การนำอาหารเพาะเชื้อกลับมาใช้เลี้ยง *Ankistrodesmus* sp. และ *Scenedesmus* sp. ในถังปฏิกรณ์ชีวภาพอากาศยก

นางสาวหทัยชนก รอดราคี

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ปีการศึกษา 2556

REUSE OF MEDIUM FOR *ANKISTRODESMUS* SP. AND *SCENEDESMUS* SP. CULTURE IN AIRLIFT PHOTOBIOREACTOR

Miss Hathaichanok Rodrakhee

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Engineering Program in Chemical Engineering
Department of Chemical Engineering
Faculty of Engineering
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Thesis Title	REUSE OF MEDIUM FOR *ANKISTRODESMUS* SP. AND *SCENEDESMUS* SP. CULTURE IN AIRLIFT PHOTOBIOREACTOR
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Field of Study	Chemical Engineering
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หทัยชนก รอดราคี : การนำอาหารเพาะเชื้อกลับมาใช้เลี้ยง *Ankistrodesmus* sp. และ *Scenedesmus* sp. ในถังปฏิกรณ์ชีวภาพอากาศยก. (REUSE OF MEDIUM FOR *ANKISTRODESMUS* SP. AND *SCENEDESMUS* SP. CULTURE IN AIRLIFT PHOTOBIOREACTOR) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร.ประเสริฐ ภวสันต์, 111 หน้า.

งานวิจัยนี้แบ่งออกเป็นสามส่วน คือ การเลี้ยงสาหร่าย *Ankistrodesmus* sp. และ *Scenedesmus* sp. ในถังปฏิกรณ์ชีวภาพอากาศยกขนาด 25 ลิตร พบว่าการเจริญเติบโตของสาหร่ายทั้งสองชนิดให้ความหนาแน่นของเซลล์สาหร่าย *Ankistrodesmus* sp. เท่ากับ $9.76\pm0.36\times10^{6}$ เซลล์ มล.$^{-1}$ และ *Scenedesmus* sp. เท่ากับ $2.95\pm0.48\times10^{6}$ เซลล์ มล.$^{-1}$ ส่วนที่สองของงานวิจัยนี้จะอธิบายถึงการเลี้ยงสาหร่ายทั้งสองชนิดในความเข้มข้นของสารอาหารที่แตกต่างกัน โดยเฉพาะการลดธาตุไนโตรเจนและธาตุฟอสฟอรัสในสูตรอาหาร BG11 จากการทดลองพบว่าการลดอาหารลงไปที่ 25% ของความเข้มข้นของสารอาหารของไนโตรเจนและฟอสฟอรัสให้การเจริญเติบโตที่ดีที่สุด โดยสาหร่าย *Ankistrodesmus* sp. ให้ความหนาแน่นเซลล์เท่ากับ $1.23\pm1.68\times10^{7}$ เซลล์ มล.$^{-1}$ และน้ำหนักมวลสาหร่ายเท่ากับ 0.70 ± 0.01 กรัม ล.$^{-1}$ ขณะที่สาหร่าย *Scenedesmus* sp. ให้ความหนาแน่นเซลล์เท่ากับ $2.89\pm0.83\times10^{6}$ เซลล์ มล.$^{-1}$ และน้ำหนักมวลสาหร่ายเท่ากับ 0.47 ± 0.01 กรัม ล.$^{-1}$ อีกทั้งการลดสารอาหารที่ความเข้มข้นที่แตกต่างกันยังส่งผลกระทบต่อองค์ประกอบทางชีวเคมีของสาหร่าย เช่น ปริมาณลิปิดของสาหร่าย *Ankistrodesmus* sp. ในการลดสารอาหารที่ 25% ได้เท่ากับ 33±2%, 31±0.2%, 30±1.0% และ 29±4.5% โดยน้ำหนักของสาหร่าย สำหรับถังควบคุม, ถังที่ลดธาตุอาหารไนโตรเจนและฟอสฟอรัส, ถังที่ลดฟอสฟอรัส และถังที่ลดไนโตรเจน ตามลำดับ และในส่วนสุดท้ายของการทดลองยังได้นำน้ำอาหารกลับที่ผ่านการเลี้ยงมาเลี้ยงสาหร่ายอีกครั้งเพื่อตรวจสอบการเจริญเติบโตของสาหร่าย โดยสาหร่าย *Ankistrodesmus* sp. ประสบความสำเร็จในการเลี้ยงในน้ำอาหารที่นำกลับมาใช้ใหม่ ซึ่งได้ค่าความหนาแน่นของเซลล์ในถังตามสูตรอาหาร BG11 เท่ากับ $9.06\pm1.40\times10^{6}$ เซลล์ มล.$^{-1}$, ถังที่นำกลับมาใช้ใหม่ครั้งที่หนึ่งเท่ากับ $1.47\pm0.28\times10^{7}$ เซลล์ มล.$^{-1}$ และถังที่นำกลับมาใช้ใหม่ครั้งที่สองเท่ากับ $8.76\pm2.79\times10^{6}$ เซลล์ มล.$^{-1}$ ส่วนค่าองค์ประกอบทางชีวเคมีของสาหร่ายทั้งสองตัวยังส่งผลกระทบที่แตกต่างกันระหว่างน้ำอาหารตามสูตรอาหาร BG11 และน้ำอาหารที่นำกลับมาใช้ใหม่อีกด้วย อีกทั้งในส่วนสุดท้ายของการวิจัยนี้ยังมีการศึกษาการประเมินผลทางวิชาเศรษฐศาสตร์และอธิบายผลการทดลองโดยการใช้สมการพื้นฐานในการอธิบายต้นทุนของการเลี้ยง เพื่อเปรียบเทียบความแตกต่างระหว่างการเลี้ยงสาหร่ายในเงื่อนไขที่ลดความเข้มข้นของสารอาหารที่แตกต่างกันและการนำน้ำอาหารกลับมาใช้ใหม่

ภาควิชา วิศวกรรมเคมี ลายมือชื่อนิสิต .....

สาขาวิชา วิศวกรรมเคมี ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

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HATHAICHANOK RODRAKHEE: REUSE OF MEDIUM FOR *ANKISTRODESMUS* SP. AND *SCENEDESMUS* SP. CULTURE IN AIRLIFT PHOTOBIOREACTOR. ADVISOR: ASSOC. PROF. PRASERT PAVASANT, Ph.D., 111 pp.

This research is divided into three sections. The first part shows the growth of two algae (*Ankistrodesmus* sp. and *Scenedesmus* sp.) which were cultivated in the 25 L non-baffled flat panel airlift photobioreactor (NB-FPAP). The result shows that the growths of *Ankistrodesmus* sp. and *Scenedesmus* sp. were $9.76\pm0.36\times10^{6}$ and $2.95\pm0.48\times10^{6}$, respectively. The second part describes the use of reduced nutrient of BG11 in the cultivation of the two algae. The cultured microalgae with 25%N&P medium provided the best growth condition with the cell density of $1.23\pm1.68\times10^{7}$ and $2.89\pm0.83\times10^{6}$ cell mL^{-1} and the dry weight of 0.70±0.01 and 0.47±0.01 g L^{-1} for *Ankistrodesmus* sp. and *Scenedesmus* sp., respectively. The various nutrient reduction conditions exerted some effect on the biochemical composition of the algae, e.g. lipid contents in *Ankistrodesmus* sp. from control batch, 25%N&P, 25%P and 25%N were 33±2%wt, 31±0.2%wt, 30±1.0%wt and 29±4.5%wt, respectively. In the last part, the remaining nutrient was reused in the algal culture to examine the growth of such culture. *Ankistrodesmus* sp. was found to be successfully cultivated in reuse mediums where the cell density from fresh, 1^{st} reuse and 2^{nd} reuse mediums were $9.06\pm1.40\times10^{6}$, $1.47\pm0.28\times10^{7}$ and $8.76\pm2.79\times10^{6}$ cell mL^{-1}, respectively. Biochemical composition of the two algae was also affected from the differences in the medium concentration. Last, this study shows basic concepts for economics assessment, and describes methodologies for the cost estimation of microalgal culture using simple costing equations which is then used to compare the different cost reduction options, e.g. reduced and reused nutrients.

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

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

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CHAPTER 1

INTRODUCTION

1.1 Motivation

Fossil fuels are now widely known as unsustainable source of energy. The diminishing supplies and the contribution of petroleum-derived fuels to the increased carbon dioxide concentrations in the environment have prompted a search of renewable sources of energy. Biologically-derived fuels have increasingly been mentioned as an important alternative. Photosynthetic plants, including rapeseed, corn, sunflower, soybean, coconut and others, produce and store lipid oils. However, the important economic and environmental impacts of using agricultural crops, especially food crops, as a feedstock for biofuels have raised crucial sustainability issue, when worldwide food supply experiences significant shortage and biofuels have been blamed as significant contributors.

Microalgae represent another potential alternative feedstock for fuel production to be a renewable source of oil. The characteristics that make microalgae such an attractive alternative are that photosynthesis mechanism in microalgae is similar to that of higher plants, therefore it is considered a natural CO_2 storage device. The growth of microalgae is significantly higher and microalgae do not compete against human food supply or water usage like other bio-sources.

Application of microalgae can be more environmentally sustainable, cost-effective and profitable, if combined with processes such as wastewater and flue gas treatment (Mata et al., 2010). Environmental applications include CO_2 emission uptake and the treatment of wastewater containing nutrient components like N and P. Microalgae fine chemicals and bioactive compounds are generally found and used as human health care products, and animal feed.

Although algae show great potential, significant economic, technical challenges remain to be solved in order to scale up for mass production. One of the major drawbacks of algal application compared with in-land plants is that if the algae are to be used in dry form, the cost of harvest and drying can be significant and seriously affects the whole feasibility of such application. In addition, the cost of nutrient can be unattractive and the search for a new cheaply available nutrient substitute is also important. This research seeks appropriate nutrient management in the cultivation of microalgae. This can be achieved through the detail examination of specific nutrient requirement of the algae followed by the formulation of the

nutrient. This has to be performed along with the management of the spent nutrients to ensure that all nutrients can be used effectively. Two microalgae (*Scenedesmus* sp. and *Ankistrodesmus* sp.) are selected as the case studies and the flat panel airlift photobioreactor is employed as a closed cultivation system.

1.2 Objectives

- To examine nutrient requirement for *Scenedesmus* sp. and *Ankistrodesmus* sp.
- To reduce cultivation cost through the management of spent nutrients

1.3 Scopes

The cultivation system works under indoor condition with defined light intensity and at room temperature.

- Green algae (*Scenedesmus* sp. and *Ankistrodesmus* sp.) were chosen for this study.
- The alga was cultivated in a batch cultivation system (25 L flat panel airlift photobioreactor).
- The air flow rate was varied varying from 0.1 to 0.4 vvm.
- The light intensity was examined in the range of 10,000 and 30,000 lux for *Scenedesmus* sp. and *Ankistrodesmus* sp. respectively.
- Residual nutrients and the nutrient compositions were examined for their most appropriate conditions that gave maximum economical benefit.

Chapter 2

Backgrounds and literature review

2.1 Microalgae

Microalgae are photosynthetic organisms found in both marine and freshwater environments. Similar to other plants, algae use photosynthesis to convert sunlight energy into chemical energy, and store in the form of proteins, carbohydrates, and lipids. Algae primarily require three components to grow: sunlight, carbon dioxide, and water. Microbiologists have categorized microalgae into a variety of classes, mainly distinguished by basic cellular structure, pigmentation, and life cycle. Microalgae are composed of prokaryote and eukaryote individuals. The most economically important microalgal families are *Cyanophyceae*, *Chlorophyceae*, *Rhodophyceae*, *Chrysophyceae* and *Bacillariophyceae*, (Figure 2.1) and thus a screening process to determine the best suitable strains for production is required e.g. biofuel, bioactive compound, human health and animal feed. The screening process certainly needs to look at what types of products are available from each biomass.

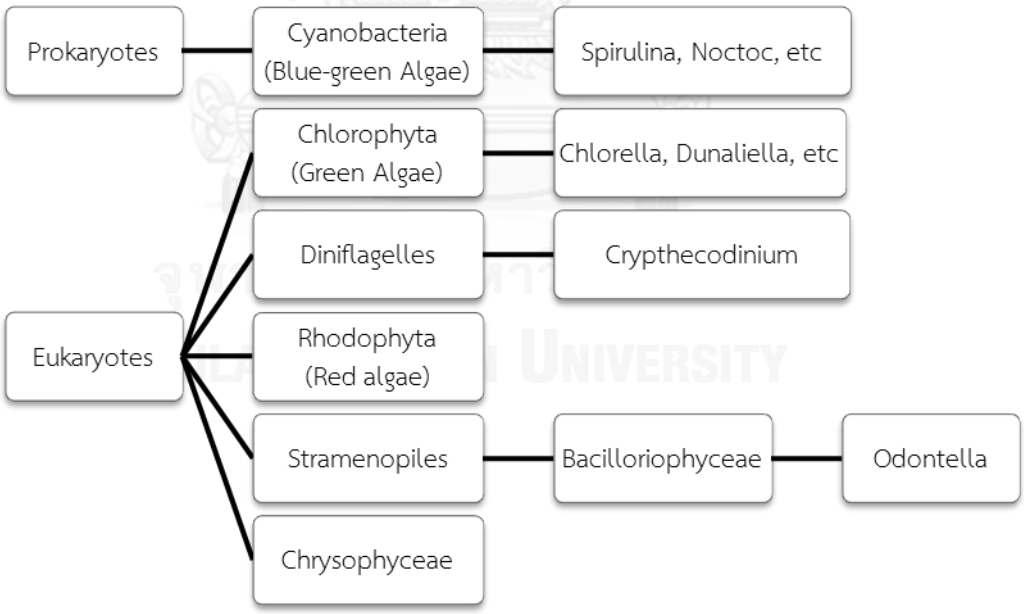


Figure 2.1 Microalgae classification

2.1.1 Green Microalgae

Green algae make up the division Chlorophyta and unicellular organisms. Some of the unifying characteristics of this division include similar photosynthetic pigments that make up the chloroplast, which includes Chlorophylls a and b, xanthophylls and primary carotenoids. Almost all green algae store their carbohydrates in the form of starch. Their cell walls are typically composed of polysaccharides, including lipolipid. The most obvious organelle of green algae is their chloroplast, which is responsible for giving them their green color.

Table 2.1 General composition of different algae (% of dry matter) (Becker, 2007)

Strain	Protein	Carbohydrates	Lipids	Nucleic acid
Scenedesmusobliquus	50-56	10-17	12-14	3-6
Scenedesmusquadricauda	47	-	1.9	-
Scenedesmusdimorphus	8-18	21-52	16-40	-
Chlamydomonasrheinhardii	48	17	21	-
Chlorella vulgaris	51-58	12-17	14-22	4-5
Chlorella pyrenoidosa	57	26	2	-
Spirogyra sp.	6-20	33-64	11-21	-
Dunaliellabioculata	49	4	8	-
Dunaliellasalina	57	32	6	-
Euglena gracilis	39-61	14-18	14-20	-
Prymnesiumparvum	28-45	25-33	22-38	1-2
Tetraselmismaculata	52	15	3	-
Porphyridiumcruentum	28-39	40-57	9-14	-
Spirulinaplatensis	46-63	8-14	4--9	2-5
Spirulina maxima	60-71	13-16	6-7	3-4.5
Synechoccus sp.	63	15	11	5
Anabaena cylindrica	43-56	25-30	4-7	-

2.1.2 *Scenedesmus* sp.

Scenedesmus sp. is a green alga under the scientific classification as detailed in Table 2.2. The cell can be either immobile or staying together as a colony. The colonies mostly have two or four cells but may occasionally have 8, 16 or 32 cells attached side by side. The shape of cell can be various, i.e. crescent, spindle-shaped or ovoid. Growth may be dense in nutrient-rich mediums but is not typically considered a nuisance. Like many other algae, *Scenedesmus* is an important primary producer and food source for higher trophic levels. Also it is a common bioindicator of physical and chemical changes in environmental conditions. The genus is commonly used to detect the presence of nutrients or toxins resulting from anthropogenic inputs to aquatic systems. *Scenedesmus* sp. was often found to be predominant species in conventional mixed culture waste water treatment plant sites (Xin *et al.*, 2010). In addition, Makarevičien *et al.* (2011) described that the biomass of *Scenedesmus* sp. was suitable to biofuel production.

Table 2.2 Scientific classification of *Scenedesmus*

Domain	Eukaryota
Kingdom	Protista
Division	Chlorophyta
Class	Chlorophyceae
Order	Chlorococcales
Family	Scenedesmaceae
Genus	Scenedesmus

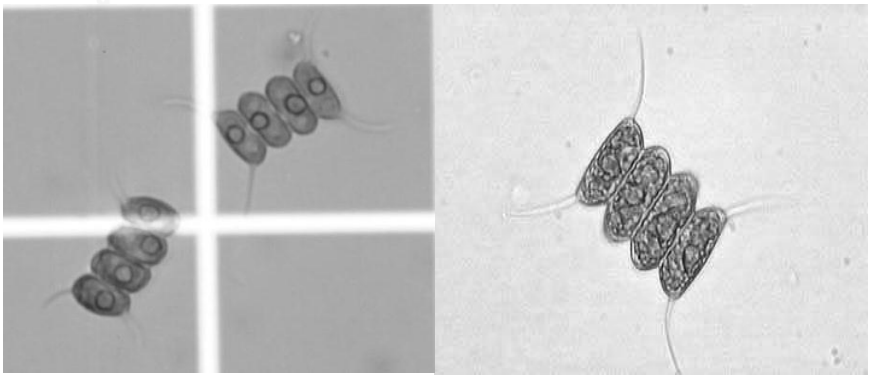


Figure 2.2 *Scenedesmus* sp.

Table 2.3 Reviews of *Scenedesmus* cultivation

Author	Strains	Reactor	Medium	Volume (L)	T (°C)	Light intensity (μmol m^{-2} s^{-1})	pH	Aeration rate (L min^{-1})	CO_2 (% of air)	Time (d)	Cell concentration (cell mL^{-1})	Biomass Concentration (g L^{-1})	Productivity (g L^{-1} d^{-1})	μ	Chemical composition
Kunikane *et al.* (1984)	*S. Dimorphus*			0.1	25±1		7	4		4					
Macedo and Pinto-Corlho (2001)	*S.armatus*			2	21±3	11-19					4.0×10^4				Lipids=7.8%
Tukaj *et al.* (2003)	*S.armatus*	Plate parallel vessel	Bristol's medium	0.6	30	21.82									
Sánchez Mirón *et al.* (2000)	*S.almeriensis*	Bubble column	Mann and Myer's medium	2	34	1,625		1					0.73		Lipid = 12% Carbohydrate = 24.6% Protein =49.4%
Yoo *et al.* (2010)	*S.*sp.		BG 11		25±1	150			10	14			0.2175		
Kim *et al.* (2011)	*S.* sp.		BG11	0.2	25±1	150	7.5		1	16					
Tang et al. (2011)	*S. obliquus*	Bubble	BG11	0.8	25±1	180	7	0.2	10	14		1.84±0.01	1.55±0.004	0.037 (max)	Lipid =19.3%
Goswami and Kalita (2011)	*S. quadricauda*	Batch	BG11	1	25	47	7.5			11		1.523			

2.1.3 *Ankistrodesmus* sp.

Ankistrodesmus is a unicellular, uninucleate green alga commonly found in the phytoplankton of small ponds. It is a photoautotrophic microorganism that utilizes the energy from the sun to produce its own energy (ATP) as well as relying on CO_2 as a carbon source. Nevertheless, if needed, it can also grow heterotrophically *Ankistrodesmus* is needle-like in shape, with gradually tapering ends. Usually it is about 3 μm in diameter at the broadest point, and it averages about 40 μm in length. Also it is known to be an excellent lipid producer and potential feedstock for biodiesel production and the chemical composition of early promising microalgae including high producers of hydrocarbons, carbohydrates, proteins, and lipids.

Table 2.4 Scientific classification of *Ankistrodesmus*

Domain	Eukaryota
Kingdom	Viridiplantae
Division	Chlorophyta
Class	Chlorophyceae
Order	Sphaeropleales
Family	Selenastraceae
Genus	Ankistrodesmus

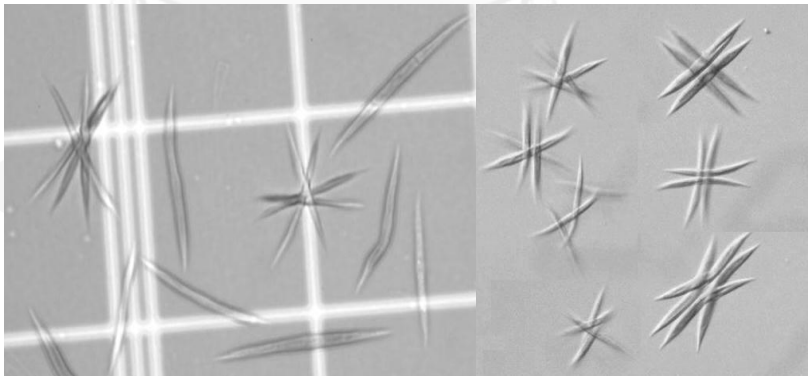


Figure 2.3 *Ankistrodesmus* sp.

Table 2.5 Reviews of *Ankistrodesmus* cultivation

Author	Strains	Reactor	Medium	Volume (L)	T (°C)	Light intensity (μmol m^{-2} s^{-1})	pH	Aeration rate	CO_2	Time (d)	Cell concentration (cell mL^{-1})	Biomass Concentration (g L^{-1})	Chemical composition
Ullrich and Glaser (1982)	*A.Braunii*	Batch	Inorganic		30	400	8			0.6			
Talbot *et al.* (1991)	*A.falcatus*	Batch	Phormidiumbohneri	0.25	20	650		1 vvm					
Nayak *et al.* (1996)	*A.falcatus*	Batch		0.2	27±2	70	7.0				150 $x10^{2}$	0.3	
Macedo and Pinto-Corlho (2001)	A.gracilis	Batch		2	21±3	11-19					4.0 $x10^{4}$		Lipids=11.2%
Habib *et al.* (2004)	*A.convolutus*		Inorganic	70			7						
Sipaúba-Tavares and Pereira (2008)	*A.gracilis*	Batch	CHU_{12}	850	22±2	21.48	6.7			17	135$x10^{4}$		Protein = 47-70% Carbohydrates=5% Lipids=7.48%
Sipauba-Tavares *et al.* (2011)	A.gracilis	Glass Fiber	Macrophyte+NPK	2 L	22±2	51.2	7.3±1			22	74.16 $x10^{5}$		Protein = 51.79±0.47 Lipids = 6.20±0.29

2.2 Main parameters for microalgae cultivation

There exist crucial considerations needed to be addressed including algal strain selection, nutrient additions, and control of multiple variables such as residence time, media pH, and light intensity to maximize production of targeted intercellular compounds. A summary of optimal culture conditions is illustrated in Table 2.6.

2.2.1 Nutrients

Primary nutrients necessary for the growth of microalgae arenitrogen and phosphorus as well as silica for diatom. The nutrients required for algae cultivation contain both trace metals and Vitamin such as thiamin (B1), cyanocobalamin (B12) and biotin.

2.2.2 Light

Algae use sunlight for photosynthesis, and therefore this is an important energy source. Artificial light sources may be used such as fluorescent light as long as it covers the wavelength required by algae.

2.2.3 pH

pH level suitable for the cultivation of algae is in the range from 7-9, for instance, *Spirulinaplatensis* ~ 9 (Chen & Zhang, 1997) and *Scenedemus* sp. ~ 7.5 (Kim *et al.*, 2011).

2.2.4 Aeration

Proper aeration can give a mixing level between algae and medium water. This is necessary to prevent the precipitation of cultured algae and to ensure an even distribution of algal biomass and nutrients. This also helps prevent the accumulation of heat in the system particularly for outdoor culture, and lastly to enhance the exchange of gases and prevent the accumulation of oxygen in the culture (Pulz, 2001a, Pulz, 2001b).

2.2.5 Temperature

Temperature is one of the most important limiting factors for culturing algae. Optimal temperature of each species is various and occasionally based on other environmental parameters such as light intensity (Kumar *et al.*, 2010).

Table 2.6 Literature reviews

Author	Strains	Reactors	Medium	Volume (L)	T (°C)	Light intensity (μmol m^{-2} s^{-1})	pH	Cell concentration (Cell mL^{-1})	Biomass concentration (g L^{-1})	Productivity (g L^{-1} d^{-1})	Specific growth rate (d^{-1})
Merchuk *et al.* (1998)	*Porphyridium* sp.	Airlift	Organic complex	35	25	300		36			0.528
			Organic complex								
		Bubble column		35	25	300		36			0.528
Sato *et al.* (2006)	*Chlorococumlittorale*	Pipe photobioreactor		70	25		7.5		1.75	0.146	
Chini Zittelli *et al.* (2006)	*Tetraselmissuecica*	Bubble column		60	14.6-30.2	48.2-327.8	7.2-9.0	$9.95x10^{6}$			
Oncel and Sukan (2008)	*Spirulinaplatensis*	Airlift	Zarrouk's medium	1.5	25	54	9.3		2.21		0.45
		Bubble column	Zarrouk's medium	1.5	25	54	9.3		1.87		0.33
Tang *et al.* (2011)	*Scenedesmusobliquus* SJTU-3	Bubble column	BG11	0.8	25±1	180	7		1.84±0.01	0.155±0.01	0.037 (Max)
	Chlorella pyrenoidosa SJTU-2	bubble column							1.55±0.01	0.144±0.011	0.041 (Max)
Xu *et al.* (2011)	*Botryococcusbraunii*	Airlift bioreactor	Chu 13	1.8	25±1	35			2.56		

2.3 Photobioreactor for microalgae cultivation

Production of algal biomass can be carried out in fully contained photobioreactors or in open channels and ponds.

2.3.1 Open ponds

Cultivation of algae in open ponds has been extensively studied. Open ponds can be categorized into natural waters (lakes, lagoons, ponds) and artificial ponds or containers. The most commonly used systems include shallow big ponds, tanks, circular ponds and raceway ponds. Major advantages of open-culture system are their low installation and operating costs. They are normally more durable than large closed reactors and with a larger production capacity when compared with closed systems (Mata et al., 2010). However, major limitations in open ponds include poor light distribution, poor mixing, evaporative losses, diffusion of CO_2 to the atmosphere, and the fluctuation in environmental conditions can affect their growth and productivity.

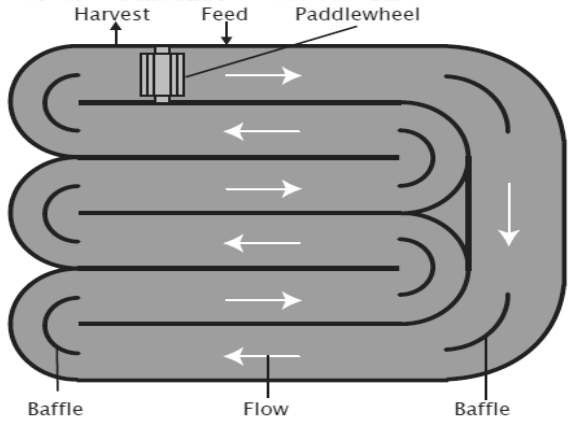


Figure 2.4 Open pond on top view (Singh et al., 2003)

2.3.2 Photobioreactors

Photobioreactors (PBRs) are closed equipments which provide a controlled environment and enable high productivity of algae. As it is a closed system, all growth requirements of algae are introduced into the system and controlled according to the requirements. There are many types of PBRs such as flat plate, tubular, bubble and airlift photobioreactors:

Flat plate photobioreactors

The flat panel reactor is basically a flat, transparent vessel in which mixing is carried out directly in the reactor with air sparging. The normal aeration level for flat panel photobioreactors is 1 liter of air per liter reactor volume per minute (Sierra *et al.*, 2008). The design examined here is the closely spaced, vertical flat panel reactor, in which light dilution is obtained by applying larger specific surface and self-shading of the panels. In this way, it is possible to achieve a higher photosynthetic efficiency, despite its higher mixing and installation costs (Norsker *et al.*, 2011).

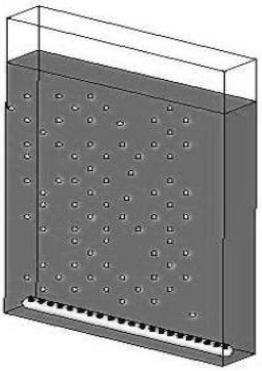


Figure 2.5 Flat plate photobioreactors (Posten & Schaub, 2009)

Tubular photobioreactors

A tubular reactor can be in form of vertical or horizontal arranged tubes and usually constructed with either borosilicate glass or transparent plastic tube. The algae-suspended fluid is able to circulate in this tubing and the circulation is maintained by a pump at the end of the system. It is one of the most suitable types for outdoor mass culture.

Figure 2.6 Tubular photobioreactors (Posten & Schaub, 2009)

Bubble column photobioreactors

A bubble column photobioreactor consists of vertical arranged cylindrical column, can be from transparent material. The gas takes place at the bottom of the column and causes a turbulent to gas exchange in the form of bubbles, comes in contact with liquid. The bubble column is simple, easy to design, provides good heat and mass transfers at low energy input. Therefore they gain wide acceptance as gas-liquid contactors.

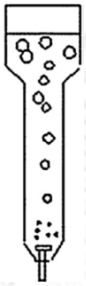


Figure 2.7 Bubble column photobioreactor (Singh *et al.*, 2003)

Airlift photobioreactors

Airlift bioreactors are similar to bubble column reactors, but they are normally vertically divided into riser and downcomer sections. This can be achieved with the installation of a draft tube for a cylindrical shape column or a separate plate for a rectangular tubular column. This geometry helps induce the circulatory effect in the reactor and prevent the accumulation of cell biomass, and in certain cases, enhance the vertical light distribution particularly for clear columns. The airlift devices clearly attain the induced liquid circulation velocity at relatively low power input for practicable culture of microalgae (Miron, 2000). This work also studies this specific type of airlift bioreactor called Flat panel airlift photobioreactor (FPAP).

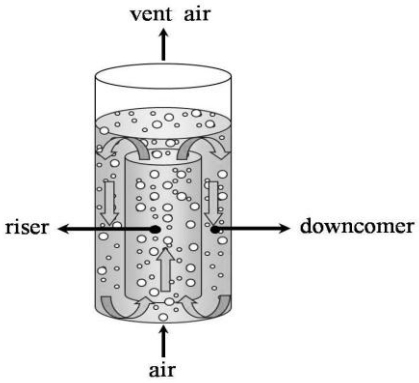


Figure 2.8 Airlift photobioreactor (Krichnavaruk *et al.*, 2005)

2.4 Reduced nutrient

Considerable variation in algal biochemical composition under conditions of nutrient limitation can be observed depending on which nutrient is limited. In general, the growth rate of algae is proportional to the uptake rate of the most limiting nutrient under optimal conditions. Nitrogen and phosphate are two important macronutrients for growth and metabolism of algal cells. Specific effects of major nutrients are discussed in Table 2.7.

Nitrogen is a fundamental element for the formation of proteins and nucleic acids. Being an integral part of essential molecules such as ATP, the energy carrier in cells, phosphate is another very important nutrient. Phosphate is also a part of the backbone of DNA and RNA, which are essential macromolecules for all living cells. Phosphorus is also a key component of phospholipids. It is not unusual for algae to become nutrient-limited (i.e., nitrogen- and phosphorus-limited) in the natural environment (Harris, 1986). Limitation of these key nutrients shifts the metabolic pathway of the organism. For example, nitrogen and phosphorus starvation shifts the lipid metabolism from membrane lipid synthesis to neutral lipid storage. This, in turn, increases the total lipid content of green algae (Hu, 2004).

Trace metals are metals present in algal cells in extremely small quantities (<4 ppm) but they are an essential component of phycophysiology. Deficiencies in trace metals can limit algal growth, whereas excesses or high metal concentrations (above the toxicity threshold) may inhibit growth, impair photosynthesis, deplete antioxidants, and damage the cell membrane.

Table 2.7 Summary of general impact of environmental factors on biochemical composition of algae.

Nutrient	Organism	Conditions	Biochemical change observed	Reference
Nitrogen	*Nannochloropsis oculata*	75% decrease in Nitrogen	Increase in lipid synthesis from 7.90% to 15.31%	Xin *et al.* (2010)
	Phaeodactylum tricornutum	Nitrogen limitation	Increase in lipid synthesis; Decrease in protein content	Morris *et al.* (1974)
	Chlorella vulgaris	75% decrease in Nitrogen	Increase in lipid synthesis from 5.90% to 16.41%	Xin et al. (2010)
	Haematococcus pluvialis	Nitrogen limitation	Increase in carotenoid formation (13% w/w)	Harker *et al.* (1996)
Phosphorus	*Chlamydomonas reinhardtii*	Limitation	Decrease in phosphatidylglycerol	Sato *et al.* (2000)
	Ankistrodesmus falcatus	Limitation	Decrease in chl *a* and protein; Increase in carbohydrate and lipids	KILHAM *et al.* (1997)
	Selenastrum minutum	Starvation	Reduced rate of respiration; Decreased photosynthetic CO_2 fixation	Theodorou *et al.* (1991)
	Scenedesmus sp.	Limitation	Increase in lipid content from 23% to 53%	Xin et al. (2010)
Iron	*Dunaliella tertiolecta*	Limitation	Decrease in cellular chlorophyll concentration	Greene *et al.* (1992)
	Chlorella vulgaris	High concentration of iron	Increase in lipid content	Liu *et al.* (2008)
	Haematococcus pluvialis	High concentration of iron	Increase in carotenoid formation	Kobayashi *et al.* (1993)

2.5 Cost of microalgae cultivation

The economic analysis is useful to review the major cost factors for production of microalgae cultivation. The microalgae production requires large volumes of water, thus the cost of water and availability must be known. In additions, water need be supplemented with nutrients. To avoid the use of freshwater and nutrients, it has been suggested that recirculation water can reduce the water consumption (and reduce nutrient cost). Moreover, in the commercial-scale production of microalgae, the reduced or reused medium is an important consideration.

The system availability and production days are most of existing studies assumed that production can be all year around, however, depending on the location there may be several months of the years that are not suitable for harvesting as the temperature inhibits growths. Systems may also need to stop operation for maintenance and system cleaning on a periodic basis. This study chose 300 day for data collected from literature (Richardson *et al.*, 2010), assumed two months are not suitable for production based on temperature and maintain system.

Table 2.8 The water cost

Literature review	$/m^3	Notes
Neenan *et al.* (1986)	0.05-0.20	Reference value of $0.067 in 1984 dollars
Weissman *et al.* (1988)	0.012-0.26	Cheaper source is saline groundwater at 800 gallons per minute and more expensive source is city water
Molina Grima *et al.* (2003)	0.0294	Water used in photobioreactor
Singh et al. (2003)	0.0100	Cost of cooling water

Table 2.9 The medium Cost

Literature review	$/m^3	Notes
Molina Grima et al. (2003)	0.5883	Takes 2.5 kg of medium to produce 1 kg of algal biomass in photobioreactor
Tapie and Bernard (1988)	0.2700	For a photobioreactor
Stepan *et al.* (2002)	0.0190	Only accounts for cost of additional nutrients; some nutrients are received from CO2 flue gas

Chapter 3

Materials and Methods

3.1 Experimental Setup

Scenedesmus sp. and *Ankistrodesmus* sp. were cultivated in a specially designed 25 L non-baffled flat panel airlift photobioreactor (NB-FPAP) (see Figure 3.1) made from clear acrylic or glass with the dimension as shown in Figure 3.2. The slope of the bottom sheet is 30°. Air flow was supplied through rotameter to porous gas sparger. Light was supplied through 8 Compact Fluorescent 20W Lamps to reactor all day which gave a constant intensity of 10,000 and 30,000 LUX for *Scenedesmus* sp and *Ankistrodesmus* sp. (Pavasant, 2011), respectively, whereas the experiment setup was maintained at 30±5°C in the room temperature.

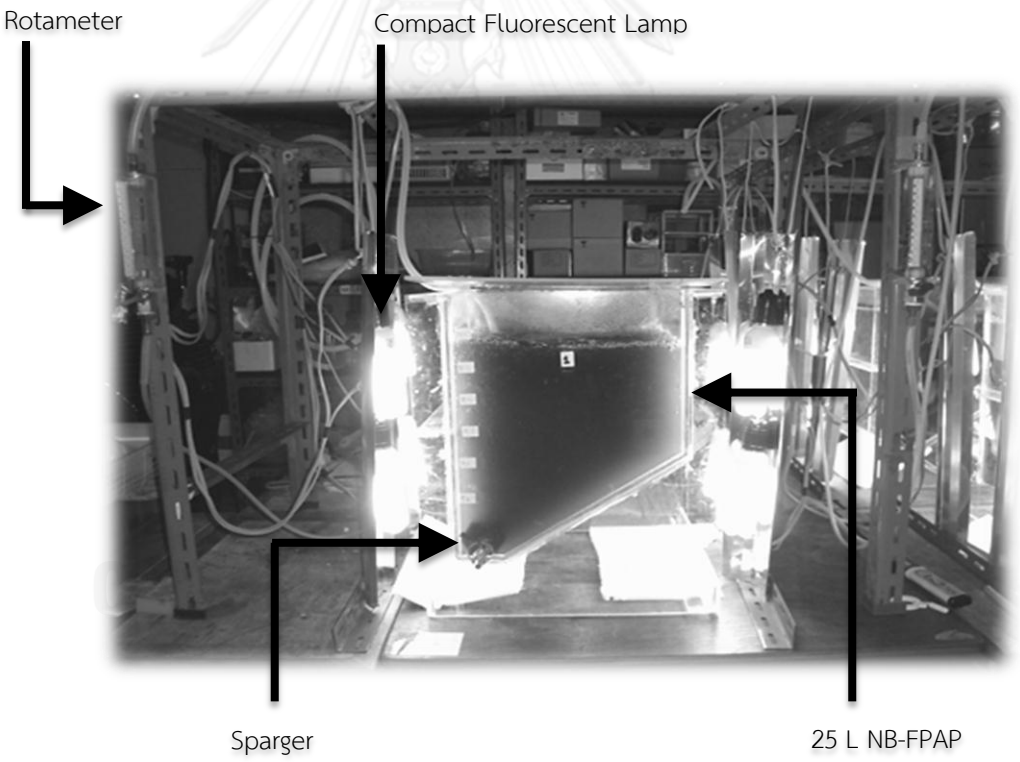


Figure 3.1 Experimental Setup

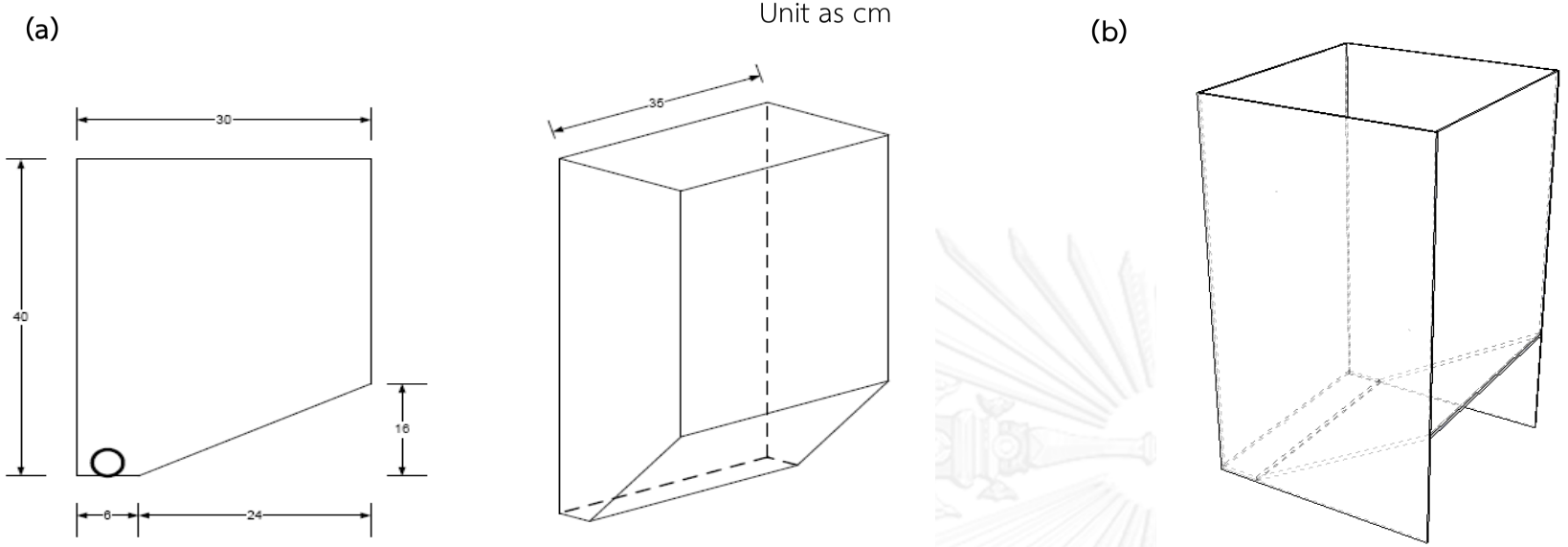


Figure 3.2 Schematic of NB-FPAP; (a) 2D view (b) 3D view

3.2 Culture medium preparation

Both green microalgae were cultivated with BG 11 medium (see composition in Table 3.1). The incubation was cultured in 2 L bottles and scaled up to the 25 L NB-FPAP

Table 3.1 composition of BG11 medium

Stock	Compositions	Concentration (gram per liter of deionized water, g L^{-1})
(1)	$NaNo_3$	15
	add 100 mL of stock (1) solution per liter of fresh water	
(2)	K_2HPO_4	4
(3)	$MgSO_4.7H_2O$	7.5
(4)	$CaCl_2.2H_2O$	3.6
(5)	Citric acid	0.6
(6)	Ammonium ferric citrate	0.6
(7)	$EDTANa_2$	0.1
(8)	Na_2CO_3	2.0
	Add 10 mL each of stock (2)-(8) solution per liter of fresh water	
(9)	Trace metal solution:	
	H_3BO_3	2.86
	$MnCl_2.4H_2O$	1.81
	$ZnSO_4.7H_2O$	0.22
	$Na2MoO_4.2H_20$	0.39
	$CuSO_4.5H_2O$	0.08

3.3 Cultivation system

3.3.1 Batch cultivation

1. Fill 25 L freshwater into reactor
2. Sterilize freshwater with sodium dichloroisocyanurate

3. Supply air through the porous sparger at the bottom of reactor for 1-2 days
4. Check for residual chlorine with potassium iodide, and if chlorine was exhausted, the water sample was clear. Otherwise a yellow solution is formed.
5. Add nutrients and initial cell from inoculum at concentration $4x10^5$ cell mL^{-1} of *Scenedesmus* sp. and repeat for *Ankistrodesmus* sp. at initial cell $1x10^6$ cell mL^{-1}
6. Supply air in the rage 0.1 vvm for *Scenedesmus* sp. and 0.2 vvm for *Ankistrodesmus* sp. and measuring light intensity and temperature
7. Take sample and count for the cell density using Haemacytometer everyday or until the exponential growth was reached
8. Repeat Steps 1-7 with air flow rate of 0.2 and 0.3 vvm for *Scenedesmus* sp., and 0.3 and 0.4 vvm for *Ankistrodesmus* sp.

Remark: this result shows in Appendix A1

3.3.2 Cultivation with reduced nutrient

1. Repeat Steps 1-4 in Section 3.3.1
2. Adjust nutrient content following these conditions: 50% Nitrogen (N), 50% Phosphorus (P), 50% Nitrogen and Phosphorus (N&P) and 100% Medium from BG11 Medium composition (% of original concentration in BG11)
3. Add initial cell from inoculum at cell concentration of $4x10^5$ and $1x10^6$ cell mL^{-1} for *Scenedesmus* sp. and *Ankistrodesmus* sp., respectively
4. Supply air at 0.2 vvm for *Scenedesmus* sp. and 0.3 vvm for *Ankistrodesmus* sp., measure light intensity and temperature (optimal air flow rate from section 3.3.1)
5. Take sample and count for the cell density using Haemacytometer everyday for 4-5 days for *Scenedesmus* sp. and 8-9 days for *Ankistrodesmus* sp.
6. Separate biomass and residual nutrient medium with centrifugal machine (1,732 xg)
7. Dewater from algae cake by freeze dry (vacuum 0.024 mbar and collector $-49^{\circ}C$)

8. Analyse for growth, biochemical composition and nutrients following methods described in Section 3.4
9. Repeat Steps 1 and 3-8 with the conditions as follows: 25%N, 25%P, 25%N-P and 100% Medium

3.3.3 Cultivation with reuse medium

1. Repeat Steps 1-5 in Section 3.3.1
2. Repeat Steps 4-6 in Section 3.3.2
3. Fill the remaining water with residual nutrient medium into 25 L reactor without sterilize
4. Add initial cell from inoculumn to remaining water at concentration of $4x10^5$ and $1x10^6$ cell mL^{-1} for *Scenedesmus* sp. and for *Ankistrodesmus* sp., respectively
5. Supply air at 0.2 vvm for *Scenedesmus* sp. and 0.3 vvm for *Ankistrodesmus* sp., measure light intensity and temperature
6. Take sample and count for the cell density using Haemacytometer everyday for 4-5 days for *Scenedesmus* sp. and 8-9 days for *Ankistrodesmus* sp.
7. Separate biomass and residual nutrient medium with centrifugal machine (1,732 xg)
8. Dewater from algae cake by freeze dry (vacuum 0.024 mbar and collector $-49^{\circ}C$)
9. Repeat 3-7 (include 3 times)
10. Analyse (include 3 times) for growth, biochemical composition and nutrients following methods described in Section 3.4

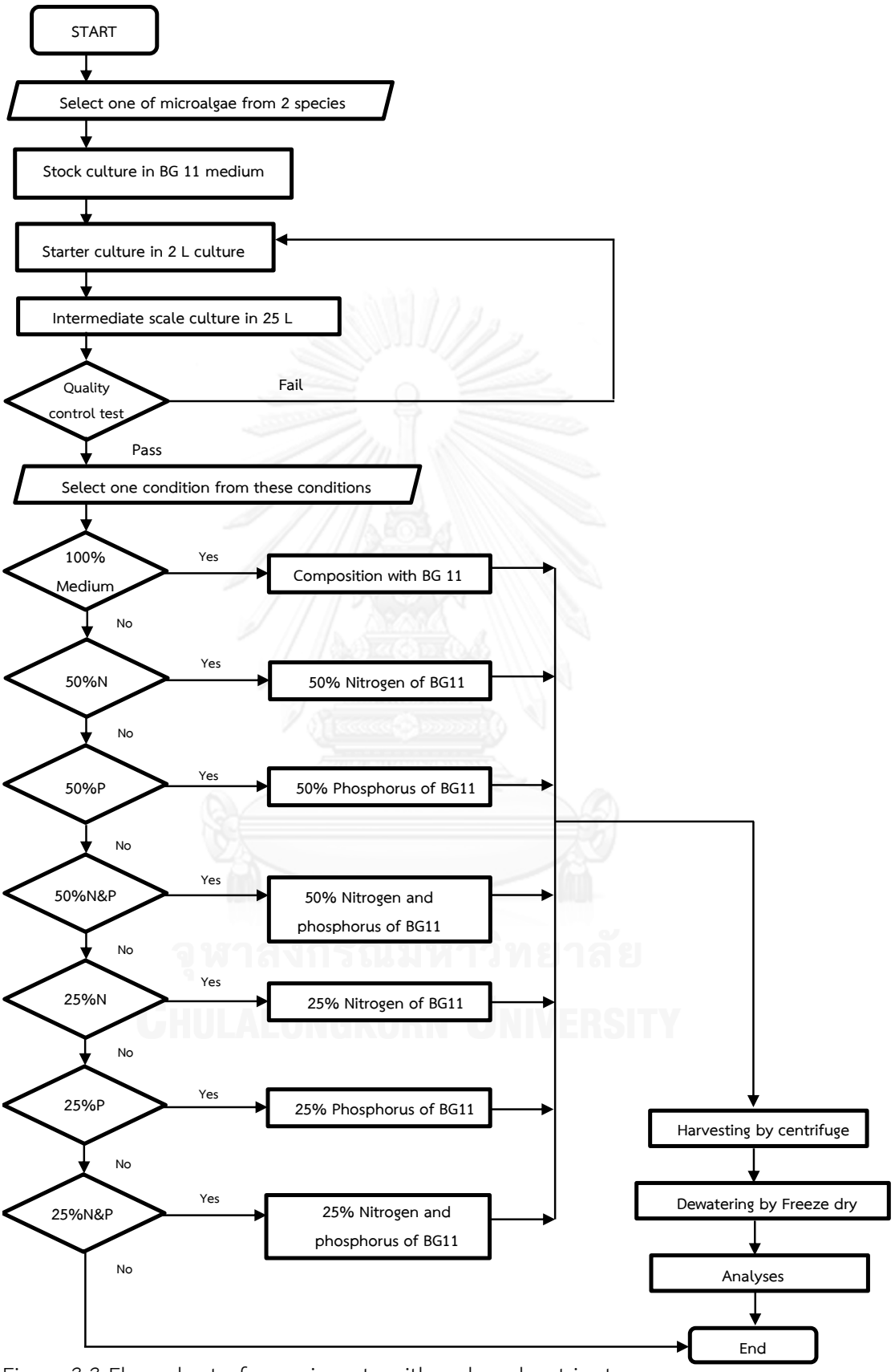


Figure 3.3 Flow chart of experiments with reduced nutrient

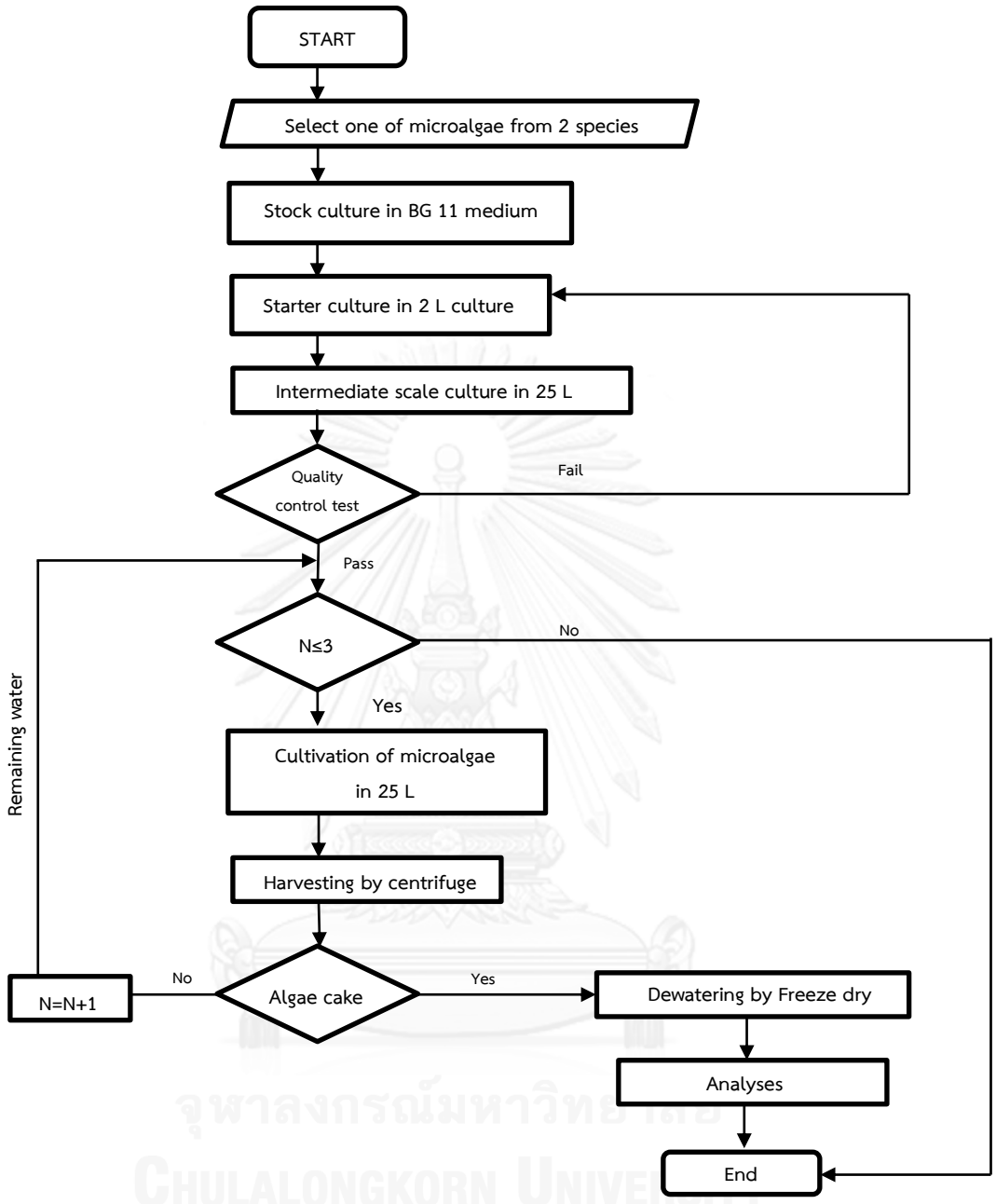


Figure 3.4 Flow chart of experiments with reused medium

3.4 Analyses and Calculations

3.4.1 Cell density

The cell density was determined using a normal blood cell counting slide. Haemcytometer, the depth of the counting grid and the medium area are 0.1 mm and 0.04 mm^2, respectively. The average cell count can be estimated from Equation 3.1 where the conversion factor ($x10^4$) is given in Equation 3.1.

1. Clean the counting slide and cover glass
2. Fill the slide with sample
3. Cover the slide with cover glass, avoid of bubbles
4. Count the cell in 25 medium squares on the grid (per 1 large square)

$$N = n \times 10^4 \qquad (3.1)$$

where

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

n = cell number was calculated from haemacytometer

3.4.2 Cell dry weight

Dry weight of algae could be determined by membrane filter and drying algae. The microalgal samples were taken and kept at a frequency of once in two days for *Ankistrodesmus* sp. and once a day for *Scenedesmus* sp. The cell concentration could be determined as follows:

$$N = \frac{n}{V} \times 1000 \qquad (3.2)$$

where

N = mass dry weight (g L^{-1})

n = mass dry weight of microalgae by filtration (g)

v = volume of sample (mL^{-1}).

3.4.3 Specific growth rate

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

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

where

μ = specific growth rate (d^{-1})

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

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

t_1 = first sampling time (d)

t_2 = second sampling time (d).

3.4.4 Productivity

The productivity is calculated by the following equation:

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

where

P = productivity (g d^{-1})

N_1 = mass dry weight at t_1 (g L^{-1})

N_2 = mass dry weight at t_2 (g L^{-1})

t_1 = first sampling time (d)

t_2 = second sampling time (d)

V = harvest volume (L).

3.4.5 Light intensity

The light intensity can be calculated from:

$$I = \frac{E}{74} \qquad (3.5)$$

where

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

E = light intensity (lux).

3.4.6 The lipid productivity

The lipid productivity is calculated from:

$$P = \frac{CL}{t} \times 1000 \qquad (3.6)$$

where

P = productivity (mg L^{-1} d^{-1})

CL = the concentration of lipid at the end of the batch run (mg L^{-1})

t = duration of the cultivation (d).

3.4.7 Determination of nutrient components

The concentrations of nitrate in medium were measured by spectrophotometer (Ulraviolet-Visible Spectrometer cary 50 series). The methods are shown in Appendix A1-1 and the concentrations of another nutrient could be measured with Induced Couple Plasma technique (ICP 700-ES series). The conditions are reported in Appendix A1-2.

3.4.8 Composition analysis

The carbon content along with nitrogen and hydrogen of *Scenedesmus* sp. and *Ankistrodesmus* sp. were measured using CHNS/O Analyzer (Perkin Elmer PE2400 Series II). Total lipids content are extracted by chloroform and methanol (2:1 by volume) with soxhlet apparatus.

3.5 Statistical analysis

ANOVA were conducted to test for result significance, using Data Analysis in Microsoft excel with α = 0.05. Any values (a, b and c) with the same letters in the same parameters indicate that the values did not differ by the tukey test at $p \leq 0.05$.

CHAPTER 4
RESULTS AND DISCUSSION

This research studies aim to reduce the cost of the two algal cultivation (*Ankistrodesmus* sp. and *Scenedesmus* sp.). The results and discussion in this chapter are divided into four sections. The first part shows the growth of two algae which were cultivated in the non-baffled flat panel airlift photobioreactor (NB-FPAP). The second part describes the use of reduced nutrient of BG11 in the cultivation of the two algae. In the third part, the remaining nutrient was reused in the algal culture to examine the growth of such culture. The last section provides economical analysis of the various cost reduction options employed in this work. Figure 4.1 illustrates the connections between the various sections in this discussion.

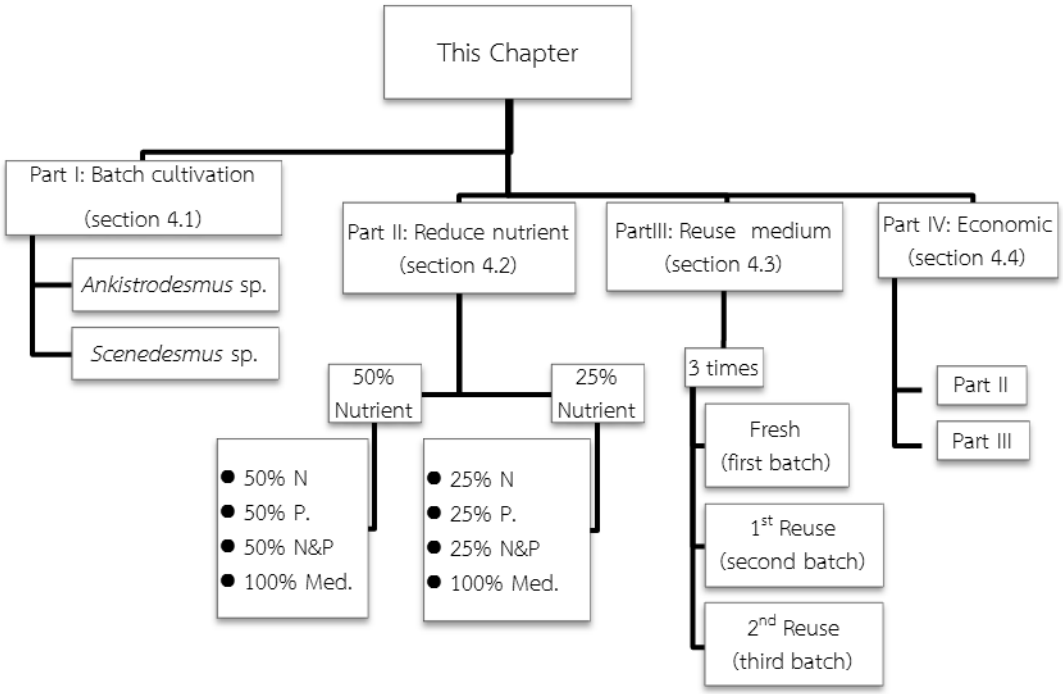


Figure 4.1 structure of results and discussion in this work

Table 4.1 optimal growth conditions

Condition	*Ankistrodesmus* sp.	*Scenedesmus* sp.
Initial cell (cell mL^{-1})	$1x10^6$	$4x10^5$
Light intensity (μmol m^{-2} s^{-1})	400	135
Air flow rate (vvm)	0.3	0.2

4.1 Batch cultivation

In this study, two microalgae (*Scenedesmus* sp. and *Ankistrodesmus* sp.) were cultivated in NB-FPAP 25 L. Both cultures consumed BG 11 as medium. The optimal growth conditions were determined by varying the growth conditions such as air flow rate and light intensity for *Scenedesmus* sp. and *Ankistrodesmus* sp. cultures and the results were identified and reported in Table 4.1.

4.1.1 Growth and biochemical composition of *Ankistrodesmus* sp. in NB-FPAP

The cultivation of *Ankistrodesmus* sp. in NB-FPAP is shown in Figure 4.2. This microalga spent one day of the lag phase, and entered the exponential growth phase, where the cells would quickly multiply themselves. After the ninth day, the cells reached their stationary growth phase, and stayed there for a period of time before decay. The maximum cell density was at $1.1\pm0.19x10^7$ cell mL^{-1} with maximum dry weight of 0.78±0.2 g L^{-1} (in Table 4.2). The temperature and pH profile of the culture are in Figure 4.3. Cells were harvested by centrifugal method in the 18^{th} day. After that, algal biomass was analysed for its biochemical composition.

%Biochemical compositions by weight (as Lipid, protein and carbohydrate) of *Ankistrodesmus* sp. are shown in Table 4.2 and Figure 4.8 (moisture and ash free). Lipid content of this microalga was 30±1%wt. Protein and carbohydrate contents were 32±1 and 27±0.2%wt, respectively. *Ankistrodesmus* was known as an excellent lipid producer and potential feedstock for biodiesel. Griffiths and Harrison (2009) reported *Ankistrodesmus falcatus* as an important microalga strained for bio-fuel production. Lipid content found in this work was within a similar range with that report by Do Nascimento *et al.* (2013). They reported that total lipid of *Ankistrodesmus* sp. cultivation in airlift photoreactor was 33.36±1.13%wt. However, depending on culture conditions biochemical content of the alga could be different.

For example, Sipauba-Tavares *et al.* (2011) only reported lipid content of 4-10%wt and protein at 26-63%wt.

4.1.2 Growth and biochemical composition of *Scenedesmus* sp. in NB-FPAP

The cultivation of *Scenedesmus* sp. in NB-FPAP is shown in Figure 4.5. The microalga was cultured for 6 days before harvesting. It spent one day of the lag phase before entering the exponential growth phase. It often reached the stationary growth phase in the fourth day and quickly decayed after that. The maximum cell density was $2.95\pm0.48\text{x}10^{6}$ cell mL^{-1} whereas the maximum dry weight was 0.25 ± 0.002 g L^{-1} (Table 4.2). The temperature and pH profiles of the culture are shown in Figure 4.6.

After centrifugation of *Scenedesmus* sp., the alga was measured for its biochemical composition as summarized in Table 4.2 and Figure 4.8. Carbohydrate content was higher than lipid and protein contents which were 37±2.7, 21±4 and 33±1, respectively. Macedo and Pinto-Corlho (2001) reported that *Scenedesmus quadricauda* displayed average lipid content at 11.1%wt which was only half the quantity found from this work. However, the cultivation of *Scenedesmus dimorphus* and *Scenedesmus quadricauda* with urea as nitrogen source could give lipid content as high as 34% and 31%wt, respectively (Goswami & Kalita, 2011) which were significantly higher than what obtained from this research. These differences could be due to the different species of the alga or different operating conditions, and still could not be concluded from this work.

4.1.3 Nutrient for *Ankistrodesmus* sp. and *Scenedesmus* sp. in NB FPAP

Concentration time profiles of nitrogen (NO_3^--N) and phosphorus (PO_3^--P) during the cultivation of *Ankistrodesmus* sp. and *Scenedesmus* sp. are shown in Figures 4.4 and 4.7, respectively. Both nutrients were only gradually decreased during the growth indicating that only small amounts of such nutrients were required for algal growth.

Table 4.3 shows %reduction of all elements in BG11 medium which revealed that *Ankistrodesmus* sp. only consumed 2.5% of the total nitrogen provided in the nutrient whereas *Scenedesmus* sp. consumed a much larger portion at 15.0%. On the other hand, *Ankistrodesmus* sp. required much more phosphorus than *Scenedesmus* sp. as phosphorus was reduced down to 80.8% for *Ankistrodesmus* sp. whereas this was down to 42.4% for *Scenedesmus* sp. The two algae also consumed other elements differently but as their concentrations were relatively small and did

not markedly affect the nutrient cost, it was not discussed in detail here. For example, Molybdenum (Mo) was important to enzyme activity such as nitrate reductase. The investigation of this element found that reductions of molybdenum for *Ankistrodesmus* sp. and *Scenedesmus* sp. were 0.6% and 37.3% of the total concentration, respectively. The iron reduction was 54.5% and 97.4% of the total supply for *Ankistrodesmus* sp. and *Scenedesmus* sp., respectively. In short, %reduction of the various nutrients depended significantly on microalgal species and perhaps the culture conditions which had to be identified case by case.

As nitrogen and phosphorus were the two major nutrients which affected the overall cost of nutrient for algal cultivation, this study only focused on the reduction and reuse of such nutrients for the effect on growth and biochemical compositions in NB-FPAP of the two microalgae (Section 4.2).

Table 4.2 Growth and biochemical composition of *Ankistrodesmus* sp. and *Scenedesmus* sp. obtained from batch cultivation in NB-FPAP

Characters	*Ankistrodesmus* sp.	*Scenedesmus* sp.
Maximum cell density (cell mL^{-1})	$1.1\pm0.19\times10^{7}$	$2.95\pm0.48\times10^{6}$
Specific growth rate (d^{-1})	0.08±0.003	0.32±0.07
Maximum dry weight (g L^{-1})	0.78±0.2	0.25±0.002
Productivity (g d^{-1})	0.66±0.11	0.45±0.04
% Lipid	30±1	21±4
% Protein	32±1	33±1
% Carbohydrate	27±0.2	37±2.7
% Moisture	2.68±1.18	0.17±0.01
% Ash	8.1±0.5	10.1±1.9
Lipid productivity (mg L^{-1} d^{-1})	9±0.6	2±0.2
Protein (mg L^{-1} d^{-1})	6±0.1	6±0.2
Carbohydrate (mg L^{-1} d^{-1})	5±0.04	7±0.51

Table 4.3 Reduction (%) of elements of batch culture by *Ankistrodesmus* sp. and *Scenedesmus* sp. in NB-FPAP

Elements	*Ankistrodesmus* sp.			*Scenedesmus* sp.		
	Concentration (mg L^{-1})		% Reduction	Concentration (mg L^{-1})		% Reduction
	Initial day	Final day		Initial day	Final day	
N	250	244	2.5	306	260	15.0
P	1.97	0.38	80.8	3.71	2.14	42.4
B	0.683	0.518	24.1	0.834	0.831	0.3
Ca	20.9	14.6	30.3	0.034	0.033	2.7
Mg	10.5	7.6	28.4	14.0	13.3	4.6
Fe	0.008	0.004	54.5	0.270	0.007	97.4
Mn	0.010	0.004	60.2	0.135	0.100	25.8
Zn	0.371	0.301	18.8	0.094	0.091	3.4
Mo	0.140	0.139	0.6	0.251	0.156	37.7
Cu	0.016	0.005	67.7	0.076	0.007	90.7
Co	0.007	0.001	83.3	0.002	0.001	40.0
K	146	133	8.8	146	120	17.7

Table 4.4 Uptake rate of available nutrients from batch cultures of *Ankistrodesmus* sp. and *Scenedesmus* sp. in NB-FPAP

Time (day)	Uptake rate for substrate ($Y_{s/x}$)			
	ΔN/ΔX		ΔP/ΔX	
	Ankistrodesmus sp.	*Scenedesmus* sp.	*Ankistrodesmus sp.*	*Scenedesmus* sp.
0				
1	$7.7x10^{-5}$	-0.167	-0.027	-0.003
2	$8.3x10^{-5}$	-0.213	-0.028	-0.004
3	$9.0x10^{-5}$	-0.304	-0.030	-0.006
4	$9.9x10^{-5}$	-0.554	-0.033	-0.010
5	$1.1x10^{-4}$	-4.419	-0.036	-0.080
6	$1.2x10^{-4}$	0.689	-0.039	0.012
7	$1.4 x10^{-4}$		-0.044	
8	$1.6 x10^{-4}$		-0.050	
9	$1.8 x10^{-4}$		-0.057	
10	$2.2 x10^{-4}$		-0.068	
11	$2.7 x10^{-4}$		-0.085	
12	$3.7 x10^{-4}$		-0.112	
13	$5.6 x10^{-4}$		-0.169	
14	$1.2 x10^{-3}$		-0.345	
15	$-1.7 x10^{-2}$		5.129	
16	$-1.0 x10^{-3}$		0.299	
17	$-5.2 x10^{-4}$		0.152	
18	$-4.3x10^{-4}$		0.137	

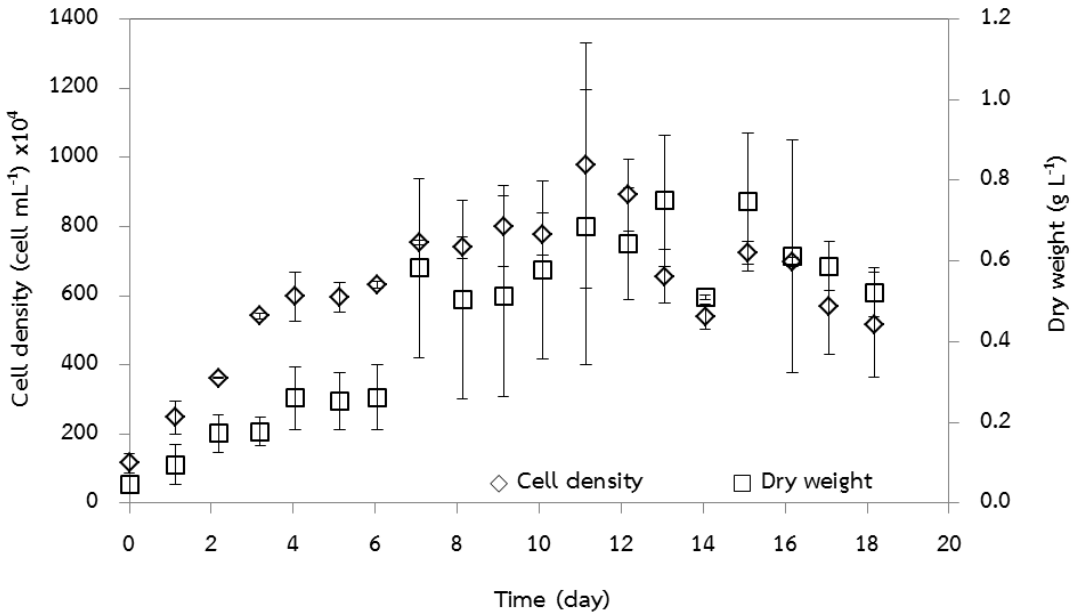


Figure 4.2 Growth of *Ankistrodesmus* sp. culture in NB-FPAP

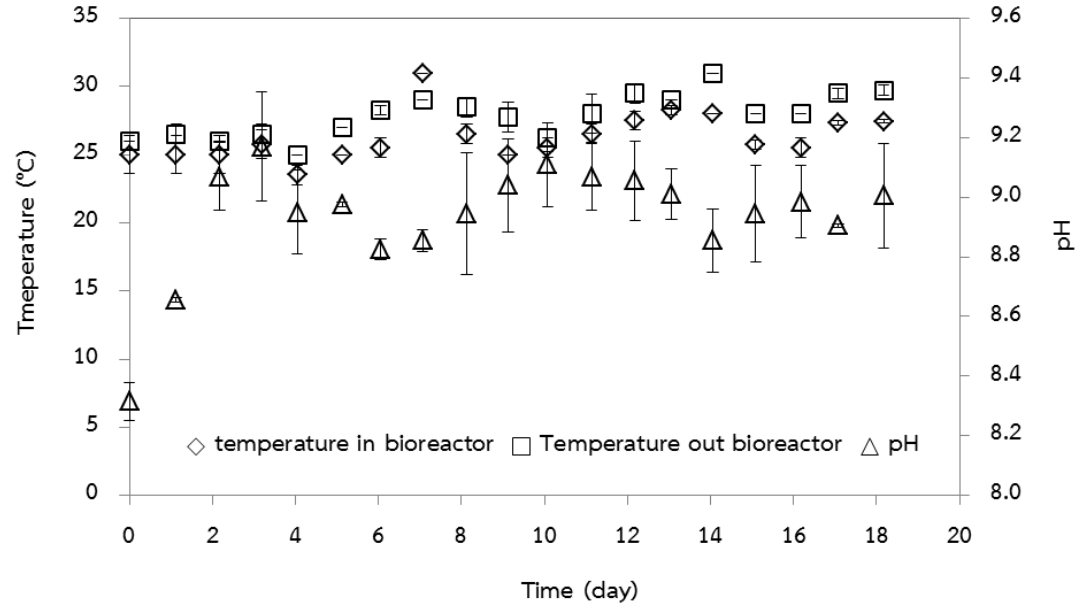


Figure 4.3 Temperature and pH of *Ankistrodesmus* sp. culture NB- FPAP

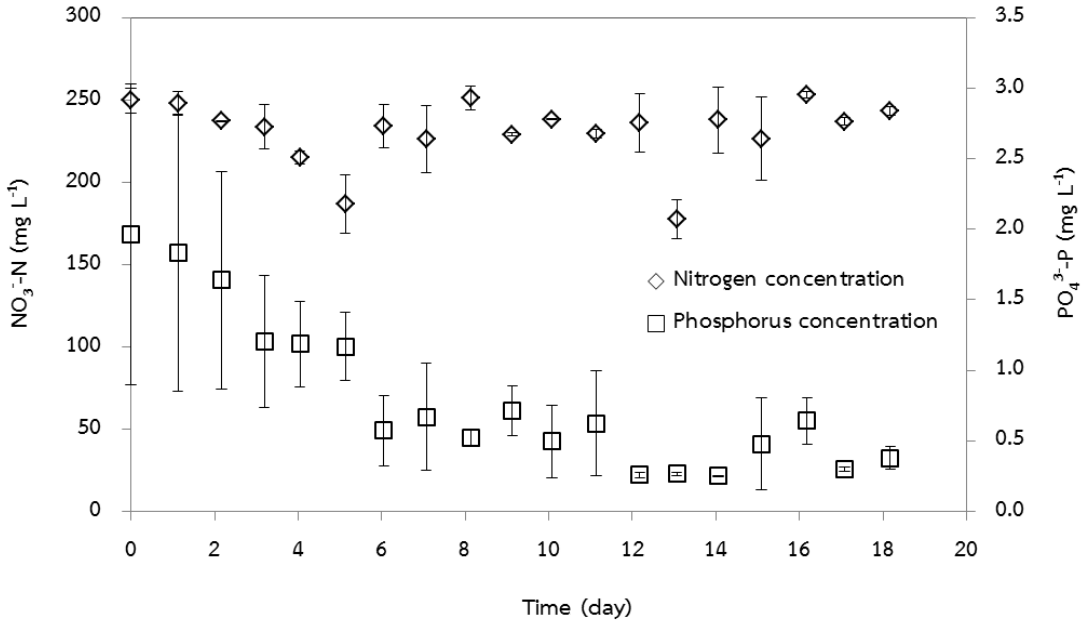


Figure 4.4 Profile of nitrogen and phosphorus concentration by *Ankistrodesmus* sp. culture in NB-FPAP

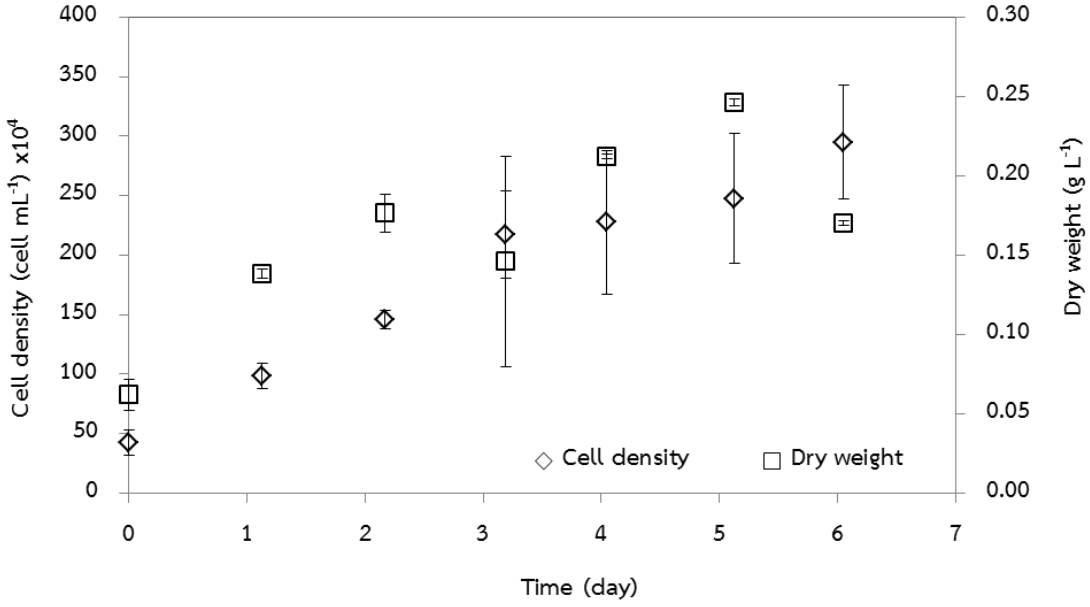


Figure 4.5 Growth of *Scenedesmus* sp. culture in NB-FPAP

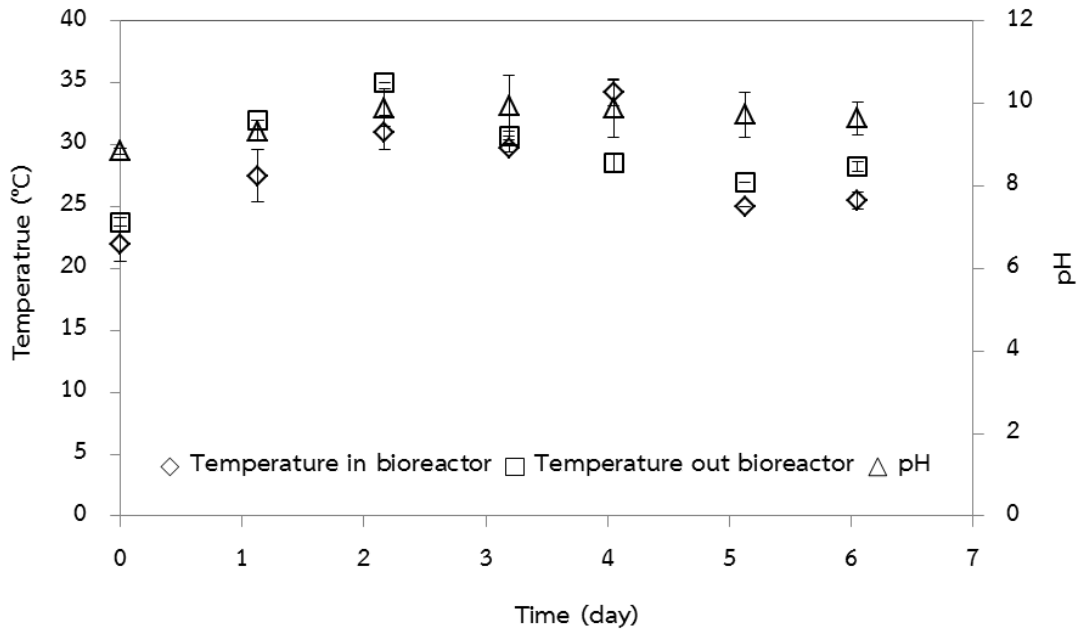


Figure 4.6 Temperature and pH of *Scenedesmus* sp. culture in NB-FPAP

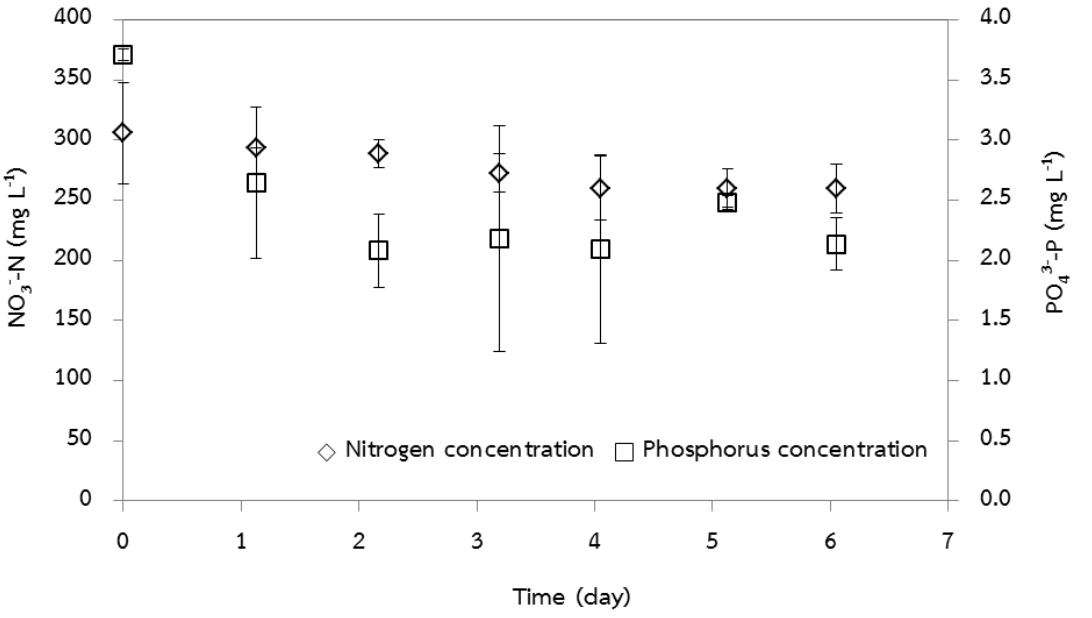


Figure 4.7 Profile of nitrogen and phosphorus concentration by *Scenedesmus* sp. culture in NB-FPAP

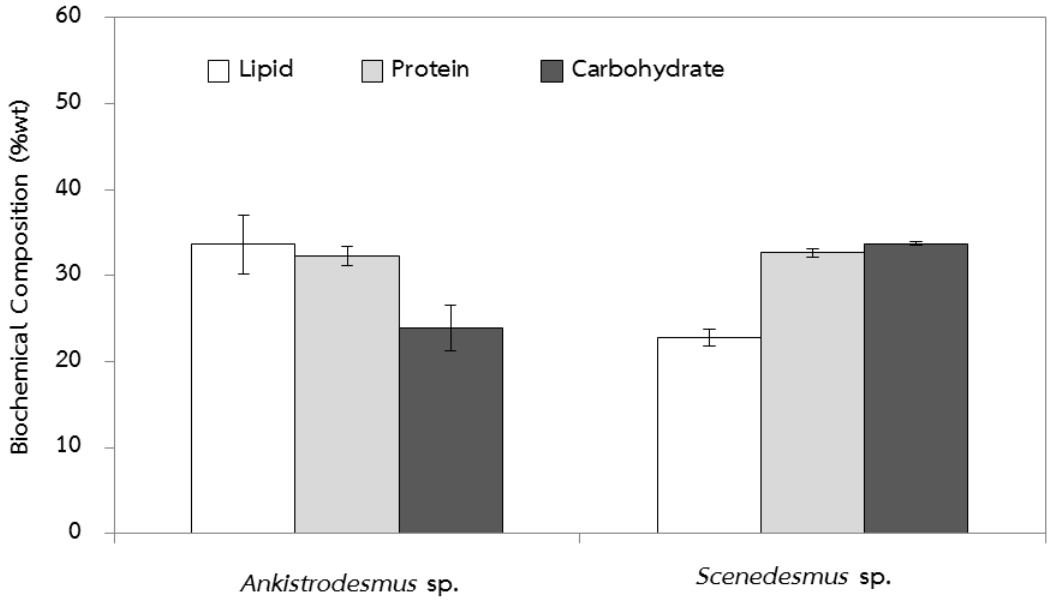


Figure 4.8 Biochemical of *Scenedesmus* sp. and *Ankistrodesmus* sp. culture in NB-FPAP

4.2 Reduced Nutrients

This section shows the effect of decreased nutrient of nitrogen and phosphorus in the BG11 medium on the growth performance and biochemical composition of the algae. The following conditions were investigated, (i) the 50% reduction in nutrient condition: 50%Nitrogen (50%N), 50%Phosphorus (50%P), 50%Nitrogen and Phosphorus (50%N&P) and 100% Medium or control batch, and (ii) the 25% reduction in nutrient: 25%N, 25%P, 25%N&P and 100%Medium (control). Each experiment was repeated twice to allow statistical test. Figure 4.9 shows the experiment setup in this section.

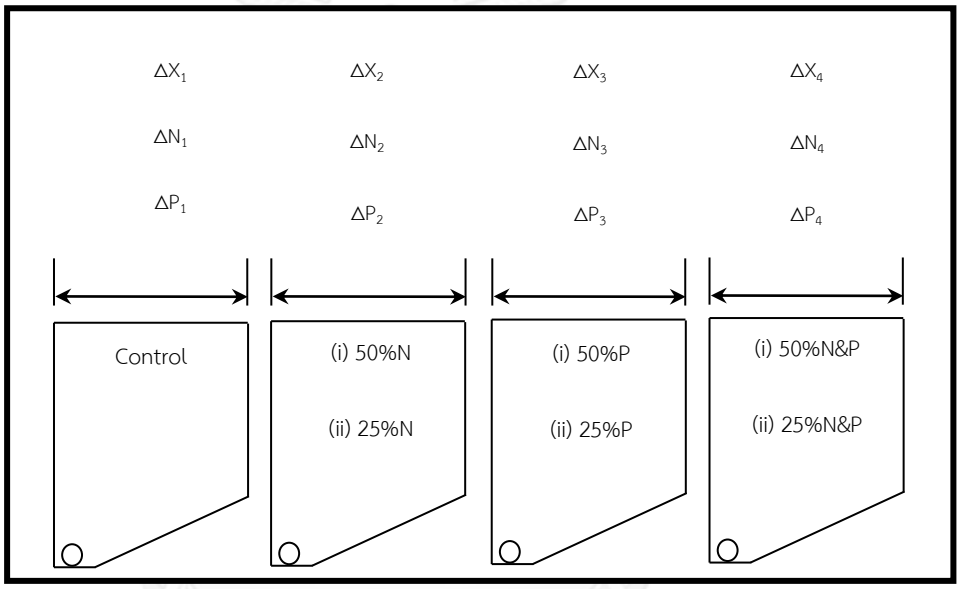


Figure 4.9 Setup for the reduced nutrient experiment

4.2.1 *Ankistrodesmus* sp.

Ankistrodesmus sp. was cultured with the conditions as stated in Table 4.1 where the cell harvest was conducted after 9 days.

Growth at 50% reduction

Figure 4.12 and Table 4.5 show the growth of *Ankistrodesmus* sp. cultured in this reduced nutrient condition. The most optimal condition for growth could be ordered from the most suitable to the least as: 50%N&P, 50%P, 50%N and 100%Medium which were $2.10\pm0.17\times10^{6}$, $1.86\pm0.09\times10^{6}$, $1.77\pm0.01\times10^{6}$ and $1.57\pm0.40\times10^{6}$ cell mL^{-1}, respectively. This microalga was cultured in half reduced nitrogen and phosphorus which they could grow well. Statistical analysis in Table 4.2 illustrates that each reduced nutrient condition provided different growth behavior

with relatively high statistical significant level ($p \leq 0.05$). The 50%N&P condition also gave the culture with the highest cell weight at 0.48±0.02 g L^{-1} followed by the control, 50%N and 50%P conditions with the cell dry weights of 0.37±0.02, 0.36±0.04 and 0.31±0.02 g L^{-1}, respectively. The difference between cell number and dry weight could be from the fact that the reduced nutrient might have an effect on morphology of microalgae. Figure 4.10 shows the cell morphology under the different mediums. The 50%N&P conditions gave the cluster of the cells which was similar to that obtained from the control experiment. The 50%P provided a relatively high cell density but no cell clustering which resulted in a lighter cell dry weight. Note that the statistical analysis suggested that the dry weights in the control batch, 50%P and 50%N were not different (same letter in Table 4.2).

In short, the half reduced nutrient of N&P conditions led to a better growth that the culture with 100%medium. This finding was opposite to that of El-Sheekh *et al.* (2013) who described that the nutrient limitation slowed down the growth of *Scenedesmus obliquus* but Mandal and Mallick (2009) described the biomass yield slightly increased in reduced nitrogen condition. This research revealed that the reduced nutrient of nitrogen and phosphorus affected the cell density and dry weight, which was the better than the growth in fresh medium or control batch. The temperature and pH profiles for this set of experiments are shown in Figures 4.14 and 4.15, respectively.

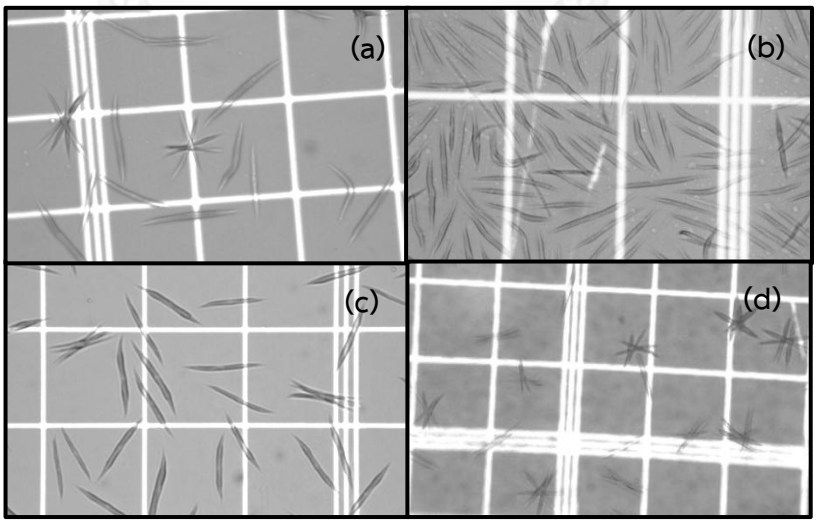


Figure 4.10 Morphology of *Ankistrodesmus* sp. cultured in different media conditions; (a) 100%Medium, (b) 50%P, (C) 50%N and (d) 50%N&P

Nutrient consumption at 50% reduction

Concentration profiles of nitrogen (NO_3^--N) and phosphorus (PO_3^--P) of *Ankistrodesmus* sp. are shown in Figures 4.16 and 4.17, respectively. In all cases, nitrogen was only gradually decreased during the growth, and phosphorus, on the other hand, diminished quite rapidly to zero.

Table 4.6 displays %reduction of all elements in the BG11 medium. The %reduction of nitrogen in 50%N was 48, and this nitrogen uptake reduced to 35% at 50%N&P condition, 14 at 50%P, and 11.1 at 100%medium. The uptake of phosphorus in 50%N&P condition was maximum at 98.8% of the total supply, and this was followed by the uptakes in 100%Medium, 50%N and 50%P which were 97.8%, 81.3% and 51.2%, respectively. Chu *et al.* (2013) explained that phosphorus was important for nitrate absorption by algal cells where the transportation of nitrate ion and protein synthesis (nitrogen needed) would be down-regulated via adenylate limitation and plasmalemma H^+-ATPase activity (Beardall *et al.*, 2005). However, the finding on phosphorus uptake in this work did not directly reflect nitrate uptake which means that there was not a linear relationship between the uptakes of phosphorus and nitrogen.

Table 4.6 summarizes the uptakes of other nutrients. Magnesium (Mg) and Iron (Fe) uptakes were found to be considerable as they are important for photosynthesis; i.e. Magnesium as positive ion is central element of the chlorophyll molecule, aggregation of ribosomes in functional units and in the formation of catalase (Encarnação *et al.*), whereas Iron is important in many metabolic functions of phytoplankton, such as electron transport in the Calvin cycle, the respiratory electron transport processes, nitrate and nitrite reductions, nitrogen fixation and the synthesis of chlorophyll. Manganese (Mn) and Zinc (Zn) as trace elements (in BG11 as B, Mn, Zn, Mo, Cu and Co) were clearly decreased in all conditions. This demonstrates that such trace elements were also significant for growth and had to be considered when reuse or recycle the mediums.

Table 4.7 demonstrates the specific uptake rate of nutrients at the various limited nutrient condition. The negative specific nutrient uptake rate in the control experiment was unexpected and still could not be described with our current knowledge. However, it is interesting to observe that the specific nitrogen uptake rate was maintained at the same magnitude regardless of the nutrient concentration, whereas the specific phosphorus uptake rate at the limiting N condition was about 10 times lower than that at the limiting P condition.

Biochemical composition at 50% reduction

Biochemical compositions of microalga cultivated under different medium concentrations (50% reduction condition) are expressed in Table 4.5 and Figure 4.18. The half reduced phosphorus led to a significantly increase in lipid content ($p \leq 0.05$). This could be due to the drastic increase in TAG levels as reported by Courchesne *et al.* (2009). Moreover, Phosphorus was also reported to be essential to the cellular processes related to energy bio-conversion (e.g. photophosphorylation). On the other hand, the half reduced nitrogen conditions did not significantly affect the lipid and carbohydrate contents when compared with that of the control batch. This, however, did not agree with the report of Chen *et al.* (2011) who found that the lipid and carbohydrate contents were significantly increased in reduced nitrate of microalgal cultivation.

25% reduction condition

Table 4.5 and Figure 4.19 display the growth of *Ankistrodesmus* sp. cultured in this reduced nutrient conditions. Statistical analysis illustrates that the various reduced nutrient conditions provided different growth behaviors with relatively high statistical significant level ($p \leq 0.05$). This experiment was conducted twice, the first batch was to compare the growth between the conditions with 50% reduction in nutrients (i.e. 50%P, 50%N, and 50%N&P as indicated in Table 4.5) with the control (100%Medium), and the second was to compare the growth between the conditions with 25% reduction in nutrients (i.e. 25%P, 25%N, and 25%N&P) with the control (100%Medium). However, although the two batches were operated at the same environmental conditions, i.e. the same temperature, and light intensity, the results from the control experiments (both with 100%Medium) were not the same. This could be due to the difference of the water quality as all experiments in this work employed sterilized tap water which could have variable quality at the various time of the year. As an overall observation, growths from reduction nutrients seemed to be better than the growth in the control experiment. Among the various conditions, the 25% reduction condition seemed to give the best growth where the 25%N&P condition gave 52.17% higher dry weight and 77.90% higher productivity than those at 100%Medium.

The biochemical compositions of *Ankistrodesmus* sp. under different nutrient concentrations are shown in Table 4.5. In terms of lipid production, the 50%N&P gave the highest lipid content in the cell. However, lipid productivity from the 25%N&P was the highest as this condition gave the best cell growth. Protein and

carbohydrate from all cases were within the same range of 28-36%wt and therefore the productivity depended primarily on cell growth.

Table 4.11 shows the ratio of the amount of biomass produced (ΔX), to the amount of substrate consumed (Δs); (g biomass/g substrate), which is defined as the growth yield. For nitrogen in particular, both UV-VIS and CHN/O analyzers were employed to indicate the level, the former for the nitrate in liquid phase, and the later for nitrogen in cell biomass. The results display that ΔX/ΔN of the control experiment (100%Medium) measured from the amount of nitrate being consumed was 7.07 but the measurement by CHN/O analyzer provided 14.88. This indicates clearly that nitrogen in liquid phase could be in various forms and only nitrate was measured for this nitrogen balance and therefore there would be an error associated with this unmeasured nitrogen compounds such as nitrite and ammonia. In this case, CHN/O was used to measure the quantity of N being uptaken into the cell biomass. The yield of biomass on phosphorus (ΔX/ΔP) was rather variable. Some condition gave a very high ΔX/ΔP, e.g. this was 735.63 at 50%P condition, but only 279.10 at 25%P and 675.68 at 25%N&P. No substantial relationship could be drawn from this results, however, there was an observation that lipid production was quite high at the condition of limiting phosphorus. The consumptions of various nutrients could be used to estimate the empirical formula of the algae as demonstrated in Table 4.12.

4.2.2 *Scenedesmus* sp.

Scenedesmus sp. was cultured with the conditions as stated in Table 4.1 where the cell harvest was conducted after 5 days.

50% reduction condition

The growth in half reduced nutrient as BG11 medium of *Scenedesmus* sp. is presented in Figure 4.22 and Table 4.8. The 50%N&P gave the culture with the highest cell density at $3.37\pm0.10\times10^{6}$ g L^{-1}, but the dry weight at 100%Medium was the greatest. The difference between cell density and dry weight could be from the fact that the reduced nutrient might have an effect on morphology of microalgae. Figure 4.11 shows the cell morphology under different mediums. The 100%Meidum condition gave cluster of flow cell in row more than reduced nutrient as follows: 50%P, 50%N and 50%N&P. Thus, this appearance affected the weight of microalgal cell. This finding was well supported by the reports from Chu et al. (2013) who cultivated *Chlorella vulgaris* in different nitrogen and phosphorus, and found that the biomass (mg L^{-1}) of control batch (100%medium) gave weight more than deficient nitrogen. In 2014 (Chu *et al.*), cultured *Scenedesmus oblique* in mediums

different nitrogen and phosphorus concentrations and found that control batch provided higher weight than those in nitrogen and phosphorus limitation conditions. In addition, Pancha *et al.* (2014) stated that nitrate limitation could slow down the metabolic activity and cell division in *Scenedesmus* sp.

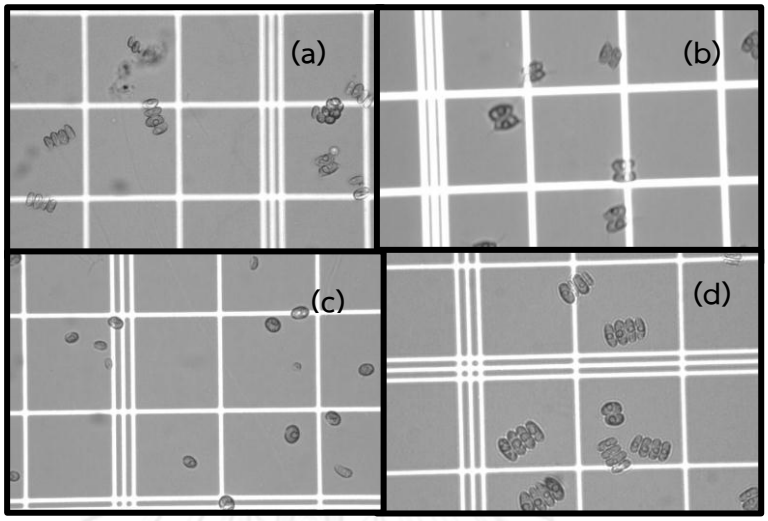


Figure 4.11 Morphology of *Scenedesmus* sp. culture in different media conditions; (a) 100%Medium, (b) 50%P, (C) 50%N and (d) 50%N&P

Biochemical compositions of microalga cultivated under different medium concentrations (50% reduction conditions) are expressed in Figure 4.27 and Table 4.8. It was quite a surprise to observe that the half reduced nitrogen led to a significantly increase in protein content ($p \leq 0.05$). The 50%N condition gave the protein content with the highest at 35±0.4%wt followed with 33±1.3%wt at 50%N&P, 33±0.7%wt at 100%Medium and 31±0.5%wt at 50%P, respectively. However, this could be easily explained as the 50%N condition allowed algal cell to uptake the nitrogen in a greater quantity than other conditions as illustrated in Table 4.10, and this led to a greater protein synthesis. This finding was supported by the statement from Syrett and Hipkin (1973) that nitrogen starvation could significantly raise nitrate assimilation and therefore protein synthesis. This increase in protein corresponded to the decrease in lipid content for this alga while maintaining a more or less constant level of carbohydrate. Similar findings were reported by Pancha et al. (2014) who stated that the removal of nitrate decreased crude protein but enhanced lipid and starch content. The 25% reduction in nutrient was found to raise the lipid content in the cell which might be due to the nutrient starvation as stated earlier.

25% reduction nutrient condition

Figure 4.25 and Table 4.8 show the growth of *Scenedesmus* sp. cultured in this reduced nutrient condition. Similar description with the case of *Ankistrodesmus* sp. is given here. The experiments were conducted in two batches, i.e. the first batch with 50% reduction in nutrients (50%P, 50%N, and 50%N&P), and the second batch with 25% reduction in nutrients (25%P, 25%N, and 25%N&P). Both batches were compared with the control experiment using 100%Medium which was carried out at the same environmental conditions and same time. Table 4.8 indicates that both control experiments yielded different results which, again, could be due to the differences in the tap water quality.

In terms of growth, the 25%N&P and the control provided very similar growth rate but the 25%N&P gave a significantly higher dry weight and productivity than the control. It is interesting to observe that the cultivation of this microalga in nutrients with only nitrogen depletion or phosphorus depletion, the cell density rapidly decreased at the last day. This could be due to the unsuitable ratio of nitrogen and phosphorus which could negatively affect the cultivation of this microalga. In the same way, Lee *et al.* (2000) reported that the optimized N: P ratio for the dominant algae could be critical in attaining higher algal growth, lipid productivity and nutrient removal efficiency. The reduced nutrients as both nitrogen and phosphorus at 75%Nutrient in this study gave higher dry weight than control batch. Thus, this result was positive which could be other choices for cultivation.

Biochemical compositions of *Scenedesmus* sp. in a quarterly reduced BG11 are shown in Figure 4.27 and Table 4.8. The protein content from the 25%N medium was the highest which was at a similar level as that from the 50%N condition. The reduced nutrient had lipid content 2-8% more than that from the control batch. The lipid productivity of 25%N&P was the highest at 21±1 mg L^{-1} d^{-1} followed by 50%P, 100%Medium and 50%N at 15±5, 11±3 and 9±2 mg L^{-1} d^{-1}, respectively.

Table 4.16 shows the growth yield which was further employed to estimate the empirical formula of this alga.

4.2.3 Concluding remarks

The experiment model studied the growth under the reduced nutrient condition (major nitrate and phosphate) and revealed its influence on the growth and biochemical composition of microalgae. The reduced nutrient at 25%N&P condition was an attractive choice for both microalgae because it gave superior biomass yield and a better productivity of biochemical constituents. In addition, the 25%N&P condition could also reduce nutrient cost for the microalgal cultivation.

Table 4.5 Growth and biochemical composition of reduced Nutrient by *Ankistrodesmus* sp.

Characters	Control	50%P	50%N	50%N&P
Final cell density (cell mL^{-1})	$1.57\pm0.40\times10^{6}$	$1.86\pm0.09\times10^{6}$	$1.77\pm0.01\times10^{6}$	$2.10\pm0.17\times10^{6}$
Specific growth rate (d^{-1})	0.07±0.02	0.08±0.02	0.10±0.00	0.08±0.01
Final Dry weight (g L^{-1})	0.37 ± 0.02^{a}	0.31 ± 0.02^{a}	0.36 ± 0.04^{a}	0.48 ± 0.02^{b}
Productivity (g d^{-1})	0.57 ± 0.02^{a}	0.36 ± 0.01^{b}	0.36 ± 0.01^{b}	0.61 ± 0.02^{a}
% Lipid	25 ± 1^{a}	31 ± 1^{b}	22 ± 1^{a}	29 ± 1^{b}
% Protein	30 ± 1^{a}	31 ± 1^{a}	35 ± 1^{b}	30 ± 1^{a}
% Carbohydrate	38±2	34±1	37±1	35±1
% Moisture	1.79	1.37	1.61	1.92
% Ash	5.48	3.64	4.92	4.07
Lipid productivity (mg L^{-1} d^{-1})	10 ± 1^{a}	8 ± 0^{a}	12 ± 2^{a}	16 ± 1^{b}
Protein productivity (mg L^{-1} d^{-1})	12 ± 0.32^{a}	12 ± 0.06^{a}	14 ± 0.06^{b}	12 ± 0.05^{a}
Carbohydrate productivity (mg L^{-1} d^{-1})	15±0.89	13±0.43	14±0.58	14±0.35
Characters	**Control**	**25%P**	**25%N**	**25%N&P**
Final cell density (cell mL^{-1})	$8.69\pm0.27\times10^{6}$	$8.88\pm0.35\times10^{6}$	$1.07\pm1.33\times10^{7}$	$1.26\pm1.68\times10^{7}$
Specific growth rate (d^{-1})	0.26±0.02	0.26±0.01	0.28±0.02	0.31±0.01
Final dry weight (g L^{-1})	0.46 ± 0.02^{a}	0.64 ± 0.01^{b}	0.52 ± 0.03^{a}	0.70 ± 0.01^{c}
Productivity (g d^{-1})	0.86±0.02	1.40±0.06	1.22±0.32	1.53±0.00
% Lipid	33±3.0	30±1.0	29±4.5	31±0.2
% Protein	31 ± 0.8^{a}	28 ± 0.4^{a}	31 ± 0.5^{b}	28 ± 0.3^{b}
% Carbohydrate	30±4.1	36±1.6	35±3.8	36±0.3
% Moisture	1.10	0.85	0.45	1.40
% Ash	5.82	4.62	5.06	4.76
Lipid productivity (mg L^{-1} d^{-1})	16 ± 2.3^{a}	21 ± 2.7^{ab}	17 ± 0.4^{a}	24 ± 0.2^{b}
Protein productivity (mg L^{-1} d^{-1})	16 ± 0.6^{a}	15 ± 0.3^{b}	16 ± 0.4^{ab}	14 ± 0.1^{b}
Carbohydrate productivity (mg L^{-1} d^{-1})	15±2.1	19±0.8	18±2.0	19±0.2

Table 4.6 Reduction (%) of elements in reduced nutrient by *Ankistrodesmus* sp.

Elements	Control batch			50%P			50%N			50%N&P		
	Concentration (mg L^{-1})		% Reduction (Δ1)	Concentration (mg L^{-1})		% Reduction (Δ2)	Concentration (mg L^{-1})		% Reduction (Δ3)	Concentration (mg L^{-1})		% Reduction (Δ4)
	Initial day	Final day		Initial day	Final day		Initial day	Final day		Initial day	Final day	
N	260	231	11.1	264	226	14	186	96	48	178	115	35
P	5.285	0.117	97.8	0.339	0.165	51.2	5.789	1.081	81.3	3.295	0.038	98.8
B	0.510	0.062	87.9	0.405	0.074	81.6	0.481	0.434	9.7	0.530	0.498	5.9
Ca	24	5	80	14	5	63	22	19	15	23	20	12
Mg	12.5	1.4	89.1	9.5	1.6	83.5	11.8	10.9	7.1	12.1	12.0	0.3
Fe	0.001	0.001	64.3	0.003	0.002	29.6	0.001	0.001	64.3	0.005	0.003	30.4
Mn	0.124	0.004	97.0	0.047	0.006	87.1	0.042	0.012	70.9	0.611	0.116	81.1
Zn	0.118	0.002	98.6	0.083	0.001	99.2	0.120	0.063	47.6	0.225	0.168	25.2
Mo	0.111	0.013	88.3	0.090	0.014	84.0	0.093	0.088	5.4	0.278	0.124	55.5
Cu	0.010	0.003	73.5	0.009	0.001	84.9	0.012	0.008	35.2	0.026	0.017	35.0
Co	0.003	0.001	67.7	0.005	0.000	95.6	0.005	0.002	48.9	0.006	0.006	0.0
K	90.2	63.0	30.2	97.2	94.3	3.0	94.4	87.5	7.3	45.6	38.4	15.7

Elements	Control batch			25%P			25%N			25%N-P		
	Concentration (mg L^{-1})		% Reduction (Δ1)	Concentration (mg L^{-1})		% Reduction (Δ2)	Concentration (mg L^{-1})		% Reduction (Δ3)	Concentration (mg L^{-1})		% Reduction (Δ4)
	Initial day	Final day		Initial day	Final day		Initial day	Final day		Initial day	Final day	
N	283	242	14.5	270	231	14.4	137	78	43.4	83	63	24.2
P	3.663	0.038	99.0	1.055	0.117	88.9	4.304	0.165	96.2	0.944	0.130	86.2
B	0.085	0.084	2.0	0.086	0.082	5.2	0.076	0.060	21.6	0.637	0.064	90.0
Ca	14.1	5.6	60.7	19.8	4.3	78.5	20.9	8.0	61.6	17.7	11.3	36.2
Mg	0.726	0.668	8.1	0.706	0.617	12.6	4.219	0.650	84.6	15.802	0.397	97.5
Fe	0.003	0.001	59.9	0.003	0.001	58.7	0.001	0.001	11.7	0.004	0.001	78.3
Mn	0.003	0.001	82.1	0.004	0.001	86.1	0.002	0.000	95.3	0.101	0.001	98.9
Zn	0.015	0.001	94.5	0.025	0.005	78.7	0.005	0.003	28.9	0.318	0.008	97.6
Mo	0.007	0.005	23.5	0.005	0.005	4.6	0.004	0.003	24.4	0.144	0.003	98.1
Cu	0.007	0.006	2.0	0.006	0.006	1.3	0.007	0.006	12.0	0.006	0.005	20.0
Co	0.002	0.002	30.0	0.003	0.003	8.4	0.006	0.001	79.5	0.008	0.003	70.2
K	50	49	2.8	57	46	18.9	79	63	20.5	70	8	88.2

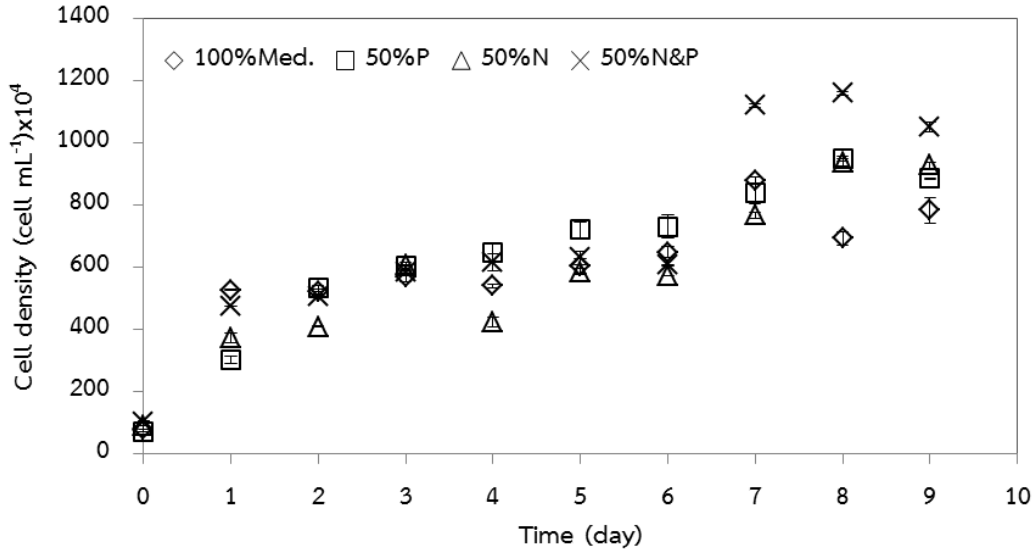


Figure 4.12 Growth of 50% nutrient by *Ankistrodesmus* sp. culture in different types of media under the conditions.

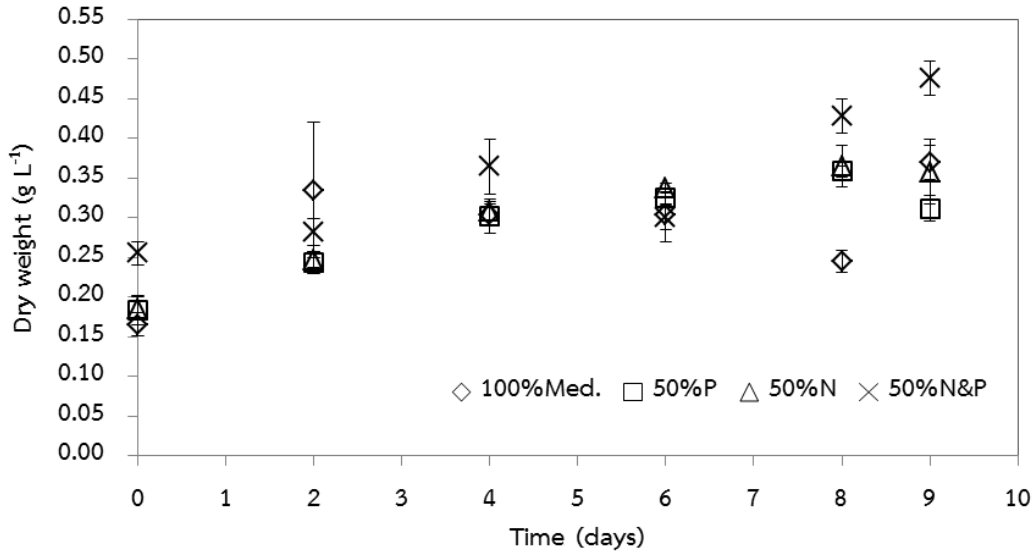


Figure 4.13 Dry weight of 50% nutrient by *Ankistrodesmus* sp. culture in different types of media under the conditions.

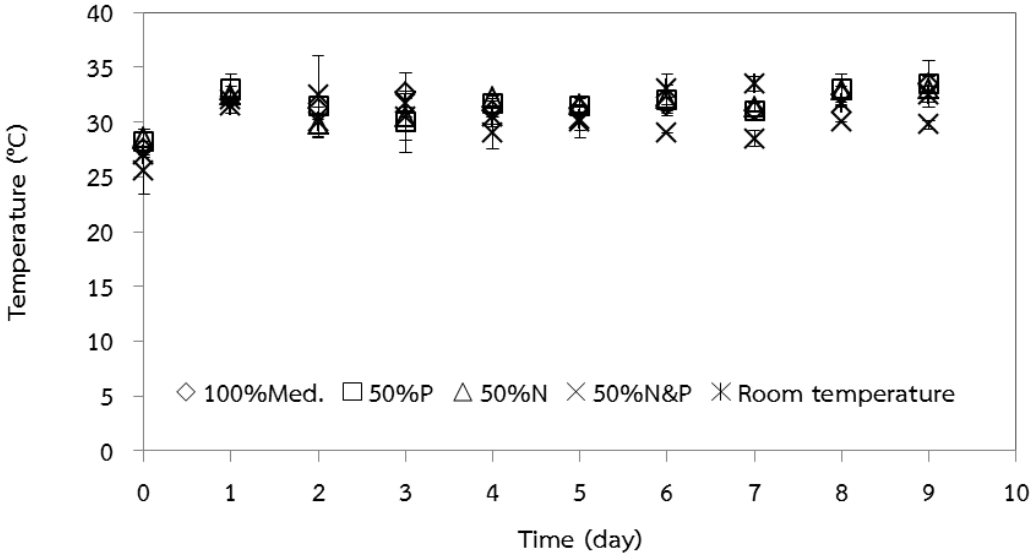


Figure 4.14 Temperature of 50% nutrient by *Ankistrodesmus* sp. culture in different types of media under the conditions.

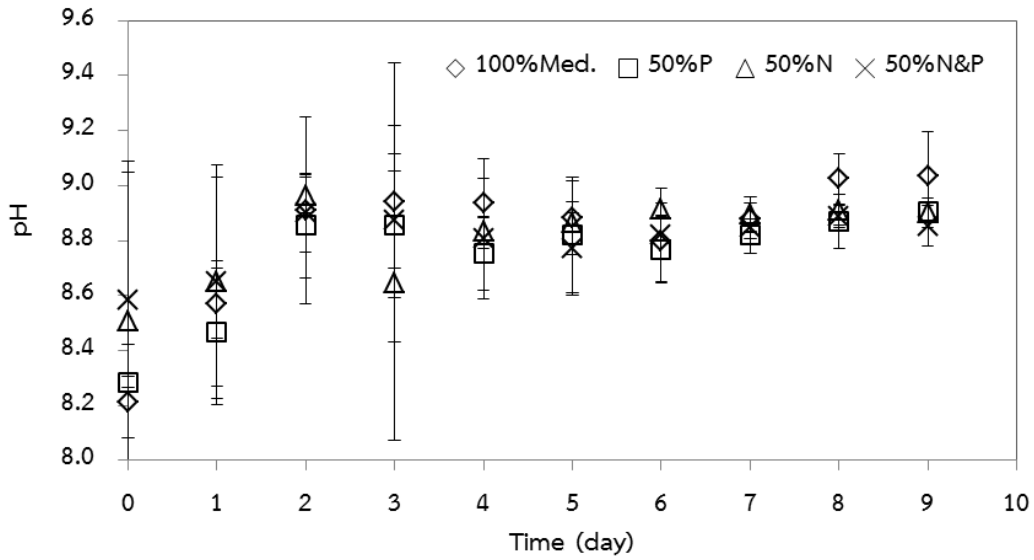


Figure 4.15 pH of 50% nutrient by *Ankistrodesmus* sp. culture in different types of media under the conditions

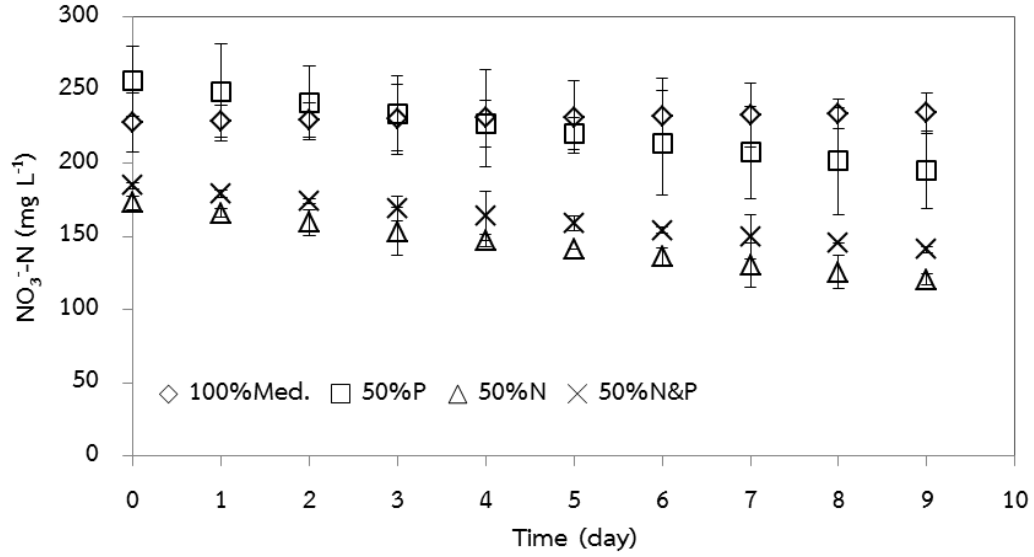


Figure 4.16 Nitrogen profile of 50% nutrient concentration by *Ankistrodesmus* sp. culture in different types of media under the conditions

Table 4.7 Uptake rate on available nutrients in reduced nutrient by *Ankistrodesmus* sp.

Time (day)	Uptake rate for substrate ($Y_{s/x}$)							
	ΔN/ΔX				ΔP/ΔX			
	Control	50%P	50%N	50%N&P	Control	50%P	50%N	50%N&P
0								
1	-0.0243	0.2007	0.2171	0.5338	0.0355	0.0303	0.0014	0.0497
2	-0.0304	0.2214	0.2535	0.3695	0.0351	0.0277	0.0013	0.0211
3	-0.0405	0.2498	0.3088	0.2787	0.0370	0.0258	0.0013	0.0097
4	-0.0603	0.2906	0.4023	0.2212	0.0438	0.0248	0.0014	0.0047
5	-0.1179	0.3539	0.5940	0.1816	0.0678	0.0250	0.0017	0.0024
6	-2.3085	0.4642	1.2039	0.1527	1.0508	0.0271	0.0028	0.0012
7	0.1322	0.7025	-13.1786	0.1307	-0.0477	0.0340	-0.0245	0.0006
8	0.0645	1.5888	-0.9631	0.1135	-0.0184	0.0635	-0.0014	0.0003
9	-0.0202	0.1849	0.1914	0.9197	0.0373	0.0338	0.0015	0.1397
Time (day)	Uptake rate for substrate ($Y_{s/x}$)							
	ΔN/ΔX				ΔP/ΔX			
	Control	25%P	25%N	25%N-P	Control	25%P	25%N	25%N-P
0								
1	0.0702	0.1156	0.1541	0.0331	0.0201	0.0192	0.0022	0.0003
2	0.0808	0.1255	0.1623	0.0350	0.0163	0.0150	0.0020	0.0003
3	0.0960	0.1394	0.1719	0.0372	0.0136	0.0120	0.0018	0.0002
4	0.1192	0.1601	0.1833	0.0400	0.0119	0.0099	0.0016	0.0002
5	0.1595	0.1932	0.1970	0.0435	0.0112	0.0086	0.0014	0.0002
6	0.2463	0.2531	0.2136	0.0480	0.0122	0.0081	0.0013	0.0002
7	0.5682	0.3914	0.2343	0.0539	0.0199	0.0090	0.0012	0.0002
8	-1.5756	1.0305	0.2608	0.0621	-0.0389	0.0170	0.0011	0.0002
9	-0.3198	-1.2198	0.2956	0.0742	-0.0056	-0.0145	0.0011	0.0003

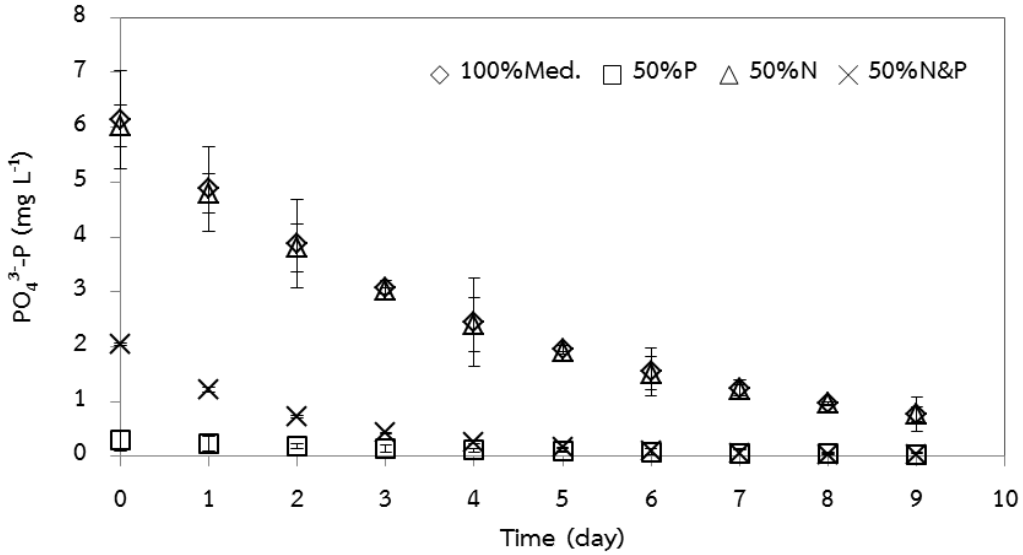


Figure 4.17 Phosphorus profile of 50% nutrient concentration by *Ankistrodesmus* sp. culture in different types of media under the conditions

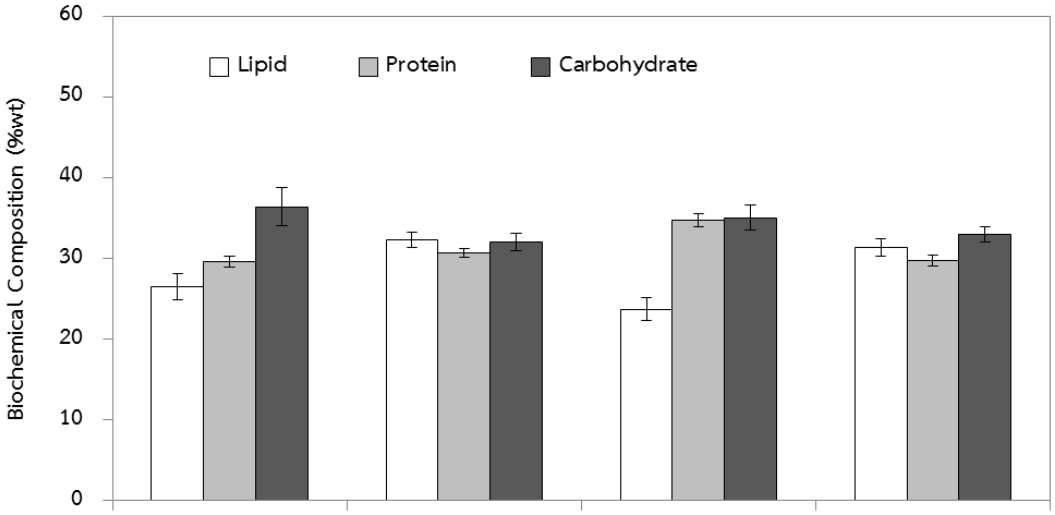


Figure 4.18 Percentage biochemical composition of 50% nutrient by *Ankistrodesmus* sp. culture in different types of media under the conditions

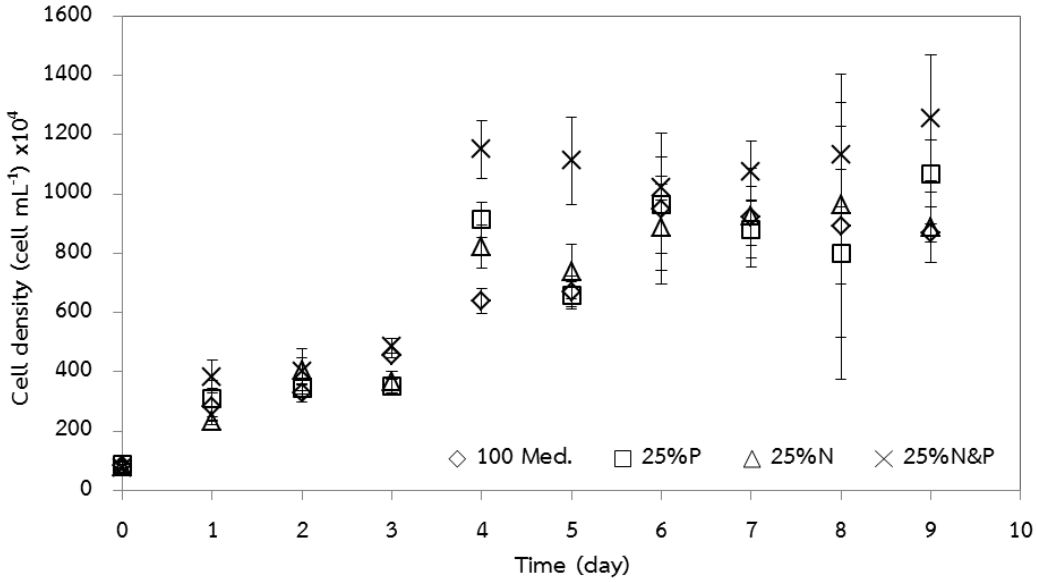


Figure 4.19 Growth of 25% nutrient by *Ankistrodesmus* sp. culture in different types of media under the conditions

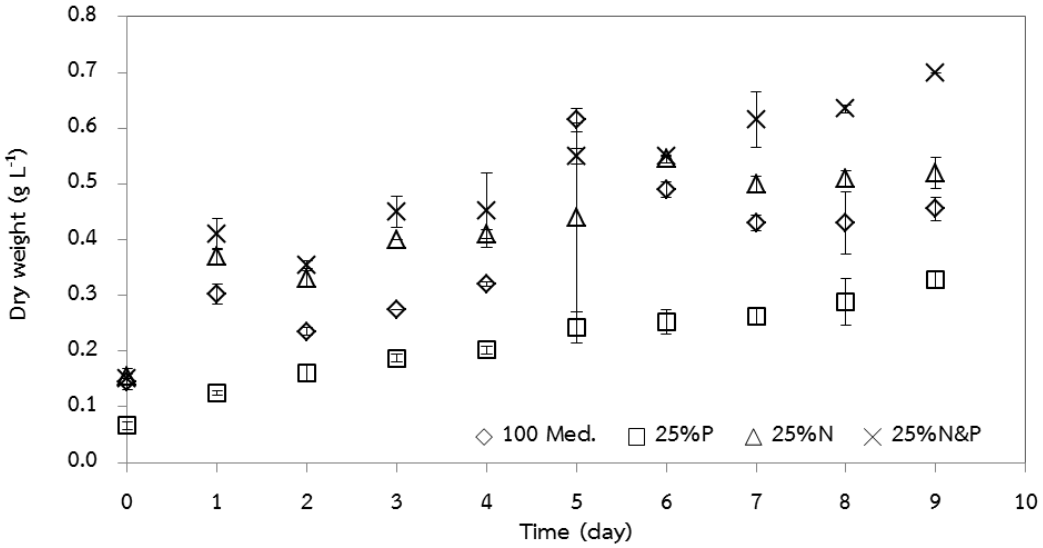


Figure 4.20 Dry weight of 25% nutrient by Ankistrodesmus sp. culture in different types of media under the conditions.

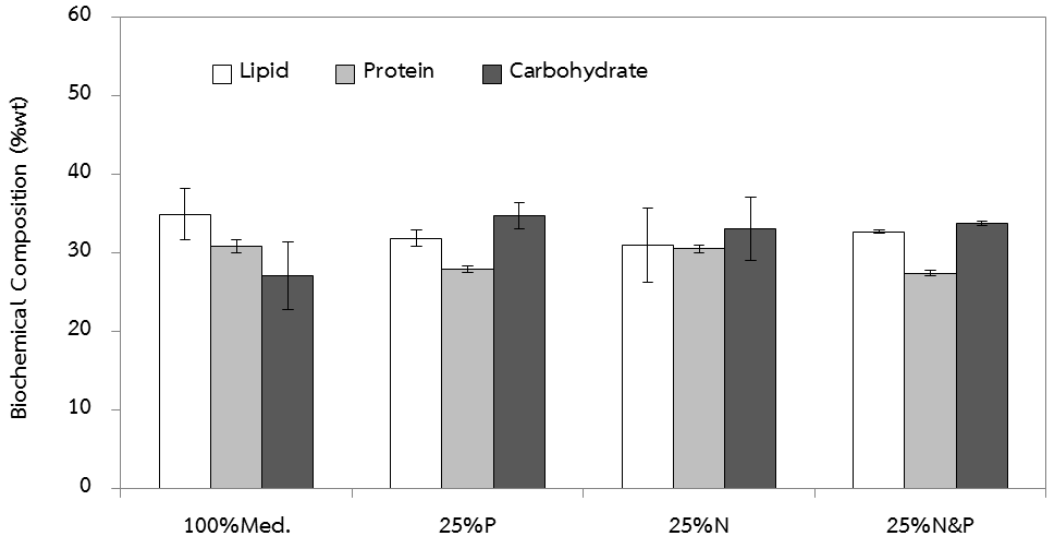


Figure 4.21 Percentage biochemical composition of 25% nutrient by *Ankistrodesmus* sp. culture in different types of media under the conditions.

Table 4.8 Growth and biochemical composition of reduced nutrient by *Scenedesmus* sp.

Characters	Control batch	50%P	50%N	50%N&P
Final cell density (cell mL^{-1})	$2.37\pm0.13x10^{6\ a}$	$3.03\pm0.09x10^{6\ a}$	$2.17\pm0.40x10^{6\ ab}$	$3.37\pm0.10x10^{6\ b}$
Specific growth rate (d^{-1})	0.37±0.026	0.35±0.019	0.30±0.049	0.36±0.001
Final Dry weight (g L^{-1})	$0.33\pm0.002^{\ a}$	$0.24\pm0.004^{\ b}$	$0.28\pm0.007^{\ c}$	$0.27\pm0.009^{\ c}$
Productivity (g d^{-1})	$1.30\pm0.01^{\ b}$	$0.95\pm0.00^{\ b}$	$1.01\pm0.04^{\ b}$	$0.96\pm0.08^{\ b}$
% Lipid	19±9	14±1	15±2	15±2
% Protein	$33\pm0.7^{\ ab}$	31 ± 0.5^{a}	$35\pm0.4^{\ b}$	$33\pm1.3^{\ b}$
% Carbohydrate	36±8.6	37±0.1	37±1.0	39±2.5
% Moisture	1.53	0.31	1.47	0.19
% Ash	10.44	17.74	11.94	11.26
Lipid productivity (mg L^{-1} d^{-1})	13±5.8	7±0.1	9±0.7	8±0.8
Protein productivity (mg L^{-1} d^{-1})	$22\pm0.02^{\ ab}$	21 ± 0.42^{a}	$23\pm0.35^{\ b}$	$23\pm0.65^{\ b}$
Carbohydrate productivity (mg L^{-1} d^{-1})	24±5.8	25±0.1	25±0.7	26±1.7
Characters	**Control batch**	**25%P**	**25%N**	**25%N&P**
Final cell density (cell mL^{-1})	$2.96\pm0.12x10^{6\ a}$	$4.0\pm2.1x10^{5\ b}$	$2.7\pm0.5x10^{5\ b}$	$2.89\pm0.83x10^{6\ a}$
Specific growth rate (d^{-1})	$0.51\pm0.01^{\ a}$	$0.11\pm0.13^{\ b}$	$0.06\pm0.02^{\ b}$	$0.51\pm0.06^{\ a}$
Final Dry weight (g L^{-1})	$0.31\pm0.01^{\ a}$	$0.31\pm0.01^{\ a}$	$0.23\pm0.03^{\ b}$	$0.47\pm0.01^{\ c}$
Productivity (g d^{-1})	$1.40\pm0.02^{\ a}$	$1.23\pm0.04^{\ a}$	$0.87\pm0.10^{\ b}$	$2.17\pm0.01^{\ b}$
% Lipid	17±5	25±8	19±1	22±1
% Protein	$33\pm0^{\ a}$	$31\pm1^{\ b}$	$34\pm0^{\ a}$	$30\pm1^{\ b}$
% Carbohydrate	38±5	34±9	37±1	41±0
% Moisture	2.67	1.23	1.76	1.64
% Ash	8.64	9.56	8.62	4.64
Lipid productivity (mg L^{-1} d^{-1})	$11\pm3^{\ ab}$	$15\pm5^{\ ab}$	9±2 a	$21\pm1^{\ b}$
Protein productivity (mg L^{-1} d^{-1})	$21\pm0.02^{\ a}$	$19\pm0.65^{\ b}$	$21\pm0.04^{\ a}$	$19\pm0.47^{\ b}$
Carbohydrate productivity (mg L^{-1} d^{-1})	24±3.1	21±6.0	23±0.6	26±0.1

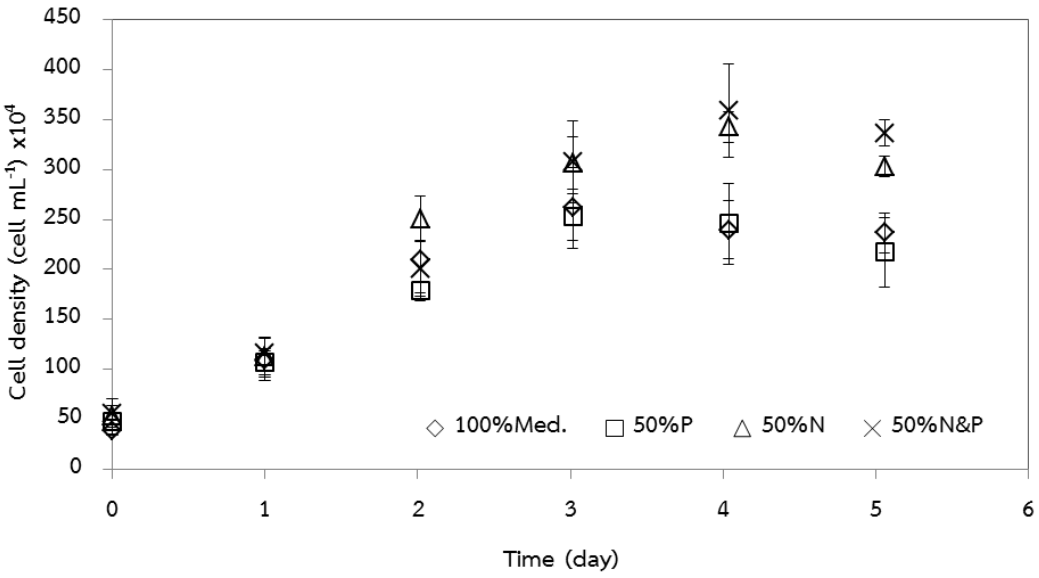


Figure 4.22 Growth of 50% nutrient by *Scenedesmus* sp. culture in different types of media under the conditions

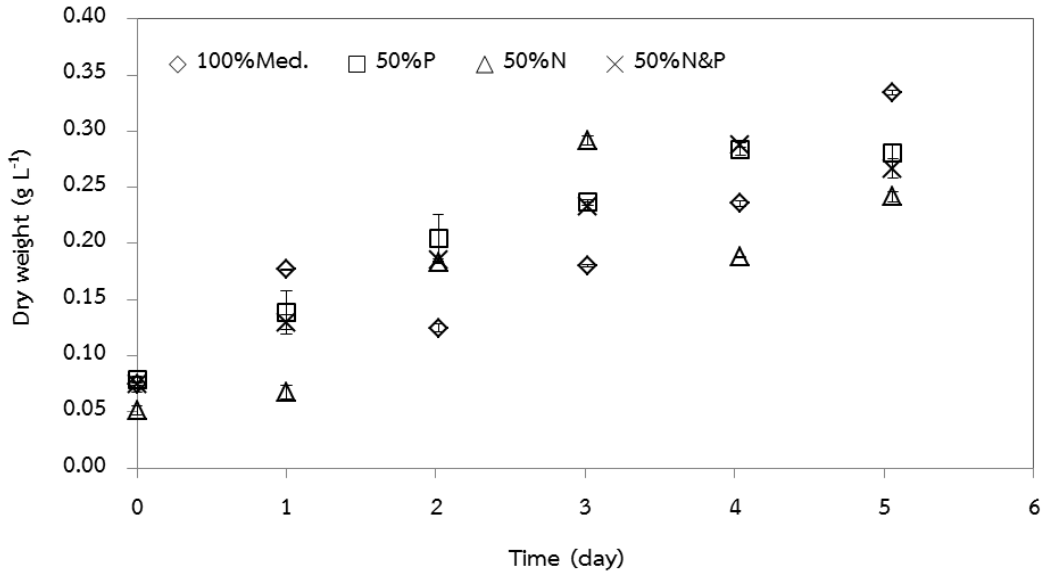


Figure 4.23 Dry weight of 50% nutrient by *Scenedesmus* sp. culture in different types of media under the conditions

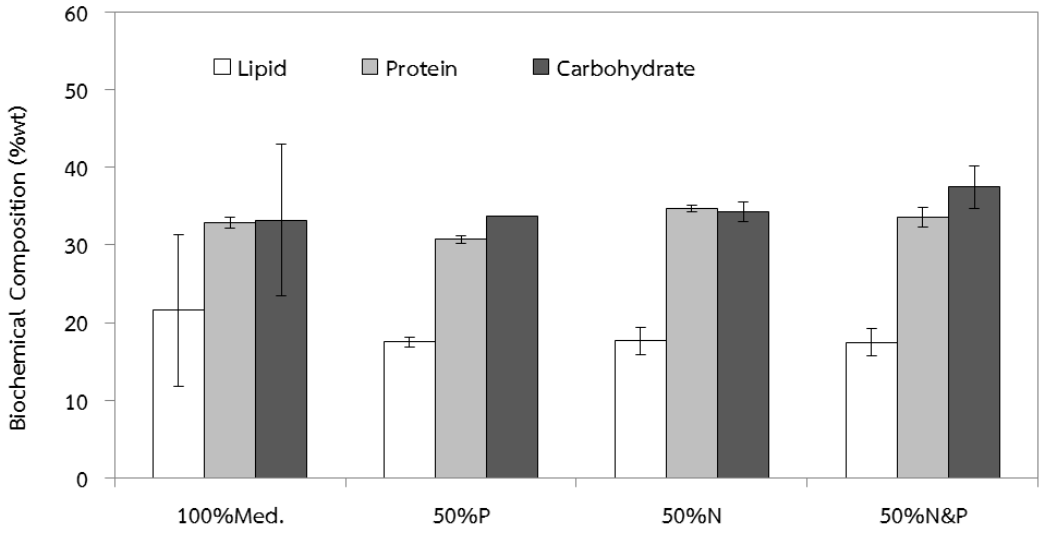


Figure 4.24 Percentage biochemical composition of 50% nutrient by *Scenedesmus* sp. culture in different types of media under the conditions

Table 4.9 Reduction (%) of elements in reduced nutrient by *Scenedesmus* sp.

Elements	Control batch			50%P			50%N			50%N&P		
	Concentration (mg L^{-1})		% Reduction (Δ1)	Concentration (mg L^{-1})		% Reduction (Δ2)	Concentration (mg L^{-1})		% Reduction (Δ3)	Concentration (mg L^{-1})		% Reduction (Δ4)
	Initial day	Final day		Initial day	Final day		Initial day	Final day		Initial day	Final day	
N	281.0	233.1	17.0	269.1	231.0	14.2	176.5	124.1	29.7	153.5	112.6	26.7
P	3.738	0.397	89.4	2.021	0.162	92.0	4.752	0.587	87.7	2.232	0.495	77.8
B	0.937	0.861	8.0	0.987	0.88	10.8	1.04	0.929	10.7	0.992	0.976	1.6
Ca	25.0	17.5	30.0	25.3	15.9	37.2	25.2	18.6	25.9	25.5	25.0	2.0
Mg	14.2	12.2	14.2	13.8	11.7	15.5	14.3	11.9	17.0	13.8	13.5	2.5
Fe	0.089	0.001	99.2	0.006	0.001	81.0	0.003	0.001	75.0	0.006	0.001	76.7
Mn	0.586	0.074	87.4	0.535	0.013	97.5	0.534	0.068	87.2	0.547	0.132	75.9
Zn	0.143	0.066	54.2	0.130	0.086	34.4	0.108	0.072	32.9	0.119	0.112	5.6
Mo	0.365	0.171	53.2	0.163	0.151	7.1	0.182	0.180	1.2	0.158	0.135	14.7
Cu	0.034	0.006	83.4	0.032	0.009	73.4	0.021	0.008	64.0	0.014	0.006	55.1
Co	0.013	0.010	22.7	0.014	0.010	27.2	0.014	0.009	35.7	0.013	0.011	20.1
K	41.2	30.6	25.6	30.0	28.9	3.5	53.0	48.0	9.4	43.7	41.2	5.8

Elements	Control batch			25%P			25%N			25%N&P		
	Concentration (mg L^{-1})		% Reduction (Δ1)	Concentration (mg L^{-1})		% Reduction (Δ2)	Concentration (mg L^{-1})		% Reduction (Δ3)	Concentration (mg L^{-1})		% Reduction (Δ4)
	Initial day	Final day		Initial day	Final day		Initial day	Final day		Initial day	Final day	
N	266.7	257.8	3.3	288.7	285.5	1.1	145.6	125.9	13.5	156.9	56.4	64.1
P	3.877	0.893	77.0	1.692	0.067	96.1	3.579	0.513	85.7	1.621	0.093	94.3
B	0.073	0.069	4.4	0.074	0.017	76.9	0.099	0.067	32.4	0.080	0.069	14.1
Ca	0.040	0.031	23.1	0.072	0.020	71.7	5.962	0.013	99.8	0.028	0.015	46.7
Mg	0.726	0.697	4.1	0.627	0.055	91.2	2.851	0.838	70.6	0.610	0.525	13.8
Fe	0.006	0.001	82.8	0.004	0.001	84.6	0.111	0.001	98.9	0.003	0.001	75.0
Mn	0.001	0.001	57.1	0.001	0.001	22.2	0.031	0.001	98.4	0.001	0.001	16.7
Zn	0.002	0.002	4.3	0.011	0.004	62.3	0.018	0.002	89.6	0.003	0.002	17.9
Mo	0.004	0.004	7.3	0.004	0.003	19.0	0.011	0.003	72.1	0.004	0.002	36.8
Cu	0.007	0.004	39.4	0.006	0.002	57.1	0.007	0.006	11.1	0.006	0.004	29.1
Co	0.004	0.003	28.2	0.004	0.004	7.3	0.003	0.001	55.6	0.004	0.003	23.8
K	759.5	743.8	2.1	899.2	743.6	17.3	782.6	767.9	1.9	782.6	767.9	1.9

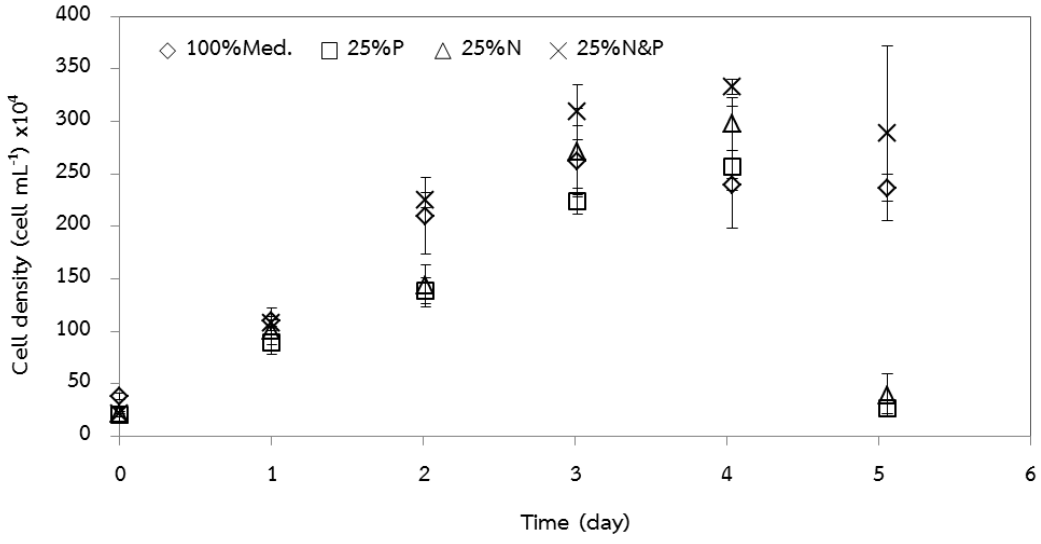


Figure 4.25 Growth of 25% nutrient by *Scenedesmus* sp. culture in different types of media under the conditions

Table 4.10 Uptake rate on available nutrients in reduced nutrient by *Scenedesmus* sp.

Time (day)	Upatake rate for substrate ($Y_{s/x}$)							
	ΔN/ΔX				ΔP/ΔX			
	Control	50%P	50%N	50%N&P	100%Med	50%P	50%N	50%N&P
0								
1	-0.6638	0.0544	0.1404	0.0528	-0.1226	0.0100	0.0229	0.0049
2	0.4647	0.0761	0.1703	0.0295	0.0617	0.0092	0.0210	0.0020
3	0.1657	0.1301	0.2253	0.0212	0.0158	0.0103	0.0210	0.0011
4	0.0985	0.4970	0.3562	0.0172	0.0068	0.0258	0.0251	0.0006
5	0.0689	-0.2567	1.0442	0.0150	0.0034	-0.0088	0.0555	0.0004
Time (day)	Uptake rate for substrate ($Y_{s/x}$)							
	ΔN/ΔX				ΔP/ΔX			
	Control	25%P	25%N	25%N&P	100%Med	25%P	25%N	25%N&P
0								
1	0.0244	0.0030	0.0176	0.4114	0.0162	0.0125	0.0085	0.0145
2	0.0262	0.0033	0.0233	0.3091	0.0132	0.0078	0.0081	0.0070
3	0.0283	0.0036	0.0348	0.2356	0.0109	0.0049	0.0087	0.0034
4	0.0308	0.0040	0.0702	0.1816	0.0090	0.0031	0.0127	0.0017
5	0.0338	0.0045	-1.7503	0.1412	0.0075	0.0020	-0.2265	0.0008

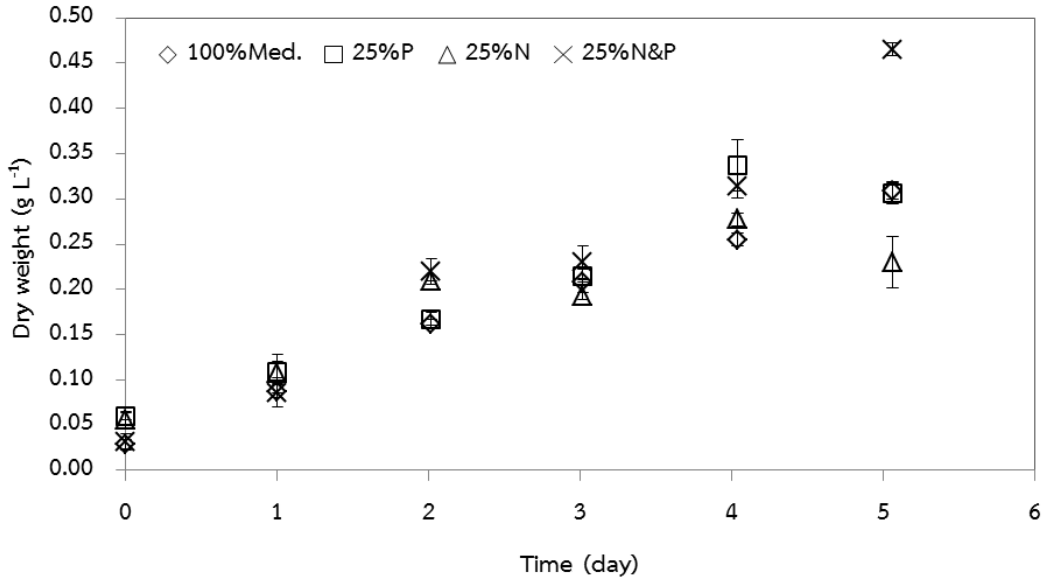


Figure 4.26 Dry weight of 25% nutrient by *Scenedesmus* sp. culture in different types of media under the conditions.

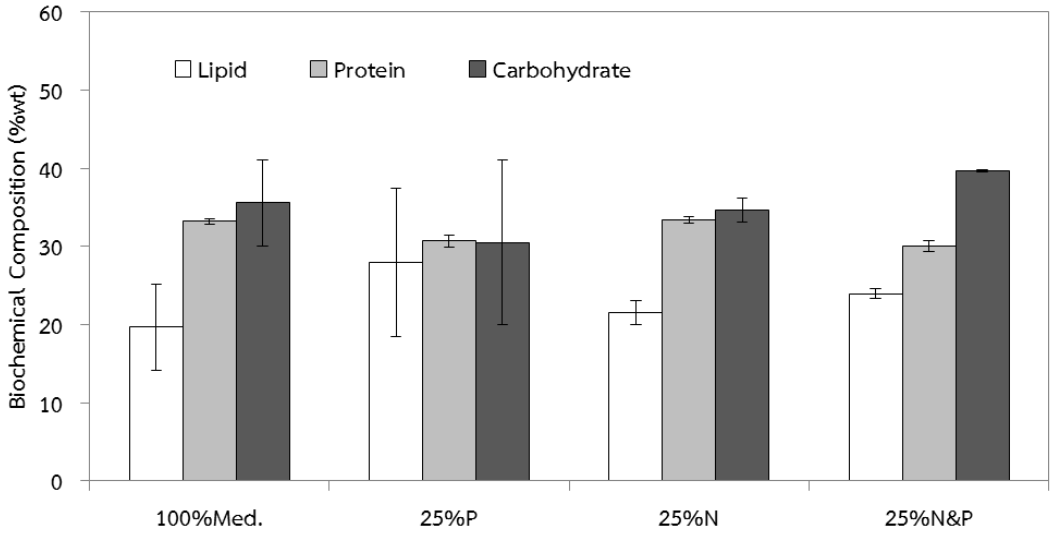


Figure 4.27 Percentage biochemical composition of 25% nutrient by *Scenedesmus* sp. culture in different types of media under the conditions.

Table 4.11 Growth yield on available nutrients in reduced nutrients

System	Growth yield for substrate ($Y_{x/s}$)		
	ΔX/ΔN		ΔX/ΔP
	UV-Vis (NO_3^- in medium)	CHN/O Analyzer (nitrogen in cell)	
***Ankistrodesmus* sp.**			
50% Nutrients			
Control batch	7.07	14.88	39.67
50%P	3.37	14.22	735.63
50%N	1.91	12.48	36.53
50%N&P	3.49	14.62	67.55
25%Nutreints			
Control batch	7.56	14.29	85.52
25%P	6.71	15.77	279.10
25%N	6.19	14.43	88.19
25%N&P	27.50	15.95	675.68
***Scenedesmus* sp.**			
50%Nutrients			
Control batch	3.37	13.39	48.29
50%P	3.58	14.31	73.32
50%N	3.92	12.76	49.31
50%N&P	5.21	13.35	122.70
25%Nutreints			
Control batch	31.46	13.25	93.83
25%P	76.88	14.37	151.38
25%N	8.83	13.09	56.75
25%N&P	4.32	14.77	284.03

Table 4.12 Empirical formula on available nutrient

Details	Empirical formula
***Ankistrodesmus* sp.**	
50%Nutrient	
Control batch	$CH_{1.9}O_{0.6}N_{0.12}P_{0.03}B_{0.005}Mn_{0.0003}Zn_{0.00022}Mo_{0.000125}Cu_{0.000014}Co_{0.0000042}Ca_{0.06}Mg_{0.1}Fe_{0.000002}K_{0.09}$
50%P	$CH_{1.9}O_{0.5}N_{0.12}P_{0.01}B_{0.005}Mn_{0.0001}Zn_{0.00022}Mo_{0.000145}Cu_{0.000023}Co_{0.0000042}Ca_{0.04}Mg_{0.1}Fe_{0.000003}K_{0.01}$
50%N	$CH_{1.8}O_{0.5}N_{0.14}P_{0.021}B_{0.006}Mn_{0.0001}Zn_{0.00012}Mo_{0.0000072}Cu_{0.00009}Co_{0.0000042}Ca_{0.01}Mg_{0.01}Fe_{0.000001}K_{0.02}$
50%N&P	$CH_{1.8}O_{0.5}N_{0.11}P_{0.011}B_{0.003}Mn_{0.0009}Zn_{0.000093}Mo_{0.000171}Cu_{0.000014}Co_{0.0000008}Ca_{0.01}Mg_{0.0004}Fe_{0.000004}K_{0.02}$
25%Nutrient	
Control batch	$CH_{2}O_{0.5}N_{0.12}P_{0.009}B_{0.00001}Mn_{0.000003}Zn_{0.000017}Mo_{0.0000016}Cu_{0.0000012}Co_{0.0000004}Ca_{0.02}Mg_{0.0002}Fe_{0.000003}K_{0.002}$
25%P	$CH_{1.9}O_{0.5}N_{0.11}P_{0.003}B_{0.00003}Mn_{0.000005}Zn_{0.000028}Mo_{0.000123}Cu_{0.0000002}Co_{0.0000003}Ca_{0.04}Mg_{0.0003}Fe_{0.000003}K_{0.03}$
25%N	$CH_{2}O_{0.5}N_{0.12}P_{0.009}B_{0.0051}Mn_{0.000002}Zn_{0.000002}Mo_{0.000123}Cu_{0.0000011}Co_{0.000006}Ca_{0.02}Mg_{0.01}Fe_{0.0000001}K_{0.03}$
25%N&P	$CH_{1.9}O_{0.5}N_{0.11}P_{0.001}B_{0.00232}Mn_{0.000080}Zn_{0.000207}Mo_{0.0000642}Cu_{0.0000007}Co_{0.000004}Ca_{0.01}Mg_{0.03}Fe_{0.000002}K_{0.07}$
***Scenedesmus* sp.**	
50%Nutrient	
Control batch	$CH_{2.2}O_{0.7}N_{0.14}P_{0.02}B_{0.001}Mn_{0.002}Zn_{0.0002}Mo_{0.0003}Cu_{0.0001}Co_{0.00001}Ca_{0.03}Mg_{0.01}Fe_{0.0003}K_{0.05}$
50%P	$CH_{1.9}O_{0.7}N_{0.14}P_{0.01}B_{0.002}Mn_{0.002}Zn_{0.0001}Mo_{0.00002}Cu_{0.0001}Co_{0.00001}Ca_{0.05}Mg_{0.02}Fe_{0.00002}K_{0.01}$
50%N	$CH_{1.9}O_{0.7}N_{0.15}P_{0.02}B_{0.001}Mn_{0.001}Zn_{0.0001}Mo_{0.000003}Cu_{0.00003}Co_{0.00001}Ca_{0.02}Mg_{0.01}Fe_{0.000005}K_{0.02}$
50%N&P	$CH_{2.0}O_{0.7}N_{0.15}P_{0.01}B_{0.0002}Mn_{0.001}Zn_{0.00001}Mo_{0.00003}Cu_{0.00002}Co_{0.000004}Ca_{0.002}Mg_{0.002}Fe_{0.00001}K_{0.01}$
25%Nutrient	
Control batch	$CH_{2.1}O_{0.7}N_{0.15}P_{0.009}B_{0.00004}Mn_{0.0002}Zn_{0.0002}Mo_{0.0001}Cu_{0.00001}Co_{0.000002}Ca_{0.00002}Mg_{0.0001}Fe_{0.00001}K_{0.04}$
25%P	$CH_{1.9}O_{0.7}N_{0.13}P_{0.006}B_{0.001}Mn_{0.000001}Zn_{0.00001}Mo_{0.000001}Cu_{0.00001}Co_{0.000001}Ca_{0.0001}Mg_{0.003}Fe_{0.00001}K_{0.43}$
25%N	$CH_{2.1}O_{0.6}N_{0.14}P_{0.02}B_{0.001}Mn_{0.0001}Zn_{0.00004}Mo_{0.00001}Cu_{0.000002}Co_{0.00001}Ca_{0.02}Mg_{0.01}Fe_{0.0003}K_{0.06}$
25%N&P	$CH_{2.0}O_{0.7}N_{0.13}P_{0.003}B_{0.0001}Mn_{0.000001}Zn_{0.000001}Mo_{0.000001}Cu_{0.000002}Co_{0.000001}Ca_{0.00002}Mg_{0.0002}Fe_{0.000002}K_{0.02}$

4.3 Reused medium

This section shows the effect of reuse nutrient of the BG11 medium on the growth performance and biochemical composition of the algae. Microalgae were cultivated in a series of photobioreactors, the first one with fresh BG11 nutrient (denoted as first batch), the second and the third with the reused nutrients from the previous batch (after removing algal cells, denoted as 1st reuse and 2nd reuse, respectively). It is noted that the nutrient after removing algae was used directly without autoclaving. Each experiment was repeated four times to allow statistical test. Figure 4.28 shows the experiment setup in this section.

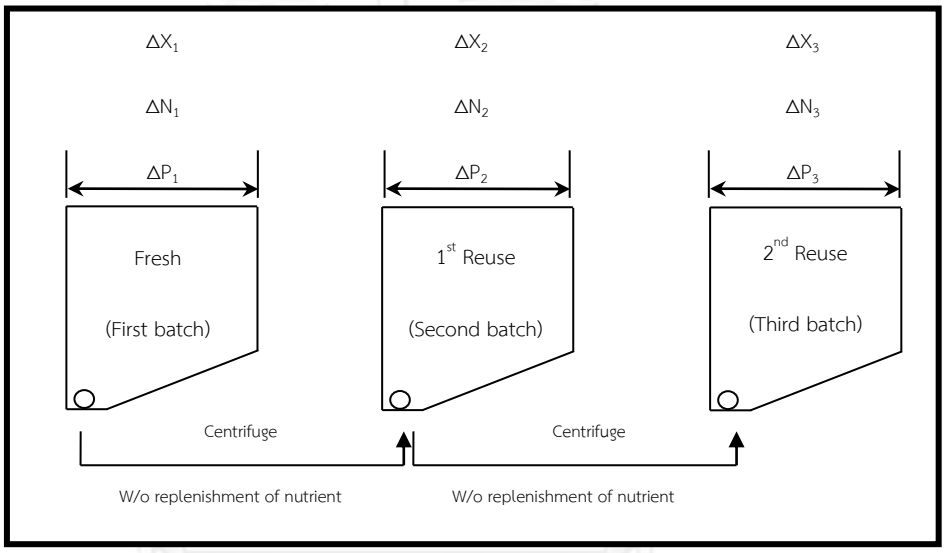


Figure 4.28 Setup for the reuse nutrient experiment

4.3.1 *Ankistrodesmus* sp. culture

Growth

Ankistrodesmus sp. was cultured with the conditions as stated in Table 4.1. The growth of all batches is shown Figure 4.29 and Table 4.13. Statistical analysis illustrates that the effect of reused nutrient condition provided different growth behavior with relatively high statistical significant level ($p \leq 0.05$). The 1st reuse condition gave the highest cell density at $1.47 \pm 0.28 \times 10^7$ cell mL^{-1} and dry weight at 0.76 ± 0.19 g L^{-1}. The second best growth condition was the culture with fresh nutrient, and the worst was the 2nd reuse, where the obtained cell densities were $9.06 \pm 1.40 \times 10^6$ and $8.76 \pm 2.79 \times 10^6$ cell mL^{-1}, and dry weight 0.55 ± 0.08 and 0.60 ± 0.13 g L^{-1}, respectively. Elemental analysis suggested that the medium still contained

adequate nutrients for the next batches. Literature demonstrates that the reuse of nutrient could have variety of effects on algal growth. For instance, Wu *et al.* (2012) stated that *Chlorella vulgaris*, *Scenedesmus* sp., *Chlorococcum* sp. exhibited similar growth pattern when cultivated in reused BG11 when compared with the fresh nutrient. However, Rodolfi *et al.* (2003) reported that *Nannochloropsis* sp. grew better in fresh nutrient when compared with reused and replenished nutrients. Similarly, Krichnavaruk et al. (2005) also indicated that the reused nutrients might contain some toxic elements which prevented the growth of the nutrient. In this particularly case, *Ankistrodesmus* sp. showed to be capable of growing in reused medium as long as it still contained adequate level of nutrients. In fact, the 1st reuse medium gave a better growth than the fresh BG11 which suggested that the initial nutrient concentration might be a little too high for an effective growth of such culture. The temperature and pH profiles for this set of experiment are shown in Figures 4.31 and 4.32, respectively.

Nutrients

Figures 4.33 and 4.34 display nitrogen and phosphorus concentration profiles from the various batches. Nitrogen concentration continuously decreased from batch to batch as some was taken up by the cells. On the other hand, there was no predefined phosphorus concentration that could be observed from the various batches. This could be due to the fact that phosphorus was not directly associated with growth, but with the storage of energy in the cell and therefore its concentration might not directly reflect the growth.

Table 4.14 demonstrates %reduction of nutrient in the medium (before and after each cultivation batch). The nitrogen reduction was found to be the highest from the second batch at 14.28% followed by first and third batches with the %reduction of 7.96% and 5.52%, respectively. This %reduction in nitrogen reflected directly the growth of the culture which is reasonable as nitrogen is an essential growth nutrient. The decreased phosphorus, on the other hand, did not follow the growth of the alga as this was found to be the highest in the first batch followed by the second and third batches (86.35%, 5.92% and 3.09%, respectively). As stated earlier, this assimilation of phosphorus could indicate the energy storage level in the algal cell and could not be described simply with the algal growth. The uptake of other trace elements for *Ankistrodesmus* sp. was reported in Table 4.14. This was not discussed in detail in this work but is given here to estimate the molecular of algal cell (in later section) and also for the sake of future reference.

Table 4.15 gives the summary of the specific uptake of nitrogen and phosphorus per unit cell mass. This shows, again, that the uptake of nitrogen per unit cell mass was quite constant (in the same magnitude) as it was associated directly with cell growth, whilst the uptake of phosphorus was not and was quite difficult to predict.

Biochemical composition

Table 4.13 and Figure 4.38 demonstrate biochemical compositions of *Ankistrodesmus* sp. Lipid contents from each batch were significantly different ($p \leq 0.05$). The lipid content was the highest at 29±2%wt from the 1st reuse medium, 24±3%wt from the fresh medium, and 19±1%wt from the 2nd reuse medium. This level of lipid was within the range reported to be obtained from the alga with the same genus, e.g. Habib *et al.* (2004) reported that the growth of *Ankistrodesmus convolutes* in wastewater gave lipid content at around 14-15%wt, and Macedo and Pinto-Corlho (2001) reported that the growth in the diet state gave a relatively high average lipid content at 22.1%wt.

Carbohydrate, on the other hand, was found to be the highest (44±12%wt) in the 2nd reuse medium, followed by the 1st and fresh mediums at 39±7% and 34±4%wt, respectively. Carvalho *et al.* (2009) reported that cells would accumulate more carbohydrate in the condition with high light intensity, which might explain the accumulation of carbohydrate in the 2nd reuse medium where the low cell density might allow more light penetration to the culture. Carbohydrate accumulations in fresh and 1st reuse mediums were not so much different which also reflected the level of light penetration to the culture (similar growth from these two batches).

Protein content in the first batch was the highest at 29±4%wt followed by the 2nd and 1st reuse medium batches with the protein content of 22±9 and 19±3%wt, respectively. As nitrogen is one major constituent of protein molecules (such as amino acids and nucleic acids) in the cell, the protein content followed very closely with the consumption or assimilation of nitrogen from the medium.

4.3.2 *Scenedesmus* sp. culture

Scenedesmus sp. was cultured with the conditions as stated in Table 4.1. Figure 4.36 and Table 4.13 show the growth of *Scenedesmus* sp. cultured in reuse mediums. For this culture, the cell could only grow in fresh nutrient with cell density of $5.19 \pm 2.76 \times 10^6$ cell mL^{-1}. Cells were not found to grow in the 1st and 2nd reuse mediums with the final cell density of $6.0 \pm 9.5 \times 10^5$ and $4.0 \pm 5.0 \times 10^4$ cell mL^{-1},

respectively. Similar findings were reported by Kim et al. (2011) who revealed that this algal species flocculated when cultivated in reused nutrient giving lower biomass yield than fresh medium. In this experiment, *Scenedesmus* sp. ceased growth after 2 days in the 1st reuse medium and did not show any sign of growth after that at all. It was possible that there were some extracellular chemicals that inhibited cell growth as suggested by Hellebust (1965) and Hii *et al.* (2011).

Biochemical composition

Although the cell density of *Scenedesmus* sp. in the 1st reuse medium was lower than the fresh batch, it is interesting to note that lipid content of the 1st reuse medium was relatively high at 22±6%wt when compared with that from the fresh batch (17±1%wt). It was possible that when cells were in an un-suitable growth condition, they started to accumulate lipid as their energy storage. This finding was supported by the work of Chu et al. (2013) who proposed that the cultivation under nutrient limitation might have badly affected biomass productivity, but could give rise to the lipid content. Protein and carbohydrate contents from the culture with fresh and 1st reuse mediums were not found to be significantly different. Having discussed so, the growth in the 1st reuse medium was meaningless in this work as cell growth was so small and could not be further applied as a productive growth condition.

4.2.3 Concluding remarks

Ankistrodesmus sp. was found to be successfully cultivated in reuse mediums where different cell properties could be obtained from the different growth conditions. On the other hand, *Scenedesmus* sp. could not be cultivated at all with reuse medium which might be due to some inhibition that might be released during the growth of this culture. From the elemental mass balance, the biochemical formulation of both algae cultivated from the various conditions could be derived as summarized in Table 4.17.

Table 4.13 Growth and biochemical composition of microalgae

Characters	Fresh	1st Reuse	2nd Reuse
***Ankistrodesmus* sp.**			
final cell density (cell mL^{-1})	$9.06\pm1.40\times10^{6}$ [a]	$1.47\pm0.28\times10^{7}$ [b]	$8.76\pm2.79\times10^{6}$ [a]
Specific growth rate (d^{-1})	0.24 ± 0.03 [a]	0.30 ± 0.01 [b]	0.22 ± 0.04 [a]
final dry weight (g L^{-1})	0.55 ± 0.08	0.76 ± 0.19	0.60 ± 0.13
Productivity (g d^{-1})	1.23 ± 0.25	1.70 ± 0.59	1.03 ± 0.60
% Lipid	24 ± 3 [a]	29 ± 2 [b]	19 ± 1 [c]
% Protein	29 ± 4	19 ± 3	22 ± 9
% Carbohydrate	34 ± 4	39 ± 7	44 ± 12
% Moisture	2.18 ± 1.5	0.65 ± 0.2	1.36 ± 0.8
% Ash	11 ± 3	12 ± 6	9 ± 4
Lipid productivity (mg L^{-1} d^{-1})	15 ± 1 [a]	24 ± 7 [b]	13 ± 3 [a]
Protein productivity (mg L^{-1} d^{-1})	18 ± 2	15 ± 2	14 ± 6
Carbohydrate productivity (mg L^{-1} d^{-1})	21 ± 3	24 ± 5	27 ± 7
***Scenedesmus* sp.**			
Final cell density (cell mL^{-1})	$5.19\pm276\times10^{6}$		
Specific growth rate (d^{-1})	0.58 ± 0.09		
Final dry weight (g L^{-1})	0.381 ± 0.1		
Productivity (g d^{-1})	1.90 ± 0.72		
% Lipid	17 ± 2		
% Protein	31 ± 2		
% Carbohydrate	39 ± 5		
% Moisture	0.15 ± 0.0		
% Ash	13 ± 4		
Lipid productivity (mg L^{-1} d^{-1})	16.5 ± 6		
Protein productivity (mg L^{-1} d^{-1})	23.2 ± 1		
Carbohydrate productivity (mg L^{-1} d^{-1})	28.9 ± 4		

Table 4.14 Reduction (%) of elements in reused medium

Elements	Fresh			1st Reuse			2nd Reuse		
	Concentration (mg L^{-1})		% Reduction (Δ1)	Concentration (mg L^{-1})		% Reduction (Δ2)	Concentration (mg L^{-1})		% Reduction (Δ3)
	Initial day	Final day		Initial day	Final day		Initial day	Final day	
***Ankistrodesmus* sp.**									
N	224.3	206.4	7.96	177.0	151.7	14.28	136.9	129.3	5.52
P	7.482	1.021	86.35	0.0456	0.0431	5.92	0.3294	0.3195	3.09
B	0.4878	0.3791	22.28	0.3612	0.2227	38.34	0.2244	0.1211	46.06
Ca	11.025	3.501	68.25	4.5949	4.5703	0.53	0.2485	0.1128	54.63
Mg	13.67	12.75	6.72	10.0343	9.1180	9.13	8.8820	4.0445	54.46
Fe	0.1243	0.0049	96.08	0.0058	0.0056	4.72	0.0172	0.0083	51.55
Mn	0.0304	0.0281	7.56	0.0191	0.0179	6.28	0.0957	0.0113	88.23
Zn	0.2630	0.2560	2.67	0.2251	0.2182	3.08	0.1437	0.1429	0.58
Mo	0.1865	0.1691	10.31	0.1106	0.0876	20.80	0.0952	0.0803	15.66
Cu	0.0021	0.0019	10.64	0.0348	0.0066	81.03	0.0077	0.0065	15.03
Co	0.0021	0.0019	10.63	0.0026	0.0025	3.81	0.0013	0.0005	61.54
K	89.35	83.89	6.10	114.8	96.84	15.65	110.9	87.79	20.80
***Scenedesmus* sp.**									
N	247.7	236.5	4.5						
P	6.0	3.7	39.2						
B	0.348	0.347	0.1						
Ca	25.0	24.4	2.6						
Mg	14.7	13.8	1.3						
Fe	0.017	0.002	88.5						
Mn	0.019	0.005	75.5						
Zn	0.204	0.139	31.9						
Mo	0.085	0.083	2.6						
Cu	0.010	0.007	32.4						
Co	0.0019	0.0018	4.5						
K	58.2	58.1	0.3						

Table 4.15 Uptake rate on available nutrients in reused medium

Time (days)	Uptake rate for substrate ($Y_{s/x}$)					
	ΔN/ΔX			ΔP/ΔX		
	Fresh	1st Reuse	2nd Reuse	Fresh	1st Reuse	2nd Reuse
Ankistrodesmus sp.						
0						
1	0.0138	0.0211	0.0097	0.0095	0.0000	0.0033
2	0.0155	0.0225	0.0108	0.0085	0.0000	0.0032
3	0.0175	0.0242	0.0122	0.0077	0.0001	0.0031
4	0.0203	0.0262	0.0141	0.0071	0.0001	0.0031
5	0.0243	0.0286	0.0166	0.0068	0.0001	0.0032
6	0.0302	0.0316	0.0203	0.0067	0.0001	0.0033
7	0.0402	0.0353	0.0261	0.0071	0.0001	0.0037
8	0.0606	0.0402	0.0368	0.0086	0.0001	0.0045
9	0.1249	0.0467	0.0629	0.0141	0.0001	0.0067
Scenedesmus sp.						
0						
1	0.0473			0.0225		
2	0.0271			0.0117		
3	0.0204			0.0079		
4	0.0168			0.0058		

Table 4.16 Growth yield on available nutrients in reused medium

System	Growth yield for substrate ($Y_{x/s}$)		
	ΔX/ΔN		ΔX/ΔP
	UV-Vis	CHN/O Analyzer	
***Ankistrodesmus* sp.**			
Fresh	24.76	15.41	69
1st reuse	24.24	21.60	245,332
2nd reuse	47.53	16.89	36,490
***Scenedesmus* sp.**			
Fresh	4.32	14.77	132.47

Table 4.17 Empirical formula on available nutrient in reused medium

Details	Empirical formula
***Ankistrodesmus* sp.**	
Fresh	$CH_{1.9}O_{0.7}N_{0.12}P_{0.012}B_{0.006}Mn_{0.000002}Zn_{0.000006}Mo_{0.00001}Cu_{0.0000002}Co_{0.0000002}Ca_{0.01}Mg_{0.002}Fe_{0.0001}K_{0.01}$
1st reuse	$CH_{1.9}O_{0.8}N_{0.09}P_{0.000004}B_{0.006}Mn_{0.000001}Zn_{0.000005}Mo_{0.00001}Cu_{0.00002}Co_{0.0000001}Ca_{0.00003}Mg_{0.002}Fe_{0.0000002}K_{0.02}$
2nd reuse	$CH_{1.7}O_{0.8}N_{0.12}P_{0.0003}B_{0.008}Mn_{0.000122}Zn_{0.000001}Mo_{0.00001}Cu_{0.000002}Co_{0.0000011}Ca_{0.00027}Mg_{0.02}Fe_{0.00001}K_{0.05}$
***Scenedesmus* sp.**	
Fresh	$CH_{1.7}O_{0.7}N_{0.13}P_{0.01}B_{0.00001}Mn_{0.00002}Zn_{0.00009}Mo_{0.000002}Cu_{0.0000041}Co_{0.0000001}Ca_{0.001}Mg_{0.003}Fe_{0.00002}K_{0.0002}$

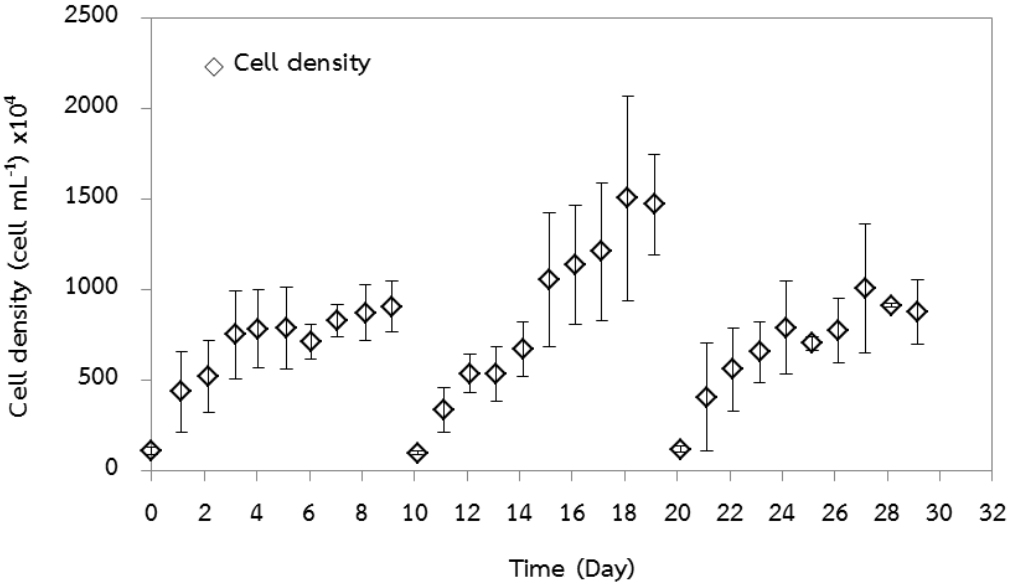


Figure 4.29 Growth of *Ankistrodesmus* sp. culture in reused medium

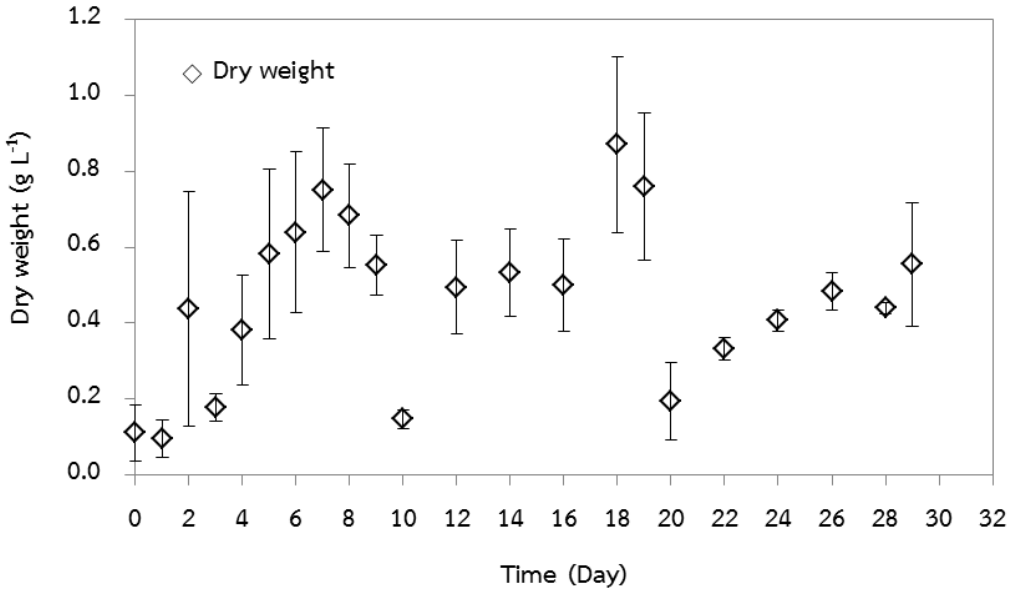


Figure 4.30 Dry weight of *Ankistrodesmus* sp. culture in reused medium

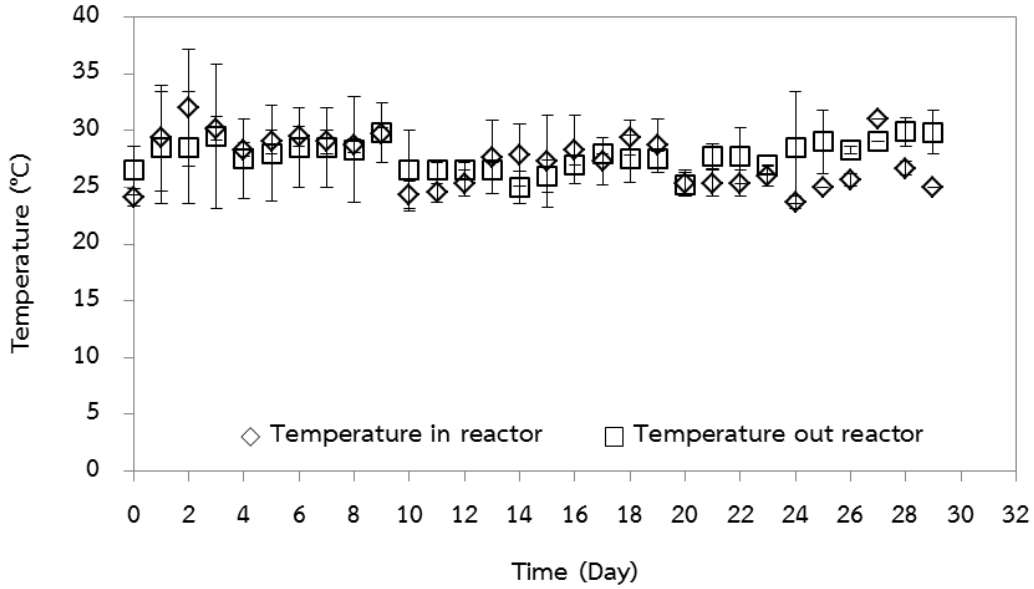


Figure 4.31 Temperature of *Ankistrodesmus* sp. culture in reused medium

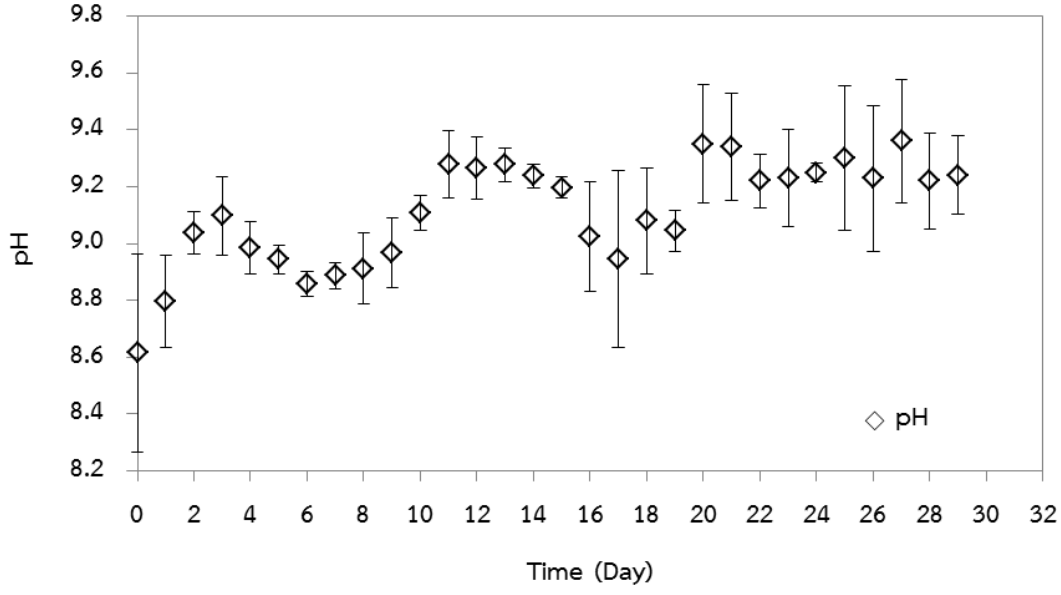


Figure 4.32 pH of *Ankistrodesmus* sp. culture in reused medium

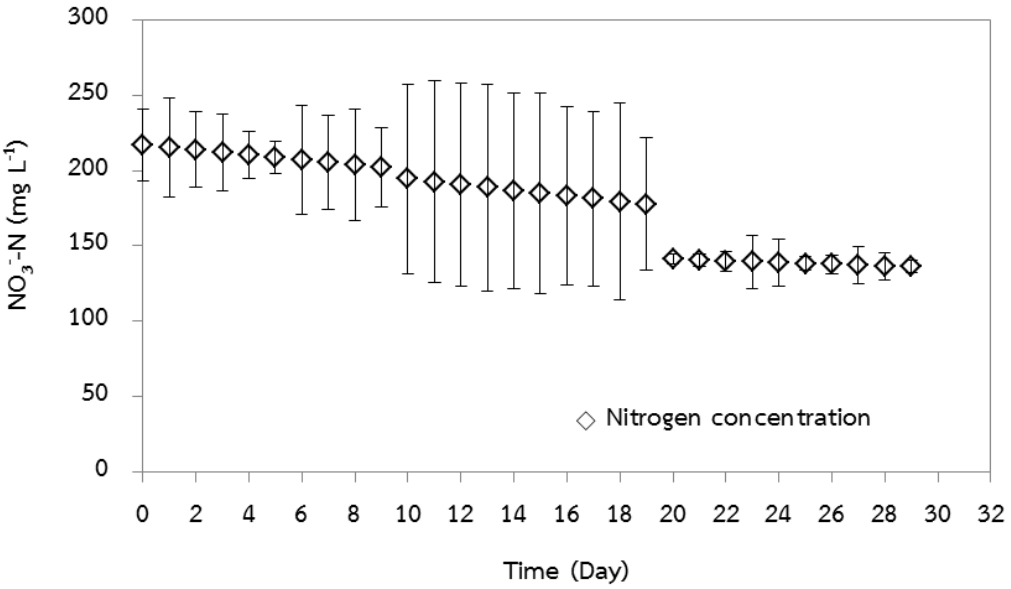


Figure 4.33 Nitrogen concentration of *Ankistrodesmus* sp. culture in reused medium

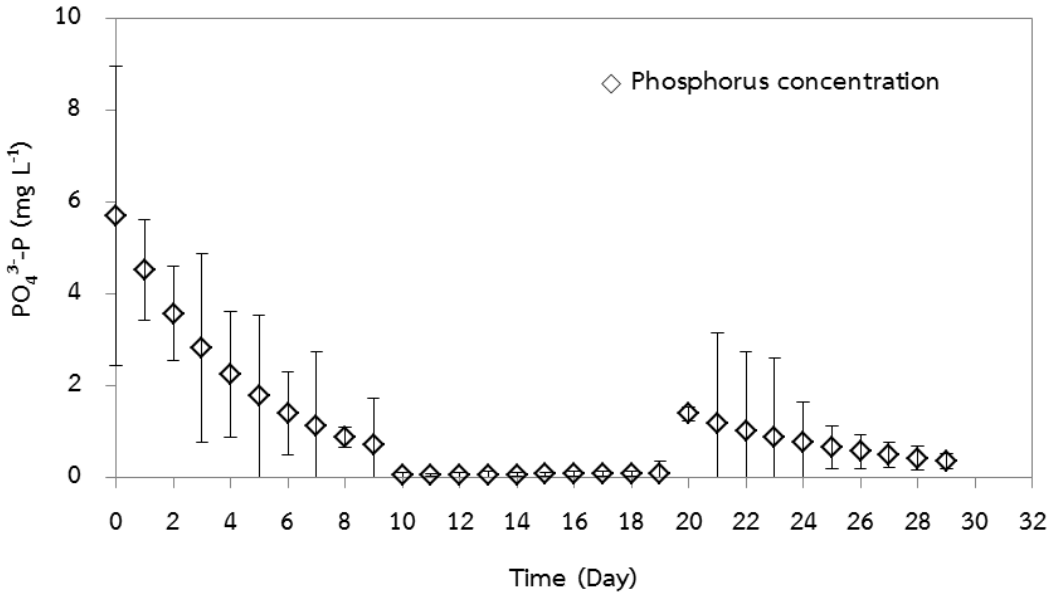


Figure 4.34 Phosphorus concentration of *Ankistrodesmus* sp. culture in reused medium

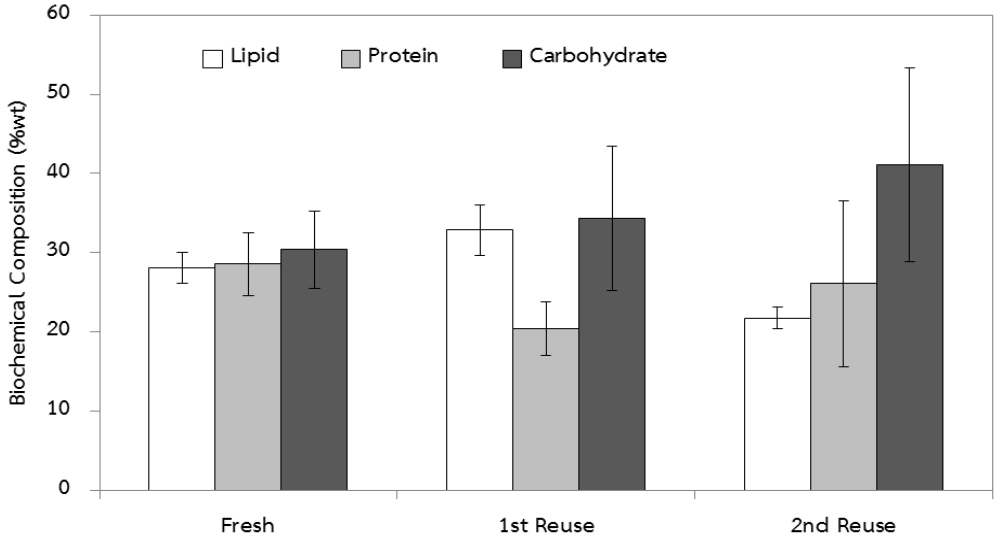


Figure 4.35 Biochemical composition of *Ankistrodesmus* sp. culture in reused medium

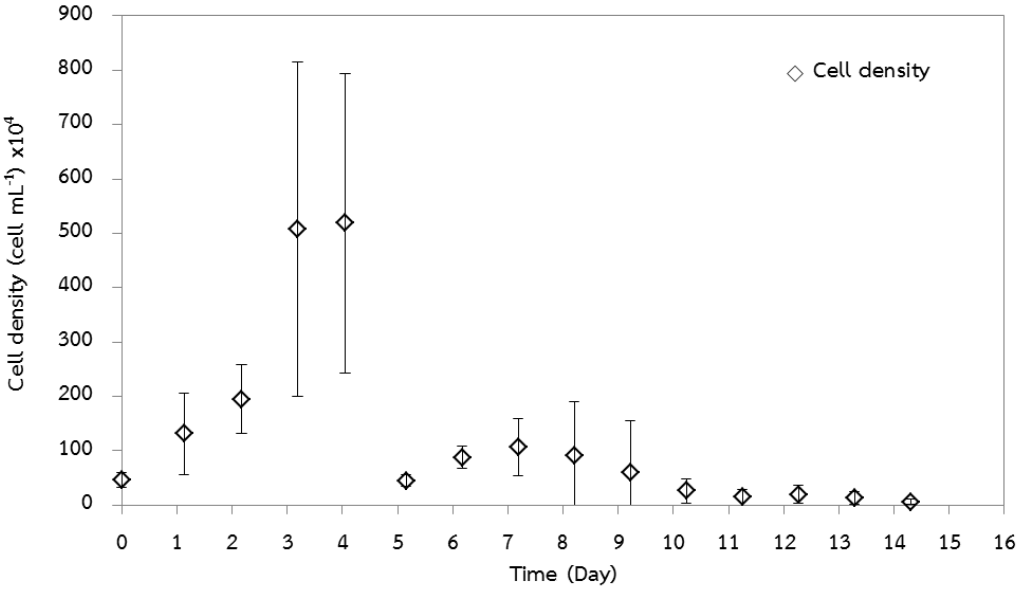


Figure 4.36 Growth of *Scenedesmus* sp. culture in reused medium

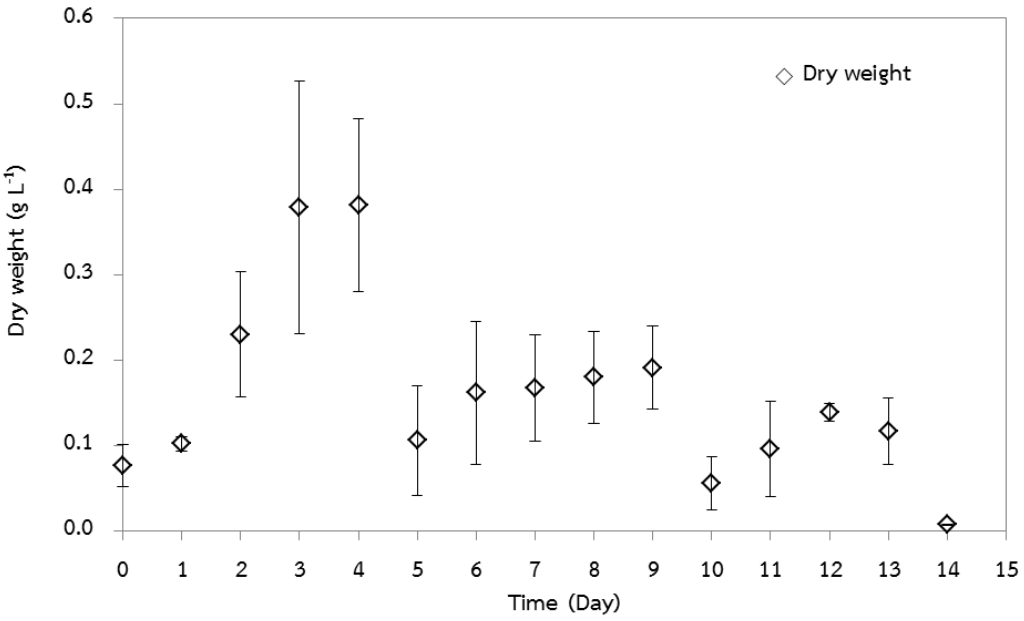


Figure 4.37 Dry weight of *Scenedesmus* sp. culture in reused medium

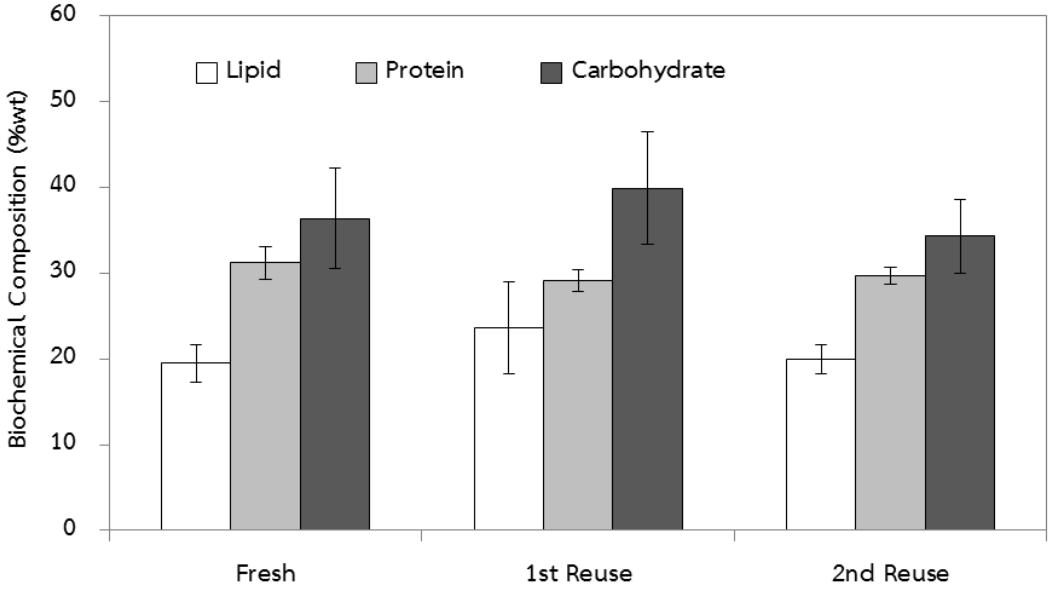


Figure 4.38 Biochemical composition of *Scenedesmus* sp. culture in reused medium

4.4 Economical assessment of algae biomass production

This section presents basic concepts for economics assessment, and describes methodologies for conducting microalgae cultivation (*Ankistrodesmus* sp. and *Scenedesmus* sp.). A simple cost model is used to compare the different cultivation scenarios (Reduced nutrients in Section 4.2 and reused nutrients in Section 4.3) for the production of algal biomass and for the minimization of the operating costs (major medium and water cost) in NB-FPAP 25 L.

Assumptions and constraints were established and installed as a part of economical evaluation. The operating costs were calculated using local utility costs in Thailand in 2014 for both microalgae cultivation. The other assumptions employed for this evaluation are listed below:

- The calculation was based on the maximum capacity of a series of reactors within the area of 1 hectare (9,375 reactor unit).
- 300 operating days per annum was applied.
- Artificial light operation was available 24 h (300 days).
- Electric charge was computed at 0.12 USD per kWh (February 2014).
- Water charge was estimated at 0.84 USD per cubic meter (February 2014).
- Exchange rate on March (2014) was 32 THB per USD.

Tables 4.18 illustrates the charges (USD/kg) at 50% and 25% reduction nutrient of *Ankistrodesmus* sp. and *Scenedesmus* sp., respectively. The reduced nutrient at 25%N&P condition provided the best economical profile as it could diminish the nutrient cost (USD/kg) by 68.29% (compared to the fresh BG11 medium). The 50% and 25% reduction could reduce the cost up to 50.83% and 56.13% for *Ankistrodesmus* sp. and *Scenedesmus* sp., respectively. Being the major cost component in BG11 (as much as 88.17%), reduction in nitrogen content could effectively reduce the cost of nutrient.

Tables 4.19 demonstrates the detail of the operation costs for the indoor cultivations of *Ankistrodesmus* sp. and *Scenedesmus* sp. at 50% and 25% reduction nutrient. The electrical contributed 47-84% to the total operation cost, whereas the water only shared 0.7-1.7 % of the total cost. Electricity was therefore the most expensive item for this cultivation which was due to the 24 hour supply of light and the use of air pump.

In the reuse part of *Ankistrodesmus* sp., the first batch always gave the highest cost as all nutrients were newly prepared. The costs of the second and third batches could clearly be reduced up to 51.74% and 67.17% of the cost of the fresh solution, respectively. This reduction was obtained because there were no additional charges from the use of remaining nutrient and water. The electrical expenses of second and third batches contributed to only 38.51% (of the total costs from Batches 1 and 2) and 27.80% (of the total costs from the three batches), respectively.

The cost of outdoor cultivation was calculated in a similar manner with the indoor except that there was no electricity charge on the supply of light. Tables 4.13 and 4.14 illustrate the charge (USD/kg) for *Ankistrodesmus* sp. and *Scenedesmus* sp., respectively. The absence of artificial light supply for this outdoor condition could reduce the cost up to 50%.

In conclusion, it was shown that the major cost for the cultivation of microalgae was the electricity. Saving the electrical consumption could be achieved by applying the outdoor culture. The second major cost component was the nutrient and water charge where reducing or reusing the nutrient helped diminish the proportion of nutrient expense. A further reduction in cost might be achieved through the development of cultivation process with other alternative nutrients such as waste fertilizer. In addition, improving algal productivity through genetic alteration might also enhance the economical status of the algal culture (Borowitzka, 1992).

Table 4.18 Compared costs in different conditions

Details	Indoor		Outdoor	
	Cost (USD kg^{-1})	Cost (USD ha^{-1})	Cost (USD kg^{-1})	Cost (USD ha^{-1})
***Ankistrodesmus* sp.**				
Reduce of 50%Nutrient Conditions				
100%Medium	298	25,877	125	10,801
50%Phosphorus	305	25,741	126	10,665
50%Nitrogen	299	21,702	91	6,626
50%Nitrogen&Phosphorus	192	21,566	58	6,490
Reduce of 25%Nutrient Conditions				
100%Medium	240	25,877	100	10,801
25%Phosphorus	171	25,673	71	10,596
25%Nitrogen	161	19,615	37	4,539
25%Nitrogen&Phosphorus	118	19,410	26	4,334
Reuse conditions				
Fresh	201	25,877	84	10,801
1^{st} reuse	97	42,084	27	11,931
2^{nd} reuse	66	58,291	15	13,062
***Scenedesmus* sp.**				
Reduce of 50%Nutrient Conditions				
100%Medium	238	18,394	130	10,019
50%Phosphorus	325	18,258	176	9,882
50%Nitrogen	217	14,220	89	5,844
50%Nitrogen&Phosphorus	231	14,083	94	5,708
Reduce of 25%Nutrient Conditions				
100%Medium	253	18,394	138	10,019
25%Phosphorus	250	18,190	135	9,814
25%Nitrogen	225	12,132	70	3,756
25%Nitrogen&Phosphorus	111	11,928	33	3,552
Reuse conditions				
Fresh	207	18,394	112	10,019

Table 4.19 Summary of operation cost for indoor cultivation in different condition

Details	Cost (USD)							
	Nutrient	% of total	Electricity	% of total	Water	% of total	Total operation	% of total
Ankistrodesmus sp.								
Reduce of 50%Nutrient Conditions								
100%Medium	315,646	36.59	540,228	62.63	6,689	0.78	862,563	100.00
50%Phosphorus	311,104	36.26	540,228	62.96	6,689	0.78	858,022	100.00
50%Nitrogen	176,485	24.40	540,228	74.68	6,689	0.92	723,403	100.0
50%Nitrogen&Phosphorus	171,944	23.92	540,228	75.15	6,689	0.93	718,862	100.00
Reduce of 25%Nutrient Conditions								
100%Medium	315,646	36.59	540,228	62.63	6,689	0.78	862,563	100.00
25%Phosphorus	308,834	36.09	540,228	63.13	6,689	0.78	855,751	100.00
25%Nitrogen	106,905	16.35	540,228	82.63	6,689	1.02	653,823	100.00
25%Nitrogen&Phosphorus	100,094	15.47	540,228	83.50	6,689	1.03	647,011	100.00
Reuse conditions								
Fresh	315,646	36.59	540,228	62.63	6,689	0.78	862,563	100.00
1st reuse	-	-	540,228	38.51	-	-	1,402,791	38.51
2nd reuse	-	-	540,228	27.80	-	-	1,943,018	27.80
***Scenedesmus* sp.**								
Reduce of 50%Nutrient Conditions								
100%Medium	568,162	51.48	523,456	47.43	12,041	1.09	1,103,659	100.00
50%Phosphorus	559,988	51.12	523,456	47.78	12,041	1.10	1,095,485	100.00
50%Nitrogen	317,674	37.23	523,456	61.35	12,041	1.41	853,170	100.00
50%Nitrogen&Phosphorus	309,500	36.63	523,456	61.95	12,041	1.42	844,996	100.00
Reduce of 25%Nutrient Conditions								
100%Medium	568,162	51.48	523,456	47.43	12,041	1.09	1,103,659	100.00
25%Phosphorus	555,901	50.93	523,456	47.96	12,041	1.10	1,091,398	100.00
25%Nitrogen	192,429	26.44	523,456	71.91	12,041	1.65	727,926	100.00
25%Nitrogen&Phosphorus	180,169	25.17	523,456	73.14	12,041	1.68	715,665	100.00
Reuse conditions								
Fresh	568,162	51.48	523,456	47.43	2,041	1.09	1,103,659	100.00

Table 4.20 Summary of operation cost for outdoor cultivation in different condition

Details	Cost (USD)							
	Nutrient	% of total	Electricity	% of total	Water	% of total	Total operation	% of total
Ankistrodesmus sp.								
Reduce of 50%Nutrient Conditions								
100%Medium	315,646	87.67	37,690	10.47	1.86	6,689	360,025	100.00
50%Phosphorus	311,104	87.52	37,690	10.60	1.88	6,689	355,484	100.00
50%Nitrogen	176,485	79.91	37,690	17.06	3.03	6,689	220,865	100.00
50%Nitrogen&Phosphorus	171,944	79.48	37,690	17.42	3.09	1.86	216,324	100.00
Reduce of 25%Nutrient Conditions								
100%Medium	315,646	87.67	37,690	10.47	6,689	1.86	360,025	100
25%Phosphorus	308,834	87.44	37,690	10.67	6,689	1.89	353,214	100
25%Nitrogen	106,905	70.66	37,690	24.91	6,689	4.42	151,285	100
25%Nitrogen&Phosphorus	100,094	69.28	37,690	26.09	6,689	4.63	144,474	100
Reuse conditions								
Fresh	315,646	87.67	37,690	10.47	6,689	1.86	360,025	100.00
1st reuse	-	-	37,690	9.48	-	-	397,716	9.48
2nd reuse	-	-	37,690	8.66	-	-	435,406	8.66
***Scenedesmus* sp.**								
Reduce of 50%Nutrient Conditions								
100%Medium	568,162	51.48	523,456	47.43	12,041	1.09	1,103,659	100.00
50%Phosphorus	559,988	51.12	523,456	47.78	12,041	1.10	1,095,485	100.00
50%Nitrogen	317,674	37.23	523,456	61.35	12,041	1.41	853,170	100.00
50%Nitrogen&Phosphorus	309,500	36.63	523,456	61.95	12,041	1.42	844,996	100.00
Reduce of 25%Nutrient Conditions								
100%Medium	568,162	51.48	523,456	47.43	12,041	1.09	1,103,659	100
25%Phosphorus	555,901	50.93	523,456	47.96	12,041	1.10	1,091,398	100
25%Nitrogen	192,429	26.44	523,456	71.91	12,041	1.65	727,926	100
25%Nitrogen&Phosphorus	180,169	25.17	523,456	73.14	12,041	1.68	715,665	100
Reuse conditions								
Fresh	568,162	51.48	523,456	47.43	2,041	1.09	1,103,659	100.00

Table 4.21 Economics for *Ankistrodesmus* sp. indoor cultivation in different media of NB-FPAP

Details	Symbol	Unit	100%Med.	% of total	50%P	% of total	50%N	% of total	50%N&P	% of total
Conditions										
Volume	A	L	25		25		25		25	
Period days	B	Day	9		9		9		9	
Dry weight	C	g/L	0.37		0.36		0.31		0.48	
productivity per reactor	D=C/B*300	g/L/Year	12		12		10		16	
Max Reactor in 1 hectare	E	unit	9,375		9,375		9,375		9,375	
productivity in 1 hectare	F=E*D	g/L/Year	115,625		112,500		96,875		150,000	
Nutrient requirement										
Nutrient cost	G	USD/L	0.0404		0.0398		0.0226		0.0220	
Nutrient charge	H=(G*E*A)*(300/B)	USD/year	315,646	36.59	311,104	36.26	176,485	24.40	171,944	23.92
Electricity requirement										
Number of light	I=E*number	unit	37,500		37,500		37,500		37,500	
Lighting	J=I*watts*24*300/1000	kWh	5,400,000		5,400,000		5,400,000		5,400,000	
Air pump	K=E*watts*24*300/1000	kWh	405,000		405,000		405,000		405,000	
total electricity	L=J+K	kWh	5,805,000		5,805,000		5,805,000		5,805,000	
Electricity charge (electricity charge = 0.12 USD per kWh)	M=L*0.12	USD/year	540,228	62.63	540,228	62.96	540,228	74.68	540,228	75.15
Water requirement										
water supply	N=E*A	L	234,375		234,375		234,375		234,375	
Water charge (water charge = 0.84 USD per m^3)	O=N*0.31	USD/year	6,689	0.78	6,689	0.78	6,689	0.92	6,689	0.93
Total operation	P=H+M+O	USD/year	862,563	100.00	858,022	100.00	723,403	100.0	718,862	100.00
Cost	Q=(P/A/E)*(B/300)	USD/L	0.1104		0.1098		0.0926		0.0920	

Chapter 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

5.1.1 Reduced nutrient

The reduced nutrient of nitrogen and phosphorus element in BG11 medium at various concentrations influenced the growth and biochemical composition of the two microalgae. This can be summarized as follows:

- The 25%N&P condition for *Ankistrodesmus* sp. provided a 34.29% higher dry weight than that with 100%Medium, whereas *Scenedesmus* sp. at 34.04%.

- The reduced nutrient affected the morphology of both microalgae, and the 25%N&P condition gave a more cluster-like culture than other conditions.

- The reduced phosphorus in BG11, 50%P of *Ankistrodesmus* sp. and 25%P of *Scenedesmus* sp., provided a 2-9% higher lipid content than other conditions.

- The reduced nitrogen in BG11 affected the accumulation of protein in the microalgae, i.e *Ankistrodesmus* sp. at nitrogen reduction in BG11 medium gave 4-5% higher protein content and 2% higher protein productivity than the other conditions, whereas *Scenedesmus* sp. gave 1-4% higher protein content and 1-2% higher protein productivity.

- The two microalgal cultivation at 25%N&P condition provided higher lipid (12-32% for *Ankistrodesmus* sp. and 28-57% for *Scenedesmus* sp.) and carbohydrate productivity (5-16% for *Ankistrodesmus* sp. and 7-19% for *Scenedesmus* sp.) than other conditions.

- The microalgal cultivation at 25%N&P gave the lowest cost, i.e. 50.83% and 56.13% lower than the cost of the control batches of *Ankistrodesmus* sp. and *Scenedesmus* sp., respectively.

5.1.2 Reused medium

- *Ankistrodesmus* sp. was found to be successfully cultivated in reuse medium which 1st reuse provided higher cell density and dry weight at 38.36% and 27.63% than that of the fresh nutrient batch.

- The cultivation in the 1st reuse nutrient for *Ankistrodesmus* sp. provided higher lipid and carbohydrate content (both at 5%) more than fresh batch, whereas carbohydrate content in the 2nd reuse nutrient condition was 10% higher than the fresh nutrient culture.

- *Scenedesmus* sp. could not be cultivated at all with reuse medium.

5.2 Recommendations

1. The empirical formula of the algae obtained from this work suggested that the algal cells only needed a small amount of nutrients for their growth (about 4-7% of the supply in fresh BG11). Further investigation should be conducted to see how much further the nutrient concentration could be reduced to.

2. This work did not attempt to monitor the biochemical composition of both microalgae on a daily basis, and therefore could not generate a conclusion on the diurnal change in cell composition which could be useful if any target components are to be focused upon. This will need to be discussed with additional experiments.

3. The reuse medium for Scenedesmus sp. was found to be unsuccessful. However, exact reasons for this will still need to be identified.

REFERENCES

Beardall, J., S. Roberts and J. A. Raven, 2005: Regulation of inorganic carbon acquisition by phosphorus limitation in the green alga Chlorella emersonii. *Canadian Journal of Botany,* **83,** 859-864.

Becker, E., 2007: Micro-algae as a source of protein. *Biotechnology advances,* **25,** 207-210.

Borowitzka, M. A., 1992: Algal biotechnology products and processes—matching science and economics. *Journal of Applied Phycology,* **4,** 267-279.

Carvalho, A. P., C. M. Monteiro and F. X. Malcata, 2009: Simultaneous effect of irradiance and temperature on biochemical composition of the microalga Pavlova lutheri. *Journal of applied phycology,* **21,** 543-552.

Chen, C.-Y., K.-L. Yeh, R. Aisyah, D.-J. Lee and J.-S. Chang, 2011: Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review. *Bioresource technology,* **102,** 71-81.

Chen, F. and Y. Zhang, 1997: High cell density mixotrophic culture of< i> Spirulina platensis</i> on glucose for phycocyanin production using a fed-batch system. *Enzyme and microbial technology,* **20,** 221-224.

Chini Zittelli, G., L. Rodolfi, N. Biondi and M. R. Tredici, 2006: Productivity and photosynthetic efficiency of outdoor cultures of< i> Tetraselmis suecica</i> in annular columns. *Aquaculture,* **261,** 932-943.

Chu, F.-F., P.-N. Chu, P.-J. Cai, W.-W. Li, P. K. Lam and R. J. Zeng, 2013: Phosphorus plays an important role in enhancing biodiesel productivity of< i> Chlorella vulgaris</i> under nitrogen deficiency. *Bioresource technology,* **134,** 341-346.

Chu, F.-F., P.-N. Chu, X.-F. Shen, P. K. Lam and R. J. Zeng, 2014: Effect of phosphorus on biodiesel production from< i> Scenedesmus obliquus</i> under nitrogen-deficiency stress. *Bioresource technology,* **152,** 241-246.

Courchesne, N. M. D., A. Parisien, B. Wang and C. Q. Lan, 2009: Enhancement of lipid production using biochemical, genetic and transcription factor engineering approaches. *Journal of biotechnology,* **141,** 31-41.

Do Nascimento, M., M. d. l. A. Dublan, J. C. F. Ortiz-Marquez and L. Curatti, 2013: High lipid productivity of an< i> Ankistrodesmus</i>–< i> Rhizobium</i> artificial consortium. *Bioresource technology,* **146,** 400-407.

El-Sheekh, M., A. E.-F. Abomohra and D. Hanelt, 2013: Optimization of biomass and fatty acid productivity of Scenedesmus obliquus as a promising microalga for

biodiesel production. *World Journal of Microbiology and Biotechnology,* **29,** 915-922.

Encarnação, T., H. D. Burrows, A. C. Pais, M. G. Campos and A. Kremer, Effect of N and P on the Uptake of Magnesium and Iron and on the Production of Carotenoids and Chlorophyll by the Microalgae Nannochloropsis sp.

Goswami, R. C. D. and M. Kalita, 2011: Scenedesmus dimorphus and Scenedesmus quadricauda: two potent indigenous microalgae strains for biomass production and CO2 mitigation—A study on their growth behavior and lipid productivity under different concentration of urea as nitrogen source. *J Algal Biomass Utln,* **2,** 42-49.

Greene, R. M., R. J. Geider, Z. Kolber and P. G. Falkowski, 1992: Iron-induced changes in light harvesting and photochemical energy conversion processes in eukaryotic marine algae. *Plant Physiology,* **100,** 565-575.

Griffiths, M. J. and S. T. Harrison, 2009: Lipid productivity as a key characteristic for choosing algal species for biodiesel production. *Journal of Applied Phycology,* **21,** 493-507.

Habib, M., F. Md Yusoff, S. Prang, M. Din and M. Shariff, 2004: Growth and Nutritional Value of a Tropical Green Alga, Ankistrodesmus convolutus Corda, in Agro-industrial Effluents. *Pertanika Journal of Tropical Agricultural Science,* **27,** 79-89.

Harker, M., A. J. Tsavalos and A. J. Young, 1996: Factors responsible for astaxanthin formation in the Chlorophyte< i> Haematococcus pluvialis</i>. *Bioresource technology,* **55,** 207-214.

Harris, G., 1986: Phytoplankton ecology-structure, function and fluctuation.

Hellebust, J., 1965: Excretion of some organic compounds by marine phytoplankton. *Limnol. Oceanogr,* **10,** 192-206.

Hii, Y., C. Soo, T. Chuah, A. Mohd-Azmi and A. Abol-Munafi, 2011: Interactive effect of ammonia and nitrate on the nitrogen uptake by Nannochloropsis sp. *Journal of Sustainability Science and Management,* **6,** 60-68.

Hu, Q., 2004: 5 Environmental Effects on Cell Composition. *Handbook of microalgal culture: biotechnology and applied phycology***,** 83.

KILHAM, S., D. KREEGER, C. GOULDEN and S. LYNN, 1997: Effects of nutrient limitation on biochemical constituents of Ankistrodesmus falcatus. *Freshwater Biology,* **38,** 591-596.

Kim, D.-G., H.-J. La, C.-Y. Ahn, Y.-H. Park and H.-M. Oh, 2011: Harvest of< i> Scenedesmus</i> sp. with bioflocculant and reuse of culture medium for subsequent high-density cultures. *Bioresource technology,* **102,** 3163-3168.

Kobayashi, M., T. Kakizono and S. Nagai, 1993: Enhanced carotenoid biosynthesis by oxidative stress in acetate-induced cyst cells of a green unicellular alga, Haematococcus pluvialis. *Applied and Environmental Microbiology,* **59,** 867-873.

Krichnavaruk, S., W. Loataweesup, S. Powtongsook and P. Pavasant, 2005: Optimal growth conditions and the cultivation of< i> Chaetoceros calcitrans</i> in airlift photobioreactor. *Chemical Engineering Journal,* **105,** 91-98.

Kumar, A., S. Ergas, X. Yuan, A. Sahu, Q. Zhang, J. Dewulf, F. X. Malcata and H. Van Langenhove, 2010: Enhanced CO< sub> 2</sub> fixation and biofuel production via microalgae: recent developments and future directions. *Trends in biotechnology,* **28,** 371-380.

Kunikane, S., M. Kaneko and R. Maehara, 1984: Growth and nutrient uptake of green alga,< i> Scenedesmus dimorphus</i>, under a wide range of nitrogen/phosphorus ratio—I. Experimental study. *Water research,* **18,** 1299-1311.

Lee, S., M. H. Jang, H. S. Kim, B. D. Yoon and H. M. Oh, 2000: Variation of microcystin content of Microcystis aeruginosa relative to medium N: P ratio and growth stage. *Journal of Applied Microbiology,* **89,** 323-329.

Liu, Z.-Y., G.-C. Wang and B.-C. Zhou, 2008: Effect of iron on growth and lipid accumulation in< i> Chlorella vulgaris</i>. *Bioresource Technology,* **99,** 4717-4722.

Macedo, C. and R. Pinto-Corlho, 2001: Nutritional status response of Daphania laevis and Moina micura from a tropical reservoir to different algal diets: Scenedesmus quadricauda and Ankistrodesmus gracilis. *Brazilian Journal of Biology,* **61,** 555-562.

Makarevičien, V., V. Andrulevičit, V. Skorupskait and J. Kasperovičien, 2011: Cultivation of Microalgae Chlorella sp. and Scenedesmus sp. as a Potentional Biofuel Feedstock. *Environmental Research, Engineering & Management,* **57**.

Mandal, S. and N. Mallick, 2009: Microalga Scenedesmus obliquus as a potential source for biodiesel production. *Applied microbiology and biotechnology,* **84,** 281-291.

Mata, T. M., A. A. Martins and N. S. Caetano, 2010: Microalgae for biodiesel production and other applications: a review. *Renewable and Sustainable Energy Reviews,* **14,** 217-232.

Merchuk, J., M. Ronen, S. Giris and S. M. Arad, 1998: Light/dark cycles in the growth of the red microalga Porphyridium sp. *Biotechnology and bioengineering,* **59,** 705-713.

Miron, A. S., 2000: Bubble Column and airlift photobioreactors for algal culture. *AIChE journal Setepber 2000 Vol. 46, No. 9***,** 1872-1887.

Molina Grima, E., E.-H. Belarbi, F. Acién Fernández, A. Robles Medina and Y. Chisti, 2003: Recovery of microalgal biomass and metabolites: process options and economics. *Biotechnology advances,* **20,** 491-515.

Morris, I., H. Glover and C. Yentsch, 1974: Products of photosynthesis by marine phytoplankton: the effect of environmental factors on the relative rates of protein synthesis. *Marine biology,* **27,** 1-9.

Nayak, S., R. Mohanty and L. Mohanty, 1996: Growth rate of Ankistrodesmus falcatus and Scenedesmus bijuga in mixed culture exposed to monocrotophos. *Bulletin of environmental contamination and toxicology,* **57,** 473-479.

Neenan, B., D. Feinberg, A. Hill, R. McIntosh and K. Terry, 1986: Fuels from microalgae: Technology status, potential, and research requirements. Solar Energy Research Inst., Golden, CO (USA).

Norsker, N.-H., M. J. Barbosa, M. H. Vermuë and R. H. Wijffels, 2011: Microalgal production—a close look at the economics. *Biotechnology Advances,* **29,** 24-27.

Oncel, S. and F. V. Sukan, 2008: Comparison of two different pneumatically mixed column photobioreactors for the cultivation of< i> Artrospira platensis</i>(< i> Spirulina platensis</i>). *Bioresource technology,* **99,** 4755-4760.

Pancha, I., K. Chokshi, B. George, T. Ghosh, C. Paliwal, R. Maurya and S. Mishra, 2014: Nitrogen stress triggered biochemical and morphological changes in the microalgae< i> Scenedesmus</i> sp. CCNM 1077. *Bioresource Technology*.

Pavasant, A. P. P., 2011: การพัฒนาระบบถังปฏิกรณ์ชีวภาพสำหรับเพาะเลี้ยงจุลสาหร่าย เพื่อใช้เป็นทางเลือก ระยะที่ 2 PTT Research

and Technology Institute 71.

Posten, C. and G. Schaub, 2009: Microalgae and terrestrial biomass as source for fuels—a process view. *Journal of biotechnology,* **142,** 64-69.

Pulz, 2001a: production systems for phototrophic microorganism. *Appl. Microbio. Biotechnol. 57***,** 287-293.

Pulz, O., 2001b: Photobioreactors: production systems for phototrophic microorganisms. *Applied Microbiology and Biotechnology,* **57,** 287-293.

Richardson, J. W., J. L. Outlaw and M. Allison, 2010: The economics of microalgae oil.

Rodolfi, L., G. C. Zittelli, L. Barsanti, G. Rosati and M. R. Tredici, 2003: Growth medium recycling in< i> Nannochloropsis</i> sp. mass cultivation. *Biomolecular engineering,* **20,** 243-248.

Sato, N., M. Hagio, H. Wada and M. Tsuzuki, 2000: Environmental effects on acidic lipids of thylakoid membranes. *Biochemical Society Transactions,* **28,** 912-914.

Sato, T., S. Usui, Y. Tsuchiya and Y. Kondo, 2006: Invention of outdoor closed type photobioreactor for microalgae. *Energy conversion and management,* **47,** 791-799.

Sierra, E., F. Acien, J. Fernandez, J. Garcia, C. Gonzalez and E. Molina, 2008: Characterization of a flat plate photobioreactor for the production of microalgae. *Chemical Engineering Journal,* **138,** 136-147.

Singh, D., E. Croiset, P. L. Douglas and M. A. Douglas, 2003: Techno-economic study of CO< sub> 2</sub> capture from an existing coal-fired power plant: MEA scrubbing vs. O< sub> 2</sub>/CO< sub> 2</sub> recycle combustion. *Energy Conversion and Management,* **44,** 3073-3091.

Sipaúba-Tavares, L. and A. Pereira, 2008: Large scale laboratory cultures of Ankistrodesmus gracilis (Reisch) Korsikov (Chlorophyta) and Diaphanosoma biergei Korinek, 1981 (Cladocera). *Brazilian Journal of Biology,* **68,** 875-883.

Sipauba-Tavares, L. H., R. N. Millan, A. Berchiell and F. M. de Souza Braga, 2011: Use of alternative media and different types of recipients in a laboratory culture of Ankistrodesmus gracilis (Reinsch) Korshikov (Chlorophyceae). *Acta Scientiarum: Biological Sciences,* **33**.

Stepan, D. J., R. E. Shockey, T. A. Moe and R. Dorn, 2002: Carbon dioxide sequestering using microalgal systems. University of North Dakota.

STRICKLAND, J. D., AND T. R. PARSON, 1972: Measurement of nitrogen concentration by spectrophotometer *A practical handbook of seawater analysis, 2nd.* Bull. Fish. Res. Bd.

Syrett, P. and C. Hipkin, 1973: The appearance of nitrate reductase activity in nitrogen-starved cells of Ankistrodesmus braunii. *Planta,* **111,** 57-64.

Talbot, P., J.-M. Thébault, A. Dauta and J. De la Noüe, 1991: A comparative study and mathematical modeling of temperature, light and growth of three microalgae potentially useful for wastewater treatment. *Water research,* **25,** 465-472.

Tapie, P. and A. Bernard, 1988: Microalgae production: technical and economic evaluations. *Biotechnology and bioengineering,* **32,** 873-885.

Theodorou, M. E., I. R. Elrifi, D. H. Turpin and W. C. Plaxton, 1991: Effects of phosphorus limitation on respiratory metabolism in the green alga Selenastrum minutum. *Plant physiology,* **95,** 1089-1095.

Weissman, J. C., R. P. Goebel and J. R. Benemann, 1988: Photobioreactor design: mixing, carbon utilization, and oxygen accumulation. *Biotechnology and bioengineering,* **31,** 336-344.

Wu, Z., Y. Zhu, W. Huang, C. Zhang, T. Li, Y. Zhang and A. Li, 2012: Evaluation of flocculation induced by pH increase for harvesting microalgae and reuse of flocculated medium. *Bioresource technology,* **110,** 496-502.

Xin, L., H. Hong-ying, G. Ke and S. Ying-xue, 2010: Effects of different nitrogen and phosphorus concentrations on the growth, nutrient uptake, and lipid accumulation of a freshwater microalga< i> Scenedesmus</i> sp. *Bioresource Technology,* **101,** 5494-5500.

Xu, L., C. Guo, F. Wang, S. Zheng and C.-Z. Liu, 2011: A simple and rapid harvesting method for microalgae by in situ magnetic separation. *Bioresource technology,* **102,** 10047-10051.

APPENDIX

Appendix A1 Standard calibration and measured condition

Appendix A1-1 Measurement of nitrogen concentration by spectrophotometer (STRICKLAND, 1972)

Blank

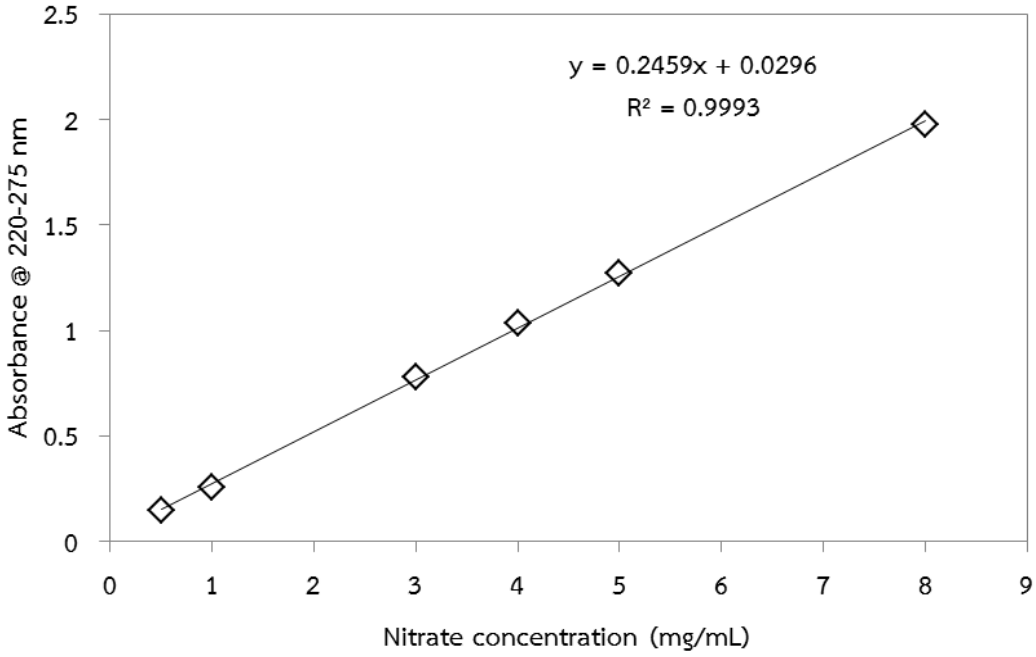
1 mL of distilled waters measured by spectrophotometer at wavelength of 220 and 275 nm and blank is set to zero.

Calibration

1. KNO_3 stock solution 100 mg NO_3-N/L is prepared by dissolved 0.7128 g of KNO_3 in 1000 mL of distilled water and keep with 1 mL of chloroform in dark glass.
2. KNO_3 stock is diluted using distilled water as 0.5, 1.0, 3.0, 4.0, 5.0, and 8.0 mg NO_3-N/L.
3. The solution is measured by spectrophotometer at wavelength of 220 and 275 nm.

Procedure

Samples are measured by wavelength of 220 nm to obtain NO_3^- reading and wavelength of 275 nm to determine interference due to dissolved organic matter.



Appendix A1-1 Standard calibration curves for nitrate concentration

Appendix A1-2 The conditions of using measurement nutrient by ICP

Condition used by: All lines

Power (kw)	1.00
Plasma flow (L/min)	15.0
Auxiliary flow (L/min)	1.5
Nebulizer flow (L/min)	0.75
Replicate read time (s)	5
Instrstabilization delay	15

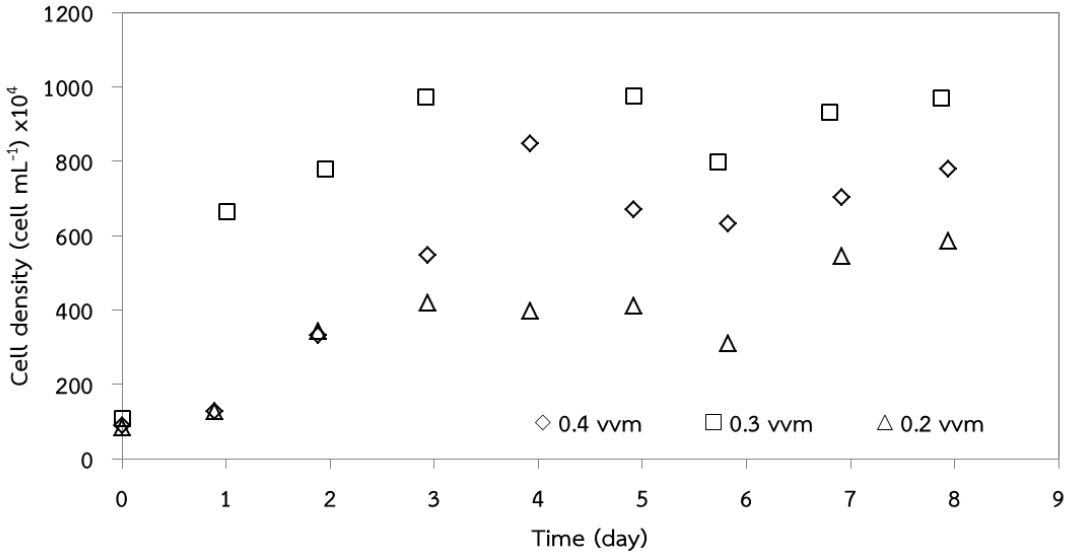
Sample introduction settings

Sample uptake delay (s)	30
Pump rate (rpm)	15
Rinse time (s)	10

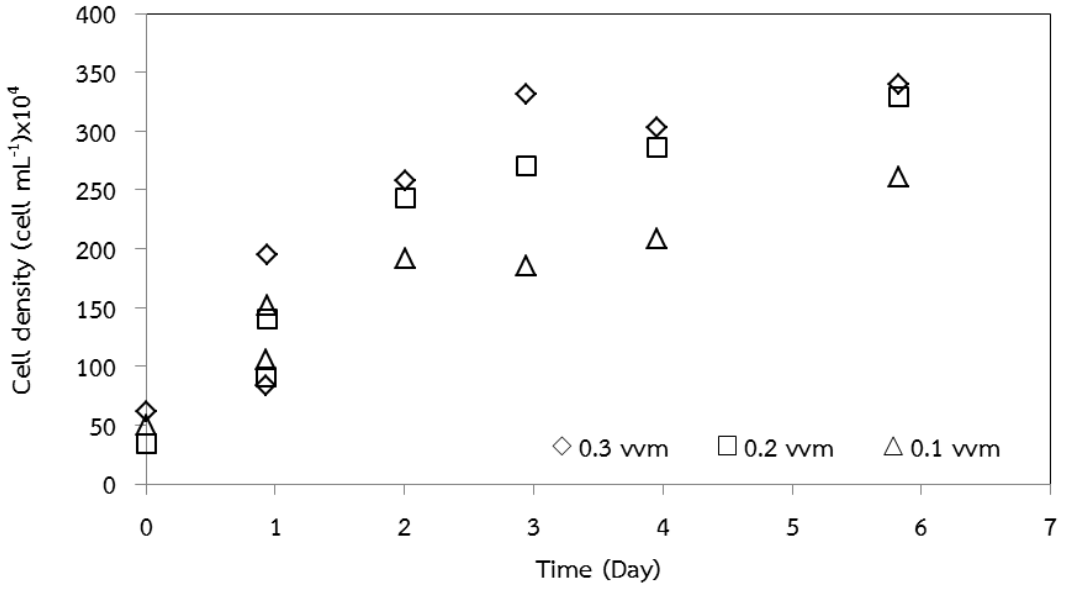
General Settings

Replicates: 3

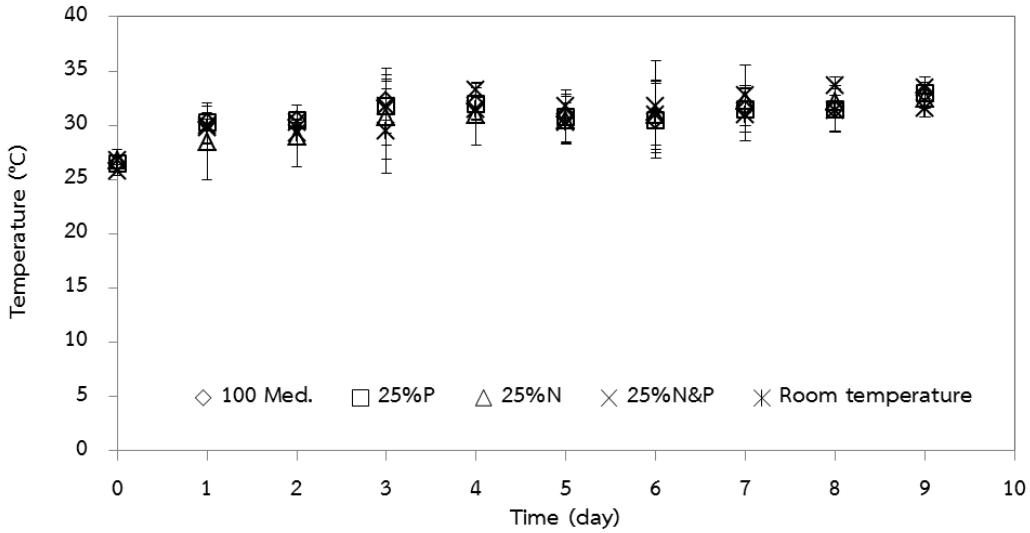
Appendix A1-3 compared run air flow rate of *Ankistrodesmus* sp.



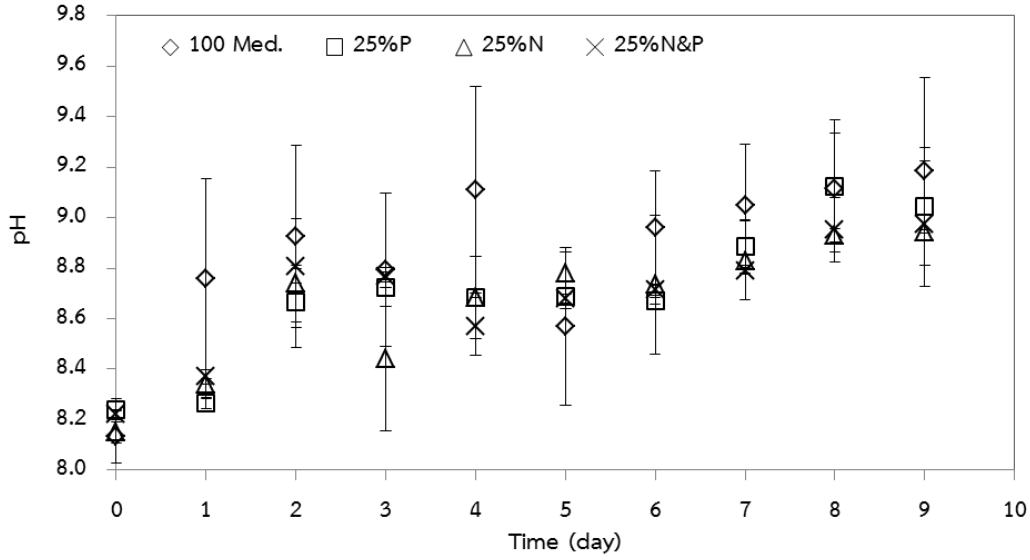
Appendix A1-4 compared run air flow rate of *Scenedesmus* sp.



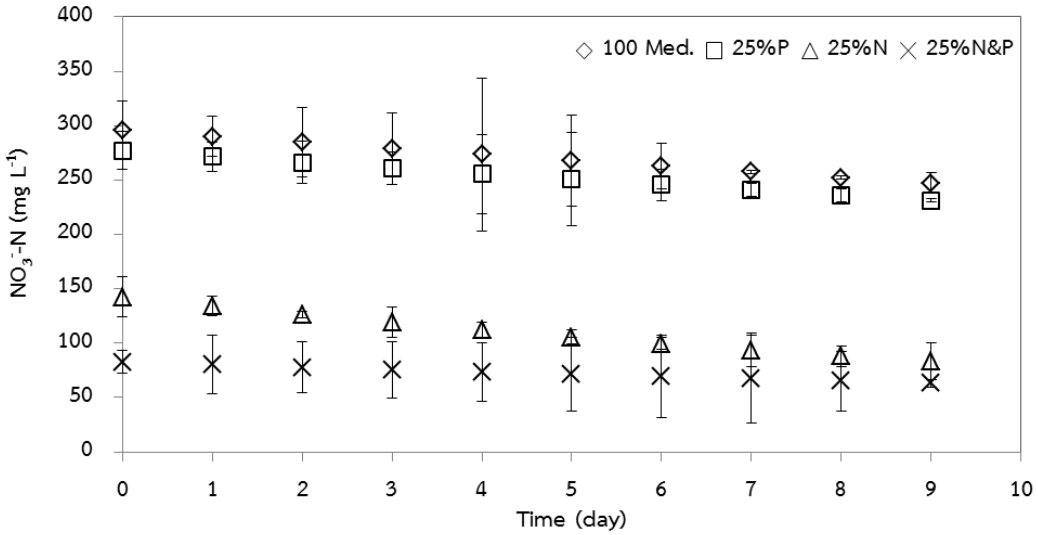
Appendix A2 reduced nutrients (Section 4.2)



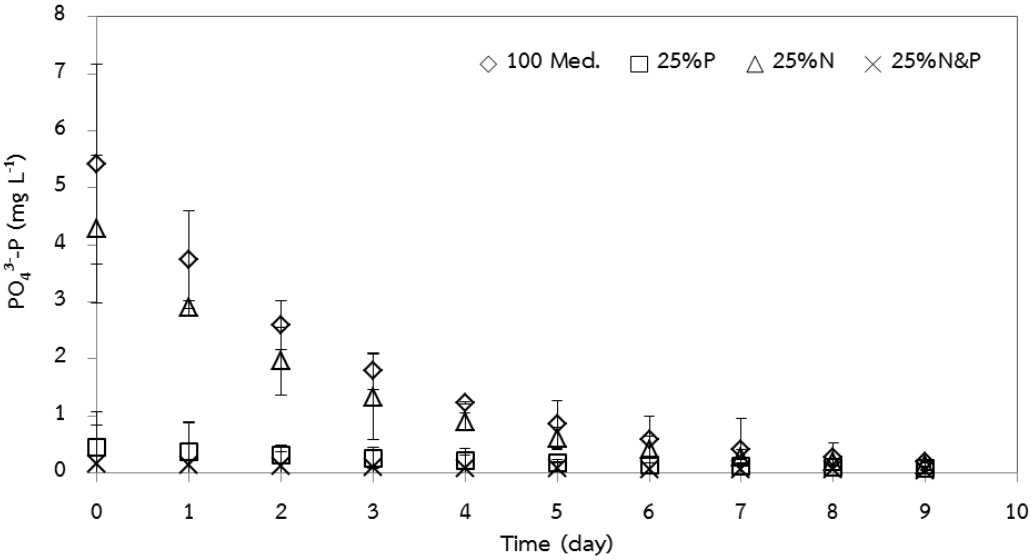
Appendix A2-1 Temperature of 25% nutrient by Ankistrodesmus sp. culture in different types of media under the conditions.



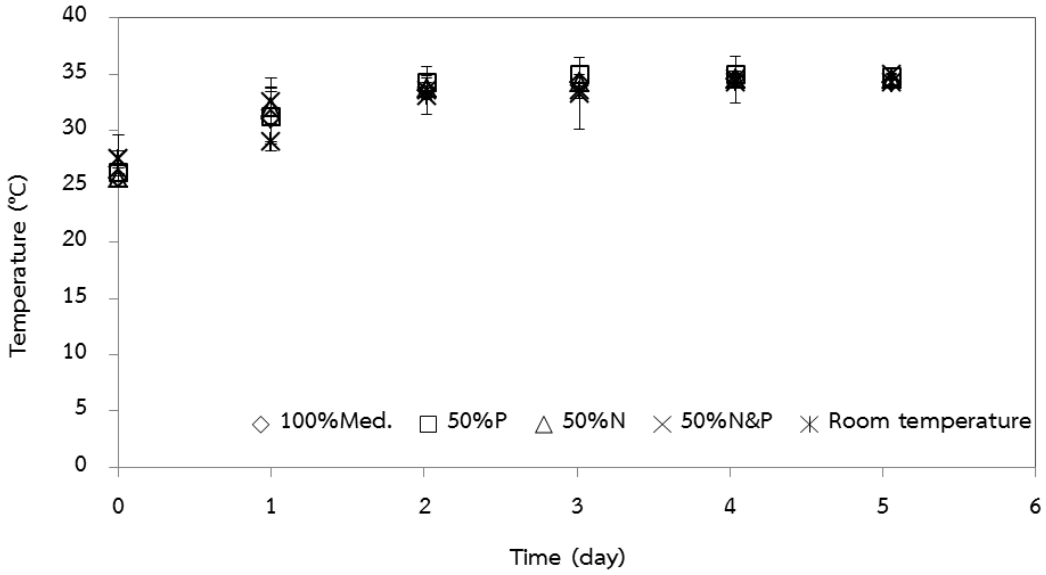
Appendix A2-2 pH of 25% nutrient by *Ankistrodesmus* sp. culture in different types of media under the conditions



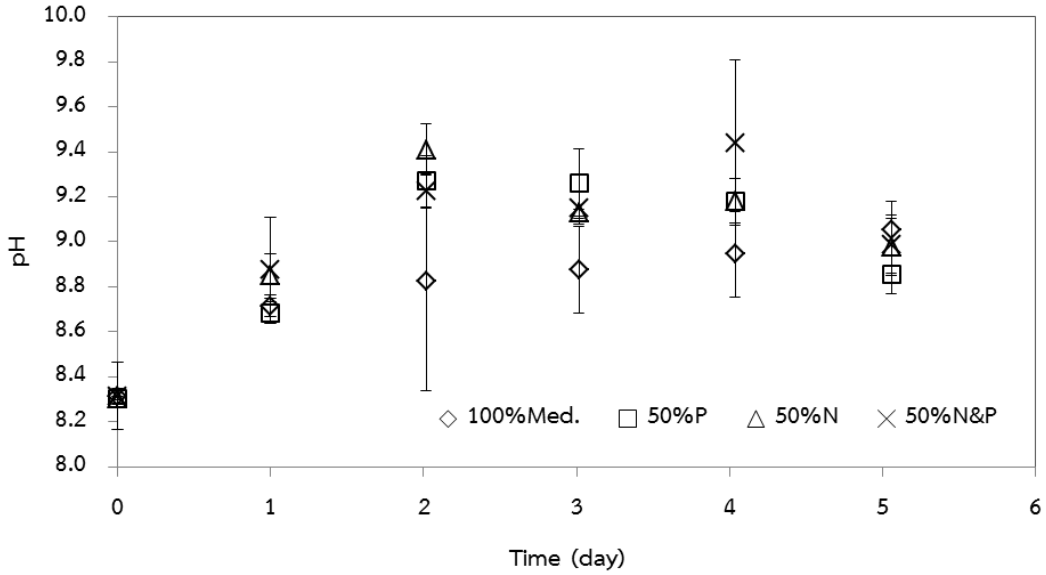
Appendix A2-3 Nitrogen profile of 25% nutrient concentration by Ankistrodesmus sp. culture in different types of media under the conditions



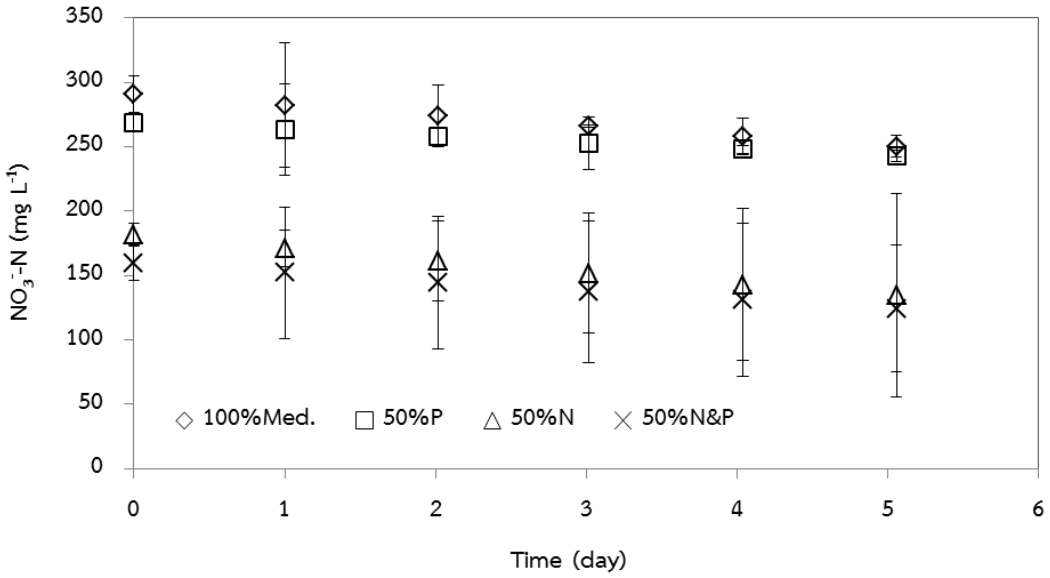
Appendix A2-4 Phosphorus profile of 25% nutrient concentration by *Ankistrodesmus* sp. culture in different types of media under the conditions



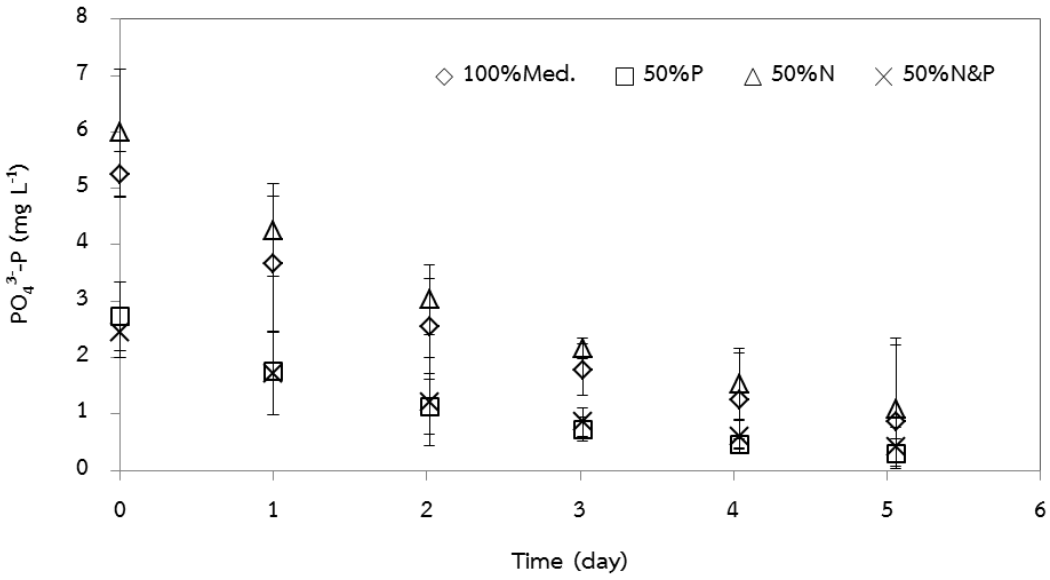
Appendix A2-5 Temperature of 50% nutrient by *Scenedesmus* sp. culture in different types of media under the conditions.



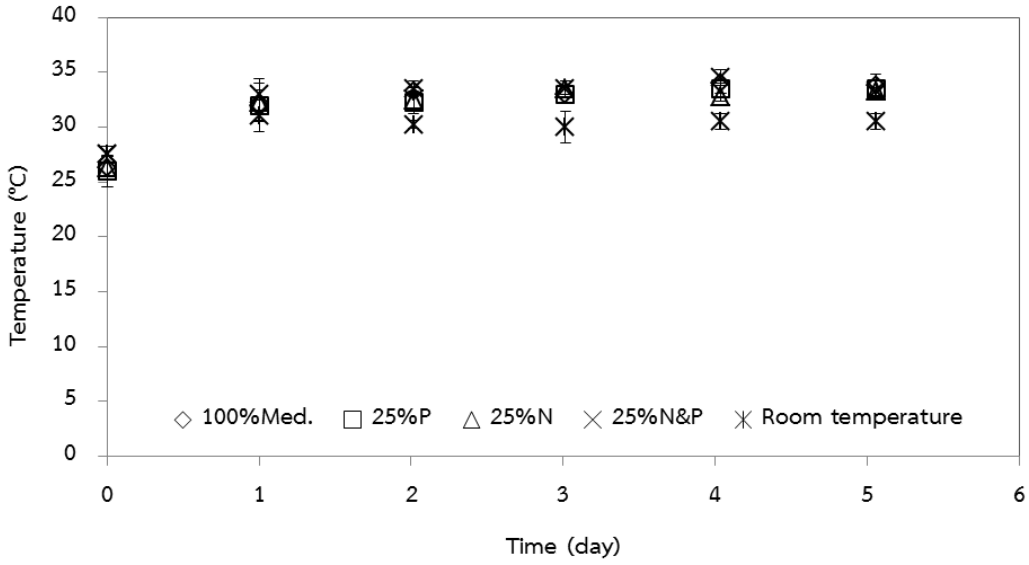
Appendix A2-6 pH of 50% nutrient by *Scenedesmus* sp. culture in different types of media under the conditions



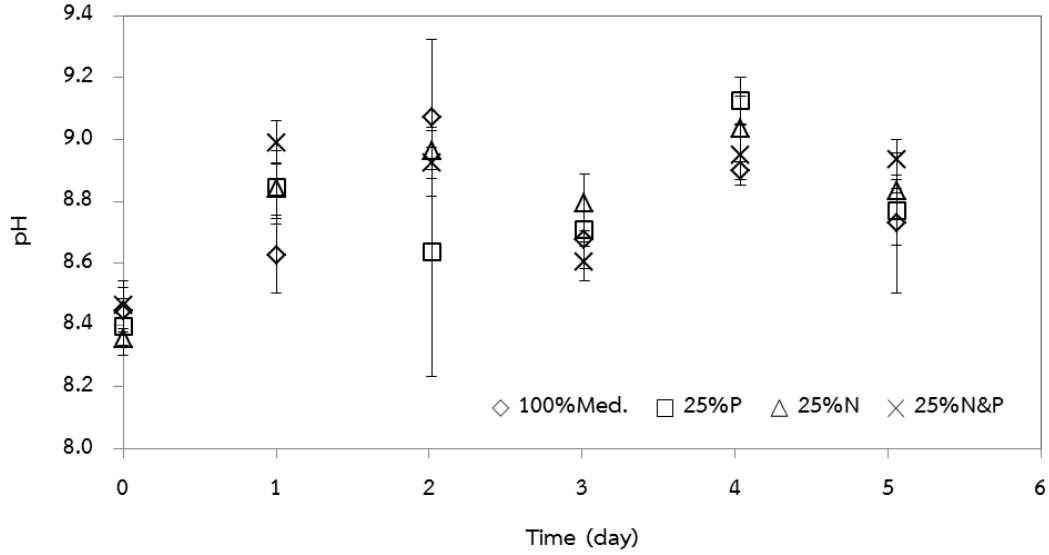
Appendix A2-7 Nitrogen profile of 50% nutrient concentration by *Scenedesmus* sp. culture in different types of media under the conditions



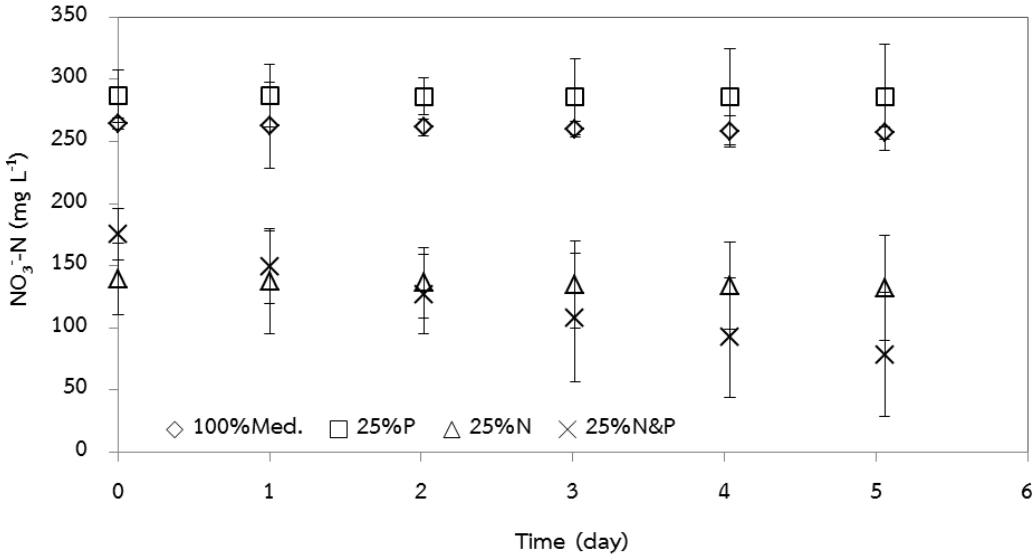
Appendix A2-8 Phosphorus profile of 50% nutrient concentration by *Scenedesmus* sp. culture in different types of media under the conditions



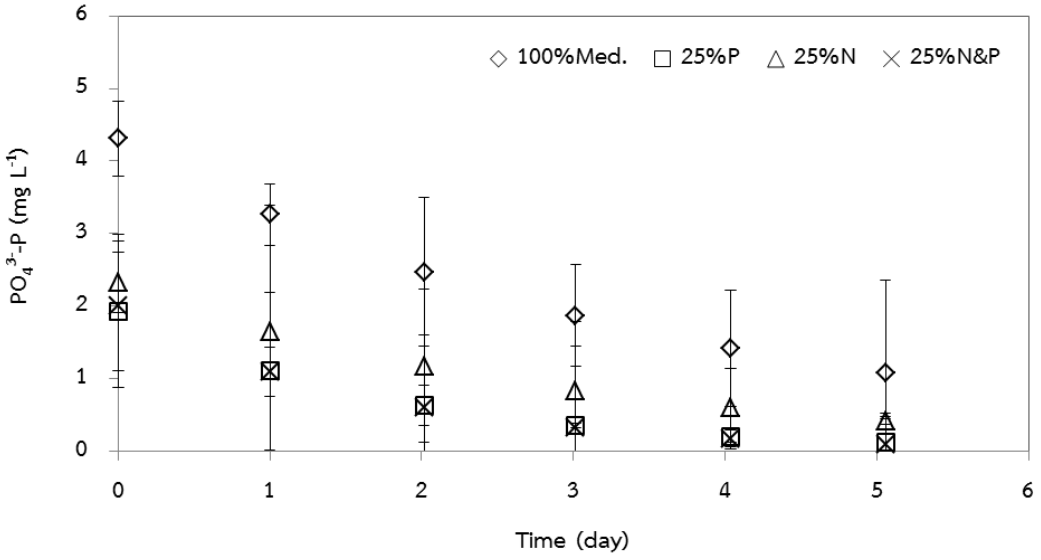
Appendix A2-9 Temperature of 25% nutrient by *Scenedesmus* sp. culture in different types of media under the conditions.



Appendix A2-10 pH of 25% nutrient by *Scenedesmus* sp. culture in different types of media under the conditions.

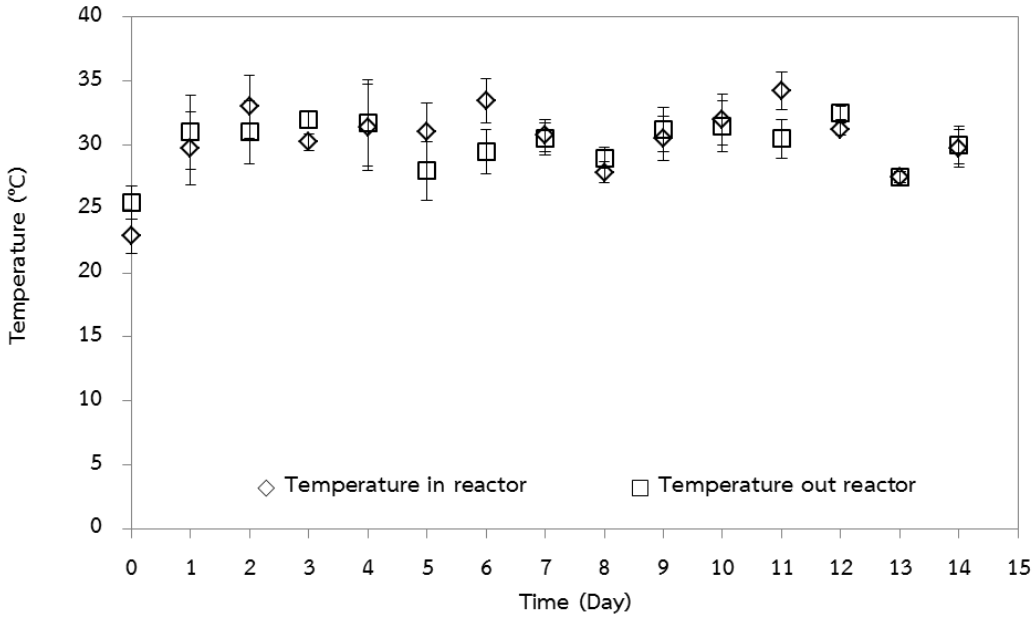


Appendix A2-11 Nitrogen profile of 25% nutrient concentration by *Scenedesmus* sp. culture in different types of media under the conditions.

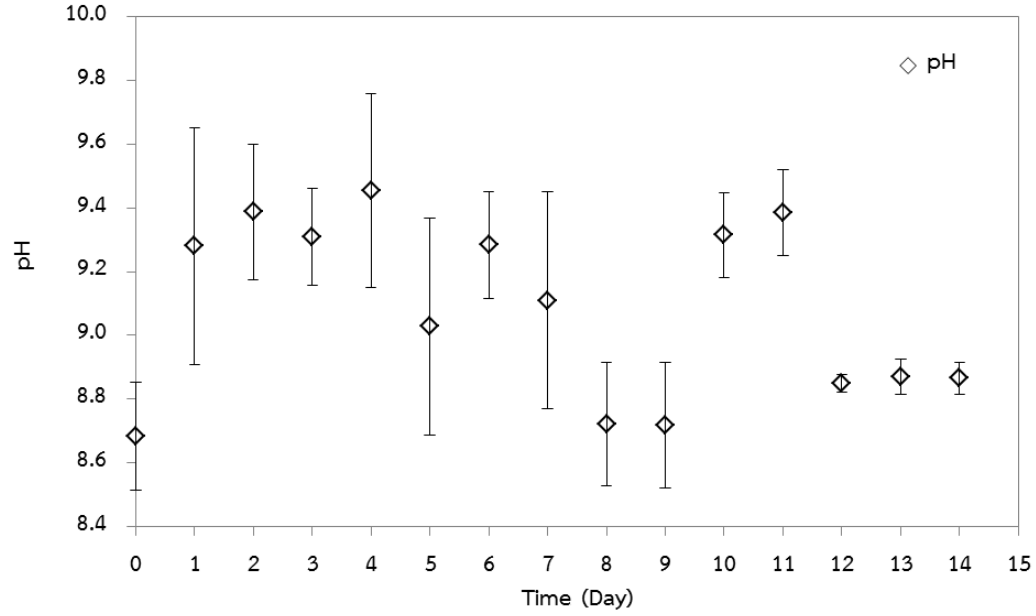


Appendix A2-12 Phosphorus profile of 25% nutrient concentration by *Scenedesmus* sp. culture in different types of media under the conditions.

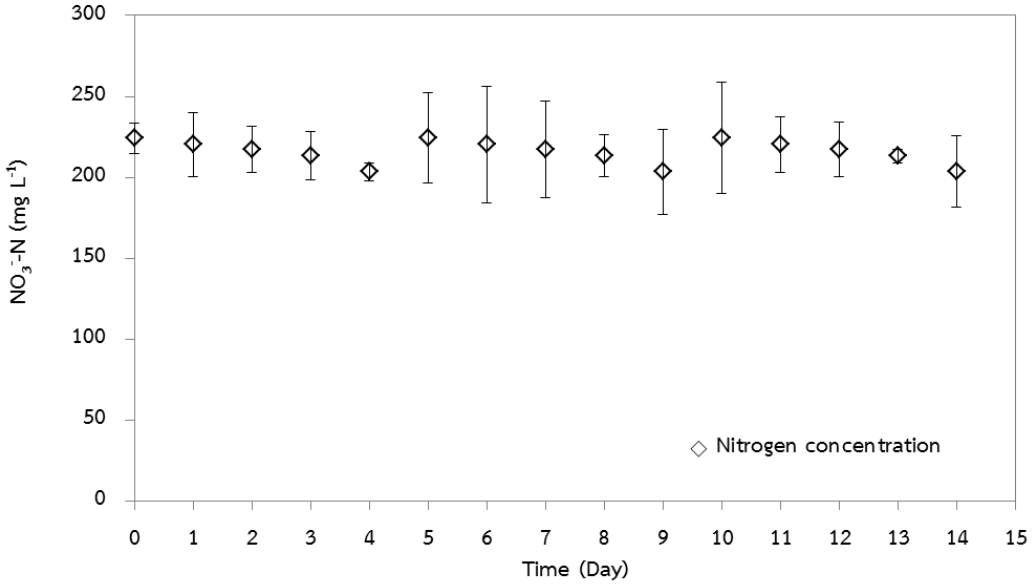
Appendix A3 reused medium (Section 4.3)



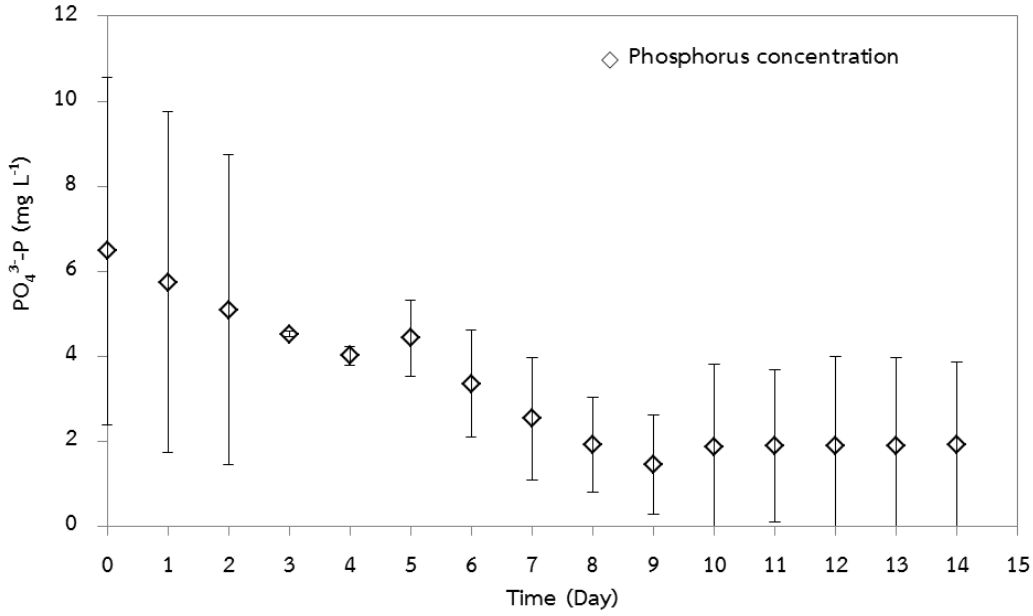
Appendix A3-1 Temperature of *Scenedesmus* sp. culture in reused medium



Appendix A3-2 pH of *Scenedesmus* sp. culture in reused medium



Appendix A3-3 Nitrogen concentration of *Scenedesmus* sp. culture in reused medium



Appendix A3-4 Phosphorus concentration of *Scenedesmus* sp. culture in reused medium

Appendix A4 Economical assessment of algae biomass production

Appendix A4-1 Economics for *Ankistrodesmus* sp. indoor cultivation in different media of NB-FPAP

Details	Symbol	Unit	100%Med.	% of total	25%N	% of total	25%P	% of total	25%N&P	% of total
Conditions										
Volume	A	L	25		25		25		25	
Period days	B	Day	9		9		9		9	
Dry weight	C	g/L	0.46		0.64		0.52		0.7	
productivity per reactor	D=C/B*300	g/L/Year	15.3		21.3		17.3		23.3	
Max Reactor in 1 hectare	E	unit	9,375		9,375		9,375		9,375	
productivity in 1 hectare	F=E*D	g/L/Year	143,750		200,000		162,500		218,750	
Nutrient requirement										
Nutrient cost	G	USD/L	0.0404		0.0395		0.0137		0.0128	
Nutrient charge	H=(G*E*A)*(300/B)	USD/year	315,646	36.59	308,834	36.09	106,905	16.35	100,094	15.47
Electricity requirement										
Number of light	I=E*number	unit	37,500		37,500		37,500		37,500	
Lighting	J=I*watts*24*300/1000	kWh	5,400,000		5,400,000		5,400,000		5,400,000	
Air pump	K=E*watts*24*300/1000	kWh	405,000		405,000		405,000		405,000	
total electricity	L=J+K	kWh	5,805,000		5,805,000		5,805,000		5,805,000	
Electricity charge (electricity charge = 0.12 USD per kWh)	M=L*0.12	USD/year	540,228	62.63	540,228	63.13	540,228	82.63	540,228	83.50
Water requirement										
water supply	N=E*A	L	234,375		234,375		234,375		234,375	
Water charge (water charge = 0.84 USD per m^3)	O=N*0.31	USD/year	6,689	0.78	6,689	0.78	6,689	1.02	6,689	1.03
Total operation	P=H+M+O	USD/year	862,563	100.00	855,751	100.00	653,823	100.00	647,011	100.00
Cost	Q=P/A/E	USD/L	0.1104		0.1095		0.0837		0.0828	

Appendix A4-2 Economics for *Ankistrodesmus* sp. indoor cultivation in different media of NB-FPAP

Details	Symbol	Unit	100%Med.	% of total	50%P	% of total	50%N	% of total	50%N&P	% of total
Conditions										
Volume	A	L	25		25		25		25	
Period days	B	Day	5		5		5		5	
Dry weight	C	g/L	0.33		0.24		0.28		0.26	
productivity per reactor	D=C/B*300	g/L/Year	19.8		14.4		16.8		15.6	
Max Reactor in 1 hectare	E	unit	9,375		9,375		9,375		9,375	
productivity in 1 hectare	F=E*D	g/L/Year	185,625		135,000		157,500		2,437.50	
Nutrient requirement										
Nutrient cost	G	USD/L	0.0404		0.0398		0.0226		0.0220	
Nutrient charge	H=(G*E*A)*(300/B)	USD/year	568,162	51.48	559,988	51.12	317,674	37.23	309,500	36.63
Electricity requirement										
Number of light	I=E*number	unit	37,500		37,500		37,500		37,500	
Lighting	J=I*watts*24*300/1000	kWh	5,400,000		5,400,000		5,400,000		5,400,000	
Air pump	K=E*watts*24*300/1000	kWh	224,775		224,775		224,775		224,775	
total electricity	L=J+K	kWh	5,624,775		5,624,775		5,624,775		5,624,775	
Electricity charge (electricity charge = 0.12 USD per kWh)	M=L*0.12	USD/year	523,456	47.43	523,456	47.78	523,456	61.35	523,456	61.95
Water requirement										
water supply	N=E*A	L	234,375		234,375		234,375		234,375	
Water charge (water charge = 0.84 USD/m^3)	O=N*0.31	USD/year	12,041	1.09	12,041	1.10	12,041	1.41	12,041	1.42
Total operation	P=H+M+O	USD/year	1,103,659	100.00	1,095,485	100.00	853,170	100.00	844,996	100.00
Cost	Q=P/A/E	USD/L	0.0785		0.0779		0.0607		0.0601	

Appendix A4-3 Economics for Scenedesmus sp. indoor cultivation in different media of NB-FPAP

Details	Symbol	Unit	100%Med.	% of total	25%P	% of total	25%N	% of total	25%N&P	% of total
Conditions										
Volume	A	L	25		25		25		25	
Period days	B	Day	5		5		5		5	
Dry weight	C	g/L	0.31		0.31		0.23		0.46	
productivity per reactor	D=C/B*300	g/L/Year	18.6		18.6		13.8		27.6	
Max Reactor in 1 hectare	E	unit	9,375		9,375		9,375		9,375	
productivity in 1 hectare	F=E*D	g/L/Year	174,375		174,375		129,375		258,750	
Nutrient requirement										
Nutrient cost	G	USD/L	0.0404		0.0395		0.0137		0.0128	
Nutrient charge	H=(G*E*A)*(300/B)	USD/year	568,162	51.48	555,901	50.93	192,429	26.44	180,169	25.17
Electricity requirement										
Number of light	I=E*number	unit	37,500		37,500		37,500		37,500	
Lighting	J=I*watts*24*300/1000	kWh	5,400,000		5,400,000		5,400,000		5,400,000	
Air pump	K=E*watts*24*300/1000	kWh	224,775		224,775		224,775		224,775	
total electricity	L=J+K	kWh	5,624,775		5,624,775		5,624,775		5,624,775	
Electricity charge (electricity charge = 0.12 USD per kWh)	M=L*0.12	USD/year	523,456	47.43	523,456	47.96	523,456	71.91	523,456	73.14
Water requirement										
water supply	N=E*A	L	234,375		234,375		234,375		234,375	
Water charge (water charge = 0.84 USD per m^3)	O=N*0.31	USD/year	12,041	1.09	12,041	1.10	12,041	1.65	12,041	1.68
Total operation	P=H+M+O	USD/year	1,103,659	100.00	1,091,398	100.00	727,926	100.00	715,665	100.00
Cost	Q=P/A/E	USD/L	0.0785		0.0776		0.0518		0.0509	

Appendix A4-4 Economics for *Ankistrodesmus* sp. indoor cultivation in reused medium of NB-FPAP

Details	Symbol	Unit	Fresh	% of total	1st reuse	% of total	2nd reuse	% of total
Conditions								
Volume	A	L	25		25		25	
Period days	B	Day	9		9		9	
Dry weight	C	g/L	0.55		1.31		1.91	
productivity per reactor	D=C/B*300	g/L/Year	18.3		43.7		63.7	
Max Reactor in 1 hectare	E	unit	9,375		9,375		9,375	
productivity in 1 hectare	F=E*D	g/L/Year	171,875		409,375		596,875	
Nutrient requirement								
Nutrient cost	G	USD/L	0.0404		0.000		0.000	
Nutrient charge	H=(G*E*A)*(300/B)	USD/year	315,646	36.59	-	-	-	-
Electricity requirement								
Number of light	I=E*number	unit	37,500		37,500		37,500	
Lighting	J=I*watts*24*300/1000	kWh	5,400,000		5,400,000		5,400,000	
Air pump	K=E*watts*24*300/1000	kWh	405,000		405,000		405,000	
total electricity	L=J+K	kWh	5,805,000		5,805,000		5,805,000	
Electricity charge (electricity charge = 0.12 USD per kWh)	M=L*0.12	USD/year	540,228	62.63	540,228	38.51	540,228	27.80
Water requirement								
water supply	N=E*A	L	234,375		-		-	
Water charge (water charge = 0.84 USD per m^3)	O=N*0.31	USD/year	6,689	0.78	-	-	-	-
Total operation	P=H+M+O	USD/year	862,563	100.00	1,402,791	38.51	1,943,018	27.80
Cost	Q=P/A/E	USD/L	0.1104		0.1796		0.2487	

Appendix A4-5 Economics for *Scenedesmus* sp. indoor cultivation in reused medium of NB-FPAP

Details	Symbol	Unit	Fresh	% of total	1st reuse	% of total	2nd reuse	% of total
Conditions								
Volume	A	L	25		25		25	
Period days	B	Day	5		5		5	
Dry weight	C	g/L	0.38		0.57		0.58	
productivity per reactor	D=C/B*300	g/L/Year	22.8		34.2		34.8	
Max Reactor in 1 hectare	E	unit	9,375		9,375		9,375	
productivity in 1 hectare	F=E*D	g/L/Year	213,750		320,625		326,250	
Nutrient requirement								
Nutrient cost	G	USD/L	0.0404		0.000		0.000	
Nutrient charge	H=(G*E*A)*(300/B)	USD/year	568,162	51.48	-	-	-	-
Electricity requirement								
Number of light	I=E*number	unit	37,500		37,500		37,500	
Lighting	J=I*watts*24*300/1000	kWh	5,400,000		5,400,000		5,400,000	
Air pump	K=E*watts*24*300/1000	kWh	224,775		224,775		224,775	
total electricity	L=J+K	kWh	5,624,775		5,624,775		5,624,775	
Electricity charge (electricity charge = 0.12 USD per kWh)	M=L*0.12	USD/year	523,456	47.43	523,456	32.17	523,456	24.34
Water requirement								
water supply	N=E*A	L	234,375		-		-	
Water charge (water charge = 0.84 USD per m^3)	O=N*0.31	USD/year	12,041	1.09	-	-	-	-
Total operation	P=H+M+O	USD/year	1,103,659	100.00	1,627,114	32.17	2,150,570	24.34
Cost	Q=P/A/E	USD/L	0.0785		0.1157		0.1529	

Appendix A4-6 Economics for *Ankistrodesmus* sp. outdoor cultivation in different media of NB-FPAP

Details	Symbol	Unit	100%Med.	% of total	50%P	% of total	50%N	% of total	50%N&P	% of total
Conditions										
Volume	A	L	25		25		25		25	
Period days	B	Day	9		9		9		9	
Dry weight	C	g/L	0.37		0.36		0.31		0.48	
productivity per reactor	D=C/B*300	g/L/Year	12		12		10		16	
Max Reactor in 1 hectare	E	unit	9,375		9,375		9,375		9,375	
productivity in 1 hectare	F=E*D	g/L/Year	115,625		112,500		96,875		150,000	
Nutrient requirement										
Nutrient cost	G	USD/L	0.0404		0.0398		0.0226		0.0220	
Nutrient charge	H=(G*E*A)*(300/B)	USD/year	315,646	87.67	311,104	87.52	176,485	79.91	171,944	79.48
Electricity requirement										
Air pump	I=E*watts*24*300/1000	kWh	405,000		405,000		405,000		405,000	
Electricity charge (electricity charge = 0.12 USD per kWh)	J=I*0.12	USD/year	37,690	10.47	37,690	10.60	37,690	17.06	37,690	17.42
Water requirement										
water supply	K=E*A	L	234,375		234,375		234,375		234,375	
Water charge (water charge = 0.84 USD per m^3)	L=(K*0.31)*(300/B)	USD/year	6,689	1.86	6,689	1.88	6,689	3.03	6,689	3.09
Total operation	M=H+J+L	USD/year	360,025	100.00	355,484	100.00	220,865	100.0	216,324	100.00
Cost	N=(M/A/E)*(B/300)	USD/L	0.0461		0.0455		0.0283		0.0277	

Appendix A4-7 Economics for *Ankistrodesmus* sp. outdoor cultivation in different media of NB-FPAP

Details	Symbol	Unit	100%Med.	% of total	25%P	% of total	25%N	% of total	25%N&P	% of total
Conditions										
Volume	A	L	25		25		25		25	
Period days	B	Day	9		9		9		9	
Dry weight	C	g/L	0.46		0.64		0.52		0.7	
productivity per reactor	D=C/B*300	g/L/Year	15.3		21.3		17.3		23.3	
Max Reactor in 1 hectare	E	unit	9,375		9,375		9,375		9,375	
productivity in 1 hectare	F=E*D	g/L/Year	143,750		200,000		162,500		218,750	
Nutrient requirement										
Nutrient cost	G	USD/L	0.0404		0.0395		0.0137		0.0128	
Nutrient charge	H=(G*E*A)*(300/B)	USD/year	315,646	87.67	308,834	87.44	106,905	70.66	100,094	69.28
Electricity requirement										
Air pump	I=E*watts*24*300/1000	kWh	405,000		405,000		405,000		405,000	
Electricity charge (electricity charge = 0.12 USD per kWh)	J=I*0.12	USD/year	37,690	10.47	37,690	10.67	37,690	24.91	37,690	26.09
Water requirement										
water supply	K=E*A	L	234,375		234,375		234,375		234,375	
Water charge (water charge = 0.84 USD per m^3)	L=(K*0.31)*(300/B)	USD/year	6,689	1.86	6,689	1.89	6,689	4.42	6,689	4.63
Total operation	M=H+J+L	USD/year	360,025	100.00	353,214	100.00	151,285	100.00	144,474	100.00
Cost	N=(M/A/E)*(B/300)	USD/L	0.0461		0.0452		0.0194		0.0185	

Appendix A4-8 Economics for *Scenedesmus* sp. outdoor cultivation in different media of NB-FPAP

Details	Symbol	Unit	100%Med.	% of total	50%P	% of total	50%N	% of total	50%N&P	% of total
Conditions										
Volume	A	L	25		25		25		25	
Period days	B	Day	5		5		5		5	
Dry weight	C	g/L	0.33		0.24		0.28		0.26	
productivity per reactor	D=C/B*300	g/L/Year	19.8		14.4		16.8		15.6	
Max Reactor in 1 hectare	E	unit	9,375		9,375		9,375		9,375	
productivity in 1 hectare	F=E*D	g/L/Year	185,625		135,000		157,500		2,437.50	
Nutrient requirement										
Nutrient cost	G	USD/L	0.0404		0.0398		0.0226		0.0220	
Nutrient charge	H=(G*E*A)*(300/B)	USD/year	568,162	94.52	559,988	94.44	317,674	90.60	309,500	90.38
Electricity requirement										
Air pump	I=E*watts*24*300/1000	kWh	224,775		224,775		224,775		224,775	
Electricity charge (electricity charge = 0.12 USD per kWh)	J=I*0.12	USD/year	20,918	3.48	20,918	3.53	20,918	5.97	20,918	6.11
Water requirement										
water supply	K=E*A	L	234,375		234,375		234,375		234,375	
Water charge (water charge = 0.84 USD per m^3)	L=(K*0.31)*(300/B)	USD/year	12,041	2.00	12,041	2.03	12,041	3.43	12,041	3.52
Total operation	M=H+J+L	USD/year	601,121	100.00	592,947	100.00	350,633	100.00	342,459	100.00
Cost	N=(M/A/E)*(B/300)	USD/L	0.0427		0.0422		0.0249		0.0244	

Appendix A4-9 Economics for *Scenedesmus* sp. outdoor cultivation in different media of NB-FPAP

Details	Symbol	Unit	100%Med.	% of total	25%P	% of total	25%N	% of total	25%N&P	% of total
Conditions										
Volume	A	L	25		25		25		25	
Period days	B	Day	5		5		5		5	
Dry weight	C	g/L	0.31		0.31		0.23		0.46	
productivity per reactor	D=C/B*300	g/L/Year	18.6		18.6		13.8		27.6	
Max Reactor in 1 hectare	E	unit	9,375		9,375		9,375		9,375	
productivity in 1 hectare	F=E*D	g/L/Year	174,375		174,375		129,375		258,750	
Nutrient requirement										
Nutrient cost	G	USD/L	0.0404		0.0395		0.0137		0.0128	
Nutrient charge	H=(G*E*A)*(300/B)	USD/year	568,162	94.52	555,901	94.40	192,429	85.38	180,169	84.54
Electricity requirement										
Air pump	I=E*watts*24*300/1000	kWh	224,775		224,775		224,775		224,775	
Electricity charge (electricity charge = 0.12 USD per kWh)	J=I*0.12	USD/year	20,918	3.48	20,918	3.55	20,918	9.28	20,918	9.81
Water requirement										
water supply	K=E*A	L	234,375		234,375		234,375		234,375	
Water charge (water charge = 0.84 USD per m^3)	L=(K*0.31)*(300/B)	USD/year	12,041	2.00	12,041	2.04	12,041	5.34	12,041	5.65
Total operation	M=H+J+L	USD/year	601,121	100.00	588,860	100.00	225,389	100.00	213,128	100.00
Cost	N=(M/A/E)*(B/300)	USD/L	0.0427		0.0419		0.0160		0.0152	

Appendix A4-10 Economics for *Ankistrodesmus* sp. outdoor cultivation in reused medium of NB-FPAP

Details	Symbol	Unit	Fresh	% of total	1st reuse	% of total	2nd reuse	% of total
Conditions								
Volume	A	L	25		25		25	
Period days	B	Day	9		9		9	
Dry weight	C	g/L	0.55		1.31		1.91	
productivity per reactor	D=C/B*300	g/L/Year	18.3		43.7		63.7	
Max Reactor in 1 hectare	E	unit	9,375		9,375		9,375	
productivity in 1 hectare	F=E*D	g/L/Year	171,875		409,375		596,875	
Nutrient requirement								
Nutrient cost	G	USD/L	0.0404		0.000		0.000	
Nutrient charge	H=(G*E*A)*(300/B)	USD/year	315,646	87.67	-	-	-	-
Electricity requirement								
Air pump	I=E*watts*24*300/1000	kWh	405,000	-	405,000	-	405,000	
Electricity charge (electricity charge = 0.12 USD per kWh)	J=I*0.12	USD/year	37,690	10.47	37,690	9.48	37,690	8.66
Water requirement								
water supply	K=E*A	L	234,375		-		-	
Water charge (water charge = 0.84 USD per m^3)	L=(K*0.31)*(300/B)	USD/year	6,689	1.86	-	-	-	-
Total operation	M=H+J+L	USD/year	360,025	100.00	397,716	9.48	435,406	8.66
Cost	N=(M/A/E)*(B/300)	USD/year	0.0461		0.0509		0.0557	

Appendix A4-11 Economics for *Scenedesmus* sp. outdoor cultivation in reused medium of NB-FPAP

Details	Symbol	Unit	1st batch	% of total	2nd batch	% of total	3rd batch	% of total
Conditions								
Volume	A	L	25		25		25	
Period days	B	Day	5		5		5	
Dry weight	C	g/L	0.38		0.57		0.58	
productivity per reactor	D=C/B*300	g/L/Year	22.8		34.2		34.8	
Max Reactor in 1 hectare	E	unit	9,375		9,375		9,375	
productivity in 1 hectare	F=E*D	g/L/Year	213,750		320,625		326,250	
Nutrient requirement								
Nutrient cost	G	USD/L	0.0404		0.000		0.000	
Nutrient charge	H=(G*E*A)*(300/B)	USD/year	568,162	94.52	-	-	-	-
Electricity requirement								
Air pump	I=E*watts*24*300/1000	kWh	224,775		224,775		224,775	
Electricity charge (electricity charge = 0.12 USD per kWh)	J=I*0.12	USD/year	20,918	3.48	20,918	3.36	20,918	3.25
Water requirement								
water supply	K=E*A	L	234,375		-		-	
Water charge (water charge = 0.84 USD per m^3)	L=(K*0.31)*(300/B)	USD/year	12,041	2.00	-	-	-	-
Total operation	M=H+J+L	USD/year	601,121	100.00	622,039	3.36	642,957	3.25
Cost	N=(M/A/E)*(B/300)	USD/year	0.0427		0.0442		0.0457	

VITA

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