen gas.

Figure 4.5 illustrates further that a reduction in P consumption (high $\Delta X/\Delta P$) could enhance lipid productivity and lower carbohydrate productivity. This can be explained with the finding of Gao *et al*. (2013) who stated that, under phosphate depriving condition, diatoms growth was interrupted where protein, chlorophyll a,

RNA and DNA accumulations were also negatively affected, but this positively affected carbohydrate productivity.

Table 4.3 illustrates that %reductions of silicate from all batches took approximately the same value in the range of 73-80%, and the experiment with fresh medium saw a slightly higher silicate reduction than the other experiments; %reduction of nitrate in the range 73 – 92% with the highest obtained from the 3rd reused medium; %reduction of phosphate in the range 67 – 72 % with 2nd reused medium taking a slightly higher level than the others. It is also interesting to observe that %Reduction of Fe was relatively high with 98.11%, 98.90%, 88.60%, and 84.12% in the fresh medium, 1st, 2nd and 3rd reuses mediums, respectively. This illustrates that Fe is an important micro-nutrient for the growth of *C.gracilis* as it helps absorb nitrate and aids the synthesis of chlorophyll *a* which is important for the process of photosynthesis. In addition, Cu was also observed to be consumed in large quantity. %Reduction of Cu obtained from the fresh medium, 1st reused, 2nd reused and 3rd reused mediums were 33.33%, 90.00%, 42.86%, 28.57% and 90.00%, respectively. Again, this strongly suggests that Cu is an essential trace-metal for the algal growth. Note that Cu participates in growth metabolism and also aids the process of photosynthesis.

Overall, Table 4.3 gives a summary of the %reduction of the other nutrients which can be used to estimate the empirical formula of the alga obtained from the cultivations with various types of nutrients as shown in Table 4.4.

4.1.3 Organic compound obtained by GC-MS

The results from this section demonstrate that the reuse of nutrients posed some negative effect on the algal growth. The reused medium was evaluated with GC-MS to find some foreign compounds which might be toxic to the algal growth. The results as shown in Figure 4.8 demonstrates that the main two emerging organic compounds were Dimethyl-silanediol and Haxanedioic acid, bis(2-ethylhexyl) ester which were found to occur and increase after the nutrients were reused. This first compound is the derivative of silicates which might occur due to the reformation of silicate to the form which is not soluble and cannot be used for cell growth. The second acidic compound might be one of the main inhibitor for algal growth as the increase of this compound always caused a decline in the cell growth. Such accumulation of the two compounds negatively affected cell growth and could well

be the reasons why the reused of nutrients always was associated with a lower cell growth.

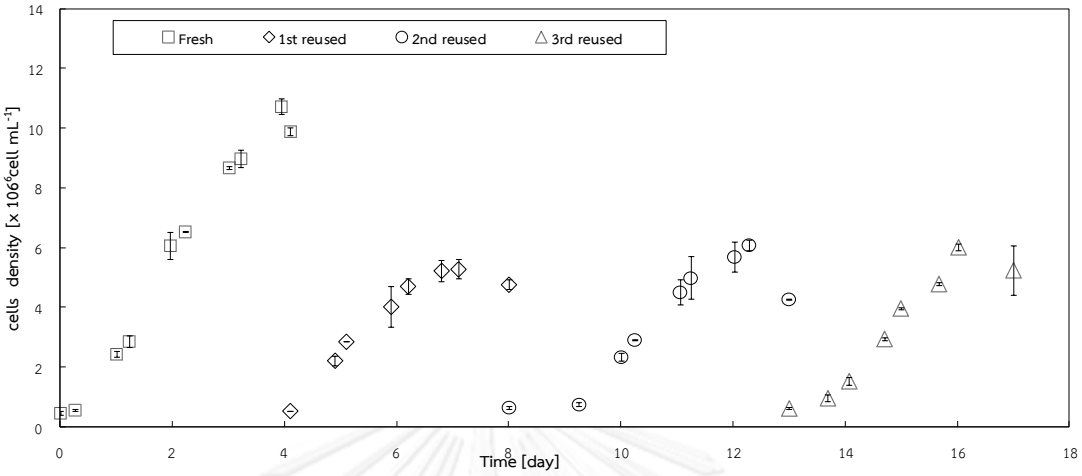

Figure 4.1 Growth behaviors of *C. gracilis* in fresh, 1st, 2nd and 3rd reused mediums

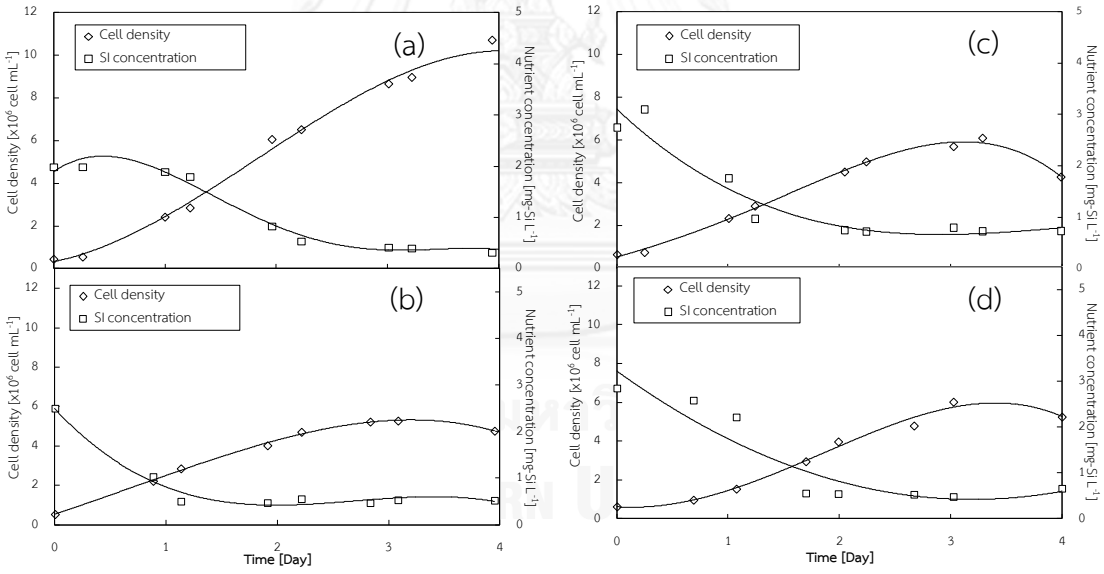

Figure 4.2 Cell density and silicate concentration for the cultivation of *C. gracilis* in (a) Fresh medium, (b) 1st reused, (c) 2nd reused and (d) 3rd reused mediums

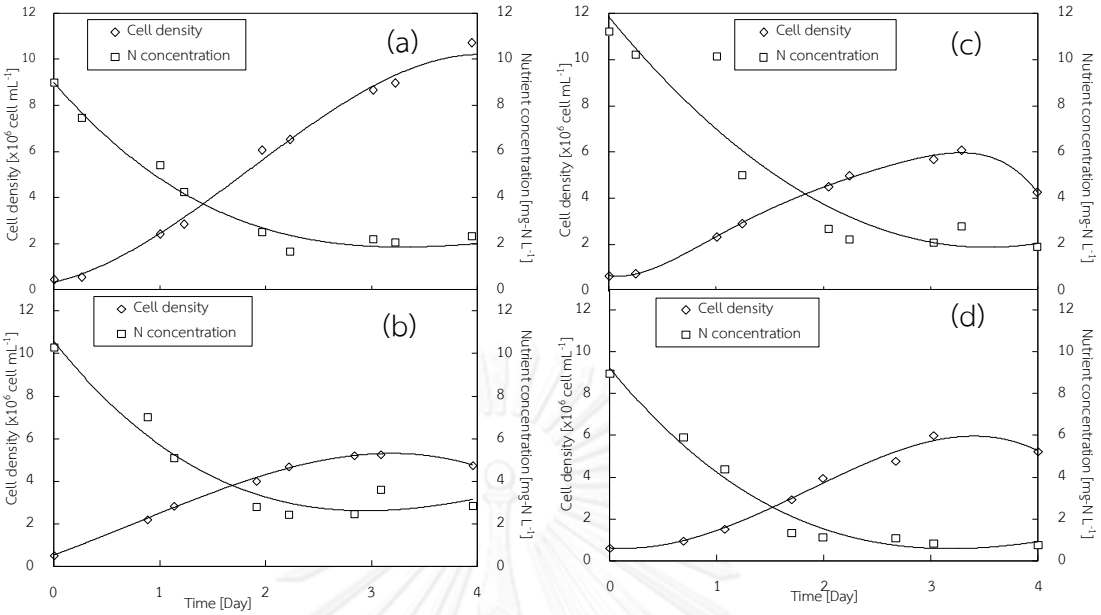

Figure 4. 3 Cell density and nitrate concentration for the cultivation of *C. gracilis* in (a) Fresh medium, (b) 1st reused, (c) 2nd reused and (d) 3rd reused mediums

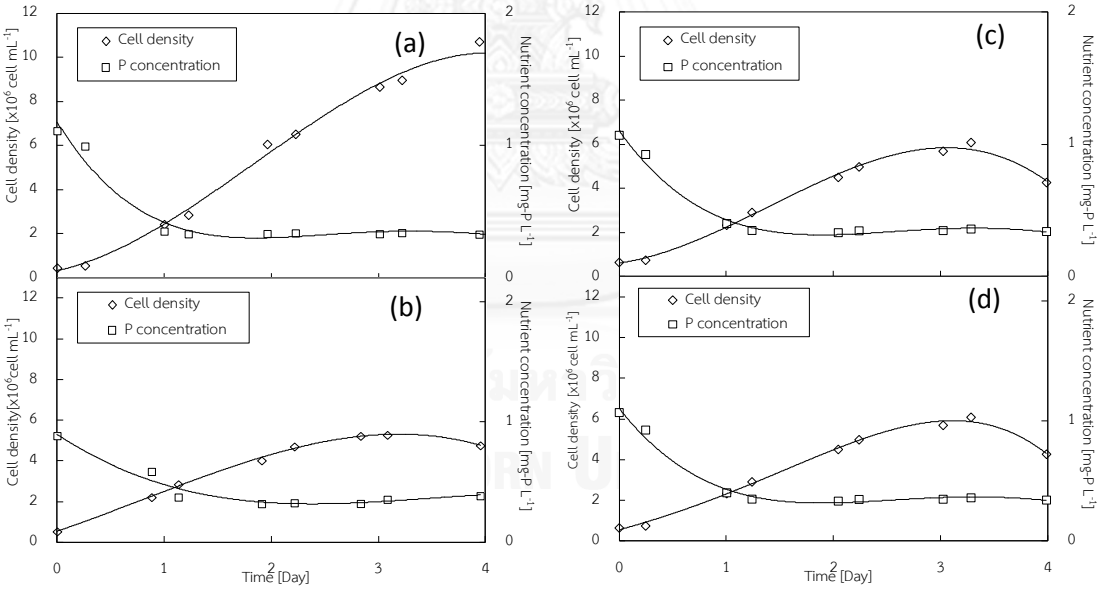

Figure 4.4 Cell density and phosphate concentration for the cultivation of *C. gracilis* in (a) Fresh medium, (b) 1st reused, (c) 2nd reused and (d) 3rd reused mediums

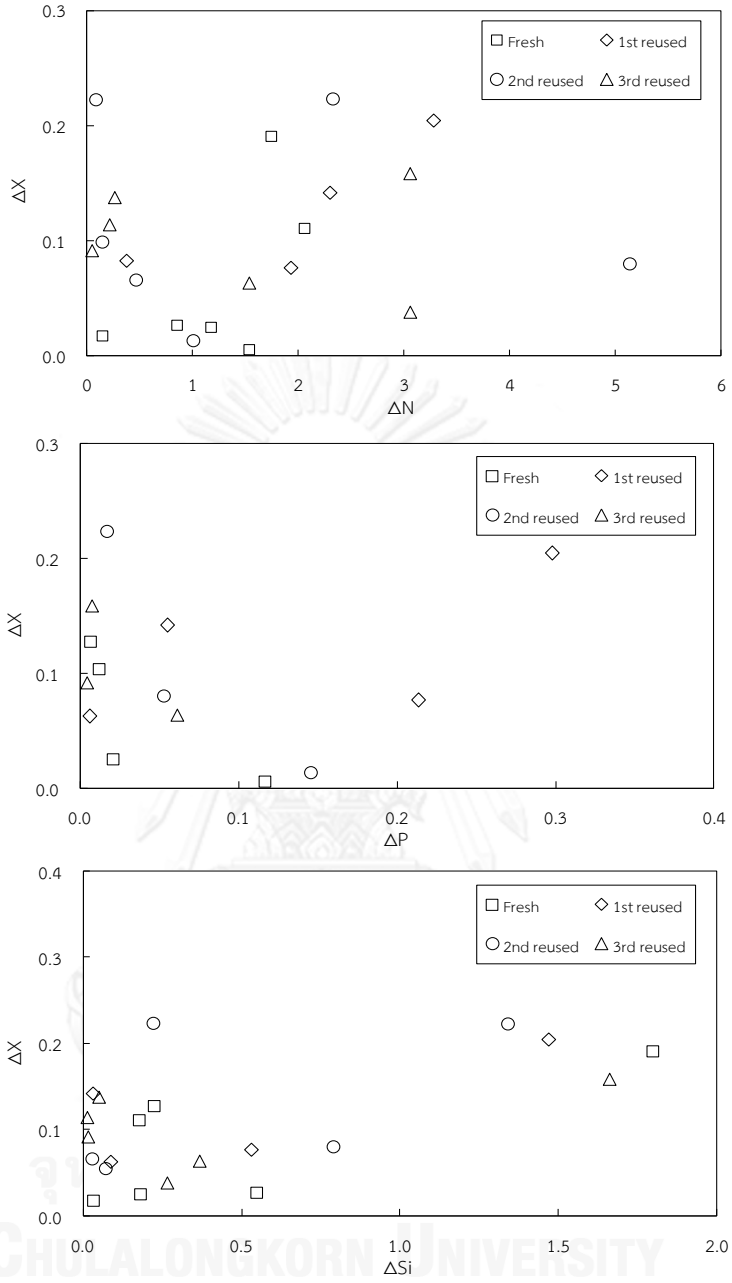

Figure 4.5 Biomass yield from cultivation with reused medium

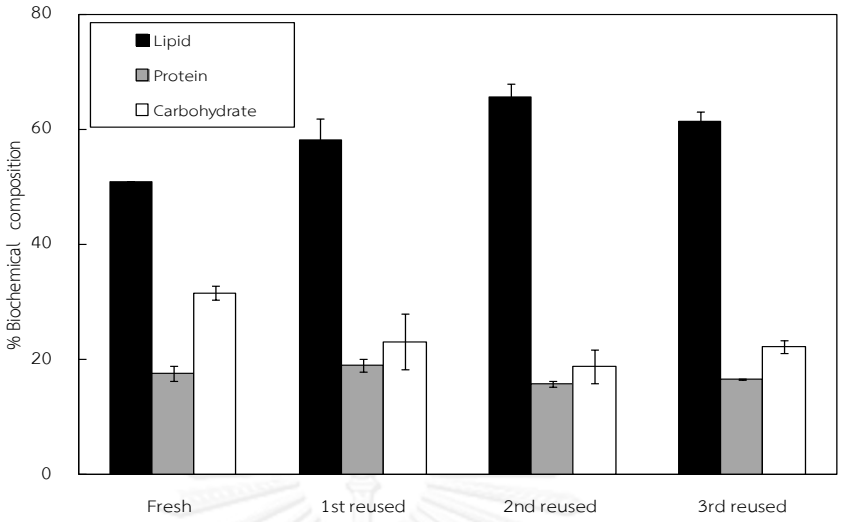

Figure 4.6 %Biochemical composition of *C. gracilis*

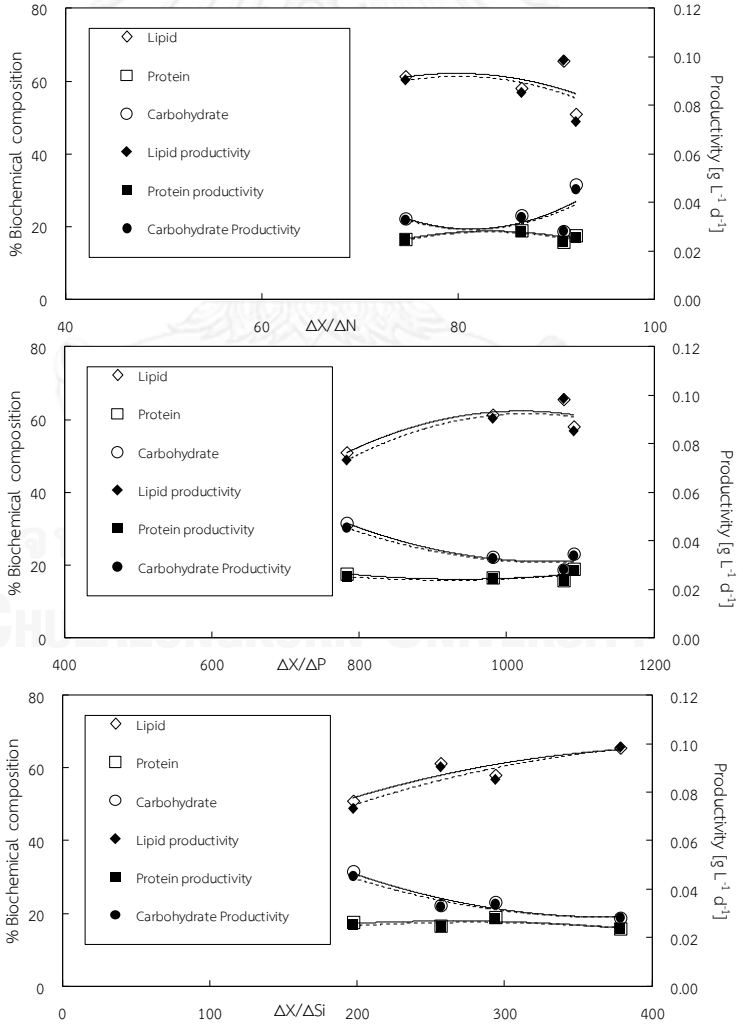

Figure 4.7 %Biochemical composition as a function of biomass yields

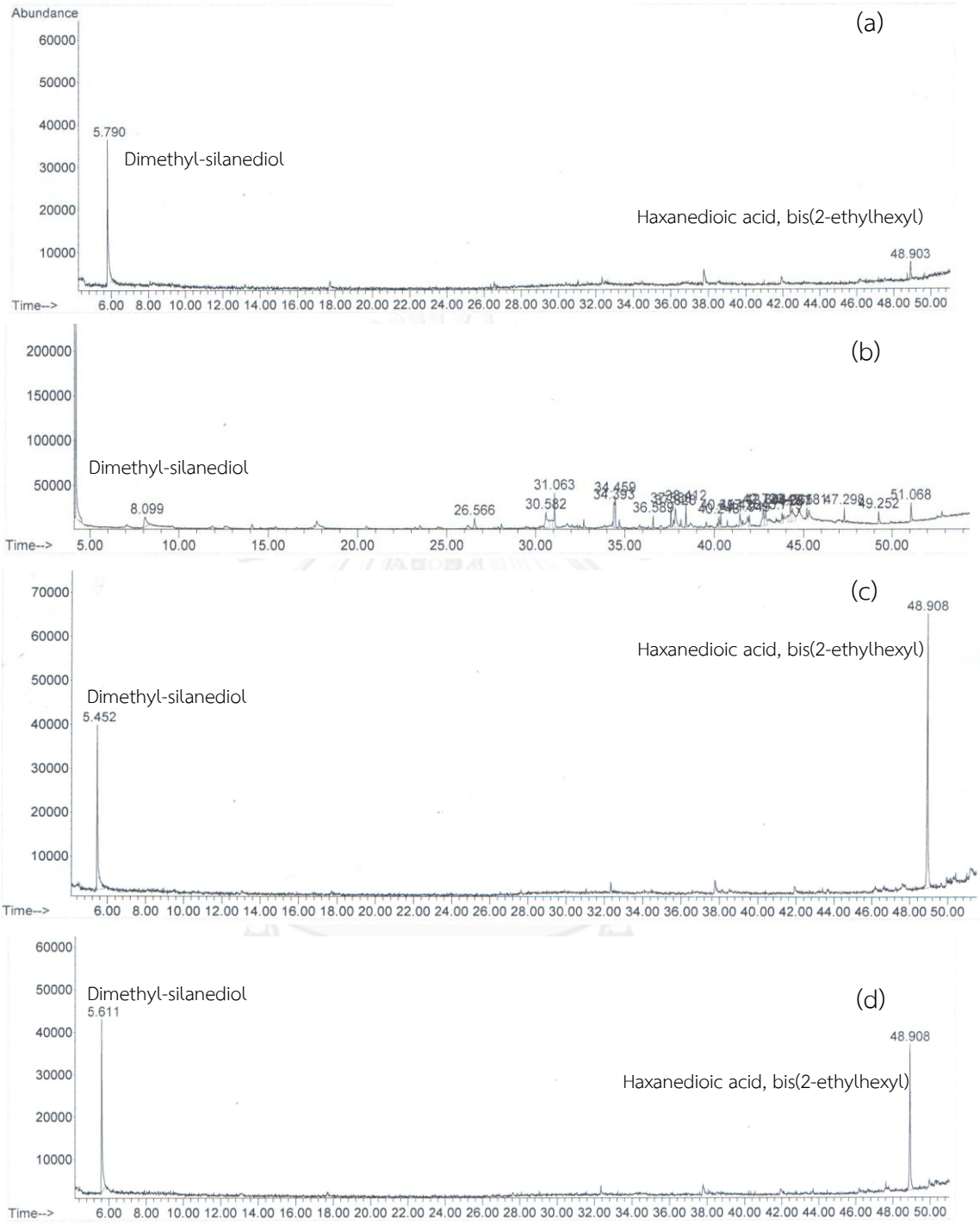

Figure 4.8 Organic compound obtained by GC-MS

(a) Initial day of Fresh medium condition

(b) Final day of Fresh medium condition

(c) Initial day of 1st reused medium condition

(d) Final day of 1st reused medium condition

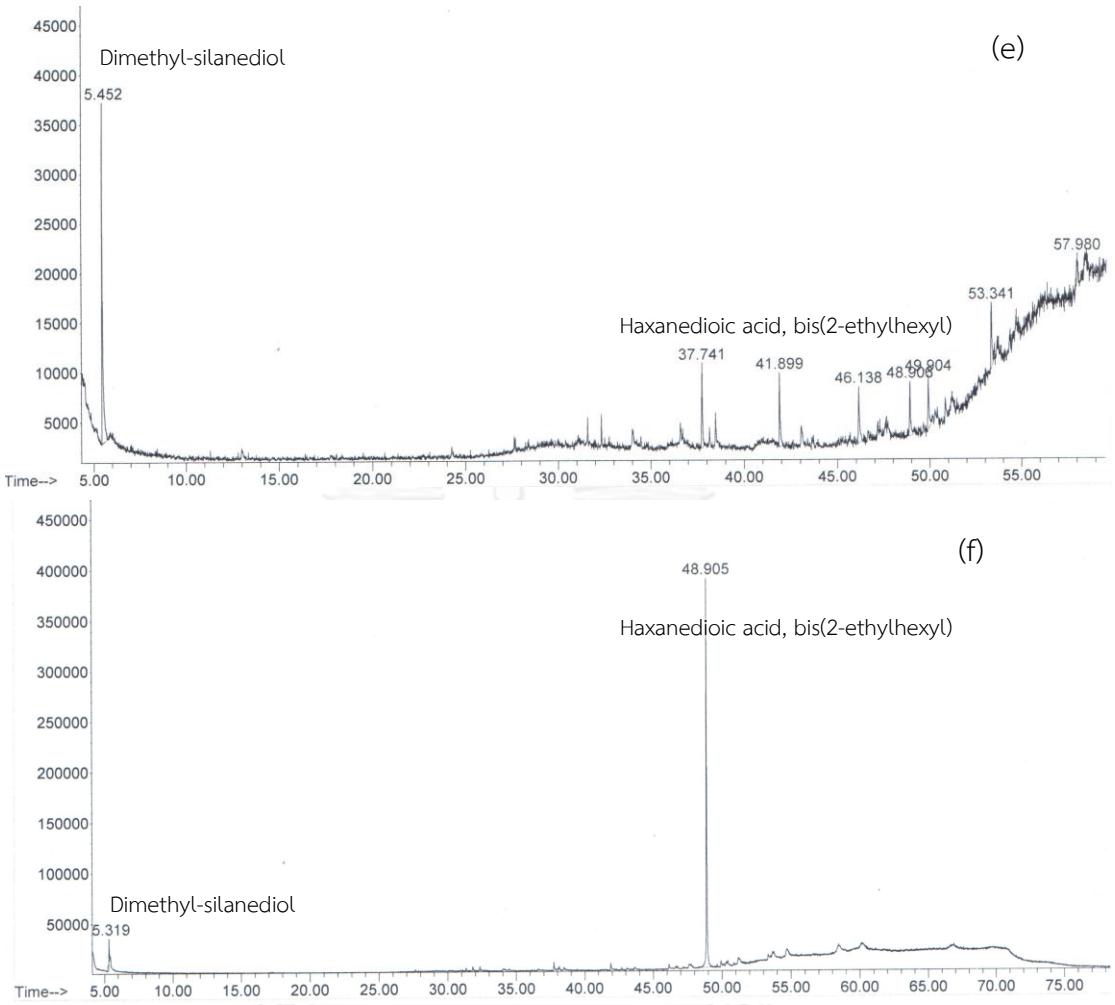

Figure 4.9 Organic compound obtained by GC-MS (Continued)

(e) Initial day of 2nd reused medium condition

(f) Final day of 2nd reused medium condition

Table 4.1 Maximum cell density, specific growth rate and productivity in fresh, 1st, 2nd and 3rd reused mediums for the cultivation of *C. gracilis*

System	Maximum cell density ($x10^6$ cell mL^{-1})	Specific growth rate (d^{-1})	Productivity ($x10^9$ cell d^{-1})	Specific productivity ($x10^9$ cell L^{-1} d^{-1})
Fresh	10.73 ± 0.25	0.79 ± 0.02	13.03 ± 0.41	2.61 ± 0.08
1st reused	5.28 ± 0.33	0.74 ± 0.01	7.69 ± 0.41	1.54 ± 0.08
2nd reused	6.09 ± 0.17	0.68 ± 0.01	8.29 ± 1.10	1.66 ± 0.22
3rd reused	6.02 ± 0.13	0.75 ± 0.02	8.94 ± 0.61	1.79 ± 0.12

Table 4.2 Uptake rate of silicate, nitrate and phosphate for the cultivation of *C. gracilis*

Time	Fresh medium			1st reused medium		
(days)	ΔSi/ΔX	ΔN/ΔX	ΔP/ΔX	ΔSi/ΔX	ΔN/ΔX	ΔP/ΔX
0						
1	1.57×10^{-3}	1.84×10^{-2}	5.77×10^{-3}	7.16×10^{-3}	2.49×10^{-2}	2.76×10^{-3}
2	9.39×10^{-3}	9.07×10^{-3}	-9.76×10^{-6}	2.10×10^{-4}	1.61×10^{-2}	3.85×10^{-4}
3	1.74×10^{-3}	-4.19×10^{-3}	4.78×10^{-5}	-1.03×10^{-2}	-1.88×10^{-1}	-5.68×10^{-3}
4	1.51×10^{-3}	-2.59×10^{-3}	1.11×10^{-4}	-1.60×10^{-4}	-1.21×10^{-2}	4.78×10^{-4}
Time	2nd reused medium			3rd reused medium		
(days)	ΔSi/ΔX	ΔN/ΔX	ΔP/ΔX	ΔSi/ΔX	ΔN/ΔX	ΔP/ΔX
0						
1	6.00×10^{-3}	3.47×10^{-4}	2.34×10^{-3}	5.72x10-3	2.39×10^{-2}	9.51×10^{-4}
2	9.84×10^{-4}	1.04×10^{-2}	7.41×10^{-5}	1.04x10-4	1.81×10^{-3}	-3.75×10^{-5}
3	-7.54×10^{-4}	1.38×10^{-3}	-1.65×10^{-5}	3.53x10-4	1.85×10^{-3}	-2.05×10^{-6}
4	2.09×10^{-5}	-3.47×10^{-3}	-7.37×10^{-5}	2.08x10-3	-8.55×10^{-4}	-2.62×10^{-5}

Table 4.3 Reduction (%) of elements for the cultivation of *C. gracilis* in fresh, 1st, 2nd and 3rd reused mediums

Elements	Fresh medium			1st reused medium		
	Concentration (mg L^{-1})		% Reduction (Δ1)	Concentration (mg L^{-1})		% Reduction (Δ2)
	Initial	Final		Initial	Final	
Si	3.6971	0.7070	80.88	2.5003	0.5228	79.09
N	9.0001	1.6279	81.91	10.3170	2.7811	73.04
P	1.1129	0.3304	70.31	0.8872	0.2904	67.27
B	0.1795	0.1775	1.11	0.2035	0.1640	19.41
Zn	0.4020	0.3605	10.32	0.4185	0.3805	9.08
Cu	0.0030	0.0020	33.33	0.0030	0.0003	90.00
Fe	0.1585	0.0030	98.11	0.2270	0.0025	98.90
K	4.4115	3.3793	23.40	4.4865	3.7665	16.05
Mn	0.0325	0.0245	24.62	0.0360	0.0235	34.72
Mo	0.0775	0.0725	6.45	0.0805	0.0725	9.94
Co	0.0040	0.0030	25.00	0.0045	0.0030	33.33

Table 4.3 Reduction (%) of elements for the cultivation of *C. gracilis* in fresh, 1st, 2nd and 3rd reused mediums (Continued)

Elements	2nd medium reuses			3rd medium reuses		
	Concentration (mg L^{-1})		% Reduction (Δ3)	Concentration (mg L^{-1})		% Reduction (Δ4)
	Initial	Final		Initial	Final	
Si	2.7447	0.7317	73.34	2.8454	0.6587	76.85
N	11.2200	1.9148	82.93	8.9900	0.7785	91.34
P	1.0700	0.2971	72.23	0.9096	0.2886	68.27
B	0.2150	0.1665	22.56	0.2000	0.1695	15.25
Zn	0.4920	0.3785	23.07	0.4660	0.3795	18.56
Cu	0.0035	0.0020	42.86	0.0035	0.0025	28.57
Fe	0.2105	0.0240	88.60	0.2235	0.0355	84.12
K	4.6855	3.8255	18.35	4.9965	3.8685	22.58
Mn	0.0395	0.0265	32.91	0.0450	0.0270	40.00
Mo	0.0845	0.0730	13.61	0.0850	0.0730	14.12
Co	0.0040	0.0030	25.00	0.0040	0.0035	12.50

Table 4.4 Empirical formula of *C. gracilis* cultivated in different mediums

System	Empirical formula
Fresh	$CH_{1.41}O_{1.09}N_{1.86}P_{0.73}Si_{0.92}B_{0.03}Zn_{0.05}Cu_{0.17}Fe_{0.56}K_{0.19}Mn_{0.14}Mo_{0.02}Co_{0.14}$
1st reused	$CH_{1.37}O_{1.17}N_{1.73}P_{0.72}Si_{0.93}B_{0.59}Zn_{0.04}Cu_{0.46}Fe_{0.59}K_{0.14}Mn_{0.21}Mo_{0.03}Co_{0.19}$
2nd reused	$CH_{1.43}O_{1.22}N_{2.00}P_{0.79}Si_{0.88}B_{0.71}Zn_{0.12}Cu_{0.23}Fe_{0.54}K_{0.16}Mn_{0.20}Mo_{0.05}Co_{0.14}$
3rd reused	$CH_{1.52}O_{1.28}N_{2.29}P_{0.77}Si_{0.94}B_{0.49}Zn_{0.09}Cu_{0.16}Fe_{0.53}K_{0.20}Mn_{0.26}Mo_{0.05}Co_{0.07}$

4.2 Fed-batch culture

The culture was further investigated to examine whether a different feeding strategy could have positive effects on algal growth. A fed batch culture with 3 levels of nutrient concentrations was set out as shown below:

- Control (Fresh medium): 100% initial fresh medium
- 50% macronutrient: The total amount of target nutrient (i.e. silicate, nitrate, and phosphate) was 50% of amount used in the fresh medium. The total feeding amount was split equally into 5 days. This was equivalent to 0.5 mL of the stock nutrient per day per 5 L of seawater.
- 100% macronutrient: The total amount of target nutrient (i.e. silicate, nitrate, and phosphate) was 100% of amount used in the fresh medium (same total amount). The total feeding amount was split equally into 5 days. This was equivalent to 0.5 mL of the stock nutrient per day per 5 L of seawater.
- 500% macronutrient: The total amount of target nutrient (i.e. silicate, nitrate, and phosphate) was 500% of amount used in the fresh medium. The total feeding amount was split equally into 5 days. This was equivalent to 0.5 mL of the stock nutrient per day per 5 L of seawater.

4.2.1 Fed batch with silicate

Figure 4.9 illustrates the result from the cultivation of *C. gracilis* where silicate feeding was separated equally into five days. It can be seen from the figure that the cultures under the 4 different medium conditions exhibited similar growth pattern, i.e. lag phase of 1 day, 2-3 days exponential phase, very short stationary phase followed by cell decay at Day 4. Table 4.5 reports the cell concentration, specific growth rate, productivity and specific productivity from the cultivation with fresh medium and mediums with silicate adjustment. A maximum cell density obtained from the fresh medium was approximately $10.27 \pm 0.21 \times 10^6$ cell mL^{-1} with a maximum specific growth rate of 0.55 ± 0.01 d^{-1}. In the 50% and 500% silicate concentrations, the attainable maximum cell densities were approximately at the same levels which were higher than that obtained from the cultivation with 100% silicate concentration, i.e. $11.08 \pm 0.35 \times 10^6$ and $11.16 \pm 0.57 \times 10^6$ cell mL^{-1}, respectively. The cultures with 50%, 100% and 500% silicate concentrations provided similar productivities of 0.57 ± 0.02, 0.50 ± 0.02 and 0.54 ± 0.02 d^{-1}. Table 4.5 shows that the fresh medium condition gave the culture with the highest productivity at $10.03 \pm 0.56 \times 10^9$ cell d^{-1} whereas the 50%, 100% and 500% silicate concentrations

provided similar productivities of 8.90 ± 0.48 x 10^9, 6.71 ± 0.74 x 10^9, 9.30 ± 0.61 x 10^9 cell d^{-1}, respectively.

Figure 4.10 illustrates how cell density changed with silicate concentration when cells were cultivated with fresh, 50%, 100% and 500% silicate concentrations. The results show that, regardless of the quantity of silicate provided, cells always grew in a similar pattern where the maximum cell density reached about 11.16 ± 0.57 x 10^6 cell mL^{-1}. This suggested that only 50% of silicate should be adequate for the proper growth of *Chaetoceros gracilis*. Figure 4.11 shows that for this case, if biomass yield (ΔX) was plotted against the consumption of major nutrients in the reactor, the highest biomass yield could be obtained with only small consumption of P and Si. Only N was needed to a proper growth as the maximum cell density was only obtained when the consumption of N was high.

Figure 4.12 displays biochemical composition of the cell and this shows that %carbohydrate always moved in the opposite direction to %total lipid, whereas %protein remained relatively constant. The maximum carbohydrate concentration was obtained with the case with 50% silicate whereas lipid was the highest in the control experiment. Biochemical composition and productivity took the same trends as illustrated in Figure 4.13.

Table 4.6 summarizes the uptake rates of the three major nutrients from this experiment whereas Table 4.7 reports the reduction in all nutrients. This led to the estimate of cell chemical formula as shown in Table 4.8.

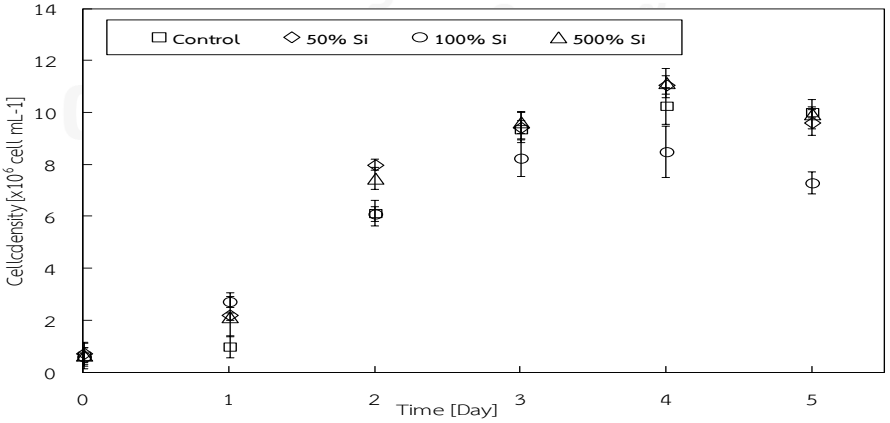

Figure 4.10 Growth behaviours of *C. gracilis* in fresh medium and mediums with silicate adjustment

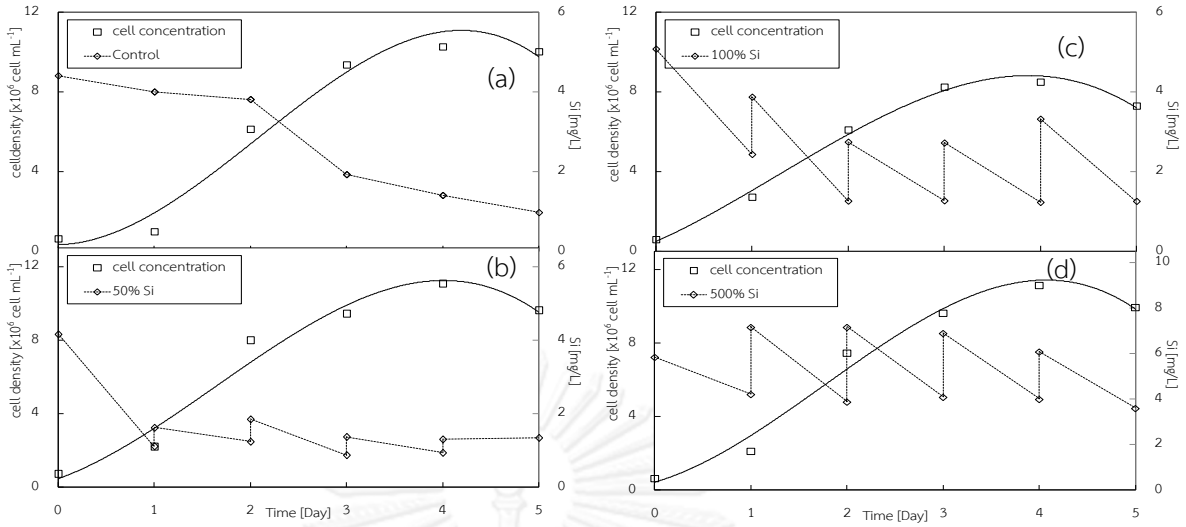

Figure 4.11Cell density and silicate concentration for the cultivation of *C. gracilis*

(a) Control, (b) 50% Si, (c) 100% Si, and (d) 500% Si concentration

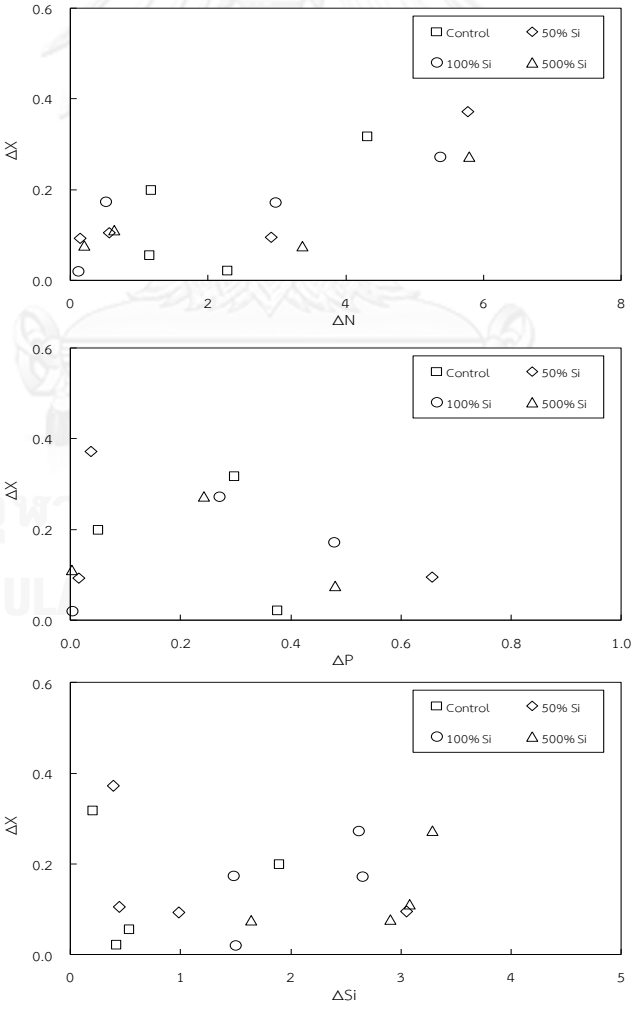

Figure 4.12 Biomass yield from cultivation with silicate adjustment

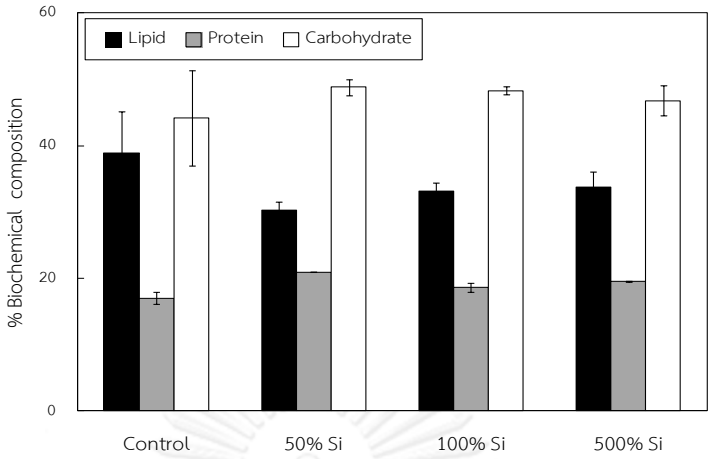

Figure 4.13 % Biochemical composition for the cultivation of *C. gracilis* in fresh medium and mediums with silicate adjustment

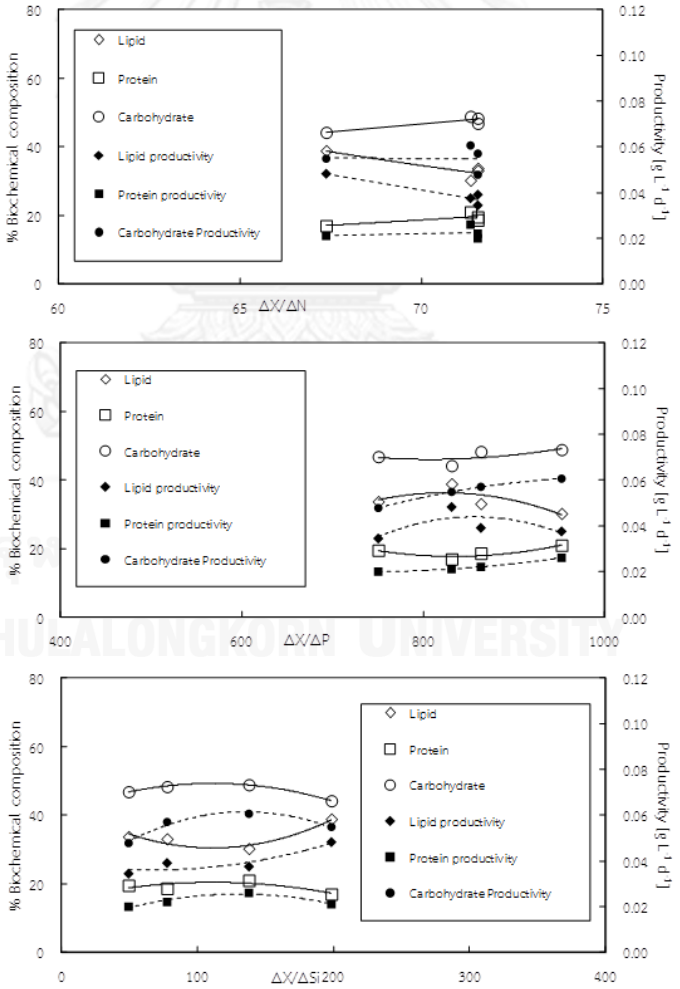

Figure 4. 14% %Biochemical composition as a function of biomass yields

Table 4.5 Maximum cell density, specific growth rate and productivity

System	Maximum cell density ($x10^6$ cell mL^{-1})	Specific growth rate (d^{-1})	Productivity ($x10^9$ cell d^{-1})	Specific productivity ($x10^9$ cell L^{-1} d^{-1})
Fresh	10.27 ± 0.21	0.55 ± 0.01	10.03 ± 0.56	2.00 ± 0.06
50% silicate	11.08 ± 0.35	0.52 ± 0.02	8.90 ± 0.48	1.78 ± 0.13
100% silicate	8.50 ± 0.99	0.50 ± 0.02	6.71 ± 0.74	1.34 ± 0.25
500% silicate	11.16 ± 0.57	0.54 ± 0.02	9.30 ± 0.61	1.86 ± 0.16

Table 4.6 Uptake rate of silicate, nitrate and phosphate for the cultivation of *C. gracilis*

Time	Fresh medium			50% silicate		
(days)	ΔSi/ΔX	ΔN/ΔX	ΔP/ΔX	ΔSi/ΔX	ΔN/ΔX	ΔP/ΔX
0						
1	$1.84x10^{-2}$	$1.03x10^{-1}$	$1.70x10^{-2}$	$3.19x10^{-2}$	$3.05x10^{-2}$	$6.87x10^{-3}$
2	$5.99x10^{-4}$	$1.35x10^{-2}$	$9.28x10^{-4}$	$1.02x10^{-3}$	$1.55x10^{-2}$	$9.48x10^{-5}$
3	$9.44x10^{-3}$	$5.77x10^{-3}$	$2.40x10^{-4}$	$1.05x10^{-2}$	$1.34x10^{-3}$	$1.42x10^{-4}$
4	$9.33x10^{-3}$	$2.02x10^{-2}$	$-4.04x10^{-6}$	$4.12x10^{-3}$	$5.21x10^{-3}$	$-4.14x10^{-5}$
Time	100% silicate			500% silicate		
(days)	ΔSi/ΔX	ΔN/ΔX	ΔP/ΔX	ΔSi/ΔX	ΔN/ΔX	ΔP/ΔX
0						
1	$1.54x10^{-2}$	$1.72x10^{-2}$	$2.77x10^{-3}$	$2.15x10^{-2}$	$4.43x10^{-2}$	$6.32x10^{-3}$
2	$9.57x10^{-3}$	$1.97x10^{-2}$	$9.86x10^{-4}$	$1.20x10^{-2}$	$2.11x10^{-2}$	$8.80x10^{-4}$
3	$8.48x10^{-3}$	$2.88x10^{-3}$	$-4.66x10^{-5}$	$2.77x10^{-2}$	$5.59x10^{-3}$	$6.11x10^{-6}$
4	$7.38x10^{-2}$	$4.93x10^{-3}$	$8.12x10^{-5}$	$3.75x10^{-2}$	$2.39x10^{-3}$	$-5.27x10^{-5}$

Table 4.7 Reduction (%) of elements for the cultivation of *C. gracilis* in fresh medium and mediums with silicate adjustment

Elements	Fresh medium			50% silicate		
	Concentration ($mg\ L^{-1}$)		% Reduction (Δ1)	Concentration ($mg\ L^{-1}$)		% Reduction (Δ2)
	Initial	Final		Initial	Final	
Si	4.4093	0.79761	81.91	4.1567	1.3417	67.72
N	11.0234	1.9112	82.66	11.2113	1.8388	83.60
P	0.9840	0.2822	71.32	0.9842	0.3044	69.07
B	0.1795	0.1775	1.11	0.2035	0.1640	19.41
Zn	0.4020	0.3605	10.32	0.4185	0.3805	9.08
Cu	0.0030	0.0020	33.33	0.0030	0.0003	90.00
Fe	0.1585	0.0030	98.11	0.2270	0.0025	98.90
K	4.4115	3.3793	23.40	4.4865	3.7665	16.05
Mn	0.0325	0.0245	24.62	0.0360	0.0235	34.72
Mo	0.0775	0.0725	6.45	0.0805	0.0725	9.94
Co	0.0040	0.0030	25.00	0.0045	0.0030	33.33

Table 4.7 Reduction (%) of elements for the cultivation of *C. gracilis* in fresh medium and mediums with silicate adjustment (continued)

Elements	100% silicate			500% silicate		
	Concentration ($mg\ L^{-1}$)		% Reduction (Δ3)	Concentration ($mg\ L^{-1}$)		% Reduction (Δ4)
	Initial	Final		Initial	Final	
Si	5.0793	1.2575	75.24	5.8533	1.2078	79.37
N	10.9108	1.8503	83.04	11.5311	1.7656	84.69
P	1.0141	0.2829	72.10	0.9899	0.2770	72.02
B	0.2150	0.1665	22.56	0.2000	0.1695	15.25
Zn	0.4920	0.3785	23.07	0.4660	0.3795	18.56
Cu	0.0035	0.0020	42.86	0.0035	0.0025	28.57
Fe	0.2105	0.0240	88.60	0.2235	0.0355	84.12
K	4.6855	3.8255	18.35	4.9965	3.8685	22.58
Mn	0.0395	0.0265	32.91	0.0450	0.0270	40.00
Mo	0.0845	0.0730	13.61	0.0850	0.0730	14.12
Co	0.0040	0.0030	25.00	0.0040	0.0035	12.50

Table 4.8 Empirical formula of *C. gracilis*

System	Empirical formula
Fresh	$CH_{1.48}O_{1.15}N_{1.94}P_{0.76}Si_{0.96}B_{0.03}Zn_{0.05}Cu_{0.17}Fe_{0.58}K_{0.19}Mn_{0.15}Mo_{0.02}Co_{0.14}$
50% silicate	$CH_{1.47}O_{1.19}N_{2.02}P_{0.76}Si_{0.82}B_{0.61}Zn_{0.05}Cu_{0.48}Fe_{0.60}K_{0.14}Mn_{0.21}Mo_{0.04}Co_{0.19}$
100% silicate	$CH_{1.44}O_{1.10}N_{1.91}P_{0.75}Si_{0.86}B_{0.67}Zn_{0.11}Cu_{0.22}Fe_{0.51}K_{0.15}Mn_{0.19}Mo_{0.05}Co_{0.14}$
500% silicate	$CH_{1.48}O_{1.23}N_{2.08}P_{0.80}Si_{0.97}B_{0.49}Zn_{0.10}Cu_{0.15}Fe_{0.52}K_{0.19}Mn_{0.25}Mo_{0.05}Co_{0.07}$

4.2.2 Fed batch with nitrate

Figure 4.14 illustrates the result from the cultivation of *C. gracilis*. It can be seen from the figure that the cultures under the 4 different medium conditions exhibited similar growth pattern with s curve. Table 4.9 illustrates the cell concentration, specific growth rate, productivity and specific productivity from the cultivation with fresh medium and mediums with nitrate adjustment. In this work, temperature 28 - 35^{o}C and pH 8.2 – 8.6 were in the proper range for the growth of *C. gracilis*. The maximum cell density obtained from the 50%, 100% and 500% nitrate concentrations were lower than that with fresh medium (11.01 ± 0.28 x 10^{6}, 10.89 ± 0.17 x 10^{6}, 10.03 ± 0.42 x 10^{6} and 12.35± 0.71 x 10^{6} cell mL^{-1}, respectively.). The specific growth rate obtained from the cultivations with 50%, 100% and 500% nitrate concentrations were slightly lower than that obtained from the cultivation with fresh medium, i.e. 0.57 ± 0.01, 0.56 ± 0.01, 0.56 ± 0.01, and 0.59 ± 0.02 d^{-1} (See Table 4.9). Laotaweesup (2003) also discovered similar evidence where a higher nitrate level resulted in a lower level of nitrate, and *Chaetoceros* could survive the growth condition without nitrate relatively well with a similar growth rate as the culture with fresh medium.

Table 4.9 shows that the fresh medium condition provided the culture with the highest productivity at 11.06 ± 0.52 x 10^{9} cell d^{-1} whereas the 50%, 100% and 500% nitrate concentrations provided similar productivities of 9.05 ± 0.39 x 10^{9}, 9.40 ± 0.35 x 10^{9}, 9.42 ± 0.46 x 10^{9} cell d^{-1}, respectively. Figure 4.15 illustrates how cell density changed with nitrate concentration when cells were cultivated with fresh, 50%, 100% and 500% nitrate concentrations. Nitrate was always consumed quickly in the culture medium and it only took two days in the cultivation with fresh medium to reach the bottom level at around 2.1974 mg-N L^{-1}. In nitrate adjustment experiments, nitrate was intermittently provided into the medium and most of the added nitrate was always completely consumed within one day.

Figure 4.16 demonstrate the relationship between biomass yield (ΔX) and major nutrient concentrations. It is interesting to see that the highest biomass yield could most of the time be obtained when only small amounts of P and N were consumed. Although a relatively large biomass yield could be obtained at low consumption of Si, the highest biomass yield was obtained when a large quantity of Si was consumed.

Figure 4.17 show that %carbohydrate was always the major component of this algal species. %Carbohydrate increased when %total lipid decreased, whereas %protein remained relatively constant throughout. Maximum carbohydrate was obtained from the culture with 50% nitrate concentration (46.48%), and at this condition, a %total lipid of 37.46%. In contrast, the maximum %total lipid was obtained from the cultivation with 500% nitrate concentration which was approximately 39.66% with a minimum %carbohydrate of 41.39%.

Figure 4.18 illustrates the result from %biochemical composition as a function of biomass yield. Biochemical composition does not seem to change significantly with biomass yield. Table 4.10 summarizes the uptake rates of the three major nutrients from this experiment. It can be observed that the uptakes of all nutrients were high in the first day and slowed down in the following days. This indicates that cells from the first day were always consumed in a greater level than the uptakes from the following day. Table 4.11 summarizes the total reductions of the nutrient from one batch culture and the information could be used to calculate the empirical formulation of the cells as given in Table 4.12.

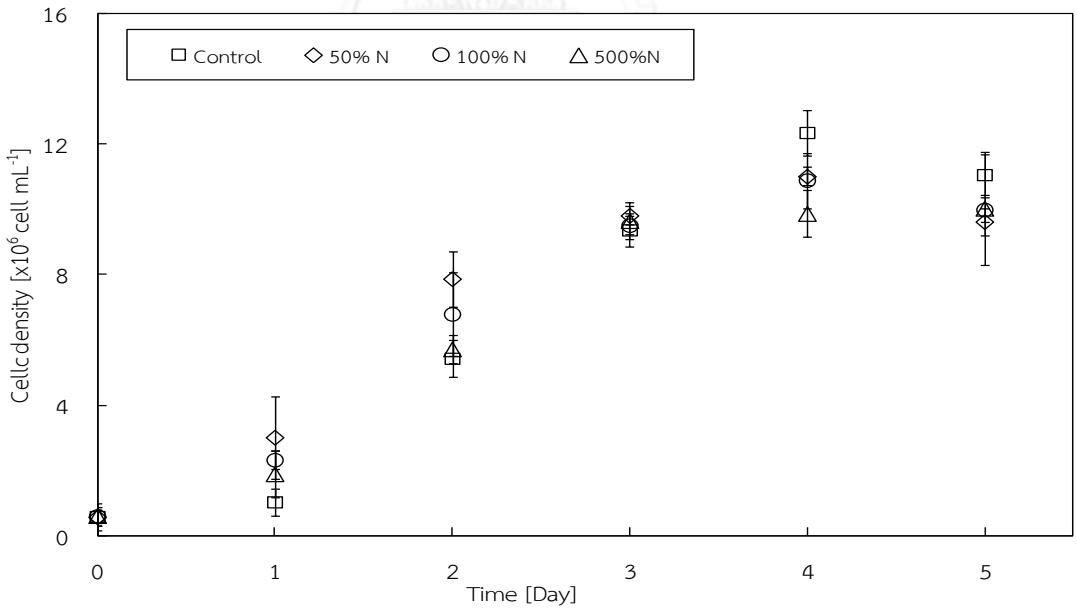

Figure 4.15 Growth behaviours of *C. gracilis* in fresh medium and mediums with nitrate adjustment

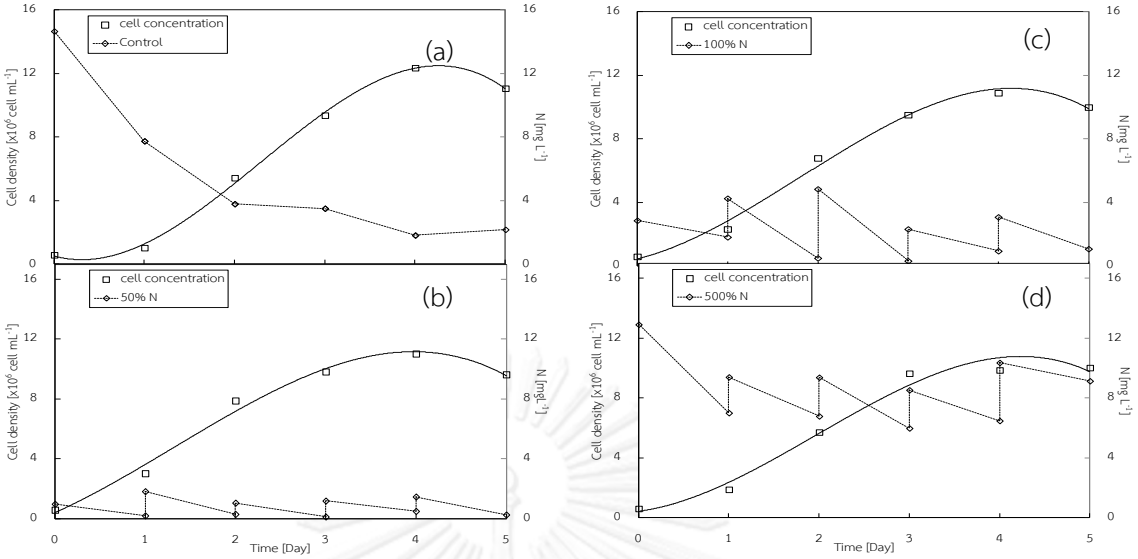

Figure 4. 16 Cell density and nitrate concentration for the cultivation of *C. gracilis*

(a) Control, (b) 50% N, (c) 100% N, and (d) 500% N concentration

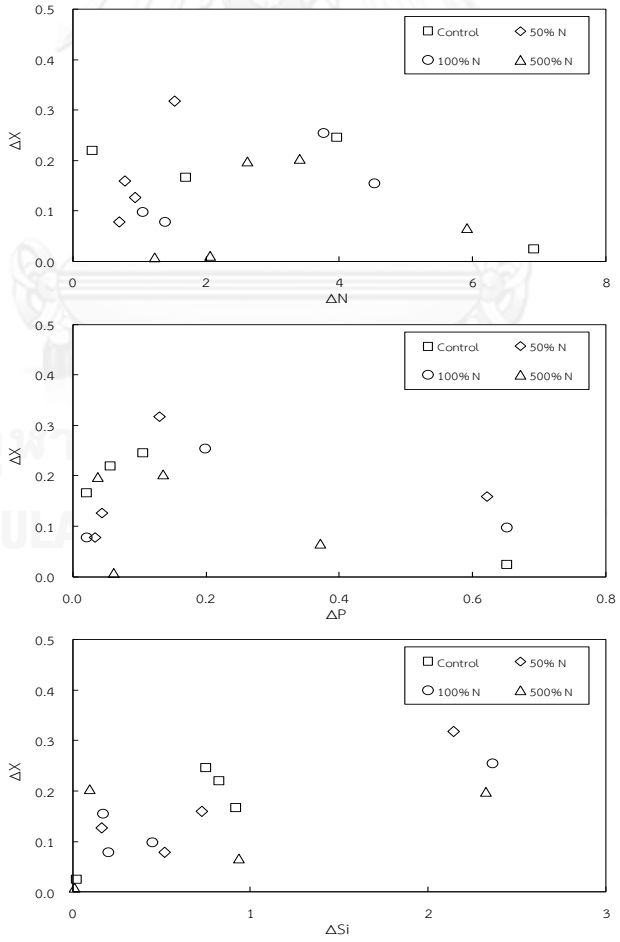

Figure 4.17 Biomass yield from cultivation with reused medium nitrate adjustment

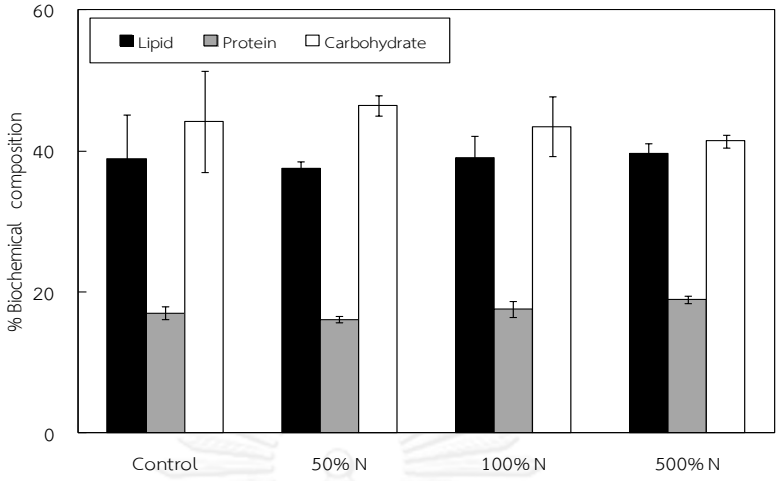

Figure 4.18 % Biochemical composition for the cultivation of *C. gracilis* in fresh medium and mediums with nitrate adjustment

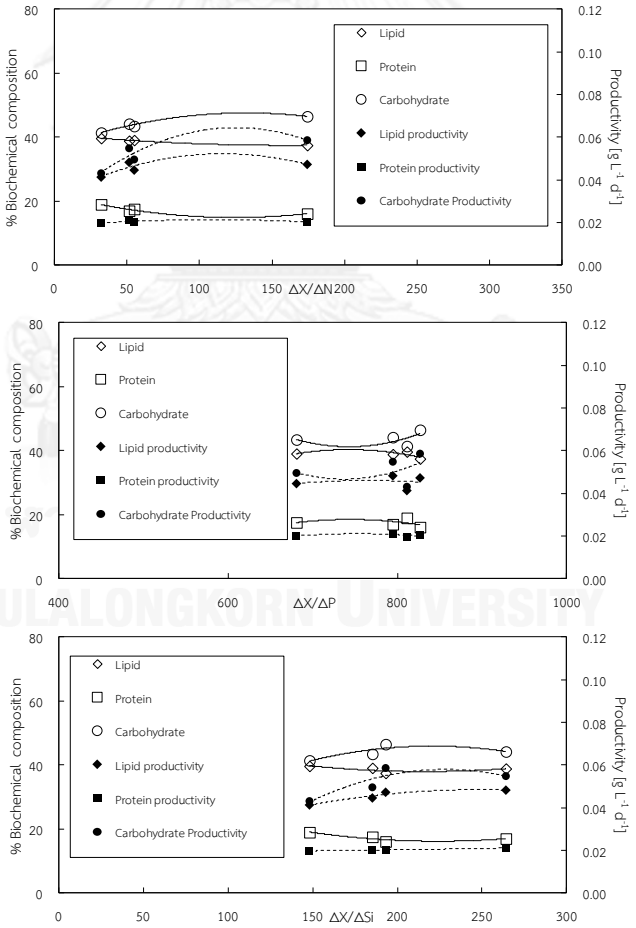

Figure 4.19 % Biochemical composition as a function of biomass yields

Table 4.9 Maximum cell density, specific growth rate and productivity

System	Maximum cell density ($x10^6$ cell mL^{-1})	Specific growth rate (d^{-1})	Productivity ($x10^9$ cell d^{-1})	Specific productivity ($x10^9$ cell L^{-1} d^{-1})
Fresh	12.35 ± 0.71	0.59 ± 0.02	11.06 ± 0.52	2..21 ± 0.08
50% nitrate	11.01 ± 0.28	0.57 ± 0.01	9.05 ± 0.39	1.81 ± 0.08
100% nitrate	10.89 ± 0.17	0.56 ± 0.01	9.40 ± 0.35	1.88 ± 0.22
500% nitrate	10.03 ± 0.42	0.56 ± 0.01	9.42 ± 0.46	1.88 ± 0.12

Table 4.10 Uptake rate of silicate, nitrate and phosphate for the cultivation of *C. gracilis*

Time	Fresh medium			50% nitrate		
(days)	ΔSi/ΔX	ΔN/ΔX	ΔP/ΔX	ΔSi/ΔX	ΔN/ΔX	ΔP/ΔX
0						
1	$7.09x10^{-4}$	$2.74x10^{-1}$	$2.58x10^{-2}$	$4.53x10^{-3}$	$4.87x10^{-3}$	$3.89x10^{-3}$
2	$3.02x10^{-3}$	$1.60x10^{-2}$	$4.23x10^{-4}$	$6.73x10^{-3}$	$4.79x10^{-3}$	$4.07x10^{-4}$
3	$3.72x10^{-3}$	$1.29x10^{-3}$	$2.52x10^{-4}$	$1.27x10^{-3}$	$7.32x10^{-3}$	$3.41x10^{-4}$
4	$5.46x10^{-3}$	$1.01x10^{-2}$	$1.19x10^{-4}$	$6.55x10^{-3}$	$8.82x10^{-3}$	$4.19x10^{-4}$
Time	100% nitrate			500% nitrate		
(days)	ΔSi/ΔX	ΔN/ΔX	ΔP/ΔX	ΔSi/ΔX	ΔN/ΔX	ΔP/ΔX
0						
1	$4.53x10^{-3}$	$1.06x10^{-2}$	$6.61x10^{-3}$	$1.41x10^{-2}$	$8.91x10^{-2}$	$5.59x10^{-3}$
2	$9.25x10^{-3}$	$1.47x10^{-2}$	$7.76x10^{-4}$	$1.17x10^{-2}$	$1.32x10^{-2}$	$1.87x10^{-4}$
3	$1.08x10^{-3}$	$2.91x10^{-2}$	$-3.90x10^{-5}$	$4.59x10^{-4}$	$1.67x10^{-2}$	$6.63x10^{-4}$
4	$2.52x10^{-3}$	$1.75x10^{-2}$	$2.58x10^{-4}$	$-5.12x10^{-3}$	$1.80x10^{-1}$	$-1.79x10^{-4}$

Table 4.11 Reduction (%) of elements for the cultivation of *C. gracilis* in fresh medium and mediums with nitrate adjustment

Elements	Fresh medium			50% nitrate		
	Concentration (mg L^{-1})		% Reduction (Δ1)	Concentration (mg L^{-1})		% Reduction (Δ2)
	Initial	Final		Initial	Final	
Si	4.0158	1.4300	64.39	4.3805	1.2203	72.14
N	14.6565	2.1794	85.13	0.9660	0.2503	74.09
P	1.1236	0.3000	73.30	1.1089	0.3010	72.86
B	0.2205	0.1762	20.09	0.2065	0.1735	15.98
Zn	0.4211	0.3645	13.44	0.4234	0.3763	11.12
Cu	0.0035	0.0020	42.86	0.0030	0.0025	16.67
Fe	0.2301	0.0035	98.48	0.2270	0.0025	98.90
K	4.9832	3.6428	26.90	4.4899	3.7813	15.78
Mn	0.0385	0.0225	41.56	0.0375	0.0250	33.33
Mo	0.0790	0.0715	9.49	0.0845	0.0755	10.65
Co	0.0045	0.0030	33.33	0.0045	0.0035	22.22

Table 4.11 Reduction (%) of elements for the cultivation of C. gracilis in fresh medium and mediums with nitrate adjustment (continued)

Elements	100% nitrate			500% nitrate		
	Concentration (mg L^{-1})		% Reduction (Δ3)	Concentration (mg L^{-1})		% Reduction (Δ4)
	Initial	Final		Initial	Final	
Si	3.9996	0.7482	81.29	4.4922	1.1938	73.43
N	2.8650	1.0786	62.35	12.9204	9.1459	29.21
P	1.1524	0.2913	74.72	1.0504	0.4484	57.31
B	0.2315	0.1730	25.27	0.2285	0.1745	23.63
Zn	0.4805	0.3964	17.50	0.4540	0.3985	12.22
Cu	0.0030	0.0020	33.33	0.0035	0.0025	28.57
Fe	0.2105	0.0240	88.60	0.2235	0.0355	84.12
K	4.9908	3.7909	24.04	4.8967	3.7895	22.61
Mn	0.0395	0.0235	40.51	0.0465	0.0295	36.56
Mo	0.0885	0.0765	13.56	0.0865	0.0725	16.18
Co	0.0040	0.0030	25.00	0.0040	0.0035	12.50

Table 4.12 Empirical formula of *C. gracilis*

System	Empirical formula
Fresh	$CH_{1.50}O_{1.06}N_{1.92}P_{0.75}Si_{0.72}B_{0.59}Zn_{0.06}Cu_{0.21}Fe_{0.56}K_{0.22}Mn_{0.24}Mo_{0.03}Co_{0.18}$
50% nitrate	$CH_{1.55}O_{1.15}N_{1.75}P_{0.78}Si_{0.85}B_{0.49}Zn_{0.06}Cu_{0.09}Fe_{0.59}K_{0.13}Mn_{0.20}Mo_{0.04}Co_{0.12}$
100% nitrate	$CH_{1.51}O_{1.06}N_{1.41}P_{0.76}Si_{0.92}B_{0.74}Zn_{0.08}Cu_{0.17}Fe_{0.50}K_{0.19}Mn_{0.23}Mo_{0.04}Co_{0.13}$
500% nitrate	$CH_{1.50}O_{1.20}N_{0.67}P_{0.60}Si_{0.84}B_{0.70}Zn_{0.06}Cu_{0.14}Fe_{0.49}K_{0.19}Mn_{0.21}Mo_{0.05}Co_{0.07}$

4.2.3 Fed batch with phosphate

Figure 4.19 shows the growth of *C. gracilis* cultured under the 4 different medium conditions. From the figure that the culture spent one day of lag phase, 2-4 days exponential phase and very short stationary phase followed by cell decay at Day 5. Table 4.13 shows that the cell concentration, specific growth rate, productivity and specific productivity from the cultivation with fresh medium and mediums with phosphate adjustment. The temperature range was 28-31^{o}C and pH range was 8.3-8.6 with all conditions. The maximum cell density was at 13.62 ± 0.89 x 10^{6} cell mL^{-1} with a maximum specific growth rate of 0.64 ± 0.02 d^{-1} for the culture with the fresh medium. The maximum cell densities from the cultivation with 50%, 100% and 500% phosphate concentrations were approximately at the same level, i.e. 11.51 ± 0.63 x 10^{6}, 10.03 ± 0.92 x 10^{6} and 9.86 ± 0.31 x 10^{6} cell mL^{-1}, respectively. Laotaweesup (2003) reported that adjusting phosphate concentration above or below the standard F/2 (Guillard's) medium adversely affected cell density. The specific growth rate of the cultures with 50%, 100% and 500% phosphate concentrations provided similar productivities of 0.60 ± 0.01, 0.57 ± 0.03 and 0.57 ± 0.01 d^{-1}. The fresh medium condition was the culture with the highest productivity at 13.62 ± 0.48 x 10^{-9} cell d^{-1} whereas the 50%, 100% and 500% nitrate concentrations provided similar productivities of 10.95 ± 0.80 x 10^{9}, 9.45 ± 0.51 x 10^{9}, 9.28 ± 0.72 x 10^{9} cell d^{-1}, respectively.

Figure 4.20 illustrates how cell density changed with phosphate concentration when cells were cultivated with fresh and phosphate adjustment. Phosphate was in most cases quickly consumed, however, when 500% phosphate was provided, a large quantity of phosphate seemed to remain in the medium. This showed that perhaps the standard phosphate level as indicated in the F/2 Standard nutrient was the most appropriate level when compared with the other conditions. However, the medium with 50% phosphate level (which was intermittently provided) seemed to be the most appropriate when considered the highest growth.

Figure 4.21 show biomass yields from cultivation with phosphate adjustment and this still shows that only a slight quantity of phosphate was required for the growth. In contrast to the experiment with nitrate adjustment, growth seemed to be better with a large consumption of nitrate and silicate. Figure 4.22 shows the biochemical composition of the cell. Again, %Carbohydrate went in the opposite direction with %total lipid whereas %protein remained constant. Maximum %carbohydrate was obtained from the condition with 50% phosphate concentration

at 46.55% while minimum %carbohydrate was obtained from the condition with 500% phosphate concentration at 43.32%. In contrast, maximum %total lipid obtained from 500% phosphate concentration was approximately 39.12% and minimum %total lipid was from 50% phosphate concentration at 37.77%.

Figure 4.23 illustrates the result from %biochemical composition as a function of biomass yield. Biochemical composition and productivity took the same trends with biomass yield. Table 4.14 summarizes the uptake rates of the three major nutrients from this experiment whereas Table 4.15 reports the reduction in all nutrients which leads to the estimate of the empirical formula as summarized in Table 4.16.

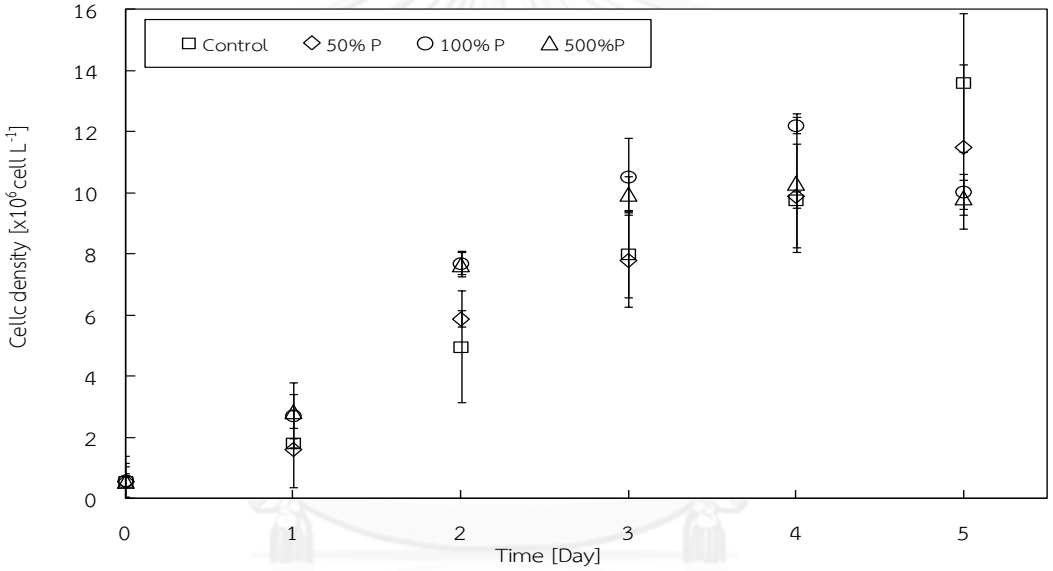

Figure 4.20 Growth behaviours of *C. gracilis* in fresh medium and mediums with phosphate adjustment

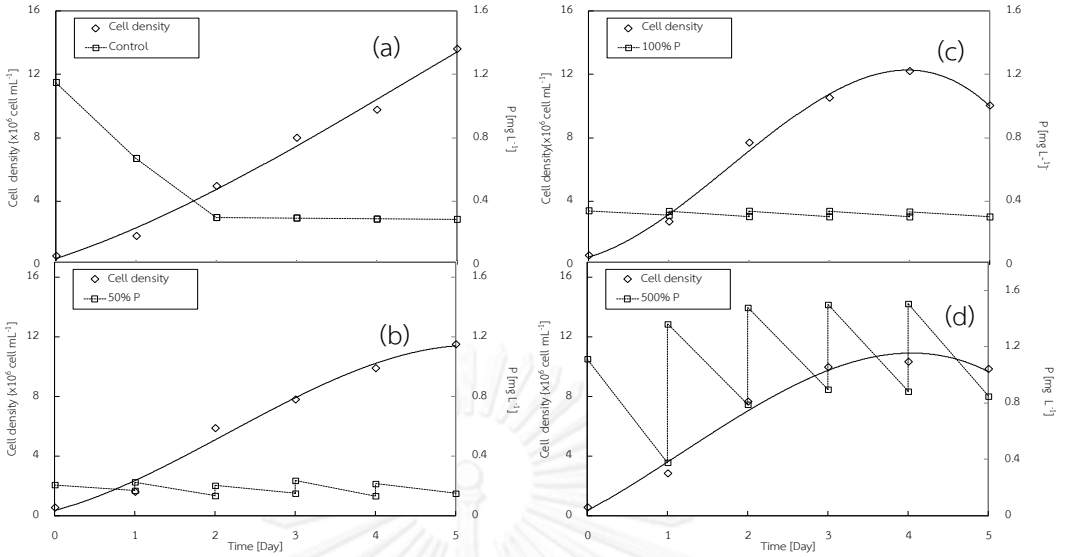

Figure 4. 21 Cell density and nitrate concentration for the cultivation of *C. gracilis*

(a) Control, (b) 50% P, (c) 100% P, and (d) 500% P concentration

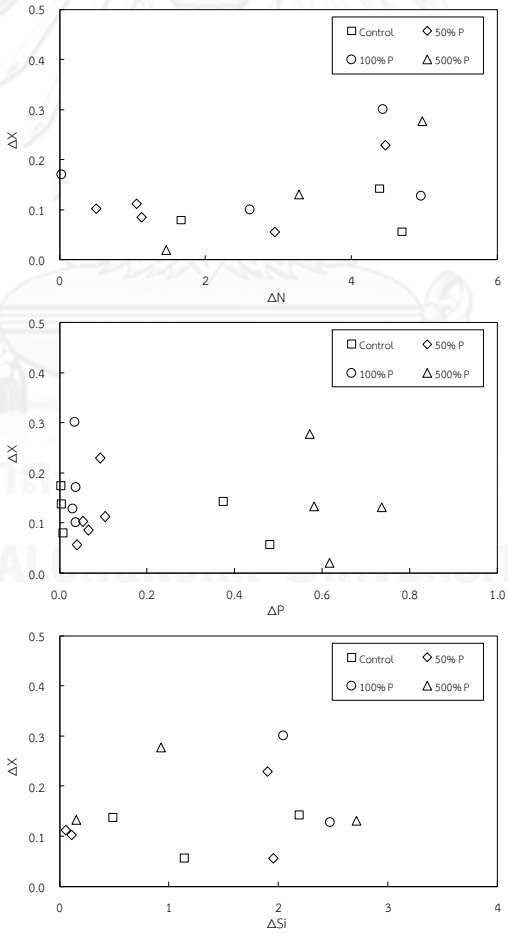

Figure 4.22 Biomass yield from cultivation with phosphate adjustment

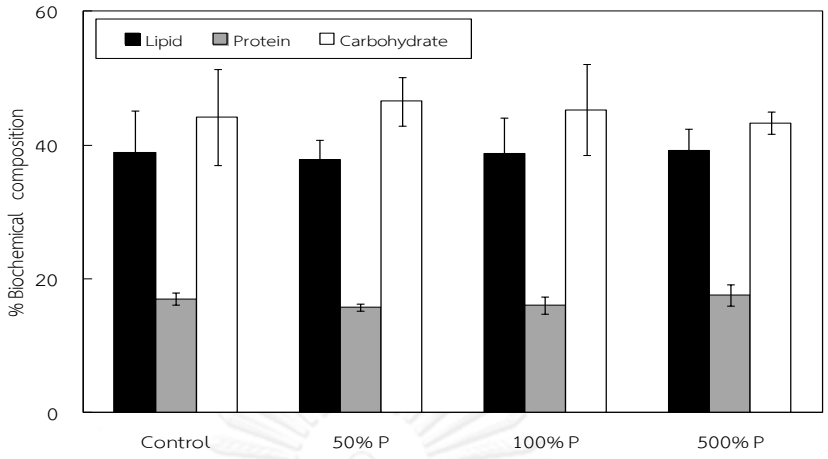

Figure 4.23 % Biochemical composition for the cultivation of *C. gracilis* in fresh medium and mediums with phosphate adjustment

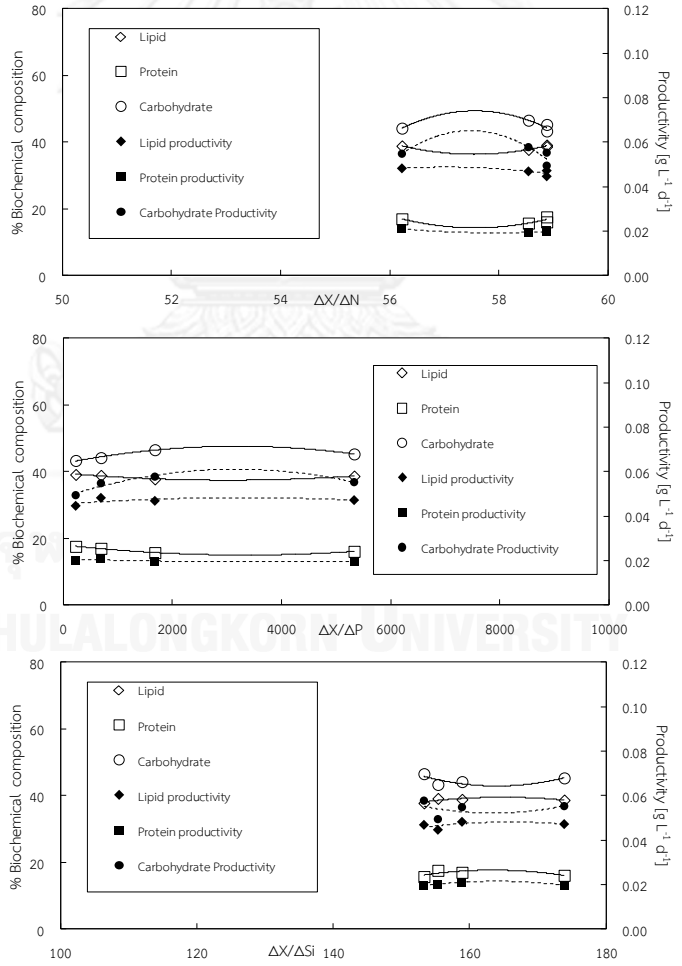

Figure 4.24 %Biochemical composition as a function of biomass yields

Table 4.13 Maximum cell density, specific growth rate and productivity

System	Maximum cell density ($x10^6$ cell mL^{-1})	Specific growth rate (d^{-1})	Productivity (x109 cell d^{-1})	Specific productivity ($x10^9$ cell L^{-1} d^{-1})
Fresh	13.62 ± 0.89	0.64 ± 0.02	11.06 ± 0.52	2..21 ± 0.08
50% phosphate	11.51 ± 0.63	0.60 ± 0.01	9.05 ± 0.39	1.81 ± 0.08
100% phosphate	12.22 ± 0.92	0.57 ± 0.01	9.40 ± 0.35	1.88 ± 0.22
500% phosphate	10.34 ± 0.31	0.57 ± 0.01	9.42 ± 0.46	1.88 ± 0.12

Table 4.14 Uptake rate of silicate, nitrate and phosphate for the cultivation of *C. gracilis*

Time	Fresh medium			50% phosphate		
(days)	ΔSi/ΔX	ΔN/ΔX	ΔP/ΔX	ΔSi/ΔX	ΔN/ΔX	ΔP/ΔX
0						
1	$1.98x10^{-2}$	$8.17x10^{-2}$	$8.35x10^{-3}$	$3.43x10^{-2}$	$5.18x10^{-2}$	$6.84x10^{-4}$
2	$1.52x10^{-2}$	$3.05x10^{-2}$	$2.60x10^{-3}$	$8.24x10^{-3}$	$1.94x10^{-2}$	$4.00x10^{-4}$
3	$3.49x10^{-3}$	$-2.28x10^{-4}$	$2.13x10^{-5}$	$1.03x10^{-3}$	$4.80x10^{-3}$	$5.08x10^{-4}$
4	$-6.11x10^{-4}$	$2.06x10^{-2}$	$8.29x10^{-5}$	$4.69x10^{-4}$	$9.28x10^{-3}$	$9.12x10^{-4}$
Time	100% phosphate			500% phosphate		
(days)	ΔSi/ΔX	ΔN/ΔX	ΔP/ΔX	ΔSi/ΔX	ΔN/ΔX	ΔP/ΔX
0						
1	$1.91x10^{-2}$	$3.83x10^{-2}$	$2.20x10^{-4}$	$2.06x10^{-2}$	$2.49x10^{-2}$	$5.58x10^{-3}$
2	$6.74x10^{-3}$	$1.46x10^{-2}$	$1.09x10^{-4}$	$3.32x10^{-3}$	$1.79x10^{-2}$	$2.05x10^{-3}$
3	$-3.62x10^{-4}$	$1.00x10^{-4}$	$2.07x10^{-4}$	$1.11x10^{-3}$	$-5.16x10^{-4}$	$4.35x10^{-3}$
4	$-3.75x10^{-3}$	$2.55x10^{-2}$	$3.46x10^{-4}$	$-7.14x10^{-3}$	$6.99x10^{-2}$	$2.96x10^{-2}$

Table 4.15 Reduction (%) of elements for the cultivation of *C. gracilis* in fresh medium and mediums with phosphate adjustment

Elements	Fresh medium			50% phosphate		
	Concentration (mg L^{-1})		% Reduction (Δ1)	Concentration (mg L^{-1})		% Reduction (Δ2)
	Initial	Final		Initial	Final	
Si	5.1470	1.4052	72.70	5.1681	1.3223	74.41
N	12.7711	2.1974	82.79	11.1073	1.0370	90.66
P	1.1500	0.2865	75.09	1.0846	0.3515	67.59
B	0.1786	0.1767	1.06	0.2145	0.1720	19.81
Zn	0.3987	0.3401	14.70	0.4275	0.3910	8.54
Cu	0.0031	0.0020	35.48	0.0030	0.0025	16.67
Fe	0.1612	0.0032	98.01	0.2325	0.0020	99.14
K	4.5983	3.4601	24.75	4.6964	3.6732	21.79
Mn	0.0315	0.0246	21.90	0.0382	0.0254	33.51
Mo	0.0723	0.0645	10.79	0.0825	0.0715	13.33
Co	0.0040	0.0030	25.00	0.0040	0.0035	12.50

Table 4.15 Reduction (%) of elements for the cultivation of *C. gracilis* in fresh medium and mediums with phosphate adjustment (continued)

Elements	100% phosphate			500% phosphate		
	Concentration ($mg\ L^{-1}$)		% Reduction (Δ3)	Concentration ($mg\ L^{-1}$)		% Reduction (Δ4)
	Initial	Final		Initial	Final	
Si	5.6620	1.1159	80.29	5.3542	1.1782	77.99
N	13.3032	1.3712	89.69	11.0255	1.4214	87.11
P	1.6852	0.1634	91.77	6.9505	3.1587	54.55
B	0.2150	0.1665	22.56	0.2000	0.1695	15.25
Zn	0.4920	0.3785	23.07	0.4660	0.3795	18.56
Cu	0.0035	0.0020	42.86	0.0030	0.0020	33.33
Fe	0.2125	0.0235	88.94	0.2285	0.0375	83.59
K	4.8895	3.8103	22.07	4.9765	3.8725	22.18
Mn	0.0375	0.0235	37.33	0.0465	0.0285	38.71
Mo	0.0865	0.0745	13.87	0.0860	0.0710	17.44
Co	0.0045	0.0035	22.22	0.0045	0.0035	22.22

Table 4.16 Empirical formula of *C. gracilis*

System	Empirical formula
Fresh	$CH_{1.51}O_{1.17}N_{1.97}P_{0.81}Si_{0.86}B_{0.03}Zn_{0.07}Cu_{0.19}Fe_{0.59}K_{0.21}Mn_{0.13}Mo_{0.04}Co_{0.14}$
50% phosphate	$CH_{1.53}O_{1.18}N_{2.16}P_{0.73}Si_{0.88}B_{0.61}Zn_{0.04}Cu_{0.09}Fe_{0.59}K_{0.19}Mn_{0.20}Mo_{0.05}Co_{0.07}$
100% phosphate	$CH_{1.41}O_{1.12}N_{2.07}P_{0.96}Si_{0.92}B_{0.68}Zn_{0.11}Cu_{0.22}Fe_{0.52}K_{0.18}Mn_{0.22}Mo_{0.05}Co_{0.12}$
500% phosphate	$CH_{1.57}O_{1.10}N_{2.01}P_{0.57}Si_{0.89}B_{0.46}Zn_{0.09}Cu_{0.17}Fe_{0.48}K_{0.18}Mn_{0.23}Mo_{0.06}Co_{0.12}$

4.3 Effects of reusing nutrient in batch culture system with fed-batch culture

To investigate effects of reducing major macronutrients, i.e. Silicate, Nitrate and Phosphate, 4 sets of experiment were conducted by varying only one of the macronutrients as follows:

- Control experiment: 100% fresh medium fed at the first day of cultivation
- 50% medium: 50% of each macronutrient was being equally separated into 4 portions and each portion was added daily to the reactor (for a total of 4 days). This was equivalent to 0.5 mL of the studies nutrient per day per 5 L of seawater.
- 1^{st} reused medium: The nutrient remaining after the first harvest was adjusted to provide the amount of nutrients equivalent to what available in the fresh medium.
- 2^{nd} reused medium: The used nutrient from the 1^{st} reused experiment was adjusted to provide the amount of nutrients equivalent to what available in the fresh medium.
- 3^{rd} reused medium: The used nutrient from the 2^{nd} reused experiment was adjusted to provide the amount of nutrients equivalent to what available in the fresh medium.

Figures 4.25, 4.26 and 4.27 illustrate cell density obtained from the cultivations based on all conditions specified above whereas the associate cell concentration, specific growth rate, productivity and specific productivity are given in Table 4.17. It can be seen that the maximum cell density obtained from the control medium and 50% medium were 2 fold that with reused nutrients in all conditions. The maximum cell density obtained from the control medium and 50% nutrients were approximately at the same level regardless of the type of macronutrients being adjusted. In other words, the maximum cell densities from the cultivation with 50% nitrate, 50%silicate and 50%phosphate were approximately at the same level, i.e. $11.64 \pm 0.11 \times 10^6$, $11.07 \pm 0.78 \times 10^6$ and $9.83 \pm 0.12 \times 10^6$ cell mL^{-1}, respectively.

For the reused experiments, the maximum cell density became lower than using fresh nutrients. The 50% reused nitrate medium provided the culture with the highest maximum cell density and productivity whereas the 50% reused silicate and 50% reused phosphate provided similar maximum cell density and productivity (see Table 4.17). The specific growth rate with control and 50% macronutrient medium was 0.74 – 0.80 d^{-1} in all conditions. However, the specific growth rate with 50% reused nutrient was in a lower range of 0.54 – 0.66 d^{-1}.

In this work, the temperature ranged between 28 - 32^{O}C and pH 8.0 – 9.0 which were in the proper range for the growth of *C. gracilis*. Samonte et al. (1993), Raghavan et al. (2008) and Hemalatha et al. (2012) reports that the temperature 25-35^{O}C and pH 8.0-9.0 were in the appropriate range for the growth of *C. gracilis*.

Figures 4.28 – 4.30 shows the relationship between cell density and macronutrient concentrations (silicate, nitrate and phosphate, respectively). It can be seen that the cultures under the control medium conditions exhibited similar growth pattern, i.e. lag phase of 1 day, 2-3 days exponential phase, very short stationary phase followed by cell decay at Day 4. In the 50% nutrient experiment, similar growth pattern was observed. Cell continued to grow in the first 4 days whereas nutrient was consumed rapidly in one day after which additional nutrient was added. Cell reached steady state but with a much lower cell density than the cultivation with fresh medium in the control experiment. The uptake rates of major nutrients in all conditions are reported in Table 4.18.

Figure 4.31 displays biochemical composition of the cell and this shows that %total lipid always moved in the opposite direction to %carbohydrate, whereas %protein remained relatively constant. In control and 50% nutrient experiments, %carbohydrate was higher than %lipid and protein. Figure 4.31 illustrates that maximum %carbohydrate of 48.09% was obtained from the condition with the 50% nitrate concentration while minimum %carbohydrate of 41.71% was obtained from the condition with the 50% silicate concentration. In contrast, %total lipid obtained from the 50% silicate concentration was maximal at approximately 37.20% and the minimum was obtained from the 50% nitrate concentration at 32.17%. The maximum %protein was obtained from the condition with control medium at 21.62% while the minimum was obtained from the 50% phosphate concentration at 18.46%.

In the reused medium, %total lipid was higher than %carbohydrate and protein. The 1st reused with 50% silicate concentration provided the highest %total lipid of 56.35%, whereas the 2nd reused and 3rd reused with 50% silicate concentration provided similar %total lipid of 54.11% and 53.82%, respectively. However, the 3rd reuse with 50% nitrate concentration gave the lowest %total lipid at 46.61%. In contrast, the 2nd reuse medium with 50% phosphate concentration offered the highest %carbohydrate of 33.19%, whereas the 3rd reused and 1st reused with 50% phosphate concentrations provided similar %carbohydrate of 31.54% and 31.32%, respectively. However, the 1st reused with 50% silicate concentration had the lowest %carbohydrate of 23.64%. In addition, the maximum %protein was

obtained from the condition with the 1st reused with 50% nitrate concentrations at 25.05% while the minimum %protein was obtained from the condition with 2nd reused with 50% phosphate concentration at 17.62%.

Regarding the biochemical composition, %total lipid was high with the reused medium with 50% silicate concentration, whereas %carbohydrate was enhanced with reused medium with 50% phosphate concentration. In contrast, high % protein content was obtained with the reused medium with 50% nitrate concentration.

Figures 4.32, 4.33, and 4.34 display the biochemical composition, and productivity of each component along with the biomass yield from the various experiments. Similar trends of these parameters were observed. Table 4.18 shows the uptake rate of the three macronutrients from the cultivation under each condition. These could lead to the reduction of the nutrients as shown in Table 4.19-4.21 and ultimately led to the anticipation of the empirical formula as reported in Table 4.22.

It should be noted, however, that, in these experiments, the cultures were cultivated under 3 macronutrients adjustment, i.e. 50% silicate, 50% nitrate and 50% phosphate. This means that the total amount of target nutrient (i.e. silicate, nitrate, and phosphate) was 50% of amount used in the fresh medium (modified standard F/2 (Guillard's)). The total feeding amount was split equally into 5 days. Krichnavaruk *et al.* (2005) reported that the growth rate of *C. calcitrans* was maximized when silicate and phosphate concentrations increased 2 fold that in the standard F/2 (Guillard's) medium. In addition, original nitrate as started in the standard F/2 (Guillard's) medium already provided the highest the specific growth rate and cell density of *C. calcitrans*. Hence, this work is like a continuing part of the previous work, where in the research, where 50% macronutrient adjustment was examined compared to the modified f/2 (Guillard's). The work did not include the experiment where 50% nutrients where supplied straight from the beginning as previous work by Krichanavaruk (2005) suggested that this would lead to the cultivation with low growth.

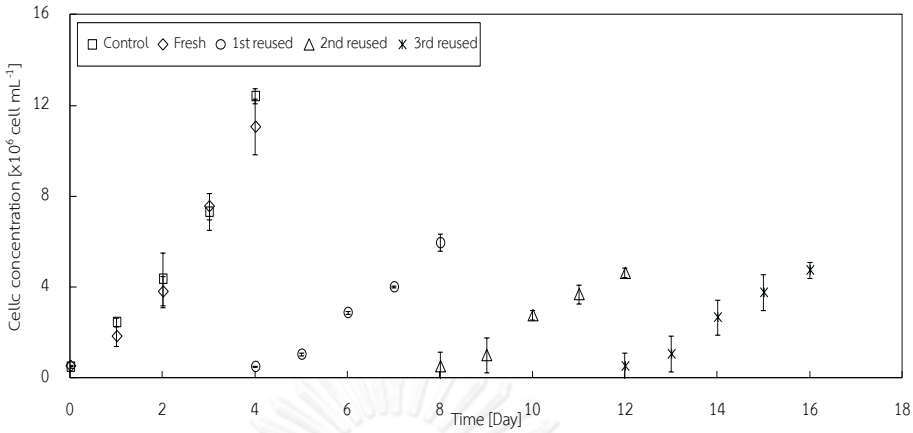

Figure 4.25 Growth behaviors of *C. gracilis* in control, fresh of 50% silicate medium, 1st, 2nd and 3rd reused of 50%silicate mediums.

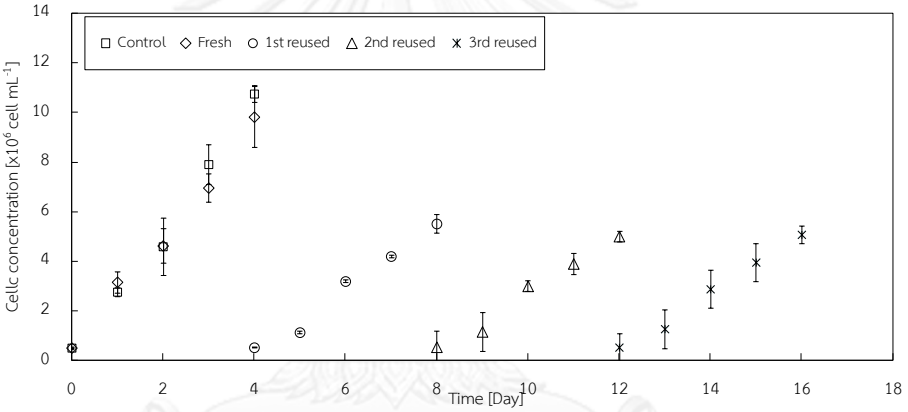

Figure 4.26 Growth behaviors of *C. gracilis* in control, fresh of 50% nitrate medium, 1st, 2nd and 3rd reused of 50%nitrate mediums.

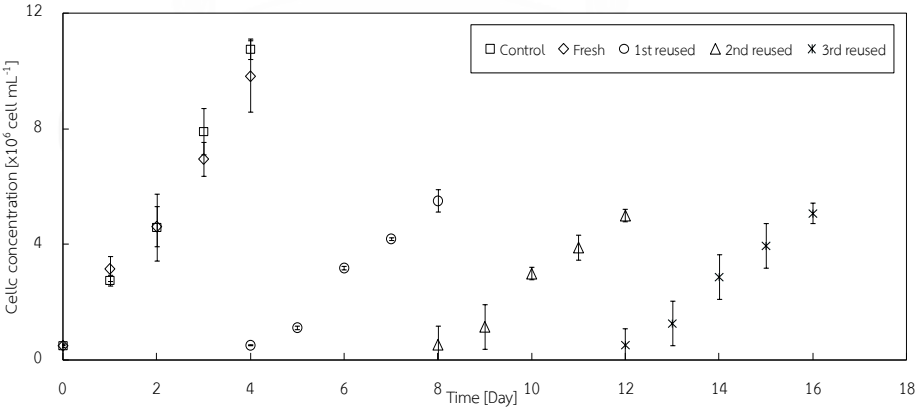

Figure 4.27 Growth behaviors of *C. gracilis* in control, fresh of 50% phosphate medium, 1st, 2nd and 3rd reused of 50%phosphate mediums.

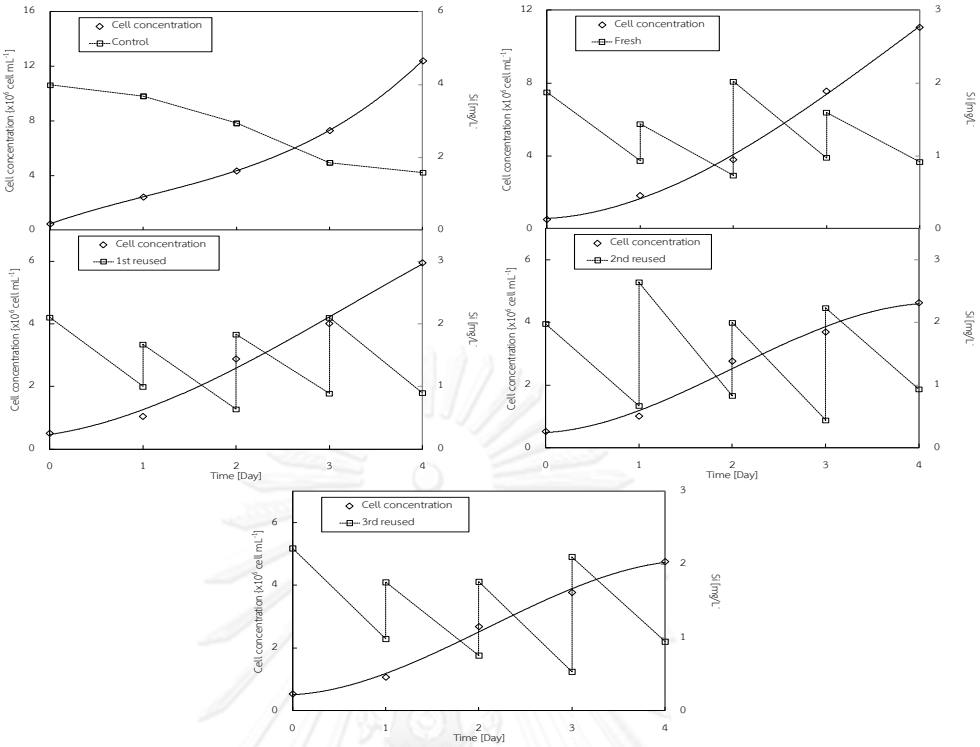

Figure 4. 28 Cell density and silicate concentration for the cultivation of *C. gracilis* (a) Control, (b) Fresh, (c) 1st reused, (d) 2nd reused and (e) 3rd reused mediums

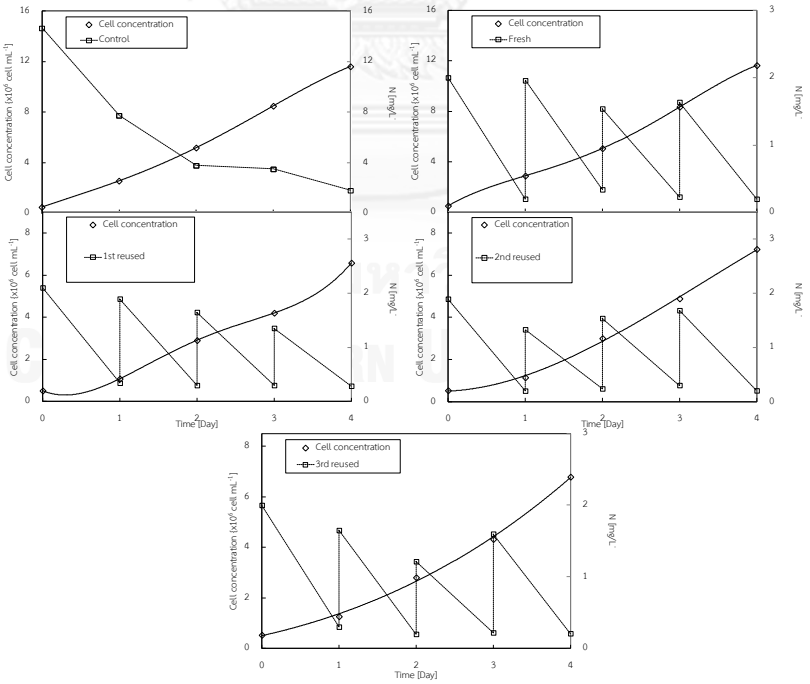

Figure 4. 29 Cell density and nitrate concentration for the cultivation of *C. gracilis* (a) Control, (b) Fresh, (c) 1st reused, (d) 2nd reused and (e) 3rd reused mediums

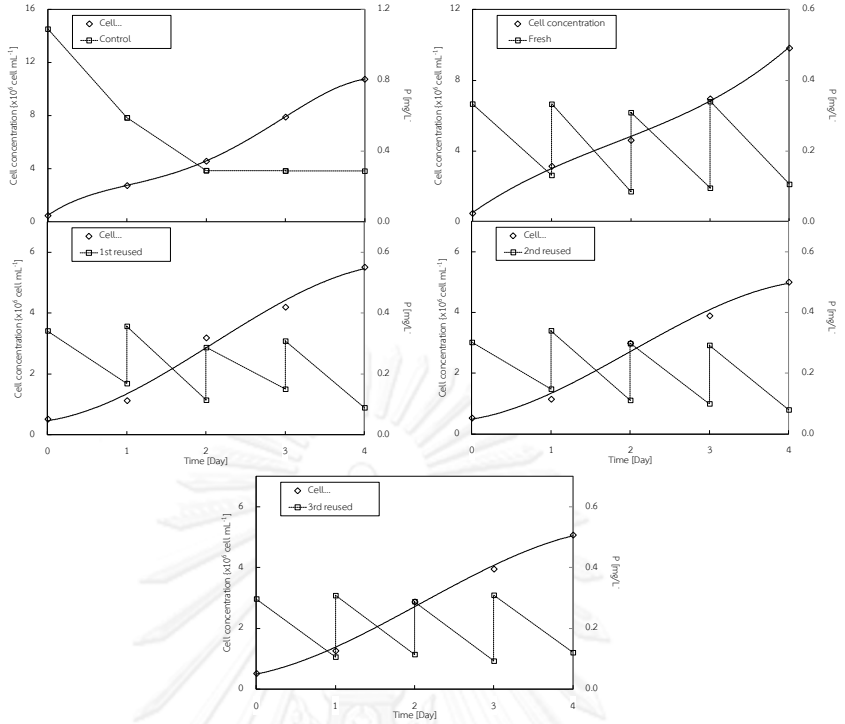

Figure 4. 30 Cell density and photphate concentration for the cultivation of *C. gracilis* (a) Control, (b) Fresh, (c) 1st reused, (d) 2nd reused and (e) 3rd reused mediums

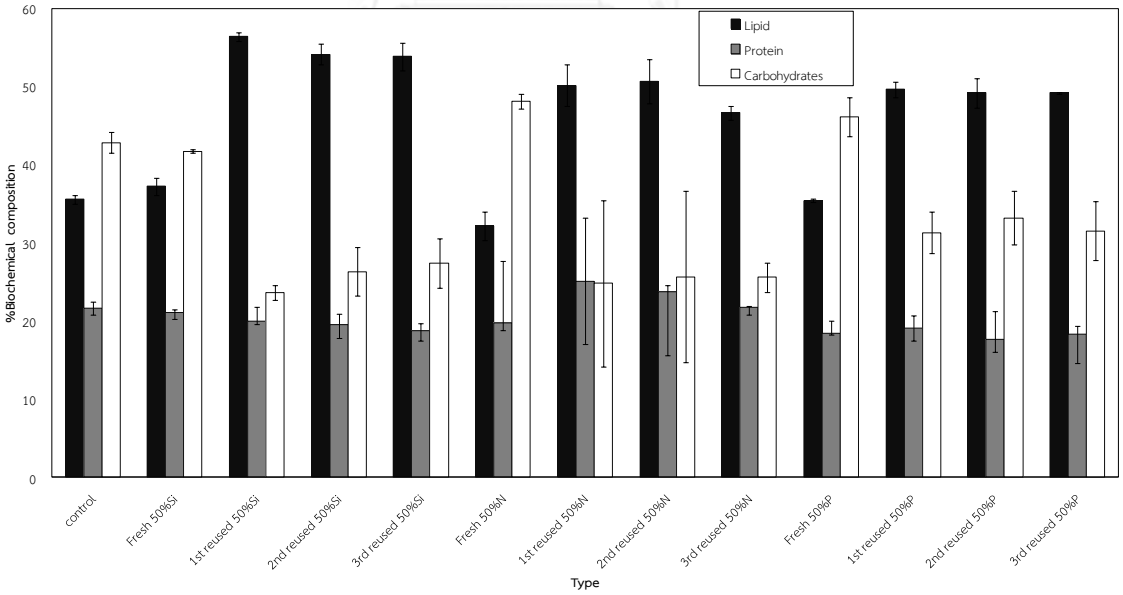

Figure 4.31 % Biochemical composition of *C. gracilis*

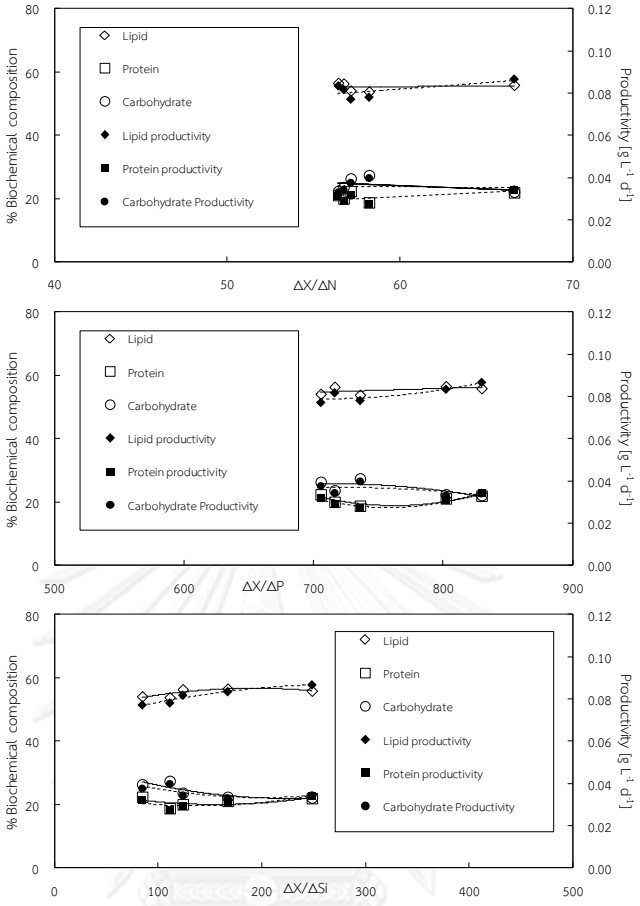

Figure 4.32 %Biochemical composition as a function of biomass yields (Reused of 50% silicate medium)

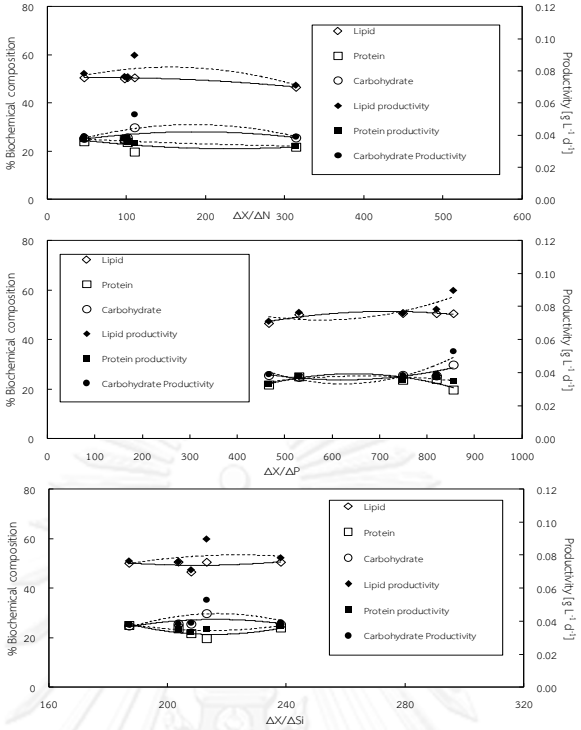

Figure 4.33%Biochemical composition as a function of biomass yields

(Reused of 50%nitrate medium)

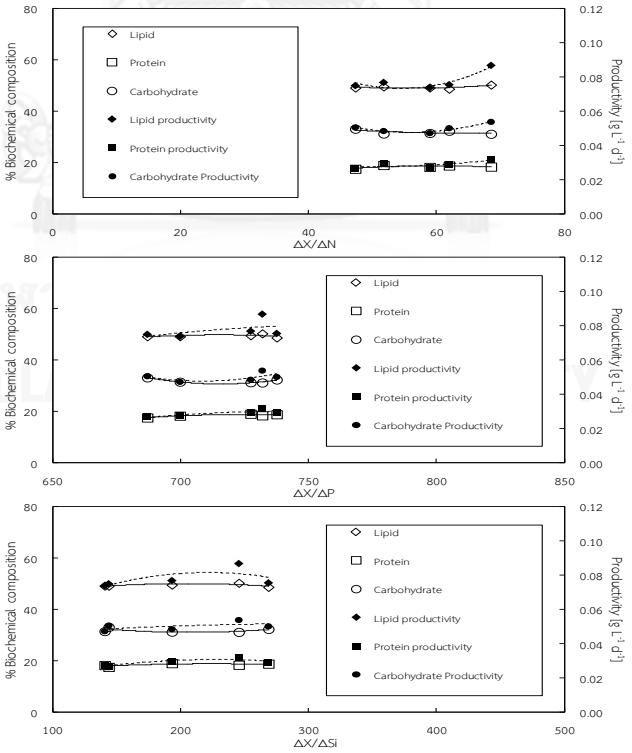

Figure 4.34 %Biochemical composition as a function of biomass yields

(Reused of 50%phosphate medium)

Table 4.17 Maximum cell density, specific growth rate and productivity in control, fresh, 1st, 2nd and 3rd reused mediums with 50%macronutrient for the cultivation of *C. gracilis*

System	Maximum cell density ($x10^6$ cell mL^{-1})	Specific growth rate (d^{-1})	Productivity ($x10^9$ cell d^{-1})	Specific productivity ($x10^9$ cell L^{-1} d^{-1})
Control 100%Si	12.42 ± 0.91	0.80 ± 0.01	15.52 ± 0.56	3.10 ± 0.06
Fresh 50%Si	11.07 ± 0.78	0.45 ± 0.02	13.83 ± 0.48	2.77 ± 0.13
1st reused 50%Si	5.96 ± 0.85	0.61 ± 0.02	7.45 ± 0.74	1.49 ± 0.25
2nd reused 50%Si	4.64 ± 0.64	0.54 ± 0.02	5.79 ± 0.61	1.16 ± 0.16
3rd reused 50%Si	4.76 ± 0.85	0.55 ± 0.02	5.95 ± 0.61	1.19 ± 0.16
Control 100%N	11.63 ± 0.88	0.78 ± 0.01	14.53 ± 0.72	2.91 ± 0.21
Fresh 50%N	11.64 ± 0.11	0.77 ± 0.02	14.55 ± 0.63	2.91 ± 0.09
1st reused 50%N	6.58 ± 0.41	0.64 ± 0.02	8.23 ± 0.79	1.65 ± 0.17
2nd reused 50%N	7.23 ± 0.14	0.66 ± 0.02	9.04 ± 0.53	1.81 ± 0.12
3rd reused 50%N	6.79 ± 0.49	0.64 ± 0.02	8.48 ± 0.80	1.70 ± 0.24
Control 100%P	10.77 ± 0.34	0.77 ± 0.01	13.46 ± 0.56	2.69 ± 0.19
Fresh 50%P	9.83 ± 0.12	0.74 ± 0.02	12.29 ± 0.48	2.46 ± 0.11
1st reused 50%P	5.52 ± 0.38	0.59 ± 0.02	6.89 ± 0.74	1.38 ± 0.18
2nd reused 50%P	5.01 ± 0.39	0.56 ± 0.02	6.26 ± 0.61	1.25 ± 0.21
3rd reused 50%P	5.08 ± 0.45	0.57 ± 0.02	6.34 ± 0.61	1.27 ± 0.28

Table 4. 18 Uptake rated of silicate, nitrate and phosphate for the cultivation of *C. gracilis*

Time	Control 100%Si			Fresh 50%Si		
(days)	ΔSi/ΔX	ΔN/ΔX	ΔP/ΔX	ΔSi/ΔX	ΔN/ΔX	ΔP/ΔX
0						
1	3.08×10^{-3}	3.02×10^{-2}	3.81×10^{-3}	1.34×10^{-2}	4.81×10^{-2}	9.38×10^{-3}
2	7.80×10^{-3}	5.64×10^{-2}	3.10×10^{-3}	6.72×10^{-3}	5.52×10^{-2}	3.37×10^{-4}
3	7.32×10^{-3}	3.40×10^{-3}	3.26×10^{-4}	5.20×10^{-3}	3.10×10^{-3}	6.61×10^{-5}
4	1.07×10^{-3}	3.92×10^{-4}	-8.90×10^{-7}	3.63×10^{-3}	9.93×10^{-4}	-2.34×10^{-5}
Time	1st reused 50%Si			1st reused 50%Si		
(days)	ΔSi/ΔX	ΔN/ΔX	ΔP/ΔX	ΔSi/ΔX	ΔN/ΔX	ΔP/ΔX
0						
1	2.10×10^{-2}	5.52×10^{-2}	9.08×10^{-3}	2.17×10^{-2}	3.76×10^{-2}	7.95×10^{-3}
2	5.79×10^{-3}	3.23×10^{-2}	1.51×10^{-3}	8.40×10^{-3}	1.99×10^{-2}	1.12×10^{-3}
3	8.57×10^{-3}	1.13×10^{-3}	-7.36×10^{-5}	1.36×10^{-2}	1.01×10^{-2}	5.94×10^{-6}
4	6.36×10^{-3}	2.91×10^{-3}	8.69×10^{-6}	1.13×10^{-2}	9.82×10^{-3}	-3.54×10^{-5}
Time	3rd reused 50%Si					
(days)	ΔSi/ΔX	ΔN/ΔX	ΔP/ΔX			
0						
1	1.90×10^{-2}	3.47×10^{-2}	1.01×10^{-2}			
2	5.09×10^{-3}	2.18×10^{-2}	1.79×10^{-4}			
3	9.27×10^{-3}	8.68×10^{-3}	9.97×10^{-5}			
4	9.66×10^{-3}	9.41×10^{-3}	-3.63×10^{-5}			

Table 4. 18 Uptake rated of silicate, nitrate and phosphate for the cultivation of C. gracilis (Continued)

Time	Control 100%N			Fresh 50%N		
(days)	ΔSi/ΔX	ΔN/ΔX	ΔP/ΔX	ΔSi/ΔX	ΔN/ΔX	ΔP/ΔX
0						
1	4.34×10^{-3}	6.24×10^{-2}	4.94×10^{-3}	5.23×10^{-3}	1.24×10^{-2}	4.23×10^{-3}
2	2.24×10^{-3}	2.83×10^{-2}	1.32×10^{-3}	1.23×10^{-2}	1.22×10^{-2}	1.40×10^{-3}
3	3.46×10^{-3}	1.62×10^{-3}	1.89×10^{-4}	3.40×10^{-3}	6.53×10^{-3}	1.70×10^{-5}
4	6.53×10^{-3}	1.01×10^{-2}	-2.41×10^{-4}	5.98×10^{-4}	7.15×10^{-3}	-4.03×10^{-5}
Time	1st reused 50%N			2nd reused 50%N		
(days)	ΔSi/ΔX	ΔN/ΔX	ΔP/ΔX	ΔSi/ΔX	ΔN/ΔX	ΔP/ΔX
0						
1	1.15×10^{-2}	3.36×10^{-2}	1.92×10^{-2}	1.23×10^{-2}	3.32×10^{-2}	1.03×10^{-2}
2	1.31×10^{-2}	9.43×10^{-3}	2.79×10^{-4}	1.30×10^{-2}	7.07×10^{-3}	1.30×10^{-3}
3	1.74×10^{-3}	1.12×10^{-2}	-2.07×10^{-4}	2.73×10^{-4}	7.90×10^{-3}	1.48×10^{-4}
4	-6.06×10^{-5}	4.85×10^{-3}	1.57×10^{-4}	2.61×10^{-4}	7.61×10^{-3}	-2.88×10^{-5}
Time	3rd reused 50%N					
(days)	ΔSi/ΔX	ΔN/ΔX	ΔP/ΔX			
0						
1	1.12×10^{-2}	2.57×10^{-2}	1.41×10^{-2}			
2	1.44×10^{-2}	1.05×10^{-2}	1.50×10^{-3}			
3	-2.74×10^{-5}	7.30×10^{-3}	1.15×10^{-4}			
4	-1.22×10^{-4}	6.27×10^{-3}	2.26×10^{-4}			

Table 4.18 Uptake rated of silicate, nitrate and phosphate for the cultivation of C. gracilis (Continued)

Time	Control 100%P			Fresh 50%P		
(days)	ΔSi/ΔX	ΔN/ΔX	ΔP/ΔX	ΔSi/ΔX	ΔN/ΔX	ΔP/ΔX
0						
1	0.65x10	$2.26x10^{-2}$	$3.85x10^{-3}$	$5.96x10^{-3}$	$1.85x10^{-2}$	$1.08x10^{-3}$
2	1.15x10	$4.24x10^{-2}$	$2.82x10^{-3}$	$1.31x10^{-2}$	$5.91x10^{-2}$	$2.40x10^{-3}$
3	$4.82x10^{-1}$	$3.92x10^{-3}$	$9.17x10^{-6}$	$-4.95x10^{-4}$	$6.99x10^{-4}$	$1.30x10^{-3}$
4	$3.14x10^{-1}$	$8.34x10^{-3}$	$5.00x10^{-6}$	$1.42x10^{-3}$	$-4.78x10^{-4}$	$1.16x10^{-3}$
Time	1^{st} reused 50%P			2^{nd} reused 50%P		
(days)	ΔSi/ΔX	ΔN/ΔX	ΔP/ΔX	ΔSi/ΔX	ΔN/ΔX	ΔP/ΔX
0						
1	$1.68x10^{-2}$	$5.75x10^{-2}$	$2.53x10^{-3}$	$3.30x10^{-2}$	$4.71x10^{-2}$	$2.03x10^{-3}$
2	$8.01x10^{-3}$	$1.94x10^{-2}$	$1.05x10^{-3}$	$5.68x10^{-3}$	$2.92x10^{-2}$	$1.02x10^{-3}$
3	$-1.54x10^{-3}$	$1.34x10^{-3}$	$1.21x10^{-3}$	$3.39x10^{-4}$	$-2.43x10^{-3}$	$1.80x10^{-3}$
4	$5.10x10^{-4}$	$1.54x10^{-2}$	$1.48x10^{-3}$	$-9.12x10^{-5}$	$1.25x10^{-2}$	$1.58x10^{-3}$
Time	3^{rd} reused 50%P					
(days)	ΔSi/ΔX	ΔN/ΔX	ΔP/ΔX			
0						
1	$2.53x10^{-2}$	$3.30x10^{-2}$	$2.17x10^{-3}$			
2	$7.72x10^{-3}$	$2.90x10E^{-2}$	$1.02x10^{-3}$			
3	$-4.79x10E^{-4}$	$6.84x10E^{-3}$	$1.53x10^{-3}$			
4	$1.48x10^{-3}$	$-1.19x10^{-3}$	$1.43x10^{-3}$			

Table 4.19 Reduction (%) of elements for the cultivation of *C. gracilis*) in control, fresh of 50%silicate medium, 1st, 2nd and 3rd reused of 50%silicate mediums

Elements	Control			Fresh medium		
	Concentration (mg L^{-1})		% Reduction (Δ1)	Concentration (mg L^{-1})		% Reduction (Δ2)
	Initial	Final		Initial	Final	
Si	3.9876	1.5931	60.05	6.9321	3.5722	48.47
N	13.0021	2.0015	84.61	12.5983	1.9002	84.92
P	1.1003	0.2791	74.63	0.9973	0.2997	69.95
B	0.1893	0.1698	10.30	0.1998	0.1731	13.36
Zn	0.4221	0.3531	16.35	0.4993	0.3979	20.31
Cu	0.0045	0.0015	66.67	0.0030	0.0001	96.67
Fe	0.1792	0.0025	98.60	0.2331	0.0031	98.67
K	4.6031	3.2987	28.34	4.4934	3.8001	15.43
Mn	0.0535	0.0216	59.63	0.0397	0.0219	44.84
Mo	0.0815	0.0704	13.62	0.0905	0.0715	20.99
Co	0.0035	0.0015	57.14	0.0045	0.0025	44.44

Table 4.19 Reduction (%) of elements for the cultivation of *C. gracilis*) in control, fresh of 50%silicate medium, 1st, 2nd and 3rd reused of 50%silicate mediums (Continued)

Elements	1st reused			2nd reused		
	Concentration (mg L^{-1})		% Reduction (Δ3)	Concentration (mg L^{-1})		% Reduction (Δ4)
	Initial	Final		Initial	Final	
Si	7.7098	3.4279	55.54	8.8487	2.8727	67.54
N	14.0091	12.0037	14.31	11.2113	1.8388	83.60
P	1.3025	0.2901	77.73	0.9903	0.2089	78.91
B	0.1803	0.1769	1.89	0.2119	0.1907	10.00
Zn	0.4135	0.3792	8.30	0.4986	0.4001	19.76
Cu	0.0040	0.0005	87.50	0.0025	0.0001	96.00
Fe	0.1997	0.0027	98.65	0.2103	0.0021	99.00
K	4.6902	3.3905	27.71	4.3998	3.6994	15.92
Mn	0.0375	0.0235	37.33	0.0390	0.0285	26.92
Mo	0.0996	0.0839	15.76	0.0831	0.0805	3.13
Co	0.0055	0.0040	27.27	0.0040	0.0030	25.00

Table 4.19 Reduction (%) of elements for the cultivation of *C. gracilis*) in control, fresh of 50%silicate medium, 1st, 2nd and 3rd reused of 50%silicate mediums (Continued)

Elements	3rd reused		
	Concentration (mg L^{-1})		% Reduction (Δ5)
	Initial	Final	
Si	7.8431	3.2103	59.07
N	12.9083	1.8959	85.31
P	1.1003	0.2673	75.71
B	0.1893	0.1790	5.44
Zn	0.4653	0.3901	16.16
Cu	0.0030	0.0025	16.67
Fe	0.1976	0.0027	98.63
K	4.9862	3.4087	31.64
Mn	0.0476	0.0293	38.45
Mo	0.0895	0.0730	18.44
Co	0.0045	0.0025	44.44

Table 4.20 Reduction (%) of elements for the cultivation of *C. gracilis*) in control, fresh of 50%nitrate medium, 1st, 2nd and 3rd reused of 50%nitrate mediums

Elements	Control 100%Si			Fresh 50%Si		
	Concentration (mg L^{-1})		% Reduction (Δ1)	Concentration (mg L^{-1})		% Reduction (Δ2)
	Initial	Final		Initial	Final	
Si	4.0454	1.5547	61.57	4.4144	1.2345	72.03
N	14.6565	1.8230	87.56	7.1322	0.9737	86.35
P	1.1687	0.4451	61.91	1.2310	0.4325	64.87
B	0.1799	0.1602	10.95	0.2001	0.1597	20.19
Zn	0.4131	0.3505	15.15	0.4295	0.3701	13.83
Cu	0.0040	0.0015	62.50	0.0030	0.0002	93.33
Fe	0.1673	0.0029	98.27	0.2135	0.0037	98.27
K	4.5001	3.3814	24.86	4.4199	3.7705	14.69
Mn	0.0405	0.0235	41.98	0.0359	0.0229	36.21
Mo	0.0791	0.0713	9.86	0.0820	0.0711	13.29
Co	0.0050	0.0035	30.00	0.0045	0.0035	22.22

Table 4.20 Reduction (%) of elements for the cultivation of *C. gracilis*) in control, fresh of 50%nitrate medium, 1st, 2nd and 3rd reused of 50%nitrate mediums (Continued)

Elements	1st reused 50%Si			2nd reused 50%Si		
	Concentration (mg L^{-1})		% Reduction (Δ3)	Concentration (mg L^{-1})		% Reduction (Δ4)
	Initial	Final		Initial	Final	
Si	4.0071	0.9997	75.05	3.8769	1.1433	70.51
N	6.9995	1.2298	82.43	6.4412	0.9375	85.45
P	1.5061	0.4450	70.45	1.1971	0.4540	62.08
B	0.2119	0.1701	19.73	0.2352	0.1876	20.24
Zn	0.4987	0.3709	25.63	0.4871	0.3699	24.06
Cu	0.0030	0.0001	96.67	0.0035	0.0015	57.14
Fe	0.2198	0.0023	98.95	0.2783	0.0257	90.77
K	4.5003	3.7997	15.57	4.7089	3.7952	19.40
Mn	0.0415	0.0243	41.45	0.0395	0.0205	48.10
Mo	0.0880	0.0732	16.82	0.0895	0.0695	22.35
Co	0.0045	0.0030	33.33	0.0045	0.0025	44.44

Table 4.20 Reduction (%) of elements for the cultivation of *C. gracilis*) in control, fresh of 50%nitrate medium, 1st, 2nd and 3rd reused of 50%nitrate mediums (Continued)

Elements	3rd reused		
	Concentration (mg L^{-1})		% Reduction (Δ5)
	Initial	Final	
Si	3.9474	1.2424	68.53
N	6.4610	0.9295	85.61
P	1.6284	0.4220	74.08
B	0.2301	0.1701	26.08
Zn	0.4901	0.3854	21.36
Cu	0.0050	0.0015	70.00
Fe	0.2703	0.0319	88.20
K	4.8879	3.8715	20.79
Mn	0.0387	0.0249	35.66
Mo	0.0824	0.0718	12.86
Co	0.0045	0.0025	44.44

Table 4.21 Reduction (%) of elements for the cultivation of *C. gracilis*) in control, fresh of 50%phosphate medium, 1st, 2nd and 3rd reused of 50%phosphate mediums

Elements	Control			Fresh medium		
	Concentration (mg L^{-1})		% Reduction (Δ1)	Concentration (mg L^{-1})		% Reduction (Δ2)
	Initial	Final		Initial	Final	
Si	3.3334	1.1327	66.02	4.0097	1.3413	66.48
N	12.0439	2.5043	79.21	12.1787	2.6219	78.47
P	1.0903	0.2887	73.52	0.3335	0.1068	67.98
B	0.1865	0.1779	4.61	0.2001	0.1902	4.95
Zn	0.4122	0.3675	10.84	0.4208	0.3799	9.72
Cu	0.0035	0.0020	42.86	0.0030	0.0025	16.67
Fe	0.1601	0.0035	97.81	0.1994	0.0023	98.85
K	4.5007	3.4692	22.92	4.4975	3.8221	15.02
Mn	0.0320	0.0235	26.56	0.0375	0.0275	26.67
Mo	0.0778	0.0722	7.20	0.0830	0.0705	15.06
Co	0.0045	0.0030	33.33	0.0045	0.0025	44.44

Table 4.21 Reduction (%) of elements for the cultivation of *C. gracilis*) in control, fresh of 50%phosphate medium, 1st, 2nd and 3rd reused of 50%phosphate mediums (Continued)

Elements	1st reused			2nd reused		
	Concentration (mg L^{-1})		% Reduction (Δ3)	Concentration (mg L^{-1})		% Reduction (Δ4)
	Initial	Final		Initial	Final	
Si	3.9989	1.0884	72.78	4.9154	1.1245	77.12
N	12.8858	2.0195	84.33	12.9346	1.4185	89.03
P	0.3419	0.0889	74.00	0.3023	0.0802	73.47
B	0.2138	0.1702	20.39	0.2318	0.1901	17.99
Zn	0.4293	0.3719	13.37	0.4875	0.3936	19.26
Cu	0.0050	0.0004	92.00	0.0047	0.0010	78.72
Fe	0.2375	0.0015	99.37	0.2467	0.0023	99.07
K	4.6953	3.9012	16.91	4.7009	3.7953	19.26
Mn	0.0329	0.0238	27.66	0.0375	0.0220	41.33
Mo	0.0835	0.0734	12.10	0.0905	0.0815	9.94
Co	0.0045	0.0025	44.44	0.0045	0.0035	22.22

Table 4.21 Reduction (%) of elements for the cultivation of *C. gracilis*) in control, fresh of 50%phosphate medium, 1st, 2nd and 3rd reused of 50%phosphate mediums (Continued)

Elements	3rd reused		
	Concentration (mg L^{-1})		% Reduction (Δ5)
	Initial	Final	
Si	5.4943	1.6639	69.72
N	10.2940	1.1575	88.76
P	0.2971	0.1204	59.47
B	0.2056	0.1593	22.52
Zn	0.4791	0.3631	24.21
Cu	0.0045	0.0025	44.44
Fe	0.2293	0.0315	86.26
K	4.8974	3.7613	23.20
Mn	0.0381	0.0253	33.60
Mo	0.0825	0.0735	10.91
Co	0.0045	0.0030	33.33

Table 4.22 Empirical formula on available nutrient

System	Empirical formula
Control 100Si	$CH_{1.57}O_{1.06}N_{1.93}P_{0.77}Si_{0.68}B_{0.30}Zn_{0.08}Cu_{0.33}Fe_{0.56}K_{0.23}Mn_{0.35}Mo_{0.05}Co_{0.31}$
Fresh 50%Si	$CH_{1.53}O_{1.05}N_{1.92}P_{0.71}Si_{0.55}B_{0.39}Zn_{0.10}Cu_{0.48}Fe_{0.56}K_{0.12}Mn_{0.26}Mo_{0.07}Co_{0.24}$
1st reused 50%Si	$CH_{1.46}O_{1.00}N_{1.31}P_{0.77}Si_{0.61}B_{0.05}Zn_{0.04}Cu_{0.42}Fe_{0.54}K_{0.22}Mn_{0.21}Mo_{0.05}Co_{0.14}$
2nd reused 50%Si	$CH_{1.50}O_{1.00}N_{1.84}P_{0.79}Si_{0.74}B_{0.29}Zn_{0.09}Cu_{0.47}Fe_{0.55}K_{0.13}Mn_{0.15}Mo_{0.01}Co_{0.13}$
3rd reused 50%Si	$CH_{1.48}O_{1.02}N_{1.89}P_{0.76}Si_{0.65}B_{0.16}Zn_{0.08}Cu_{0.08}Fe_{0.55}K_{0.25}Mn_{0.22}Mo_{0.06}Co_{0.23}$
Control 100%N	$CH_{1.50}O_{0.99}N_{1.92}P_{0.62}Si_{0.67}B_{0.31}Zn_{0.07}Cu_{0.30}Fe_{0.54}K_{0.20}Mn_{0.24}Mo_{0.03}Co_{0.16}$
Fresh 50%N	$CH_{1.49}O_{0.99}N_{1.91}P_{0.65}Si_{0.79}B_{0.58}Zn_{0.07}Cu_{0.45}Fe_{0.54}K_{0.12}Mn_{0.20}Mo_{0.04}Co_{0.12}$
1st reused 50%N	$CH_{1.50}O_{0.97}N_{1.83}P_{0.71}Si_{0.83}B_{0.57}Zn_{0.12}Cu_{0.47}Fe_{0.55}K_{0.12}Mn_{0.23}Mo_{0.05}Co_{0.18}$
2nd reused 50%N	$CH_{1.52}O_{1.00}N_{1.89}P_{0.62}Si_{0.78}B_{0.58}Zn_{0.11}Cu_{0.28}Fe_{0.50}K_{0.15}Mn_{0.27}Mo_{0.07}Co_{0.23}$
3rd reused 50%N	$CH_{1.49}O_{1.02}N_{1.91}P_{0.75}Si_{0.76}B_{0.76}Zn_{0.10}Cu_{0.34}Fe_{0.49}K_{0.17}Mn_{0.20}Mo_{0.04}Co_{0.24}$
Control 100%P	$CH_{1.46}O_{1.03}N_{1.76}P_{0.74}Si_{0.73}B_{0.13}Zn_{0.05}Cu_{0.21}Fe_{0.55}K_{0.18}Mn_{0.15}Mo_{0.02}Co_{0.18}$
Fresh 50%P	$CH_{1.48}O_{1.00}N_{1.72}P_{0.67}Si_{0.73}B_{0.14}Zn_{0.05}Cu_{0.08}Fe_{0.54}K_{0.12}Mn_{0.15}Mo_{0.05}Co_{0.23}$
1st reused 50%P	$CH_{1.49}O_{1.03}N_{1.89}P_{0.75}Si_{0.81}B_{0.59}Zn_{0.06}Cu_{0.45}Fe_{0.56}K_{0.14}Mn_{0.16}Mo_{0.04}Co_{0.24}$
2nd reused 50%P	$CH_{1.43}O_{1.03}N_{1.98}P_{0.74}Si_{0.97}B_{0.52}Zn_{0.09}Cu_{0.39}Fe_{0.55}K_{0.15}Mn_{0.23}Mo_{0.03}Co_{0.12}$
3rd reused 50%P	$CH_{1.48}O_{1.03}N_{2.00}P_{0.61}Si_{0.97}B_{0.66}Zn_{0.12}Cu_{0.22}Fe_{0.49}K_{0.19}Mn_{0.19}Mo_{0.04}Co_{0.18}$

4.4 Economics of cultivation systems for *C. gracilis*

This section examines preliminary economic analysis of the management of culture medium for the cultivation of *Chaetoceros gracilis* according to the results as presented above.

The analysis was based on the following conditions:

- 30 days of cultivation times
- The production of $1X10^{11}$ cells
- Light supply 24 h
- Air supplied 24 h
- Water charge = 0.294 Baht per liter (water + salt: local prices as listed in November 2013)
- Electric charge = 3.5 Baht per kWh (Thailand grid price as listed in November 2013)

Table 4.23 displays the comparative results between the costs of the cultivation with fresh medium and reused medium. It shows that the total operating cost with fresh medium was lower than reused mediums. In fresh medium, the total cost was the lowest at 28.37THB $L^{-1}d^{-1}$, whereas the 1^{st} reused, 2^{nd} reused and 3^{rd} reused mediums provided similar total operating costs of 55.73, 48.68 and 49.07THB $L^{-1}d^{-1}$, respectively. The total operating cost of fresh medium was less than the total operating cost of reused medium by 40-50%.

Table 4.24 shows that the economics for *C. gracilis* with silicate adjustment. In the control fresh medium and 50% silicate condition, the total operating costs were approximately the same which were lower than that obtained from the total operating cost of 100% and 500% silicate concentration, i.e. 29.64 and 27.11THB $L^{-1}d^{-1}$. Note that the total operating cost of 50% silicate concentration was 8% lower than that of the control fresh medium.

Table 4.25 illustrates the economics of *C. gracilis* cultivation with nitrate adjustment. The 500% nitrate concentration had the highest operating cost of approximately 32.84THB $L^{-1}d^{-1}$, which was 33% higher than the control experiment (24.65THB $L^{-1}d^{-1}$). The total operating costs of the 50%nitrate and 100%nitrate conditions were quite similar, which was 12% higher than that obtained from the control medium.

Similarly, Table 4.26 illustrates that comparison of the total operating costs of control experiment and the phosphate adjustment experiments. It was shown that the control culture was cheaper than the phosphate adjustment experiments. The total operating cost of phosphate adjustments (50%, 100% and 500%phosphate concentration) increased from the control medium range at 18-40%.

Table 4.27 shows the results from the economic analysis of the control medium, fresh medium with 50% silicate concentration and reused medium with 50% silicate concentration. In control medium and fresh medium with 50% silicate concentration, the total operating cost was lower than with reused medium with 50% silicate concentration. The total cost of control medium and fresh medium with 50%silicate concentration were 24.51 and 27.49THB $L^{-1}d^{-1}$, respectively. The total operating cost of reused medium with 50% silicate concentration increased from the control experiment for about 90-150%.

Table 4.28 depicts that the total operating costs of the control medium and fresh medium with 50% nitrate concentration were the same, i.e. 26.17 and 26.15THB $L^{-1}d^{-1}$. The reused medium with 50% nitrate concentration was 50-65% higher than that from the control medium.

Table 4.29 shows that the total operating cost of the control medium was 26.50THB $L^{-1}d^{-1}$ whereas the fresh medium with 50% phosphate concentration cost 29.04THB $L^{-1}d^{-1}$. Due to its low cell productivity, the reused medium with 50% phosphate concentration was 92-112% more expensive than the control experiment.

Figures 4.35 – 4.41 demonstrate the distribution of the operating cost for *C. gracilis* cultivation in all conditions. The cost of electricity contributed around 85-98% to the total operation cost, whereas the cost of water contributed 0.3-2% to the total operation cost. In addition, the cost of nutrient contributed 1-8% to the total operation cost, but the cost of nutrient of 500%silicate concentration condition was higher than the cost of electricity (see Figure 4.36). The cost of electricity was always the most expensive for the cultivation indicating that the cultivation required quite a significant amount of electricity which was mainly used for light supply and air pump. Further analysis by deducting the light cost showed that this could reduce the electricity cost by up to 67% which implies that the high electricity costs must have come from the use of the air pump.

In short, Tables 4.23 – 4.29 demonstrate that the cultivation in the control and fresh medium with 50% nutrient concentration gave the best economical

profiles when compared to the other conditions. The high cost was derived from the fact that the alga was badly affected by the reused medium which badly lowered the productivity.

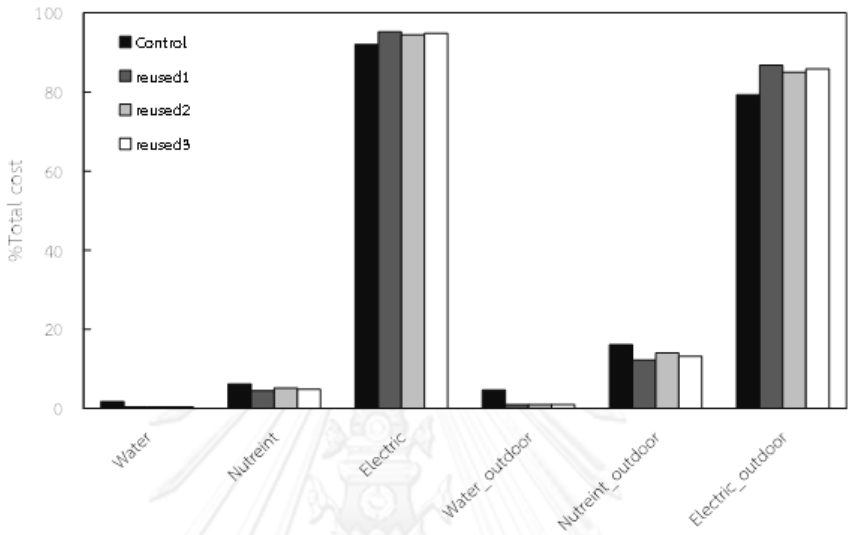

Figure 4.35 The operating cost for *Chaetoceros gracilis* cultivation in fresh, 1st, 2nd and 3rd reused mediums

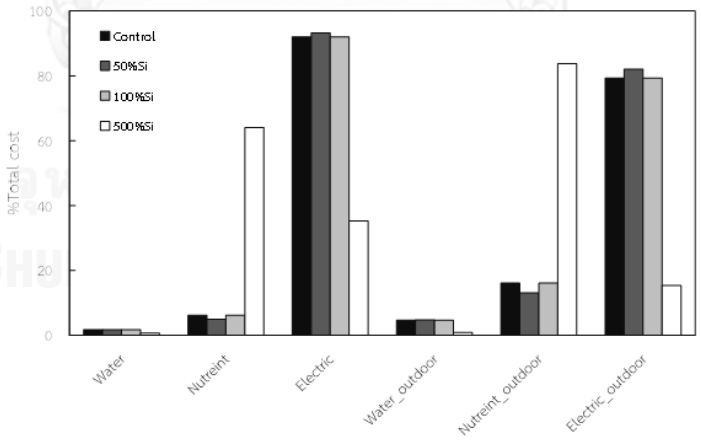

Figure 4.36 The operating cost for *Chaetoceros gracilis* cultivation in fed-batch with silicate concentration

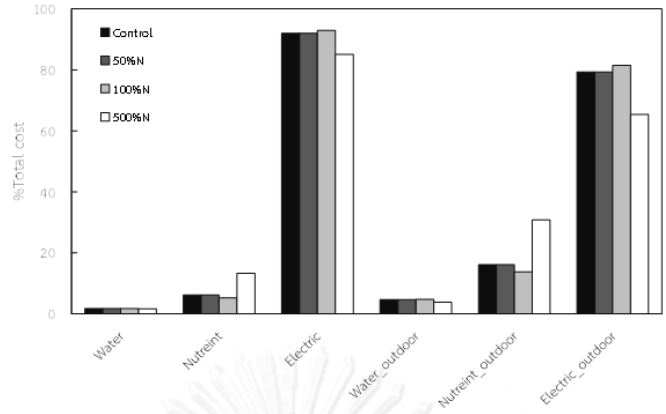

Figure 4.37 The operating cost for *Chaetoceros gracilis* cultivation in fed-batch with nitrate concentration

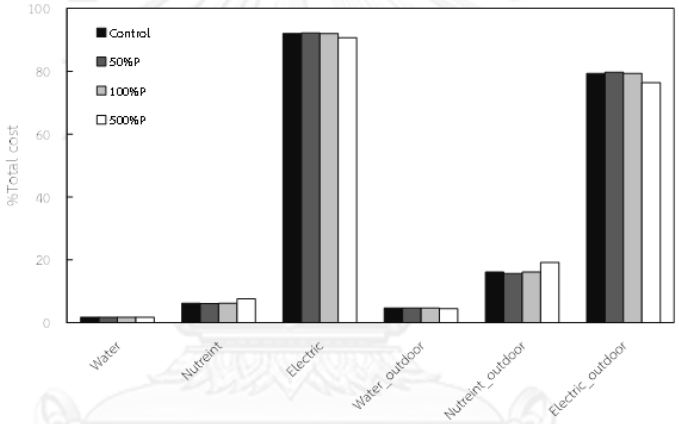

Figure 4.38 The operating cost for *Chaetoceros gracilis* cultivation in fed-batch with phosphate concentration

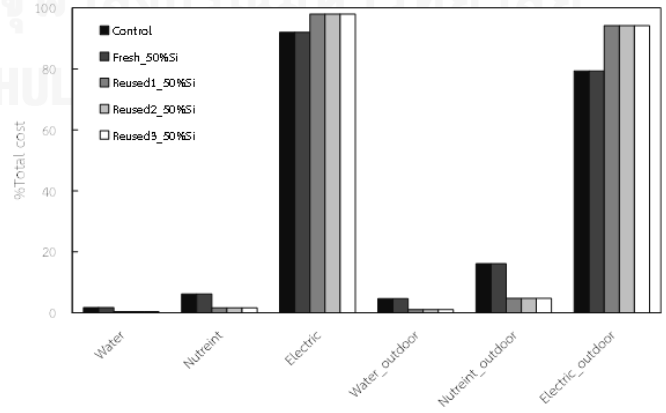

Figure 4.39 The operating cost for *Chaetoceros gracilis* cultivation in reused fed-batch with 50%silicate concentration

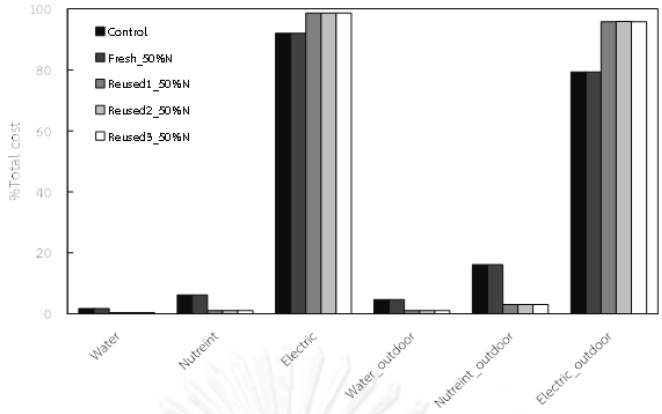

Figure 4.40 The operating cost for *Chaetoceros gracilis* cultivation in reused fed-batch with 50%nitrate concentration

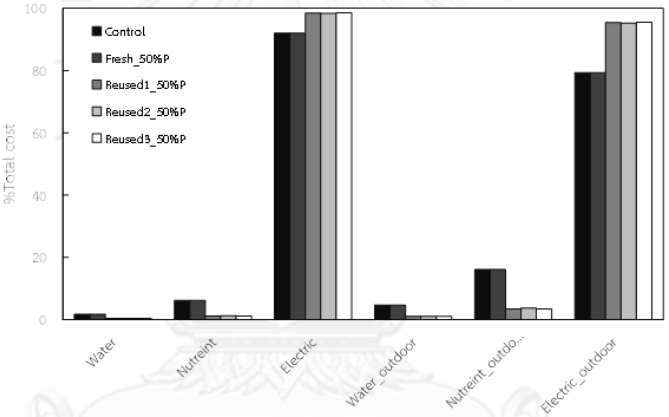

Figure 4.41 The operating cost for *Chaetoceros gracilis* cultivation in reused fed-batch with 50%phosphate concentration

Table 4.23 Economics for *C.gracilis* cultivation in fresh, 1st, 2nd and 3rd reused mediums

Type	Symbol	Component	Unit	Fresh	1st reused	2nd reused	3rd reused
A	A	Effective Volume	L	5	1	1	1
B	B	Cultivation time	d	5	5	5	5
C	C	cell density	cell mL^{-1}	$1.07x10^{7}$	$5.28x10^{6}$	$6.09x10^{6}$	$6.02x10^{6}$
D	D=C/(B*A)	Productivity (per reactor)	cell d^{1}	$1.07x10^{10}$	$1.06x10^{9}$	$1.22x10^{9}$	$1.20x10^{9}$
E	E=D/B	Specific productivity	cell $L^{-1}d^{-1}$	$2.15Ex10^{9}$	$1.06x10^{9}$	$1.22x10^{9}$	$1.20x10^{9}$
F	F	Product requirement	cell	$1x10^{11}$	$1x10^{11}$	$1x10^{11}$	$1x10^{11}$
G	G=F/E	Reactor requirement	units	1,553	3,157	2,737	2,769
H	H	Cultivation time requirement	d	30	30	30	30
I	I=(A*C*H*G)/1000	Power of light source	kWh	22,367	45,455	39,409	39,867
J	J=(B*C*H*G)/1000	Power of air pump	kWh	11,184	22,727	19,704	19,934
K	K=I+J	Total electrical unit	units	33,551	68,182	59,113	59,801
L	L=K*3.5	Cost of electricity	THB M^{-1}	117,428	238,636	206,897	209,302
M	M=A*G	Total volume of seawater	L	7,766	3,157	2,737	2,769
N	N	Cost of nutrient	THB L^{-1}	0.170263	0.118576	0.138449	0.128861
O	O=M*N	Total cost of nutrient	THB	7,934	11,229	11,367	10,703
P	P=M*N*0.294	Cost of water	THB M^{-1}	2,285	929	805	815
Q	Q=L+P	Total operating cost	THB M^{-1}	127,647	250,794	219,069	220,820
R	R=Q/(B*H)	Total operating cost	THB L^{-1} M^{-1}	851	1,672	1,460	14,72
S	S=R/H	Total operating cost	THB $L^{-1}d^{-1}$	28.37	55.73	48.68	49.07

Table 4.24 Economics *for C.gracilis* cultivation with silicate adjustment

Type	Symbol	Component	Unit	Control	50% Si	100% Si	500% Si
A	A	Effective Volume	L	5	5	5	5
B	B	Cultivation time	d	5	5	5	5
C	C	cell density	cell mL^{-1}	$1.03x10^{7}$	$1.11x10^{7}$	$8.50x10^{6}$	$1.12x10^{7}$
D	D=C/(B*A)	Productivity (per reactor)	cell d^{1}	$1.03x10^{10}$	$1.11x10^{10}$	$8.50x10^{9}$	$1.12x10^{10}$
E	E=D/B	Specific productivity	cell $L^{-1}d^{-1}$	$2.05x10^{9}$	$2.22x10^{9}$	$1.70x10^{9}$	$2.23x10^{9}$
F	F	Product requirement	cell	$1x10^{11}$	$1x10^{11}$	$1x10^{11}$	$1x10^{11}$
G	G=F/E	Reactor requirement	units	1,623	1,504	1,961	1,493
H	H	Cultivation time requirement	d	30	30	30	30
I	I=(A*C*H*G)/1000	Power of light source	kWh	23,369	21,661	28,235	21,505
J	J=(B*C*H*G)/1000	Power of air pump	kWh	11,685	10,830	14,118	10,753
K	K=I+J	Total electrical unit	units	35,054	32,491	42,353	32,258
L	L=K*3.5	Cost of electricity	THB M^{-1}	122,687	113,718	148,235	112,903
M	M=A*G	Total volume of seawater	L	8,114	7,521	9,804	7,467
N	N	Cost of nutrient	THB L^{-1}	0.170263	0.134263	0.170263	4.582627
O	O=M*N	Total cost of nutrient	THB	8,289	6,059	10,015	205,315
P	P=M*N*0.294	Cost of water	THB M^{-1}	2,387	2,213	2,884	2,197
Q	Q=L+P	Total operating cost	THB M^{-1}	133,364	121,990	161,135	320,415
R	R=Q/(B*H)	Total operating cost	THB L^{-1} M^{-1}	889	813	1,074	2,136
S	S=R/H	Total operating cost	THB $L^{-1}d^{-1}$	29.64	27.11	35.81	71.20

Table 4.25 Economics for *C.gracilis* cultivation with nitrate adjustment

Type	Symbol	Component	Unit	Control	50% N	100% N	500% N
A	A	Effective Volume	L	5	5	5	5
B	B	Cultivation time	d	5	5	5	5
C	C	cell density	cell mL^{-1}	$1.24x10^{7}$	$1.10x10^{7}$	$1.09x10^{7}$	$1.00x10^{7}$
D	D=C/(B*A)	Productivity (per reactor)	cell d^{1}	$1.24x10^{10}$	$1.10x10^{10}$	$1.09x10^{10}$	$1.00x10^{10}$
E	E=D/B	Specific productivity	cell $L^{-1}d^{-1}$	$2.47x10^{9}$	$2.20x10^{9}$	$2.18x10^{9}$	$2.01x10^{9}$
F	F	Product requirement	cell	$1x10^{11}$	$1x10^{11}$	$1x10^{11}$	$1x10^{11}$
G	G=F/E	Reactor requirement	units	1,350	1,514	1,530	1,662
H	H	Cultivation time requirement	d	30	30	30	30
I	I=(A*C*H*G)/1000	Power of light source	kWh	19,433	21,798	22,039	23,928
J	J=(B*C*H*G)/1000	Power of air pump	kWh	9,717	10,899	11,019	11,964
K	K=I+J	Total electrical unit	units	29,150	32,698	33,058	35,892
L	L=K*3.5	Cost of electricity	THB M^{-1}	102,024	114,441	115,702	125,623
M	M=A*G	Total volume of seawater	L	6,748	7,569	7,652	8,308
N	N	Cost of nutrient	THB L^{-1}	0.170263	0.142138	0.170263	0.395263
O	O=M*N	Total cost of nutrient	THB	6,893	7,732	6,526	19,704
P	P=M*N*0.294	Cost of water	THB M^{-1}	1,985	2,227	2,251	2,444
Q	Q=L+P	Total operating cost	THB M^{-1}	110,903	124,400	124,480	147,771
R	R=Q/(B*H)	Total operating cost	THB L^{-1} M^{-1}	739	829	830	985
S	S=R/H	Total operating cost	THB $L^{-1}d^{-1}$	24.65	27.64	27.66	32.84

Table 4.26 Economics for *C.gracilis* cultivation with phosphate adjustment

				Control	50% P	100% P	500% P
A	A	Effective Volume	L	5	5	5	5
B	B	Cultivation time	d	5	5	5	5
C	C	cell density	cell mL^{-1}	$1.36x10^{7}$	$1.15x10^{7}$	$1.01x10^{7}$	$9.86x10^{6}$
D	D=C/(B*A)	Productivity (per reactor)	cell d^{1}	$1.36x10^{10}$	$1.15x10^{10}$	$1.01x10^{10}$	$9.86x10^{9}$
E	E=D/B	Specific productivity	cell $L^{-1}d^{-1}$	$2.72x10^{9}$	$2.30x10^{9}$	$2.01x10^{9}$	$1.97x10^{9}$
F	F	Product requirement	cell	$1x10^{11}$	$1x10^{11}$	$1x10^{11}$	$1x10^{11}$
G	G=F/E	Reactor requirement	units	1,224	1,448	1,658	1,690
H	H	Cultivation time requirement	d	30	30	30	30
I	I=(A*C*H*G)/1000	Power of light source	kWh	17,621	20,851	23,881	24,341
J	J=(B*C*H*G)/1000	Power of air pump	kWh	8,811	10,426	11,940	12,170
K	K=I+J	Total electrical unit	units	26,432	31,277	35,821	36,511
L	L=K*3.5	Cost of electricity	THB M^{-1}	92,511	109,470	125,373	127,789
M	M=A*G	Total volume of seawater	L	6,118	7,240	8,292	8,452
N	N	Cost of nutrient	THB L^{-1}	0.170263	0.165163	0.170263	0.211063
O	O=M*N	Total cost of nutrient	THB	6,250	7,175	8,471	10,703
P	P=M*N*0.294	Cost of water	THB M^{-1}	1,800	2,130	2,439	2,486
Q	Q=L+P	Total operating cost	THB M^{-1}	100,562	118,775	136,283	140,978
R	R=Q/(B*H)	Total operating cost	THB L^{-1} M^{-1}	670	792	909	940
S	S=R/H	Total operating cost	THB $L^{-1}d^{-1}$	22.35	26.39	30.29	31.33

Table 4.27 Economics for *C.gracilis* cultivation in control, fresh of 50%silicate and reused of 50%silicate

Type	Symbol	Component	Unit	Control	Fresh	1st reused	2nd reused	3rd reused
A	A	Effective Volume	L	5	5	1	1	1
B	B	Cultivation time	d	5	5	5	5	5
C	C	cell density	cell mL^{-1}	$1.24x10^{7}$	$1.11x10^{7}$	$5.96x10^{6}$	$4.64x10^{6}$	$4.76x10^{6}$
D	D=C/(B*A)	Productivity (per reactor)	cell d^{1}	$1.24x10^{10}$	$1.11x10^{10}$	$1.19x10^{9}$	$9.28x10^{8}$	$9.52x10^{8}$
E	E=D/B	Specific productivity	cell $L^{-1}d^{-1}$	$2.48x10^{9}$	$2.21x10^{9}$	$1.19x10^{9}$	$9.28x10^{8}$	$9.52x10^{8}$
F	F	Product requirement	cell	$1x10^{11}$	$1x10^{11}$	$1x10^{11}$	$1x10^{11}$	$1x10^{11}$
G	G=F/E	Reactor requirement	units	1,342	1,506	2,796	3,592	3,501
H	H	Cultivation time requirement	d	30	30	30	30	30
I	I=(A*C*H*G)/1000	Power of light source	kWh	19,324	21,680	40,268	51,724	50,420
J	J=(B*C*H*G)/1000	Power of air pump	kWh	9,662	10,840	20,134	25,862	25,210
K	K=I+J	Total electrical unit	units	28,986	32,520	60,403	77,586	75,630
L	L=K*3.5	Cost of electricity	THB M^{-1}	101,449	113,821	211,409	271,552	264,706
M	M=A*G	Total volume of seawater	L	6,710	7,528	2,796	3,592	3,501
N	N	Cost of nutrient	THB L^{-1}	0.170263	0.136514	0.211063	0.211063	0.211063
O	O=M*N	Total cost of nutrient	THB	6,854	7,690	3,541	4,549	4,434
P	P=M*N*0.294	Cost of water	THB M^{-1}	1,974	2,215	823	1,057	1,030
Q	Q=L+P	Total operating cost	THB M^{-1}	110,278	123,726	215,773	277,157	270,170
R	R=Q/(B*H)	Total operating cost	THB L^{-1} M^{-1}	735	825	1,438	1,848	1,801
S	S=R/H	Total operating cost	THB $L^{-1}d^{-1}$	24.51	27.49	47.95	61.59	60.04

Table 4.28 Economics for *C.gracilis* cultivation in control, fresh of 50%nitrate and reused of 50%nitrate

Type	Symbol	Component	Unit	Control	Fresh	1st reused	2nd reused	3rd reused
A	A	Effective Volume	L	5	5	1	1	1
B	B	Cultivation time	d	5	5	5	5	5
C	C	cell density	cell mL^{-1}	$1.16x10^{7}$	$1.16x10^{7}$	$6.58x10^{6}$	$7.23x10^{6}$	$6.79x10^{6}$
D	D=C/(B*A)	Productivity (per reactor)	cell d^{1}	$1.16x10^{10}$	$1.16x10^{10}$	$1.32x10^{9}$	$1.45x10^{9}$	$1.36x10^{9}$
E	E=D/B	Specific productivity	cell $L^{-1}d^{-1}$	$2.33x10^{9}$	$2.33x10^{9}$	$1.32x10^{9}$	$1.45x10^{9}$	$1.36x10^{9}$
F	F	Product requirement	cell	$1x10^{11}$	$1x10^{11}$	$1x10^{11}$	$1x10^{11}$	$1x10^{11}$
G	G=F/E	Reactor requirement	units	1,433	1,432	2,533	2,305	2,455
H	H	Cultivation time requirement	d	30	30	30	30	30
I	I=(A*C*H*G)/1000	Power of light source	kWh	20,636	20,619	36,474	33,195	35,346
J	J=(B*C*H*G)/1000	Power of air pump	kWh	10,318	10,309	18,237	16,598	17,673
K	K=I+J	Total electrical unit	units	30,954	30,928	54,711	49,793	53,019
L	L=K*3.5	Cost of electricity	THB M^{-1}	108,340	108,247	191,489	174,274	185,567
M	M=A*G	Total volume of seawater	L	7,165	7,159	2,533	2,305	2,455
N	N	Cost of nutrient	THB L^{-1}	0.170263	0.180298	0.136514	0.133889	0.136882
O	O=M*N	Total cost of nutrient	THB	7,320	7,314	2,075	1,852	2,016
P	P=M*N*0.294	Cost of water	THB M^{-1}	2,108	2,106	745	678	722
Q	Q=L+P	Total operating cost	THB M^{-1}	117,769	117,667	194,309	176,804	188,305
R	R=Q/(B*H)	Total operating cost	THB L^{-1} M^{-1}	785	784	1,295	1,179	1,255
S	S=R/H	Total operating cost	THB $L^{-1}d^{-1}$	26.17	26.15	43.18	39.29	41.85

Table 4.29 Economics for *C.gracilis* cultivation in control, fresh of 50%phosphate and reused of 50%phosphate

Type	Symbol	Component	Unit	Control	Fresh	1st reused	2nd reused	3rd reused
A	A	Effective Volume	L	5	5	1	1	1
B	B	Cultivation time	d	5	5	5	5	5
C	C	cell density	cell mL^{-1}	$1.08x10^{7}$	$9.83x10^{6}$	$5.52x10^{6}$	$5.02x10^{6}$	$5.08x10^{6}$
D	D=C/(B*A)	Productivity (per reactor)	cell d^{1}	$1.08x10^{10}$	$9.83x10^{9}$	$1.10x10^{9}$	$1.00x10^{9}$	$1.02x10^{9}$
E	E=D/B	Specific productivity	cell $L^{-1}d^{-1}$	$2.15x10^{9}$	$1.97x10^{9}$	$1.10x10^{9}$	$1.00x10^{9}$	$1.02x10^{9}$
F	F	Product requirement	cell	$1x10^{11}$	$1x10^{11}$	$1x10^{11}$	$1x10^{11}$	$1x10^{11}$
G	G=F/E	Reactor requirement	units	1,548	1,695	3,019	3,320	3,281
H	H	Cultivation time requirement	d	30	30	30	30	30
I	I=(A*C*H*G)/1000	Power of light source	kWh	22,284	24,415	43,478	47,809	47,244
J	J=(B*C*H*G)/1000	Power of air pump	kWh	11,142	12,208	21,739	23,904	23,622
K	K=I+J	Total electrical unit	units	33,426	36,623	65,217	71,713	70,866
L	L=K*3.5	Cost of electricity	THB M^{-1}	116,992	128,179	228,261	250,996	248,031
M	M=A*G	Total volume of seawater	L	7,738	8,477	3,019	3,320	3,281
N	N	Cost of nutrient	THB L^{-1}	7,904	8,660	2,772	3,287	2,960
O	O=M*N	Total cost of nutrient	THB	0.170263	0.194672	0.153009	0.164992	0.150389
P	P=M*N*0.294	Cost of water	THB M^{-1}	2,276	2,494	888	977	965
Q	Q=L+P	Total operating cost	THB M^{-1}	119,268	130,673	229,149	251,973	248,997
R	R=Q/(B*H)	Total operating cost	THB L^{-1} M^{-1}	795	871	1,528	1,680	1,660
S	S=R/H	Total operating cost	THB $L^{-1}d^{-1}$	26.50	29.04	50.92	55.99	55.33

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

5.1.1 The cultivations with fresh medium with modified F/2 medium and fresh medium with 50% major nutrients always gave the highest cell density. The reuse of medium could not match the growth of the fresh medium regardless of the adjustment of the macronutrient components, i.e. only 50% of the total cell density could be obtained with the reuse medium when compared with the new medium.

The maximum cell density from fresh medium with modified F/2 medium and fresh medium with 50% major nutrients were approximately 10.73 - 12.42 x 10^6cell mL^{-1}. The maximum cell density from the reuse of medium was approximately 4.64 - 7.23 x 10^6cell mL^{-1}.

5.1.2 The adjustment of macronutrients (50%, 100%, and 500% of the modified F/2 medium) did not seem to give any positive effect on cell growth and density as long as freshly prepared mediums were used for the cultivation and not the reuse nutrients. The results of this experiment can be summarized as follows:

Fed-Batch with the continual addition of silicate, the maximum cell densities were approximately 10.27 - 11.16 x 10^6cell mL^{-1}.

Fed-Batch with the continual addition of nitrate, the maximum cell densities were approximately 10.03 - 12.35 x 10^6cell mL^{-1}.

Fed-Batch with the continual addition of phosphate, the maximum cell densities were approximately 10.34 - 13.62 x 10^6cell mL^{-1}.

5.1.3 The reuse of medium always encountered the accumulation of two main organic compounds, i.e. Dimethyl-silanediol and Haxanedioic acid, bis(2-ethylhexyl) ester. The first nonsoluble silicate compound was expected to be derived from sodium metasilicate supplied in the fresh nutrient, but this form could not be used by the alga. The second one was believed to have inhibitory effect on cell growth. This finding explained why the reuse of medium always gave a inferior growth when compared with the fresh medium.

5.1.4 Biochemical components from *Chaetoceros gracilis* cultivated with reused nutrients were rich in total lipid but poor in carbohydrate whereas protein remained approximately constant. %carbohydrate was maximized when cultivated

with fresh medium but with 50% macronutrients. Typical biochemical composition could be summarized as:

Fresh medium:

Carbohydrate: 43 - 49 %

Protein: 15 - 21 %

Lipid: 30 - 50 %

Reused medium:

Carbohydrate: 23 - 38 %

Protein: 18 - 22 %

Lipid: 48 - 60 %

5.1.5 Economic analysis suggested that the reused experiments incurred higher operating cost when compared with control and experiments with fresh medium. The major cost contributor was electricity followed by water and nutrients. The cost of electricity contributed around 85 - 98% to the total operating cost, whereas the cost of water 0.3 - 2%, and the cost of nutrient 1 - 8% to the total operating cost.

The total operating cost of the fresh medium with the modified F/2 medium and fresh medium with 50% major nutrients were the same, whereas the total operating cost of reused medium was 50% higher than the fresh medium.

5.2 Recommendations

5.2.1 A more fine adjustment of nutrient concentration (e.g. below 50%) should be further investigated to find the optimal food condition for *Chaetoceros gracilis*.

5.2.2 A co-factor on the effect of reducing nutrients should be examined. For instance, the effect of reducing nitrate together with silicate (and/or perhaps phosphorus) should be investigated.

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APPENDIX

APPENDIX A

Measurement of nutrient concentration

Appendix A-1: Measurement of silicate concentration

Prepared of reagent for quantitative analysis of silicate in culture diatoms by Strickland and Parson (1972)

A. Reagent

1. Molybdate solution

$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ 4 g dissolved in 300 ml D.I.water and add 12 ml of HCl concentration. Mixed and adjust the volume to 500 ml by D.I.water. Store the Molybdate solution in a plastic bottle where it is stable indefinitely.

2. Metol-sulphite solution

Na_2SO_3 6 g dissolved in 500 ml D.I.water and add 10 g of Metol (P-metylaminophenol sulphate). Filter the solution with filter paper No.1. Store the Metol-sulphite solution in a glass bottle tightly stopped by rubber bung and the solution is stable for many months.

3. Oxalic acid solution

Oxalic acid dehydrate $((COOH)_2\cdot 2H_2O)$ 50 g dissolved in 500 ml D.I.water. Store the Oxalic acid solution in a glass bottle tightly stopped by rubber bung.

4. Sulphuric acid solution

Sulpuric acid 99.9% 250 ml dissolved in 250 D.I.water. Keep until the cooling solution and adjust the volume to 500 ml by D.I.water. Store the Sulphuric acid solution in a glass bottle tightly stopped by rubber bung.

5. Mixed reagent

Mixed 100 ml of Metol-sulphite solution with 60 ml of Oxalic acid solution. Add 60 ml Sulphuric acid 50% solution and adjust the volume 300 ml by D.I.water.

B. Prepared silicate stock solution (concentration is 14.935 mg-Si L^{-1})

Weighed 0.1 g of Sodium silico fluoride (Na_2SiF_6) dissolved in 1000 ml D.I.water. Store in a glass bottle.

C. Prepared of an artificial seawater for analyzed standard solution

Weighed 27 g of NaCl and 8 g of $MgSO_4 \cdot 7H_2O$ dissolved in 1000 ml D.I.water. Store in a plastic bottle.

D. Prepared of calibration solution

Prepared standard silicate from silicate solution concentration (Na_2SiF_6 stock) is 14.935 mg-Si L^{-1}. (Diluted by artificial seawater)

1. Dilute 500 fold of Na_2SiF_6 stock, Concentration is 0.02987 mg-Si L^{-1}
2. Dilute 250 fold of Na_2SiF_6 stock, Concentration is 0.05974 mg-Si L^{-1}
3. Dilute 100 fold of Na_2SiF_6 stock, Concentration is 0.14935 mg-Si L^{-1}
4. Dilute 50 fold of Na_2SiF_6 stock, Concentration is 0.29870 mg-Si L^{-1}
5. Dilute 10 fold of Na_2SiF_6 stock, Concentration is 1.49350 mg-Si L^{-1}
6. Dilute 5 fold of Na_2SiF_6 stock, Concentration is 2.98700 mg-Si L^{-1}

E. Blank test

1. Add 0.5 ml of D.I.water to 0.2 ml of molybdate solution, allow 10 min for reaction time.

2. Add 0.3 ml of mixed reagent, allow 2-3 hr for reaction time.

3. Measure the solution by UV-Visible spectrophotometer at wavelength of 810 nm. (Set blank equal zero)

F. Procedure

1. Add 0.5 ml of Sample (calibration solution) to 0.2 ml of molybdate solution, allow 10 min for reaction time.

2. Add 0.3 ml of mixed reagent, allow 2-3 hr for reaction time.

3. Measure the solution by UV-Visible spectrophotometer at wavelength of 810 nm.

4. The value obtained from the measurements to the standard curve to calculated the concentration of silicate.

Appendix A-2: Measurement of nitrogen concentration

Prepared of reagent for quantitative analysis of nitrogen in culture diatoms by Strickland and Parson (1972)

A. Prepared nitrogen stock solution (concentration is 100 mg-N L^{-1})

Weighed 0.7128 g of KNO_3 (Through the oven at temperature of 105^0C for 24 hr.) dissolved in 1000 ml D.I.water. Store in a dark glass bottle.

B. Prepared of calibration solution

Prepared standard solution from nitrogen stock solution concentration (KNO_3 stock) is 0.7128 mg-Ni L^{-1}. (Diluted by D.I.water)

1. Dilute 200 fold of KNO_3 stock, Concentration is 0.5 mg-N L^{-1}
2. Dilute 100 fold of KNO_3 stock, Concentration is 1.0 mg-N L^{-1}
3. Dilute 50 fold of KNO_3 stock, Concentration is 2.0 mg-N L^{-1}
4. Dilute 30 fold of KNO_3 stock, Concentration is 3.0 mg-N L^{-1}
5. Dilute 25 fold of KNO_3 stock, Concentration is 4.0 mg-N L^{-1}
6. Dilute 20 fold of KNO_3 stock, Concentration is 5.0 mg-N L^{-1}
7. Dilute 15 fold of KNO_3 stock, Concentration is 8.0 mg-N L^{-1}
8. Dilute 10 fold of KNO_3 stock, Concentration is 10 mg-N L^{-1}

C. Blank test

2 ml of D.I.water, Measured by UV-Visible spectrophotometer at wavelength of 220 and 275 nm. (Set blank equal zero)

E. Procedure

Measured the sample (calibration solution) by UV-Visible spectrophotometer at wavelength of 220 nm. obtain N reading and wavelength of 275 nm. to determine interference due to dissolved organic matter. The value obtained from the measurements to the standard curve to calculated the concentration of nitrogen.

Appendix A-3: Measurement of phosphate concentration

Prepared of reagent for quantitative analysis of phosphate in culture diatoms by Strickland and Parson (1972)

A. Reagent

1. Ammonium molybdate solution

$(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 15 g dissolved in 500 ml D.I.water. Store the Ammonium molybdate solution in a plastic bottle where it is stable indefinitely.

2. Sulphuric acid solution

Sulpuric acid 99.9% 140 ml dissolved in 900 D.I.water. Store the Sulphuric acid solution in a glass bottle tightly stopped by rubber bung and keep refrigerated.

3. Ascorbic acid solution

Ascorbic acid 27 g dissolved in 500 ml D.I.water. Store the Ascorbic acid solution in a plastic bottle tightly stopped by rubber bung and keep refrigerated.

4. Potassium antimonyl-tartrate solution

Potassium antimonyl-tartrate 0.34 g dissolved in 250 ml D.I.water. Store the Potassium antimonyl-tartrate solution in a glass bottle. (or plastic bottle)

5. Mixed reagent

Mixed 2 ml of Ammonium molybdate solution, 5 ml of Sulphuric acid solution, 2 ml of Ascorbic acid solution and 1 ml of Potassium antimonyl-tartrate solution. Prepared afresh each day.

B. Prepared phosphate stock solution (concentration is 186 mg-P L^{-1})

Weighed 0.816 g of KH_2PO_4 dissolved in 1000 ml D.I.water. Store in a dark glass bottle.

C. Prepared of calibration solution

Prepared standard solution from phosphate stock solution concentration (KH_2PO_4 stock) is 186 mg-P L^{-1}. (Diluted by D.I.water)

1. Dilute 10000 fold of KH_2PO_4 stock, Concentration is 0.0186 mg-P L^{-1}

2. Dilute 2000 fold of KH_2PO_4 stock, Concentration is 0.0930 mg-P L^{-1}

3. Dilute 1000 fold of KH_2PO_4 stock, Concentration is 0.1860 mg-P L^{-1}

4. Dilute 500 fold of KH_2PO_4 stock, Concentration is 0.3720 mg-P L^{-1}

D. Blank test

1. Add 1 ml of D.I.water to 0.1 ml of mixed reagent, allow 30 min for reaction time.

2. Measure the solution by UV-Visible spectrophotometer at wavelength of 885 nm. (Set blank equal zero)

E. Procedure

1. Add 1 ml of Sample (calibration solution) to 0.1ml of mixed reagent, allow 30 min for reaction time.

2. Measure the solution by UV-Visible spectrophotometer at wavelength of 885 nm.

4. The value obtained from the measurements to the standard curve to calculated the concentration of phosphate.

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