การเพาะเลี้ยงสาหร่าย *Scenedesmus armatus* สภาวะกลางแจ้งด้วยถังปฏิกรณ์ชีวภาพเชิงแสงแบบอากาศยกขนาดใหญ่

นางสาววทัตตา ฤทธิ์เจริญ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิศวกรรมศาสตรดุษฎีบัณฑิต
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LARGE SCALE OUTDOOR CULTIVATION OF MICROALGA *SCENEDESMUS ARMATUS* WITH AIRLIFT PHOTOBIOREACTOR

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A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Engineering Program in Chemical Engineering
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วทัตตา ฤทธิ์เจริญ : การเพาะเลี้ยงสาหร่าย *Scenedesmus armatus* สภาวะกลางแจ้งด้วยถังปฏิกรณ์ชีวภาพเชิงแสงแบบอากาศยกขนาดใหญ่ (LARGE SCALE OUTDOOR CULTIVATION OF MICROALGA *SCENEDESMUS ARMATUS* WITH AIRLIFT PHOTOBIOREACTOR) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร. ประเสริฐ ภวสันต์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ดร.สรวิศ เผ่าทองศุข, 115 หน้า.

วัตถุประสงค์ของงานวิจัยนี้คือ การศึกษาผลของรูปแบบถังปฏิกรณ์ชีวภาพเชิงแสงแบบอากาศยก และการป้อนก๊าซคาร์บอนไดออกไซด์ต่อการเจริญเติบโตของจุลสาหร่ายสีเขียวชนิด *Scenedesmus armatus* รวมถึงผลของการขยายขนาดการเพาะเลี้ยงจากระบบขนาดเล็กในร่มเป็นระบบขนาดใหญ่กลางแจ้ง สภาวะการเพาะเลี้ยงที่เหมาะสมสำหรับระบบขนาดเล็กในร่ม คือ อุณหภูมิ 35องศาเซลเซียส ความเข้มแสง 10 กิโลลักซ์ และความเร็วของอากาศ 1 เซนติเมตรต่อวินาที ซึ่งได้อัตราการผลิตชีวมวลจำเพาะเท่ากับ 106±6.6 มิลลิกรัมต่อลิตรต่อวัน ปริมาณไขมัน โปรตีน และคาร์โบไฮเดรตเท่ากับ 21.7±1.0, 32.9±2.6, 45.4±1.6 เปอร์เซ็นต์โดยน้ำหนัก ตามลำดับ การเพาะเลี้ยงขนาดใหญ่กลางแจ้งถูกดำเนินการแบบกะด้วยถังปฏิกรณ์ชีวภาพเชิงแสงแบบอากาศยกแบบแบนปริมาตร 100 ลิตร ความเร็วของอากาศ 0.35 เซนติเมตรต่อวินาที หรืออัตราการให้อากาศ 0.2 โดยปริมาตรอากาศต่อปริมาตรน้ำเลี้ยงต่อนาที ภายใต้สภาวะอากาศของประเทศไทย ผลการเพาะเลี้ยงแสดงให้เห็นว่าเซลล์สามารถเจริญเติบโตและมีความต้านทานต่อความเข้มแสงและอุณหภูมิสูง โดยที่อัตราการเจริญเติบโตสูงสุดแท้จริงเกิดขึ้นในช่วงเวลา 9.00น.ถึง15.00น. กับค่า pH ในช่วงกว้าง 6.4 ถึง 11 ซึ่งมีอัตราการผลิตชีวมวลเฉลี่ยจำเพาะเท่ากับ 39.2 ถึง 55.5 มิลลิกรัมต่อลิตรต่อวัน การป้อนก๊าซคาร์บอนไดออกไซด์เข้าในระบบการเพาะเลี้ยงกลางแจ้งระหว่างวัน ส่งผลเพียงเล็กน้อยต่อการเจริญเติบโต แต่มีอิทธิพลต่อการสะสมสารชีวเคมี เมื่อป้อนก๊าซคาร์บอนไดออกไซด์ในช่วงความเข้มข้น 2 ถึง 15 เปอร์เซ็นต์โดยปริมาตรของอัตราการให้อากาศ (0.2 โดยปริมาตรอากาศต่อปริมาตรน้ำเลี้ยงต่อนาที) พบว่า ปริมาณไขมันและโปรตีน เพิ่มขึ้นจาก 19.8 เป็น 25.6 และ 37.8 เป็น 48.2 เปอร์เซ็นต์โดยน้ำหนัก ตามลำดับ ในขณะที่ ปริมาณคาร์โบไฮเดรต ลดลงจาก 42.5 เป็น 26.2 เปอร์เซ็นต์โดยน้ำหนัก การเพาะเลี้ยง *S. armatus* ด้วยการป้อนก๊าซคาร์บอนไดออกไซด์ 2 เปอร์เซ็นต์โดยปริมาตร ทำให้ได้อัตราการผลิตชีวมวลเฉลี่ยจำเพาะสูงสุด 91.3 มิลลิกรัมต่อลิตรต่อวัน อัตราการตรึงก๊าซคาร์บอนไดออกไซด์สูงสุด 165 มิลลิกรัมคาร์บอนไดออกไซด์ต่อลิตรต่อวัน และอัตราการผลิตไขมัน โปรตีนและคาร์โบไฮเดรตเฉลี่ยสูงสุดเท่ากับ 22.2 38.3 และ 30.8 มิลลิกรัมต่อลิตรต่อวัน นอกจากนี้ก๊าซคาร์บอนไดออกไซด์ยังถูกใช้เป็นตัวควบคุมค่า pH โดยที่ ค่า pH 7 ถึง 7.7 ให้ผลการเจริญเติบโตคล้ายกับการป้อนก๊าซคาร์บอนไดออกไซด์เข้มข้น 2 เปอร์เซ็นต์โดยปริมาตร การออกแบบถังปฏิกรณ์ชีวภาพเชิงแสงแบบอากาศยกแบบใหม่ชนิดไร้แผ่นกั้น 2 รูปแบบคือ ถังปฏิกรณ์ชีวภาพเชิงแสงแบบอากาศยกชนิดไร้แผ่นกั้นแบบกรวย และถังปฏิกรณ์ชีวภาพเชิงแสงแบบอากาศยกชนิดไร้แผ่นกั้นแบบแบน แสดงให้เห็นว่าสามารถนำมาเพาะเลี้ยงจุลสาหร่ายได้ โดยที่อัตราการเจริญเติบโตของเซลล์ไม่ขึ้นกับองศาของมุมที่ก้นถังปฏิกรณ์ชีวภาพเชิงแสงแบบอากาศยกชนิดไร้แผ่นกั้นแบบกรวย (30 45 และ 53 องศา) โดยที่ถังกรวยมุมก้นถัง30 องศา เหมาะกับการเพาะเลี้ยง *S. armatus* กลางแจ้ง เนื่องจากมีปริมาตรสูงสุดเมื่อพื้นที่การเพาะเลี้ยงเท่ากัน ในขณะที่การเพาะเลี้ยง *S. armatus* ด้วยถังปฏิกรณ์ชีวภาพเชิงแสงแบบอากาศยกชนิดไร้แผ่นกั้นแบบแบนที่ความกว้างของถังคือ 20 30 40 และ 50 เซนติเมตร และความสูงของน้ำเลี้ยง 40 50 และ 60 เซนติเมตร พบว่า ความสูงของน้ำเลี้ยงที่เหมาะสม คือ 40 และ 50 เซนติเมตร และความกว้างของถัง 50 เซนติเมตร โดยที่อัตราการผลิตชีวมวลจำเพาะเฉลี่ยอยู่ในช่วง 44.6 ถึง 45.5 มิลลิกรัมต่อลิตรต่อวัน

ภาควิชา วิศวกรรมเคมี	ลายมือชื่อนิสิต .....
สาขาวิชา วิศวกรรมเคมี	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....
ปีการศึกษา 2557	ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

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KEYWORDS: SCENEDESMUS / AIRLIFT PHOTOBIOREACTOR / OUTDOOR / CARBON DIOXIDE / PRODUCTIVITY

WATADTA RITCHAROEN: LARGE SCALE OUTDOOR CULTIVATION OF MICROALGA *SCENEDESMUS ARMATUS* WITH AIRLIFT PHOTOBIOREACTOR. ADVISOR: ASSOC. PROF.PRASERT PAVASANT, Ph.D., CO-ADVISOR: SORAWIT POWTONGSOOK, Ph.D., 115 pp.

The aim of this work was to investigate the effect of the airlift photobioreactor configurations and CO_2 supplement on the cultivation of the green microalga *Scenedesmus armatus.* The scale-up from small scale indoor to large scale outdoor cultivations was also examined. The optimal condition for small scale indoor cultivation was 35°C, 10 kLux and superficial gas velocity (u_{sg}) 1 cm s^{-1}, this was equivalent to the specific biomass productivity of 106±6.6 mg $L^{-1}d^{-1}$. The associate biochemical compositions of lipid, protein and carbohydrate were 21.7±1.0, 32.9±2.6, 45.4±1.6 %w/w, respectively. The outdoor large scale operation was conducted in 100 L flat panel airlift photobioreactors (FPAPs) with u_{sg} 0.35 cm s^{-1} or aeration rate of 0.2 vvm in the batch mode under Thailand climate. The results indicated that cells could tolerate high light intensity and relatively high temperature where the highest actual growth rate occurred at around 9:00 AM to 3:00 PM with a wide pH range of 6.4 to 11. The average specific biomass productivity was around 39.2 to 55.5 mg $L^{-1}d^{-1}$. The supply of CO_2 had a slight influence on growth characteristics but did exert some observable effects on biochemical accumulations. Adding CO_2 from 2 to 15% by volume in the aeration (0.2 vvm) during daytime caused an increase in lipid and protein from 19.8 to 25.6 and 37.8 to 48.2% w/w, respectively, whereas carbohydrate decreased from 42.5 to 26.2% w/w. *S. armatus* cultivated with 2% CO_2 enriched air provided the highest the average of the average biomass productivity of 91.3 mg $L^{-1}d^{-1}$ which corresponded to a CO_2 fixation of 165 mg CO_2 $L^{-1}d^{-1}$ with the average lipid, protein and carbohydrate productivities of 22.2, 38.3 and 30.8 mg $L^{-1}d^{-1}$. CO_2 was also used to control pH in the range of 7-7.7 and similar results with the addition of 2%CO_2 were obtained. A novel large scale airlift systems without the physical gas separating baffle, i.e Non-baffled cone airlift photobioreactors (NB-CAPs) and Non-baffled flat panel airlift photobioreactors (NB-FPAPs), could be successfully used to cultivate the microalgal culture. Growth was independent of the slope of the cone bottom NB-CAPs (30°, 45° and 53°) where the 30° cone bottom NB-CAP was suggested for *S. armatus* outdoor cultivation as it provided the highest volume using the same area. *S. armatus* was cultivated in NB-FPAPs with various the widths of the reactor from 20, 30, 40 and 50 cm and the unaerated medium heights from 40, 50 and 60 cm. The optimal medium height of NB-FPAPs was 40-50 cm whereas the width was 50 cm. The specific biomass productivities were around 44.6-45.5 mg $L^{-1}d^{-1}$.

Department:	Chemical Engineering	Student's Signature	.....
Field of Study:	Chemical Engineering	Advisor's Signature	.....
Academic Year:	2014	Co-Advisor's Signature	.....

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Chapter I
Introduction

1.1 Motivations

It has been widely accepted that the content of greenhouse gases especially carbon dioxide has continuously increased over the years. This problem has undoubtedly been created anthropogenically particularly fuel burning in industries and urban transportation. Although this chronic problem will require long term mitigating measures, an enormous number of research works have now been devoted to lessening this problem by reducing the amount of greenhouse gases emission. One of the potential approaches is to make use of photosynthetic activities, either in large scale plants or in more effective microorganisms like microalgae. There are evidences that many types of microalgae could uptake carbon dioxide as their carbon source and many of them could withstand high carbon dioxide condition, e.g. in the range of 5 to 20% (Ho et al., 2010b, Jacob-Lopes et al., 2010, Sydney et al., 2010, Tang et al., 2011). It was reported that CO_2 has affected biochemical composition in algae, for example, lipid and carbohydrate accumulation were enhanced when *Chlorella sorokiniana* and *Dunaliella salina* were cultured in air enriched-CO_2 (Muradyan et al., 2004, Tanadul et al., 2014). Polyunsaturated fatty acid in *Scenedesmus obliquus* and *Chlorella pyrenoidosa* increased with increasing feeding CO_2 concentrations (Tang et al., 2011). Carbohydrate accumulation in *Chlamydomonas reinhardtii* decreased but protein increased with high CO_2 condition (Thyssen et al., 2001).

Literature reveals that stains of *Scenedesmus* exhibited a possibility to remove carbon dioxide and effectively produced useful substances like carbohydrates, lipid and proteins. Examples are *Scenedesmus obliquus* CNW-N (Ho et al., 2010b), *Scenedesmus* sp. (Yoo et al., 2010), *Scenedesmus caribeanus* (Westerhoff et al., 2010), *Scenedesmus obliquus* SJTU-3 (Tang et al., 2011).

Large scale cultivation of microalgae poses one of the most intriguing research aspects as the future development of all microalgal applications will need to have a reliable and economical industrial scale cultivation process with reasonably high biomass yield. There are always trade-offs between using an open pond that utilizes sunlight and a well controlled photobioreactor, as the former requires lesser resources but a reliable operation is doubtful whereas the latter operates with high level of confidentiality, flexibility and reliability, but with a cost of more expensive controlling systems. For example, dry weight per one kilogram of *Chaetoceros muelleri* under indoor condition was 2.5 times more expensive than that obtained from outdoor condition (López-Elías et al., 2005). This high cost is compensated by high productivity inherited from the well controlled photobioreactor, e.g. the cultivation of microalgae in large scale airlift photobioreactor gave 1.5-4 times better productivity than the open pond systems (Issarapayup et al., 2009, Krichnavaruk et al., 2007).

Airlift systems have been proven to be an effective alternative for the cultivation of microalgae as reported by Krichnavaruk et al. (2007) for *Chaetoceros*, Kaewpintong et al. (2007) and Issarapayup et al. (2009) for *Haematococcus*. This research focuses on the design of large scale airlift photobioreactor with adequate, low cost environmental control for the culture of *Scenedesmus amartus* TISTR 8591. This algal species is well acclimatized to Thai environmental conditions, and can be cultured outdoor with small contamination risk. The harvest is also simple as the cells can be separated from the medium just by gravity sedimentation. In addition, its relatively high protein and carbohydrate contents render it suitable as supplementary animal feed and bio-energy. For this work, the effect of the configurations of reactor and the supply of carbon dioxide on growth, productivity and biochemical accumulation were investigated.

1.2 Objectives

- To develop a large scale airlift cultivation system of green alga (*Scenedesmus amartus*) under outdoor condition

- To examine airlift photobioreactor configurations for large scale algal culture

1.3 Scopes

- The large scale cultivation system under outdoor condition with sunlight and atmospheric temperature.

  - Green alga (*Scenedesmus armatus*) was chosen for this study.
  - The alga was cultivated in a batch cultivation system with flat panel airlift photobioreactor (FPAP).
  - Working volume of system was 100 L.
  - The superficial gas velocity (u_{sg}) and air flow rate were in the range of 0.18-0.52 cm s^{-1} and 0.1-0.3 vvm (volume gas per working volume per minute) of FPAP.
  - Carbon dioxide fixation was examined by supplying carbon dioxide concentration in the range of 2-15% by volume of aeration rate.
  - Carbon dioxide was supplied to culture systems for a light: dark period of 12:12.
  - pH was controlled using supplying CO_2.
  - The effect of scale up from scale indoor to outdoor was investigated.

- To examine airlift photobioreactor configurations for large scale algal culture

  - Two types of non-baffled airlift photobioreactors were employed, i.e.
    - Non-baffled flat panel airlift photobioreactor (NB-FPAP)

- Non-baffled cone airlift photobioreactors (NB-CAP) with variable bottom slopes (30, 45 and 53 degrees with x-plane)

- Air flow rate was in the range of 0.1-0.3 vvm of NB-CAP.
- Unaerated medium height in NB-CAP was 34-75 cm.
- Widths of NB-FPAPs were 20, 30, 40 and 50 cm.
- Unaerated medium heights in NB-FPAPs were 30, 40 and 50 cm.

Chapter II
Backgrounds and literature review

2.1 Microalgae

Microalgae are a source of valuable nutrients for human and animals such as protein, fatty acids, carbohydrate and vitamin as well as antioxidants. In addition, algae extracts can be used in various applications such as biodiesel, fertilizer, supplementary food and animal feed. This chapter provides necessary detail on the algae and their cultivating systems.

2.1.1 Green microalgae

Green microalgae are among protists in the protista kingdom and Chlorophyta phylum. Green microalgae can take unicellular, colony or filamentous forms with chloroplast and pigment groups (chlorophylls, carothenoids, and Phycobilins) used for photosynthesis process. Microalgae are found in both freshwater and marine systems, examples include *Chlorella*, *Scenedesmus*, *Chlorococcoum*, and *Tetraselmis*. Chemical compositions of the algae are different from species to species as illustrated in Table 2.1. Utilizations of these chemical compositions are variables, e.g. used as raw material for biodiesel or bioethanol productions, or as protein supplement in animal feed.

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 shapes of cell are various, i.e. crescent, spindle-shaped or ovoid. Utilization of *Scenedesmus* includes the use as animal feed due to its relatively high protein content. *Scenedesmus* sp. and *Scenedesmus obliquus* have been studied as

a carbon dioxide mitigation means with a lipid production pathway. Yoo et al. (2010) and Tang et al. (2011) reported that *Scenedesmus* sp. could grow as a dense culture using carbon dioxide in the range of 5-20%. *Scenedesmus almeriensis* can produce high value antioxidant, i.e. lutein at approximately 0.53% of its dry weight which could reach a high productivity of 3.8 mg of lutien $L^{-1}day^{-1}$ (Sánchez et al., 2008). This makes *S. almeriensis* a potential candidate for commercial lutein production. Other reviews are summarized in Table 2.3. For outdoor cultivation, the selecting strain should grow reasonably well under normal environmental conditions. For Thailand, *Scenedesmus armatus* TISTR 8591 exhibits interesting properties as it is a rapid growing local species with minimum requirement for system maintenance.

Table2.1 Chemical compositions of green microalgae (Singh et al., 2011)

Strain	Protein (%)	Carbohydrate (%)	Lipid (%)
Scenedesmus obliquus	50-56	10-17	12-14
Scenedesmus quadricauda	47	-	1.9
Scenedesmus dimorphus	8-18	21-52	16-40
Chlamydomonas rheinhardii	48	17	21
Chlorella vulgaris	51-58	12-17	14-22
Chlorella pyrenoidosa	57	26	2
Spirogyra sp.	6-20	33-64	11-21
Dunaliella bioculata	49	4	8
Dunaliella salina	57	32	6
Tetraselmis maculata	53	15	3

Table2.2 Scientific classification of *Scenedesmus* sp.

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

Table2.3 Reviews of *Scenedesmus* cultivation

Author (year)	Strains	reactor	medium	Vol.	T	Light intensity	pH	Aeration rate		CO_2	Time	biomass concentration	productivity	μ	Chemical composition
				(L)	(°C)	(Lux)		vvm	$Lmin^{-1}$	(% of air)	(d)	($g\ L^{-1}$)	($g\ L^{-1}d^{-1}$)	(d^{-1})	
Tukaj et al. (2003)	*S. armartus*	plate-parallel vessel	Bristol's medium	0.6	30	21.82				2					
Rodjaroen et al. (2007)	*S.amartus* TISTR 8591		BG11	0.3	28±1	4,440					20	0.05			Starch = 15.4%
Sánchez et al. (2008)	*S.almeriensis*	bubble column	Mann and Myer's medium	2	34	120,250		0.5	1				0.73		Lipid = 12% Carbohydrate= 24.6% Protein = 49.4%
Ho et al. (2010b)	*S.obliquus* CNW-N	stirr tank	Detmer's medium	1	28	4,440	6	0.003		10	12	3.51	0.29	1.19	Lipid = 12.3%
Ho et al. (2010a)	*S.obliquus* CNW-N	stirr tank	BG11	1	28	4,440	6.2			20	12	2.63	0.2	1.019	
	S. obliquus AS-6-1											1.9	0.15	1.065	
Yoo et al. (2010)	*Scenedesmus*. sp.		BG11		25±1	11,100				10	14		0.2175		
	Chlorella vulgaris		BG11		25±1	11,100				10	14		0.10476		
	Botryococcus braunii		modified Chu 13		25±1	11,100				10	14		0.02655		

Table 2.3 (Cont.) Reviews of *Scenedesmus* cultivation

Author, year	Strains	reactor	medium	Vol.	T	Light intensity	pH	Aeration rate		CO_2	Time	biomass concentration	productivity	μ	Chemical composition
				(L)	(°C)	(Lux)		vvm	$Lmin^{-1}$	(% of air)	(d)	($g\ L^{-1}$)	($g\ L^{-1}d^{-1}$)	(d^{-1})	
Westerhoff et al. (2010)	mixed *Scenedesmus caribeanus* and *Chlorella vulgaris*	helical tubular	BG11	9	31	3,256			1.25	10 to 12	14		0.056 gC/L-d		
		flat-plate	BG11	10		17,760				10 to 12	16		0.42 gC/L-d		
		flat-plate	BG11	10		sunlight (<1,350 to 135,000)				10 to 12	64		0.14 gC/L-d		
Kim et al. (2011)	*Scenedesmus* sp.	stirr tank	BG11	0.2	25±1	11100	7.5 (in.)				16				
Tang et al. (2011)	*S. obliquus* SJTU-3	bubble	modified BG11	0.8	25±1	13,320	7.0 (in.)	0.25	0.2	10	14	1.84±0.01	0.155±0.004	0.037 (Max)	Lipid = 19.3%
	Chlorella pyrenoidosa SJTU-2	bubble	modified BG11	0.8	25±1	13,320	7.0 (in.)	0.25	0.2	10	14	1.55±0.01	0.144±0.011	0.041 (Max)	Lipid = 24.3%
(Ketheesan and Nirmalakhandan, 2011)	*Scenedesmus* sp.	airlift-raceway	Bols's Basal	20	24-26	4,000			0.6		24		0.16±0.03		

2.2 Photosynthesis

2.2.1 Pigment groups

Microalgae consist of three main pigment groups; chlorophylls, phycobilins and carotenoids which can absorb light at different wavelengths as demonstrated in Table 2.4. The type of Chlorophyll are typically various as *a*, *b*, *c* and *d* which absorb light in the range 450-475 and 630-675 nm. Structure of chlorophyll contains a central magnesium atom and long chain terpenoid alcohol. Chlorophyll *a* has a major role in photosynthesis process as it is responsible for light absorption and the transfer of energy to the photosynthetic pathway. Carotenoids are two hexacarbon rings joined with long chain hydrocarbon. The molecule of carotenoids is either long chain hydrocarbon called carotenes (α-carotene, β-carotene) or oxygenated hydrocarbons called xanthophylls (lutein, violaxanthin). Carotenoids absorb light in the range 400-550 nm which has a role as accessory light-harvesting pigments transfer to chlorophyll *a*. Phycobilins are water-soluble which differ from chlorophyll and carotenoids and absorb light in the range 500-650 nm.

Table2.4 Characteristics of pigment (Carvalho et al., 2011)

Pigment group	Color	Range of absorption bands (nm)
Chlorophylls	Green	450-475
(e.g. Chlorophyll *a*, Chlorophyll *b*)		630-675
Phycobilins	Blue, Red	500-650
(e.g. Phycocyanin, Phycoerythrin)		
Carotenoids	Yellow,	400-550
(e.g. β-Carotene, Lutein)	Orange	

2.2.2 Photosynthesis process

Photosynthesis is the process that uses light energy with carbon dioxide and water to produce carbohydrate (sugar) and oxygen for growth. The chemical equation of reaction is shown below:

$$6CO_2 + 6\ H_2O + \text{light energy} \rightarrow C_6H_{12}O_6 + 6O_2 \qquad (2.1)$$

The photosynthesis process occurs in chloroplast as a two step mechanism, viz. light reaction and dark reaction. Light reaction occurs in thylakoid membrane where Chlorophylls absorb light energy and split water to electron, proton and oxygen. Electron transfers through Photosystem I (PS I, 700 nm) and Photosystem II (PS II, 680 nm) to yield chemical energy accumulated in forms of ATP and NADPH; these accumulated energy forms are used in the dark reaction. The dark reaction or known as the Calvin cycle is the process of carbon dioxide fixation which occurs in the stoma outside the thylakoid before being further converted to other useful chemicals such as carbohydrate (sugar). The schematic of overview photosynthesis is illustrated in Figure 2.1.

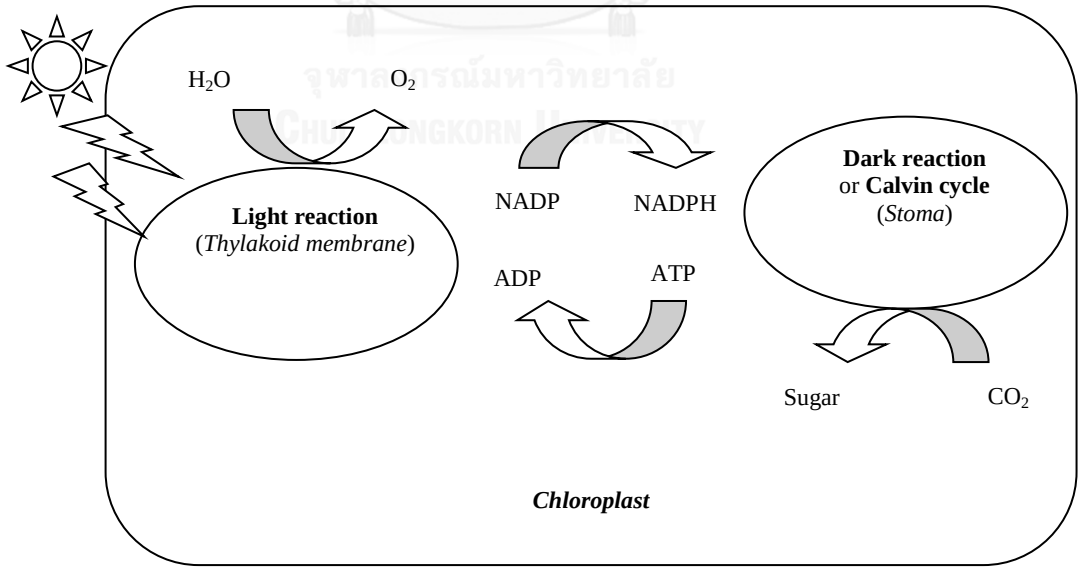

Figure2.1 Schematic overview of photosynthetic process

The correlation between the rate of photosynthesis (P) and light intensity (I) is illustrated in Figure 2.2. At low light intensity (light-limited region), the photosynthetic rate is proportional with light intensity where the initial slope (**α**) represents light utilization efficiency. Note that the rate of photosynthesis equals zero as light intensity falls below the compensation point (Ic). When increasing light intensity, the rate of photosynthetic gradually increases and reaches the at the light saturation intensity (Is). Finally, the photosynthetic rate declines due to photo-inhibition at the intensity of Ih.

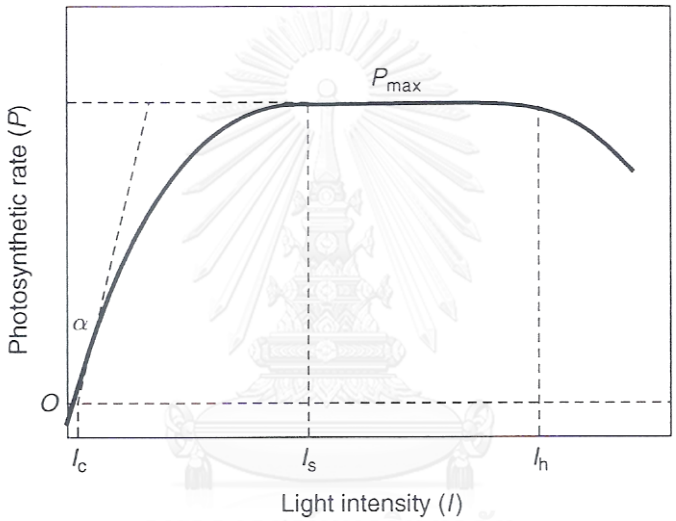

Figure2.2 Light response curve of photosynthesis (Richmond, 2007)

Photo-inhibition occurs when the culture receives excess light intensity for a long time. Indicators for photo-inhibition phenomena include slow oxygen evolution and chlorophyll fluorescence. Actually, oxygen evolution from photosynthesis is expressed in terms of μmol or mg O_2 per mg^{-1} (Chl) h^{-1} or per cell h^{-1}. Excess dissolved oxygen in the culture can also generate inhibition effect that leads to cell death. Chlorophyll fluorescence is a convenient method that indicates the performance of photochemical process in PS II. The difference between the maximum fluorescence (Fm) and the minimum fluorescence (Fo) is the variable fluorescence (Fv). The photochemical yield of PS II is estimated by the ratio of

Fv/Fm. For active green algae, Fv/Fm is in the range of 0.65-0.80 (Richmond, 2007). Fv/Fm below 0.65 could indicate photo-inhibition.

2.2.3 Photosynthetic efficiency

The photosynthetic efficiency (PE) is the effectiveness of light energy converted into chemical energy during photosynthesis which defines as the energy stored in biomass per unit of light energy impinging on the culture. PE is calculated from areal productivity (P_a) multiplied with energy content in biomass (E_b) and divided by solar irradiance input (I_i) as follows:

$$PE = \frac{P_a\ (g\,m^{-2}\,d^{-1}) \times E_b\ (kJ\,g^{-1})}{I_i\ (kJ\,m^{-2}\,d^{-1})} \qquad (2.2)$$

PE is a function of average irradiance, Fv/Fm ratio and air flow rate (Fernández et al., 2003). The PE increases when Fv/Fm and air flow rate increase. In the contrast, the PE decreases when the average irradiance increase which PE of the outdoor cultivation was lower than indoor cultivation due to high light intensity (Van Bergeijk et al., 2010).

2.3 Main parameters for microalgal cultivation

Many parameters are important to growth of microalgae, these include nutrients, CO_2 or carbon sources, temperature, light intensity, pH, aeration and etc.

- Nutrients

Primary nutrients which are important to growth of microalgae are carbon, nitrogen and phosphorus as well as silica for diatom. Autotrophic culture system use carbon source from inorganic sources viz. carbon dioxide (CO_2), bicarbonate ($NaHCO_3$) and carbonate (Na_2CO_3). Some reported that both bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions could be more easily consumed than carbon dioxide as they were more

soluble in water (Yeh and Chang, 2010). Nitrogen is associated with the primary metabolism of microalgae and also constitutes cell protein. Phosphorus is essential as it constitutes energy storage compounds. Typical nitrogen and phosphorus sources are nitrate (NO_3^-) and phosphate (PO_4^{2-}). Furthermore, trace metal elements and vitamin are added for effective cultivation.

- Carbon dioxide concentration

Carbon dioxide is a carbon source for the growth of algae. It is a precursor in the photosynthesis. Carbon dioxide sources include air (approximately 0.03% CO_2), industrial exhaust gas (e.g. flue gas about 15-20%CO_2) and soluble carbonates (e.g. $NaHCO_3$ and Na_2CO_3). The carbon dioxide fixation efficiency of microalgae is about 10-50 times faster than terrestrial plants (Costa et al., 2000) and the cultivation system is environmentally friendly. Hence, most literatures search for strains which can tolerant high carbon dioxide and effective CO_2 fixation through high biomass productivity. Literature reviews are summarized in Table 2.5.

- Temperature

Temperature has major effects to cell growth and biochemical composition such as fatty acid types and accumulated quantity (Hu et al., 2008). The optimal temperature of each species is various and occasionally based on other environmental parameters such as light intensity (Kumar et al., 2010). The optimal temperature for most microalgae is around 22 to 28 $^{\circ}C$ (Ong et al., 2010). Note that photo-inhibition can occur providing that the temperature is lower or higher than the optimal level (Richmond, 2007).

- Light intensity

Microalgae need light as energy source for photosynthesis. Each type of microalgae has its own optimal light intensity above or below which photo-oxidation

and photo-inhibition or growth limiting could take place (Carvalho et al., 2011). Furthermore, light has effect on the lipid content since light affects the ultra-structure of cell organelles where lipid is an important component of their membrane composition (Hu et al., 2008).

- Photoperiod

Light/Dark cycle can affect photosynthesis. This consists of two stages as light dependent and dark phase (light independent). A long lighting period can increase biomass concentration including the amount of proteins, carbohydrates, and lipids in cell (Fábregas et al., 2002, Khoeyi et al., 2012).

- pH

Generally, microalgae grow well at neutral pH but some species can grow at higher pH such as *Spirulina platensis* (pH 9) (Sydney et al., 2010) or lower pH such as *Chlorococcum littorale* (pH 5.5) (Iwasaki et al., 1996). In order to ensure high growth rate, many cultivation systems are controlled at optimal pH by carbon dioxide or acid and base. Microalgae are effective as natural carbon dioxide removal mechanism as they use CO_2 as their carbon source, but increasing carbon dioxide concentration could lower the pH of the culture and alter the growth behavior. Nevertheless, carbon dioxide tolerant microalgae are still very interesting and attract research attention in a wide scale.

- Aeration rate

Aeration is necessary to induce mixing in microalgal culture and to prevent cell sedimentation. The air is supplied through device such as porous gas sparger for bubble and airlift photobioreactors, or through paddle wheel for race way pond. Increasing aeration rate generally leads to a higher liquid velocity and a more turbulent-like flow (Fernández et al., 2001) which improves mass transfer between

gas and liquid and effective light utilization resulting in a better growth (Krichnavaruk et al., 2005). However, inducing aeration rate could cause cell damage and also implies high electricity cost.

The summary of the cultivation systems and their operating conditions along with the growth parameters is illustrated in Table 2.6.

Table2.5 Performance of carbon dioxide removal by microalgae

Author	Strains	Reactors	Vol (L)	CO_2 (% of air)	Biomass concentration (g L^{-1})	Productivity (g $L^{-1}d^{-1}$)	Specific growth rate (d^{-1})	Maximum CO_2 consumption rate (g $L^{-1}d^{-1}$)	CO_2 fixed rate in biomass
Jacob-Lopes et al. (2009)	*Aphanothece* microscopic Nägeli (cyanobacteria)	Bubble column		15	5.1 (24:0)	0.770 (24:0)		1.44(24:0)	99.7% (24:0)
					2.06 (12:12)	0.301 (12:12)		0.56 (12:12)	39% (12:12)
Widjaja et al. (2009)	*Chlorella vulgaris*	Stirr tank		0.03-3.33	0.86				
Chiu et al. (2009)	*Chlorella* sp. NCTU-2	Bubble column	4	5	2.37		0.18		
		Airlift with draft tube	4	5	2.53		0.23		
		Airlift with porous draft tube	4	5	3.46		0.25		
Ong et al. (2010)	*Chlorella* sp.	Bubble column		5			0.4-0.1 (25-40 ^oC)		11.8-15.52 mg/min (0.25-0.5 vvm)
	Chlorella sp. mutant MT-7						0.58-0.36 (25-40 ^oC)		12.45-18.24 mg/min (0.25-0.5 vvm)
	Chlorella sp. mutant MT-15						0.74-0.69 (25-40 ^oC)		10.92-17.13 mg/min (0.25-0.5 vvm)

Table 2.5 (Cont.) Performance of carbon dioxide removal by microalgae

Author	Strains	Reactors	Vol. (L)	CO_2 concentration (% of air)	Biomass concentration (g L^{-1})	Productivity (g $L^{-1}d^{-1}$)	Specific growth rate (d^{-1})	Maximum CO_2 consumption rate (g $L^{-1}d^{-1}$)	CO_2 fixed rate in biomass
López et al. (2010)	*Aphanothece* microscopic Nägeli	Bubble column		15	2.02 (24:0) 1.8 (12:12)			26.9 (24:0, gas phase) 24.6 (24:0, liquid phase)	3%
Sydney et al. (2010)	*Chlorella vulgaris* LEB-104	Bubble column		5	1.94	0.31	0.29	0.250	
	Botryococcus braunii SAG-30.81				3.11		0.24	0.500	
	Spirulina platensis LEB-52				2.18	0.73	0.22	0.320	
	Dunaliella tertiolecta SAG-13.86				2.15	0.42	0.21	0.270	
Ho et al. (2010b)	*Scenedesmus obliquus* CNW-N	Stirr tank		10	3.51	0.29	1.19	0.55	
Westerhoff et al. (2010)	mixed *Scenedesmus caribeanus* and *Chlorella vulgaris*	helical tubular		10 to 12		0.056 (gC $L^{-1}d^{-1}$)			2 to 3%
		flat-plate		10 to 12		0.42 (gC $L^{-1}d^{-1}$)			8 to 10%
Ho et al. (2010a)	*Scenedesmus obliquus* CNW-N	stirr tank		20	1.9	0.15	1.065	0.39	
	Scenedesmus obliquus AS-6-1	stirr tank		20	1.9	0.15	1.065	0.29	
Tang et al. (2011)	*Scenedesmus obliquus* SJTU-3	bubble column		10	1.84±0.01	0.155±0.004	0.037 (Max)	0.288	
	Chlorella pyrenoidosa SJTU-2	bubble column			1.55±0.01	0.144±0.011	0.041 (Max)	0.26	

Table2.6 Literature reviews

Author (Year)	Strains	Reactors	Vol. (L)	T (°C)	Light (Lux)	pH	Cell concentration ($x10^6$ cells 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	35	25	22,200		36			0.528
		Bubble column	35	25	22,200		36			0.528
Renaud et al. (2002)	*Rhodomonas* sp.	Flask	1.5	25-27	5920	8.3	0.44-0.59			0.3-0.35
	Prymnesiophyte NT19			27-30			4.51-4.97			0.54-0.56
	Cryptomonas sp.			27-30			0.68-1.05			0.27-0.33
	Chaetoceros sp.			27-35			1.95-3.98			0.74-0.87
	Isochrysysis sp.			27-30			3.55-4.94			0.89-0.97
Xu et al. (2002)	*Undaria pinnatifida*	Airlift	2.5	25	2,960		-	0.71	0.12	
		Bubble column	2.5	25	2,960		-	0.59	0.08	
Yim *et* al. (2003)	Gyrodinium impudicum strain KG03	Airlift	2	22.5	11,100	8		1.35		0.69
Krichnavaruk et al. (2005)	*Chaetoceros calcitrans*	Airlift	17	30	29,600		8.88			1.78
		Bubble column	17	30	29,600		5.80			0.91
Vega-Estrada et al. (2005)	*Haematococcus pluvialis*	Airlift	2.2	26	12,580		1.1			0.23

Table 2.6 (Cont.) Literature reviews

Author (Year)	Strains	Reactors	Vol. (L)	T (°C)	Light (Lux)	pH	Cell concentration ($x10^6$ cells mL^{-1})	Biomass concentration ($g\ L^{-1}$)	Productivity ($g\ L^{-1}d^{-1}$)	Specific growth rate (d^{-1})
Converti et al. (2006)	*Spirulina platensis*	Open pond	65	30	4,070	9.5			0.005	0.12
		Tubular	5.5	30	8,880			10.6	0.062	0.19
Kaewpintong et al. (2007)	*Haematococcus pluvialis*	Airlift	3	27	1,480	7.1 (in.)	0.795			0.45
		Bubble column	3	27	1,480		0.420			0.36
Krichnavaruk et al. (2007)	*Chaetoceros calcitrans*	Airlift	17	30	25,900		8.88		4.8×10^9 cells $L^{-1}d^{-1}$	1.78
		Airlift	170	30	25,900		2.96		7.5×10^8 cells $L^{-1}d^{-1}$	0.62
		Bubble column	17	30	25,900		4.96		1.9×10^9 cells $L^{-1}d^{-1}$	0.69
		Bubble column	170	30	25,900		2.51		6.2×10^8 cells $L^{-1}d^{-1}$	0.59
Oncel and Sukan (2008)	*Spirulina platensis*	Airlift	1.5	25	4,000	9.3	-	2.21		0.45
		Bubble column	1.5	25	4,000	9.3	-	1.87		0.33
Issarapayup et al. (2009)	*Haematococcus pluvialis*	Flat panel airlift	17	25	1,480	7	0.41			0.52
			90	25	1,480	7	0.40			0.39
Yeh and Chang (2010)	*Chlorella vugaris*	Stirred	1	25		6.2		0.55		1.41

2.4 Photobioreactors for microalgae cultivation

Microalgal culture systems have been optimized in order to obtain maximum growth. Typical culture systems can be separated into two types, i.e. open systems (pond, tank and race way) and close systems (flat plate, tubular, bubble and airlift photobioreactors). Close photobioreactors have attracted more than open systems due to the ease of control and the attainment of high biomass productivity (Ugwu et al., 2008). Both open and close systems are explained as follows.

2.4.1 Open pond

Microalgal culture in open ponds is good for mass cultivation. The most commonly used systems include shallow big ponds, tanks, circular ponds and raceway ponds (Ugwu et al., 2008). The major advantage of open pond systems is its simplicity for both construction and operation. However, the disadvantage of this system is low productivity, low light utilization and easy contamination. Thin-layer technology can overcome drawback of open pond due to decreasing microalgal suspension layer, nevertheless this system suffers high energy consumption for operation (Doucha and Lívanský, 2006).

2.4.2 Flat plate photobioreactors

Flat plate or flat panel photobioreactor is interesting for photosynthetic of microorganism due to their large illumination surface area that is suitable for outdoor culture (Ugwu et al., 2008). The flat panel photobioreactors are normally made from transparent materials for maximum utilization of solar light energy. The accumulation of dissolved oxygen in this reactor is relatively low compared to horizontal tubular photobioreactor but this might not have notable effects on algal growth. Sierra et al. (2008) characterized fluid-dynamic and mass transfer of the flat panel photobioreactor and concluded that the main advantages of this reactor were low power consumption (53W m^{-3}) and high mass transfer capacity (0.007 L s^{-1}), but on the other hand, the major disadvantage is high stress damage from aeration.

2.4.3 Tubular photobioreactors

Tubular photobioreactor is one of the suitable types of bioreactors for outdoor cultivation as it gives a large illumination surface area. Normally, tubular photobioreactor combines with pump or airlift system for circulation. The airlift system is preferable as it helps avoid cell damage from mechanical pumping, and also provides efficient oxygen removal (Fernández et al., 2001). The limitations of this reactor are poor mass transfer and difficult to control temperature including gradient of pH, dissolved oxygen and carbon dioxide along the tubes. The scale up of tubular photobioreactor requires large area and many problems can occur for example increasing diameter of tube, the illumination surface area to volume ratio would decrease (Ugwu et al., 2008).

2.4.4 Bubble column photobioreactors

Bubble column photobioreactor is a pneumatic type which provides good mixing by air bubbling, easy to operate, low shear stress and high mass transfer. However, the scale up of this reactor has some limitation for example the photobioreactor is scaled up by increasing the diameter of reactor, and this lowers the surface area for light illumination. In addition, increasing column height could mean a higher energy consumption for mixing.

2.4.5 Airlift photobioreactors

Airlift photobioreactor is one type of pneumatic reactors that is similar to bubble column reactors. The major difference is that they contain a draft tube which improves circulation and oxygen transfer in the reactor. Airlift systems consist of two sections; riser and downcomer, and the aeration only takes place in riser. Airlift photobioreactors can be classified into two major types as the internal loop, which the circulation between riser and downcomer arise in the same cylindrical tube, and external loop which has two separated columns operating as riser and downcomer with conduit arms connecting the two columns together near the top and the

bottom. Some problems may occur during the scale up particularly for the cylinder-type column as an increase in the diameter of the column might not have linear relationships with other reactor characteristics such as area-to-volume ratio. Issarapayup et al. (2009) found that novel flat panel airlift photobioreactor (FP-ALPBR) can overcome this problem as the scale up could be achieved without losing growth behavior just by enhancing the length of the tube.

2.5 Large scale outdoor cultivation

Outdoor large scale microalgae cultivation systems have been studied extensively as the use of sunlight renders this the most economical method for mass cultivation. The scale up of photobioreactors was often achieved by multiplying the number of single units including multi-step of volume expansion. Several evidences of similar work both uncontrolled and well controlled conditions and this will be summarized as shown below.

2.5.1 Effect of type of photobioreactors to cell growth

The main design parameters for the algal cultivation system are the design to allow effective light utilization and to give good mixing/mass transfer. Tubular photobioreactors are typically used for outdoor microalgae production as they provide better light utilization and good mixing(Carlozzi, 2003, Carlozzi & Sacchi, 2001, Fernández et al., 2001, Hu et al., 2008, López et al., 2006, Sato et al., 2006). Fernández et al. (2001) illustrated that the diameter of tube should be suitable in order to achieve high productivity, and the volumetric productivity decreased whereas the areal productivity increased with an increase in diameter. Airlift systems are often combined with tubular to increase effectiveness of cell production as the airlift section helps eliminate dead zone and dark zone, not mentioning its oxygen removal promotion potential (Carlozzi, 2003, Fernández et al., 2001). Examples include the work of López et al. (2006) who reported that the growth rate and cell productivity of *Haematococcus pluvialis* in tubular photobioreactor were better than

bubble column because the diameter of horizontal tubular photobioreactor was small which enhanced the irradiance on surface. In this work, as high as 37 folds of high value astaxanthin, could be produced in the tubular system when compared with the bubble column. Several other types of bioreactors have been proposed with a larger working volume. For instance, a very high productivity of *Chlorella sp.* (4.3 g DW $L^{-1}d^{-1}$) and cell density (40 gDW L^{-1}) was obtained from thin-layer photobioreactor 2,000 L (Doucha and Lívanský, 2009).

2.5.2 Effect of environmental condition to growth and biochemical products

Literature has widely reported the effects of various parameters on growth of microalgae such as temperature, light intensity and season. For instance, *Chaetoceros muelleri* gave a lower cell concentration in winter than in spring and summer seasons and suggested that spring provided a more suitable temperature and light conditions than winter (Becerra-Dórame et al., 2010, López-Elías et al., 2005, Voltolina et al., 2008). On the other hand, lipid seems to increase at low temperature and irradiance, i.e. the lipid composition is the highest to lowest in winter, spring and summer, respectively. Similarly, carbohydrate is higher in winter and lower in summer (López-Elías et al., 2005, Voltolina et al., 2008). Note that the occurrence of protein exhibits an inverse trend to that of lipid. *Rhodopseudomonas palustris*, is a bacteria cultured in the tubular photobioreactor where the temperature was controlled by submerge tubular in water (Carlozzi & Sacchi, 2001). The experiments showed that protein, carbohydrate, and poly-beta-hydroxybutyrate (PHB) obtained during January to July varied where carbohydrate and PHB slightly increased over time, whereas protein took the reverse trend.

The summary of the outdoor cultivation systems and their operating conditions along with the growth parameters is summarized in Table 2.7.

Table2.7 Literature reviews of outdoor cultivation

Author (Year)	Strains	Reactor	Conditions	Results
Fernández et al. (2001)	*Phaeodactylum tricornutum*	Airlift and tubular solar receiver	*Continuous system* Volume = 200 L Nutrient = Mann and Myers medium Temp. controlled in a water pond = 20±2 ^{o}C Avg. light intensity = 1289 μEm^{-2} s^{-1} pH controlled = 7.7 (adjust by CO_2) Liquid velocity = 0.50 ms^{-1} Superficial gas velocity = 0.3 ms^{-1} Dilution rate = 0.05 h^{-1}	Spring season Biomass concentration = 2.38 g L^{-1} Biomass productivity = 1.19 gL^{-1}d^{-1} Areal productivity of biomass = 19.8 g m^{-2}d^{-1}
Carlozzi and Sacchi (2001)	*Rhodopseudomanas palustris*	Tubular photobioreactor	*Batch system* Volume = 53 L Nutrient = modified van Niel medium Temp. controlled at 30±1 ^{o}C Avg. light intensity = 1243 μEm^{-2} s^{-1} Initial cell concentration = 1 g L^{-1} pH controlled = 7.o±0.05 (adjust by 1 N HCl)	Biomass productivity = 1.42 gL^{-1}d^{-1} Reactor productivity = 75 g reactor^{-1} d^{-1}
Mirón et al. (2002)	*Phaeodactylum tricornutum*	- Bubble column - airlift bioreactors (a split-cylinder and a draft-tube airlift device)	*Fed-bauch* Volume = 60 L Nutrient = modified Ukeles medium Temp. controlled at 22±1 ^{o}C Avg. light intensity = 1150 ± 52 μEm^{-2} s^{-1} pH controlled = 7.6 (adjust by CO_2)	August Biomass concentration = ~4 g L^{-1} Specific growth rate (avg.) = 0.021 h^{-1} Carbohydrate accumulation (up to ~13% w/w)

Table 2.7 (Cont.) Literature reviews of outdoor cultivation

Author (Year)	Strains	Reactor	Conditions	Results
Fernández et al. (2003)	*Phaeodactylum tricornutum*	Helical photobioreactor	*Batch system* Volume = 75 L Nutrient = Mann and Myers medium Temp. controlled at 28 $^{\circ}C$ Avg. light intensity = 1100 $\mu Em^{-2} s^{-1}$ pH controlled = 7.7 (adjust by CO_2) Air flow rate in the riser = 11 $Lmin^{-1}$ Cultivation time =7 d	Biomass concentration = 3.0 g L^{-1} Biomass productivity = 1.3 g$L^{-1} d^{-1}$ Specific growth rate = 0.05 h^{-1}
Carlozzi (2003)	*Arthrospira platensis*	Airlift and tubular photobioreactor	*Continuous system* Volume = 11 L Nutrient = Zarrouk's medium Temp. controlled in a water pond = 31±1.0 $^{\circ}C$ Avg. light intensity = 717 $\mu Em^{-2} s^{-1}$ pH controlled = 9.4 ± 0.1 (adjust by CO_2) Dilution rate = 0.013 h^{-1}	Biomass concentration (sunrise) = 3.1 g L^{-1} Biomass productivity = 1.59 g$L^{-1} d^{-1}$
Moreno et al. (2003)	*Anabaena* sp. ATCC33047	Raceway pond	*Semi-continuous system* Volume = 100 L Temp. controlled at 30 $^{\circ}C$ pH controlled = 8.5-9 (adjust by CO_2) Solar irradiance= 18.7 MJ $m^{-2} d^{-1}$ (spring), 22.1 MJ $m^{-2} d^{-1}$ (summer), 9.7 MJ $m^{-2} d^{-1}$ (autumn), 8.6 MJ $m^{-2} d^{-1}$ (winter)	Biomass concentration = 0.18 g L^{-1} (spring), 0.23 g L^{-1} (summer), 0.14 g L^{-1} (autumn), 0.11 g L^{-1} (winter) Productivity= 18.5 g $m^{-2} d^{-1}$(spring), 23.5 g $m^{-2} d^{-1}$ (summer), 10.6 g $m^{-2} d^{-1}$ (autumn), 9.4 g $m^{-2} d^{-1}$ (winter) Photosynthetic efficiency= 2.22% (spring), 2.38%(summer), 2.45% (autumn), 2.45% (winter)

Table 2.7 (Cont.) Literature reviews of outdoor cultivation

Author (Year)	Strains	Reactor	Conditions	Results
López-Elías et al. (2005)	*Chaetoceros muelleri*	Bubble column	*Batch system* Volume = 300 and 3000 L Nutrient = f/2 medium Temp. = 22.1-29.4 ^{o}C and 20.3-26.4 ^{o}C pH = 7.3-8.3 and 9.0-9.5	*Spring season* 300 L: Cell concentration = 2.94x10^{6} cell mL^{-1} 3000 L: Cell concentration = 1.75x10^{6} cell mL^{-1} Lipid content = 16.6-36.1%
López et al. (2006)	*Haematococcus pluvialis*	Airlift- tubular and bubble column photobioreactor	*Batch system* Fast characterization method Volume = 55 L Temp. controlled at 20 ^{o}C Avg. light intensity = 1600 µEm^{-2}s^{-1} (airlift-tubular) and 425 µEm^{-2}s^{-1} (bubble column) pH controlled = 8.0 (adjust by CO_2) Air flow rate= 55 Lmin^{-1} (1 vvm) Cultivation time =16 d	airlift- tubular: Biomass concentration of green cell= 7.0 g L^{-1} Biomass productivity = 0.41 gL^{-1}d^{-1} Cell density of green cell= 5.5 x 10^{6} cell mL^{-1} Specific growth rate = 0.040 h^{-1} Astaxanthin content = 1.1 %wt Astaxanthin productivity= 4.4 mg L^{-1}d^{-1} Bubble column: Biomass concentration of green cell = 1.4 g L^{-1} Cell density of green cell = 3.8 x 10^{6} cell mL^{-1} Biomass productivity = 0.06 gL^{-1}d^{-1} Specific growth rate = 0.021 h^{-1} Astaxanthin content = 0.25 %wt Astaxanthin productivity= 0.12 mg L^{-1}d^{-1}

Table 2.7 (Cont.) Literature reviews of outdoor cultivation

Author (Year)	Strains	Reactor	Conditions	Results
Sato et al. (2006)	*Chlorococum littorale*	Pipe photobioreactor	*Batch system* Volume = 70 L Temp. controlled at 25 ^{o}C pH controlled = 7.5 Air flow rate=31 Lmin^{-1} Cultivation time =12 d	Biomass concentration = 1.75 g L^{-1} Biomass productivity = 0.146 gL^{-1} d^{-1} Growth rate per land area = 20.5 gm^{-2}d^{-1}
Zittelli et al. (2006)	*Tetraselmis suecica*	Bubble column	*Batch system* Volume = 120 L Nutrient = f medium Temp. controlled at 27 ^{o}C pH controlled = 8.0±0.2 (adjust by CO_2) Avg. light intensity = 1129 µEm^{-2} s^{-1} Air flow rate= 27.6 Lmin^{-1} (0.23 vvm) Cultivation time = 16 d	Biomass productivity = 0.46 gL^{-1}d^{-1} Growth rate per land area = 36.3 gm^{-2}d^{-1}
Hu et al. (2008)	*Pavlova viridis*	Tubular photobioreactor	*Batch system* Autumn and early winter 2005 Volume = 60 L Avg.temp. = 14.6-30.2 ^{o}C pH = 7.2-9.0 Avg. light intensity = 48.2-327.8 µEm^{-2} s^{-1} Cultivation time = 10 d	Cell concentration = 9.95x10^{6} cell mL^{-1} Total lipid =10% dry wt.

Table 2.7 (Cont.) Literature reviews of outdoor cultivation

Author (Year)	Strains	Reactor	Conditions	Results
Voltolina et al. (2008)	*Chaetoceros muelleri*	Bubble column	*Batch system* Volume = 300 L Nutrient = f/2 medium Initial cell concentration = $0.2x10^6$ cells mL^{-1} pH controlled = 8.0±0.5 (adjust by CO_2) Cultivation time = 3 d	Winter: Cell concentration = $0.95x10^6$ cells mL^{-1} Biomass concentration =0.038 g L^{-1} Specific growth rate = 0.04 h^{-1} Lipid = 30.3%, Protein = 49.3% Carbohydrate = 18.0% Spring: Cell concentration = $4.15x10^6$ cells mL^{-1} Biomass concentration =0.195 g L^{-1} Specific growth rate = 0.007 h^{-1} Lipid = 28.2%, Protein = 56.3% Carbohydrate = 15.2% Summer: Cell concentration = $3.59x10^6$ cells mL^{-1} Biomass concentration =0.145 g L^{-1} Specific growth rate = 0.0025 h^{-1} Lipid = 22.1%, Protein = 64.4% Carbohydrate = 12.7%
Doucha and Lívanský (2009)	*Chlorella* sp.	Thin layer photobioreactor	*Fed-batch system* Volume = 2000 L pH controlled = 6.8-8.0 Avg. light intensity = 62.74 $\mu Em^{-2}s^{-1}$ Nutrient = f/2 medium Cultivation time = 9 d	Biomass concentration =40 g L^{-1} Max. specific growth rate = 0.09 h^{-1}

Table 2.7 (Cont.) Literature reviews of outdoor cultivation

Author (Year)	Strains	Reactor	Conditions	Results
Van Bergeijk et al. (2010)	*Isochrysis* aff. *Galbana* (T-iso)	Airlift and tubular photobioreactor	*Batch system* Volume = 400 L Initial cell concentration: 5 x10^{6} cell mL^{-1} Cultivation time = 14 d	Specific growth rate = 0.39 d^{-1} Productivity= 0.075 g L^{-1} d^{-1} Areal productivity= 7.91 g m^{-2} d^{-1} Photosynthetic efficiency=2.51%
Becerra-Dórame et al. (2010)	*Chaetoceros muelleri* and *Dunaliella* sp.	Tank with recirculation cascade	*Batch system* Volume = 200 L Initial cell concentration: 0.2 x10^{6} cell mL^{-1} (*C. muelleri*) 0.1 x10^{6} cell mL^{-1} (*Dunaliella* sp.) Nutrient = f medium Cultivation time = 4 d Winter season Light intensity= 383-1405 µmol m^{-2} s^{-1} (*C. muelleri*), 779-1532 µmol m^{-2} s^{-1} (*Dunaliella* sp.) Temp.= 9.8-22.3 °C pH= 7.9-8.8 (*C. muelleri*), 7.5-8.7 (*Dunaliella* sp.) Spring season Light intensity= 1213-1405 µmol m^{-2} s^{-1} (*C. muelleri*), 996-1405 µmol m^{-2} s^{-1} (*Dunaliella* sp.) Temp.= 18.8-34.8 °C pH= 8.2-10.0 (*C. muelleri*), 8.8-9.9 (*Dunaliella* sp.)	*C. muelleri* Cell concentration = 0.85 x 10^{6} cell mL^{-1} (Winter), 2.8 x 10^{6} cell mL^{-1} (Spring) Biomass concentration = 0.12 g L^{-1} (Winter), 0.35 g L^{-1} (Spring) Organic matter = 0.069 g L^{-1} (Winter), 0.085 g L^{-1} (Spring) Ash = 0.045 g L^{-1}(Winter), 0.26 g L^{-1} (Spring) *Dunaliella* sp. Cell concentration = 0.23 x 10^{6} cell mL^{-1}(Winter), 0.78 x 10^{6} cell mL^{-1} (Spring) Biomass concentration = 0.17 g L^{-1} (Winter), 0.27 g L^{-1} (Spring) Organic matter = 0.064 g L^{-1} ^{1} (Winter), 0.12 g L^{-1} (Spring) Ash = 0.096 g L^{-1} ^{1} (Winter), 0. g L^{-1} (Spring)

Table 2.7 (Cont.) Literature reviews of outdoor cultivation

Author	Strains	Reactor	Conditions	Results
Chinnasamy et al. (2010)	Mixotrophic algal strains (1:1): *Chlamydomanas globosa, Scenedesmus bijaga*	Raceway (RW), Vertical tank reactor (VTR) and polybags (PB)	*Batch system* Volume = 500 L (RW), 100 L (VTR) and 20 L (PB) Nutrient = untreated carpet industry effluent Avg.temp. = 23.4°C (RW), 27°C (VTR) and 32.°C (PB), Avg. light intensity = 883 $\mu Em^{-2}s^{-1}$ (RW), 1068 $\mu Em^{-2}s^{-1}$ (VTR) and 947 $\mu Em^{-2}s^{-1}$ (PB), Air flow rate= 10 $Lmin^{-1}$ (RW), 2 $Lmin^{-1}$ (VTR), 0.4-0.8 $Lmin^{-1}$ (PB), CO_2 flow rate= 500-600 $mLmin^{-1}$ (RW), 100-120 $mLmin^{-1}$ (VTR) and 20-48 $mLmin^{-1}$ (PB) Cultivation time = 8 d	Biomass productivity = 0.057 $gL^{-1}d^{-1}$ (RW), 0.044 $gL^{-1}d^{-1}$ (VTR) and 0.07 $gL^{-1}d^{-1}$ (PB) Lipid content = 5.3%
Oh et al. (2010)	*Chlorella minutissina*		*Perfusion system* Volume = 200 L , Nutrient = thermal plume from nuclear power plants, Initial cell concentration = 0.5 g L^{-1}, Avg.temp. = 30°C , Avg. light intensity = $2.19x10^{15}$ $\mu Em^{-2}s^{-1}$, CO_2 flow rate= 300 $mLmin^{-1}$ Cultivation time = 80 d	Biomass concentration= 3.4 g L^{-1} Specific growth rate = $2.7x10^{-4}$ h^{-1} Lipid content = 17% wt.
Ong et al. (2010)	*Chlorella* sp. MT-15	Bubble column	*Batch system* Volume = 40 L , Avg.temp. = 41±1°C Avg. light intensity = 81000 $\mu Em^{-2}s^{-1}$ (day time) and 300 $\mu Em^{-2}s^{-1}$ (night time) Air flow rate = 10 L min^{-1}, CO_2 flow rate= 500 $mLmin^{-1}$, Cultivation time = 8 d	Biomass concentration= 0.35 g L^{-1} Specific growth rate = 0.01 h^{-1}

2.6 Online monitoring system for microalgae cultivation

Growth of microalga under outdoor cultivation is affected significantly by environmental conditions. A well controlled system is therefore necessary to maintain the growth. Typical growth monitoring technique includes the cell number and dry weight, but this technique suffers disadvantages of tedious and time consuming which may not be suitable for large cultivation system. The online monitoring of growth should be developed to allow a more effective measure of the algal cells. An example of continuous biomass monitoring method includes the oxygen evolution technique, for example, Cogne et al. (2001) measured and correlated the pressure inside a closed photobioreactor and the oxygen evolution rate for the onlined measure of the growth of *Spirulina platensis*. Another method for the measurement of cell density is to use the optical density (OD) measured with turbidumetric technique. For instance, the biomass of *Pavlova lutheri* was monitored by continuously drawing the cells from the photobioreactor and analyzed for its optical density/turbidity which was then converted to the cell number or cell mass (Meireles et al., 2002). The same author later proposed the use of optical density in automatic monitoring system for *Pavlova lutheri* where the signal was employed in the feedback control for the lighting of the system (Meireles et al., 2008). Similar technique was employed by Sandnes et al. (2006) who investigated the effectiveness of the near infrared (NIR) optical density sensor for online and controlled cell density under large scale cultivation of *Nannochloropsis oceanic* (200 L tubular photobioreactor). The summary of advantages and disadvantages of both methods are shown in Table 2.8.

2.7 Cost of microalgae cultivation

Cost of cultivation is a very important factor determining the potential of the cultivation system in large scale production. Grima et al. (2003) illustrated the economics of recovery of eicosapentaeoic acid (EPA), which is an omega-3 fatty acid, from *Porphyridium cruentum.* The biomass culture was an outdoor tubular

photobioreactor. The annual costs of biomass production were mostly distributed to the fixed capital investment at about 39%, raw material and labor at about 13% and 17%, respectively. Two downstream processes are the extraction-esterification of crude microalgal oil and EPA purification had raw material as major cost about 85% and 59%. The final cost of EPA recovery from processes i.e. biomass production, the extraction-esterification and purification shared 43%, 53% and 4%, respectively. It can be seen that the biomass productivity stage always contributed significantly to the overall cost and it was suggested that one option to reduce cost was to enhance biomass productivity with a better design of photobioreactor. The determination of cultivation system types, either open or close could dictate the production cost. Chisti (2007) evaluated the annual production cost of 100 tons of algal biomass from the raceway pond and photobioreactor systems where it was shown that one kilogram of biomass cost 2.95 $ kg^{-1} in the photobioreactor and 3.80 $ kg^{-1} in the raceway pond. This was because volumetric productivity of photobioreactor could be as high as 13 folds when compared with the race way system. The size of the culture system can also affect the production cost. Issarapayup et al. (2009) demonstrated that the cultivation of *Haematococcus pluvialis* in the 90 L flat panel airlift photobioreactor under indoor condition (for 18 g of alga) could save 21$ when compared with the similar 17 L system. There are options for minimizing production cost, such as using outdoor culture, reused medium, wastewater medium and choosing suitable strain and so on.

Electricity cost could be significant if the culture system is indoor. López-Elías et al. (2005) cultured *Chaetoceros muelleri* under outdoor condition using sunlight as light source and reported that the outdoor cultivation for the production of biomass 1 kg of DW cost 2.5 folds less than the indoor cultivation. Using reused medium could reduce nutrients cost but often is reported to lower the growth. For instance, the indoor cultivation of *Scenedesmus* sp. grown with the reused BG 11 medium after one cycle saw an 8% reduction in the growth yield compared with the growth using the fresh medium. However, adding some fresh medium could help maintain high the growth where 20% or 50% supplementation of fresh medium could lead to

an increase in the growth yield of about 10-11%. This could be due to the remaining of some effective substances like glucose and ferrous ions (Kim et al., 2011). In addition, using wastewater as a nutrient source under outdoor condition could be more economical, particularly for mixotrophic algal strains. *Chlamydomonas globosa, Chlorella minutissima* and *Scenedesmus bijuga* have been reported to be able to grow in untreated carpet industry (Chinnasamy et al., 2010), and *Chlorella minutissima* grown in spent sea water from nuclear power plant (Oh et al., 2010). If the location of outdoor cultivation is on subtropical zone, the selection thermal tolerant cell could save cost of cooling system. (Ong et al., 2010) found that mutant of *Chlorella* sp. MT-15 grown under high temperature over 40°C better than *Chlorella* sp. (wide type).

Table2.8 Advantages and disadvantages of monitoring methods (Meireles et al., 2002)

Method	Advantages	Disadvantages
Oxygen evolution (Dissolved oxygen)	- Measuring directly from the photosynthesis reaction	- Probes are vulnerable in long term operation. - Relationship between DO and biomass concentration can be complicating. - Sterilization of biosensor is difficult.
Optical density	- non-invasive biomass production - easy to set up	- OD does not correlate linearly with biomass in the whole growth range. - fouling by dead cell could pose a problem.

Chapter III
Materials and methods

3.1 Flow chart of work

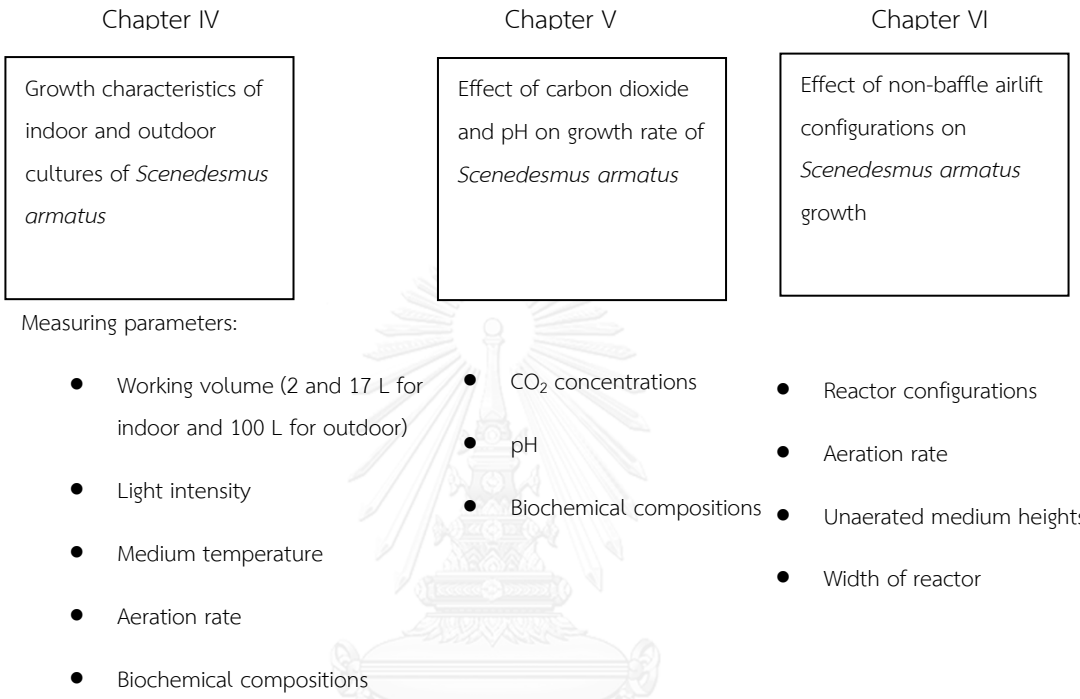

Figure3.1 Connection between the various stages of experiments carried out in this work

Figure 3.1 delineates the connections between the various stages of experiments designed for this work and the various designs and operating conditions were to be optimized. Principally, the work was divided into 3 parts. The first part was to cultivate the alga in indoor and scale up to the 100L outdoor flat panel airlift photobioreactors (FPAPs). The second part was to study effect of CO_2 and pH on growth of *S. armatus* with 100 L of FPAPs where CO_2 concentrations and pH level were examined. The third part was to propose a novel large scale airlift system without the separator with two models as non-baffled cone airlift photobioreactors (NB-CAPs) and non-baffled flat panel airlift photobioreactors (NB-FPAPs).

3.2 Culture medium preparation (Chapter IV, V, and VI)

Green microalga, *Scenedesmus armatus* TISTR 8591, obtained from Thailand Institute of Scientific and Technological Research (TISTR), was cultivated with BG11 medium (see compositions in Table 3.1). The incubation was carried out in 2 L clear glass bottles at room temperature, surface light intensity of about 10 kLux (light was supplied with four compact 20W fluorescent lamps), for 4 days. This culture was used an inoculum for indoor cultivation.

The 2 L inoculum was then up-scaled in a 30 L indoor flat panel airlift photobioreactor made from clear acrylic plastic with the dimension of 35x40x40 (WxLxH) cm^3. The illumination was given by eight 20W compact fluorescent lamps with the surface light intensity of about 10 kLux. The aeration was supplied using an air compressor (LP 100, Resun®, China) with the aeration rate fixed at 0.2 vvm (6 L min^{-1}). Finally, the inoculum was transferred to large scale airlift photobioreactors.

3.3 Experimental setup

3.3.1 Design for indoor cultivation (Chapter IV)

3.3.1.1 Light intensity and temperature control chamber

The experiments were carried out using 6 bottles of 2 L glass bubble column where were placed in a temperature and light intensity controlled chamber. The control chamber includes twelve compact fluorescence light bulbs (20 Watts) as a light source and a temperature control devices. The temperature was controlled using an evaporative cooling system and heater system (Figure 3.2). The surface light intensity was adjusted by moving light bulb on acrylic sheet into or out from the surface of the photobioreactor. Air was supplied through calibration rotameters to a porous sparger in the range of velocity from 0.5-1.5 cm s^{-1} at the bottom of the bubble columns. Light intensity and temperature were in the range from 28-40°C and 10-30 klux.

Table3.1 Composition of BG11 medium (Stanier et al., 1971)

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 (1)- (9) 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
	$Na_2MoO_4.2H_2O$	0.39
	$CuSO_4.5H_2O$	0.08
	$Co(NO_3)_2.6H_2O$	0.05
	Add 1 mL of stock (9) solution per liter of fresh water	

3.3.1.2 Indoor cultivation

A 17 L flat panel airlift photobioreactor (FPAP) was made from clear acrylic plastic with the dimension of 20x20x50 (WxLxH) cm^3 as shown in Figure 3.3. The separator plate, located 10 cm from the column bottom, was used to separate the reactor to riser and downcomer. The ratio of downcomer cross sectional area (A_d) and riser cross sectional area (A_r) was set at 1.5. The illumination was given by four 20W compact fluorescent lamps with the surface light intensity of about 10,000 Lux. The aeration was supplied using an air compressor (LP 100, Resun®, China) through three porous spargers with velocity of 1 cm s^{-1}.

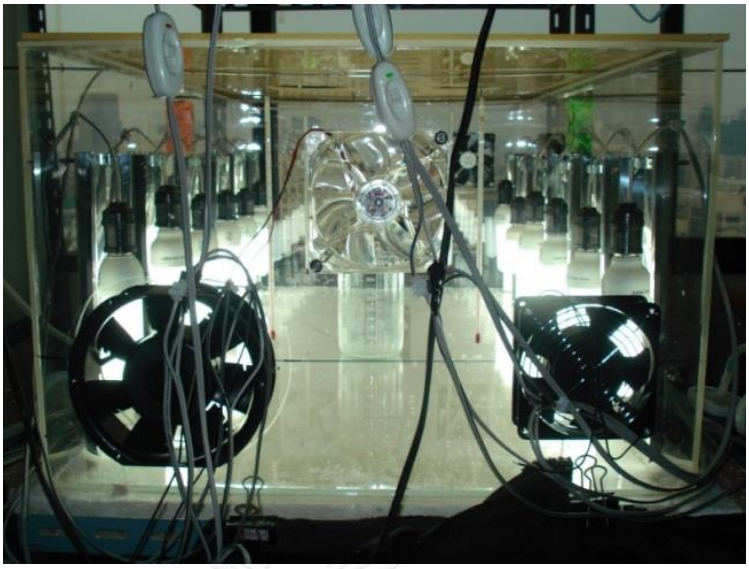

Figure3.2 Experimental set up in temperature and light intensity controlled chamber

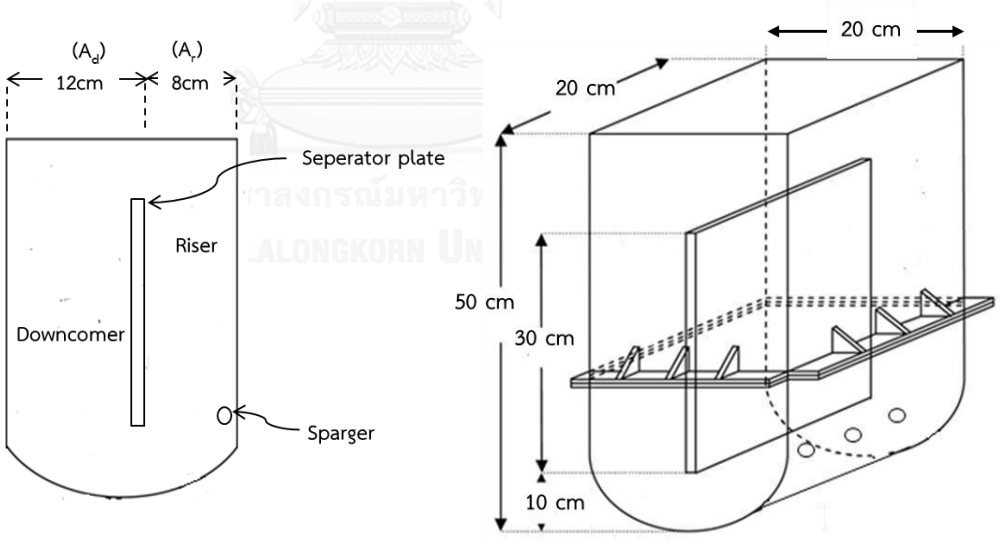

Figure3.3 Schematic of 17 L flat panel airlift photobioreactor (FPAP)

3.3.2 Photobioreactor design for outdoor cultivation (Chapter IV, V, and VI)

Three configurations of airlift photobioreactor were chosen for microalgal cultivation, i.e. flat panel airlift photobioreactors (FPAPs), non-baffled flat panel airlift photobioreactors (NB-FPAPs), and non-baffled cone airlift photobioreactors (NB-CAPs). The components of FPAP were made from clear acrylic with the dimension as shown in Figure 3.4, i.e. wall thickness of 8 mm, 100 cm height, 120 cm length, and 20 cm width. The separator plate, located 8 cm from the column bottom, was used to separate the reactor to riser and downcomer. The ratio of area of downcomer (A_d) and riser (A_r) was set at 1.5. NB-FPAPs were made from fiber glass with the following dimension: 80 cm height, 100 cm length, and different widths, i.e. 20, 30, 40 and 50 cm, where the bottom is sloped 30° with horizontal plane (Figure 3.5). The NB-CAPs were made from clear acrylic sheet. The configurations of NB-CAPs were shown in Figure 3.6, having the diameter of 100 cm, the height of 80 cm, and the wall thickness of 8 mm where the slope of the bottom sheet varies from 30, 45 and 53°. Air flow was supplied through rotameter (Dwyer series RMA RATE-MASTER® Flowmeter) to a porous gas sparger. The light intensity and temperature were measured and recorded (HOBO® Pendant Temperature/Light Data Logger, United States).

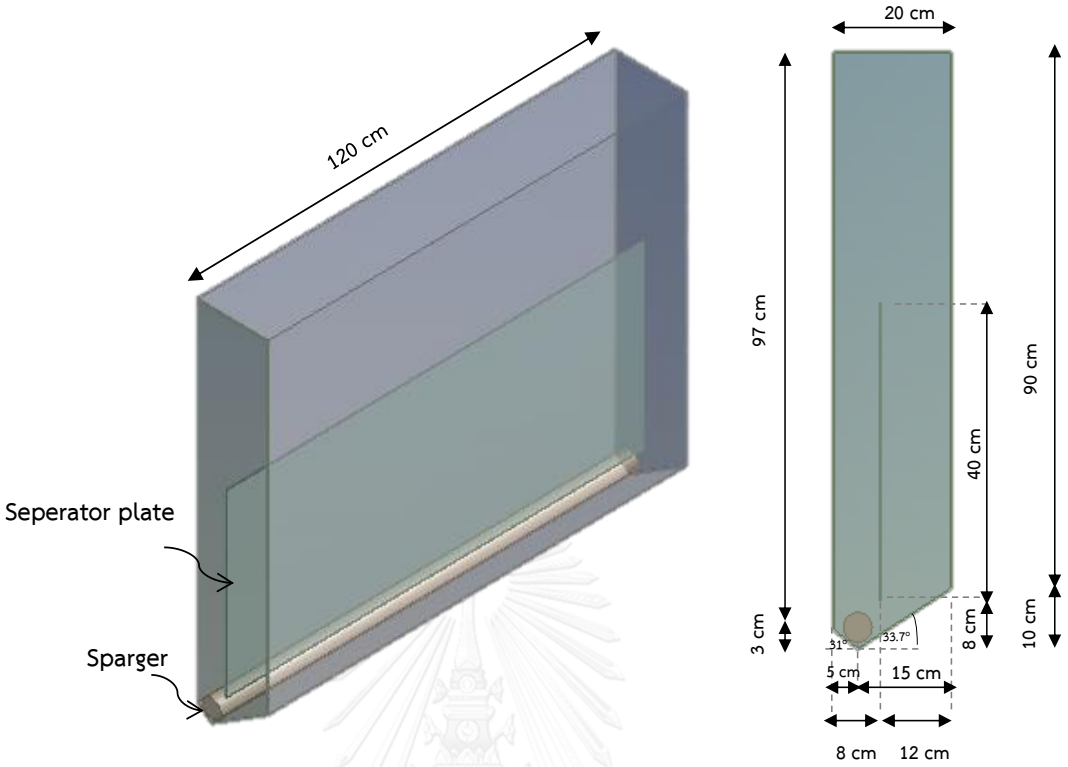

Figure3.4 Schematic of flat panel airlift photobioreactor (FPAP)

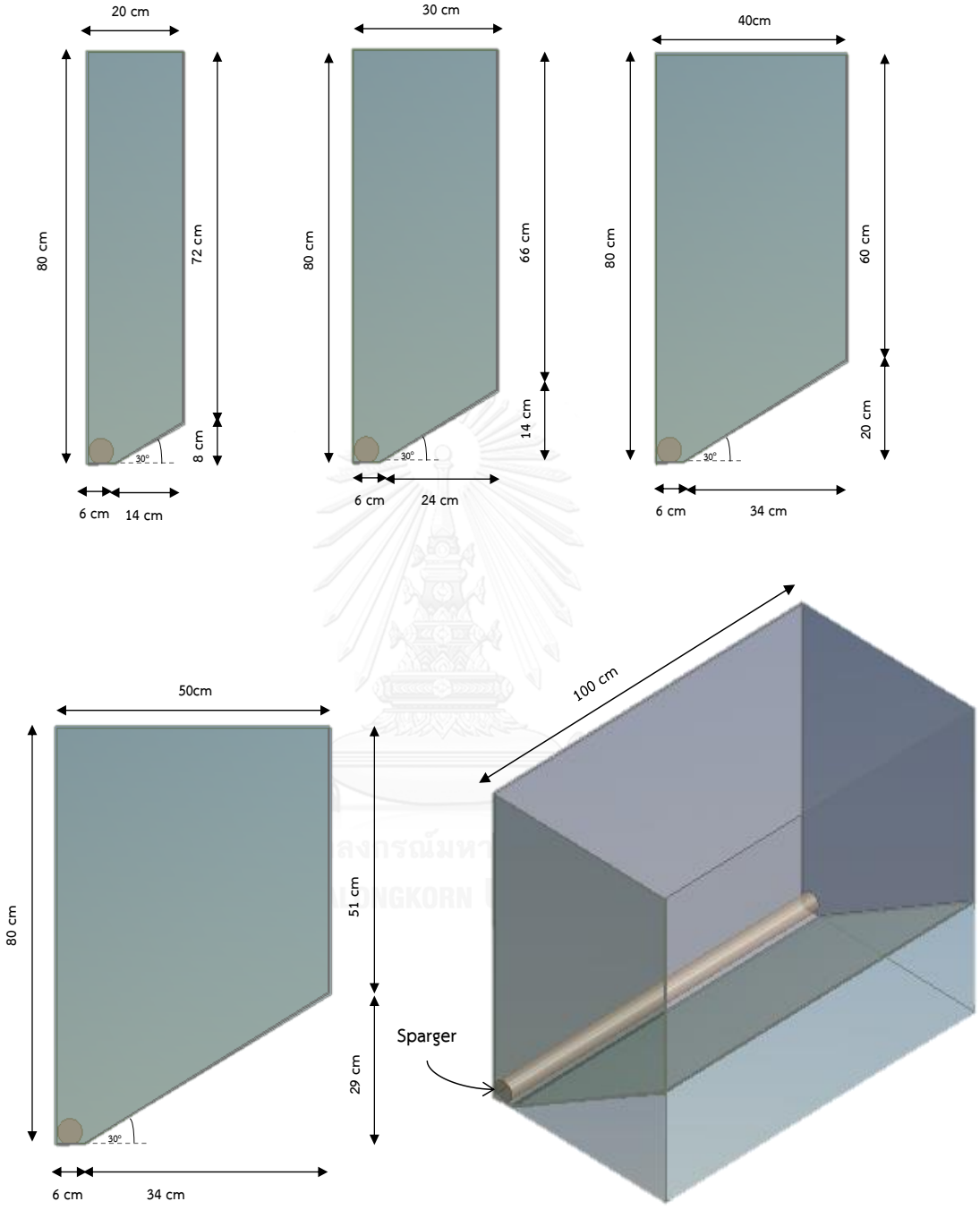

Figure3.5 Schematic of non-baffled flat panel airlift photobioreactors (NB-FPAPs)

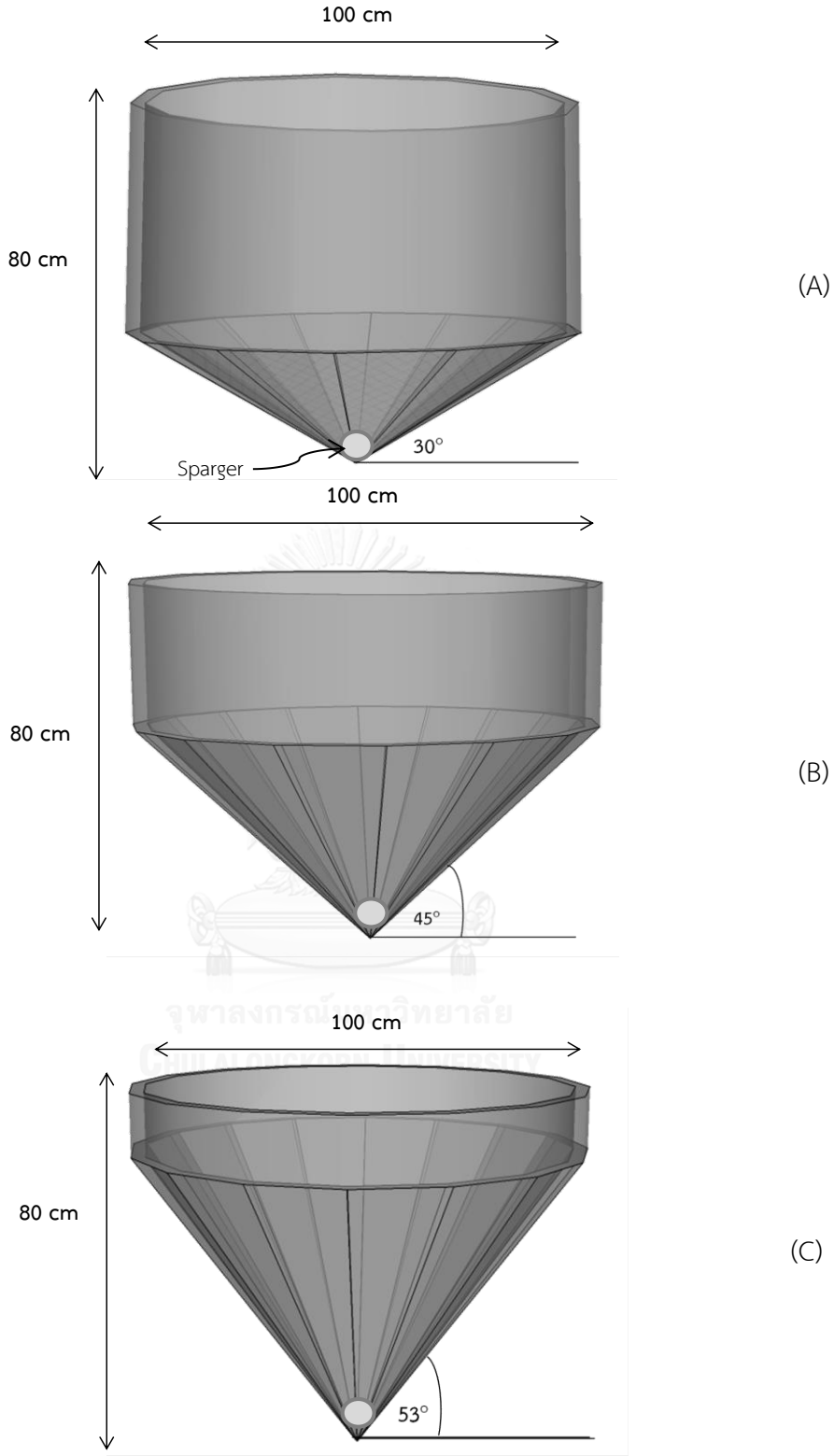

Figure3.6 Schematic of non-baffled cone airlift photobioreactors (NB-CAPs): (A) 30°, (B) 45°, and (C) 53° with x-plane

3.4 Microalgae cultivation

3.4.1 Effect of temperature (Chapter IV)

1. Fill 2 L freshwater into all glass bubble columns and sterilize with steam (Autoclave) at 121ºC for 15 min
2. Add the nutrients and inoculum at initial cells concentration $10x10^{4}$ cells mL^{-1}
3. Control air temperature constant at 30±0.5 ºC with a temperature controller
4. Adjust light intensity to approximately 10 kLux
5. Supply sterilized compressed air (treat with the air filter size 0.2 µm) through a porous gas sparger and control superficial gas velocity at 1 cm s^{-1}
6. Take samples once a day (add fresh water into the photobioreactor by the water level was beginning before taking samples) and count for the cell density using haemacytometer
7. Harvest cells from all bubble columns when the culture reaches its stationary phase
8. Separate cells from water by centrifuge at a speed of 4,000 rpm for 15 min
9. Remove residual medium in the algal slurries by freeze and dryer machine (0.024 bar and -40°C) for a period of 2-3 days
10. Analyze for lipid, protein, carbohydrates, moisture and ash in the dry biomass
11. Calculate the specific growth rate using Equation 3.8 and the specific productivity using Equation 3.9
12. Repeat Steps 1 to 11, but change the temperature in Step 3 to 35 ºC and 40ºC, respectively

3.4.2 Effect of light intensity (Chapter IV)

1. Repeat Experiments in Section 3.4.1 with the optimal temperature
2. Change the light intensity from 10 to 15-30 klux

3.4.3 Effect of superficial gas velocity (Chapter IV)

1. Repeat Experiments in Section 3.4.1 with the optimal temperature and light intensity
2. Change the superficial velocity from 1 to 0.5 and 1.5 cm s^{-1}

3.4.4 Batch indoor culture (Chapter IV)

1. Fill 17 L freshwater into FPAPs
2. Sterilize freshwater with sodium dichloroisocyanurate (1.7 g/tablet)
3. Supply air through the porous sparger at the bottom of reactor for 24 h at u_{sg} 1 cm s^{-1}
4. Check for residual chlorine with potassium iodide, and if chlorine is exhausted, the water sample is clear, otherwise a yellow solution is formed.
5. Add the nutrients and inoculum at initial cells concentration $40x10^{4}$ cells mL^{-1}
6. Take sample 10 mL of culture and filter through GF/C filter (47 mm of diameter, Whatman), wash with distilled water, and dry to the final weight at 80°C for two days at a once a day frequency until the stationary growth was reached.
7. Harvest cells when the culture reaches its stationary phase
8. Separate cells from residual medium by centrifugal machine at a speed of 4,000 rpm for 15 min
9. Dewater from algal cake by freeze dryer machine (0.024 bar and -40°C) for 2-3 days
10. Analyze for lipid, protein, carbohydrates, moisture and ash in the dry biomass
11. Calculate the specific growth rate using Equation 3.8 and the specific productivity using Equation 3.9

3.4.5 Outdoor cultivation (Chapter IV, V, VI)

3.4.5.1 Batch culture (Chapter IV, V, and VI)

1. Fill 100 L freshwater into FPAPs
2. Sterilize freshwater with sodium dichloroisocyanurate (1.7 g/tablet)
3. Supply air through the porous sparger at the bottom of reactor for 24 h
4. Check for residual chlorine with potassium iodide, and if chlorine is exhausted, the water sample is clear, otherwise a yellow solution is formed.
5. Add the nutrients and inoculum at initial concentration $40x10^{4}$ cells mL^{-1}
6. Supply air at aeration rate in the range of 0.1-0.3 vvm (0.18-0.52 cm s^{-1}) and record light intensity and temperature
7. Take sample 40 mL of culture and filter through GF/C filter (47 mm of diameter, Whatman), wash with distilled water, and dry to the final weight at 80°C for two days at a once a day frequency until the stationary growth was reached.
8. Harvest cells when the culture reaches its stationary phase
9. Separate cells from residual medium by continuous centrifugation (7,392xg) (Alfa Laval DX203B-34, Spain)
10. Remove residual water from algal cake by freeze dryer machine (0.024 bar and -40°C) for 2-3 days
11. Analyze for lipid, protein, carbohydrates, moisture and ash in the dry biomass
12. Calculate the specific growth rate using Equation 3.8, the specific productivity using Equation 3.9 and nitrogen uptake using Equation 3.12
13. Repeat Steps 1–12 with NB-CAPs configurations with air flow rate 0.1-0.3 vvm

3.4.5.2 Cultivation with air and carbon dioxide mixture (Chapter V)

1. Repeat steps 1-5 in Section 3.4.5.1
2. Sparge a mixture of air flow rate of 0.2 vvm and CO_2 in the concentrations of 2, 5 and 15% by volume of aeration rate (20 L min-1) for the dark:light period of 12:12 (Light:dark)
3. Repeat steps 7-11 in Section 3.4.5.1
4. Calculate the specific growth rate using Equation 3.8, the specific productivity using Equation 3.9, carbon dioxide fixation using Equation 3.10, carbon dioxide utilization efficiency using Equation 3.11 and nitrogen uptake using Equation 3.12

3.4.5.3 Cultivation with controlled pH (Chapter V)

1. Repeat steps 1-5 in Section 3.4.5.1
2. Supply air at aeration rate of 0.2 vvm
3. Control pH in medium at the set point (pH_{sp}) of 7±0.5 and 7.7±0.5 with pH controller
4. Supply pure CO_2 at the flow rate of 400 mL min^{-1} (2%CO_2 by vol.) mixed with air when pH was higher than pH_{sp}.
5. Repeat steps 7-11 in Section 3.4.5.1
6. Repeat steps 4 in Section 3.4.5.2

3.4.5.4 Effect of width and height of reactor (Chapter VI)

1. Fill freshwater into the NB-FPAP with width of reactor 20, 30, 40 and 50 cm at the medium height range of 40, 50 and 60 cm
2. Repeat steps 2-5 in Section 3.4.5.1
3. Supply air at aeration rate of 0.2 vvm

4. Repeat steps 7-12 in Section 3.4.5.1
5. Repeat steps 1-4 with NB-CAPs at the medium height 75 cm

3.5 Online growth monitoring of *S. armatus*

Biomass concentration was measured by an optical density technique. The culture was continuously drawn out of the photobioreactor using centrifugal pump (3-MD-SC, Little Gaint Pump Company, United States) and flown through the sensor before recirculated back to the bioreactor (Figure 3.7). The light sensor consisted of the near-infrared LED and photo-transmitter. The output voltage signals were recorded at every 10 min interval with data logger and this voltage was then inversely proportional to the pre-calibrated biomass concentration.

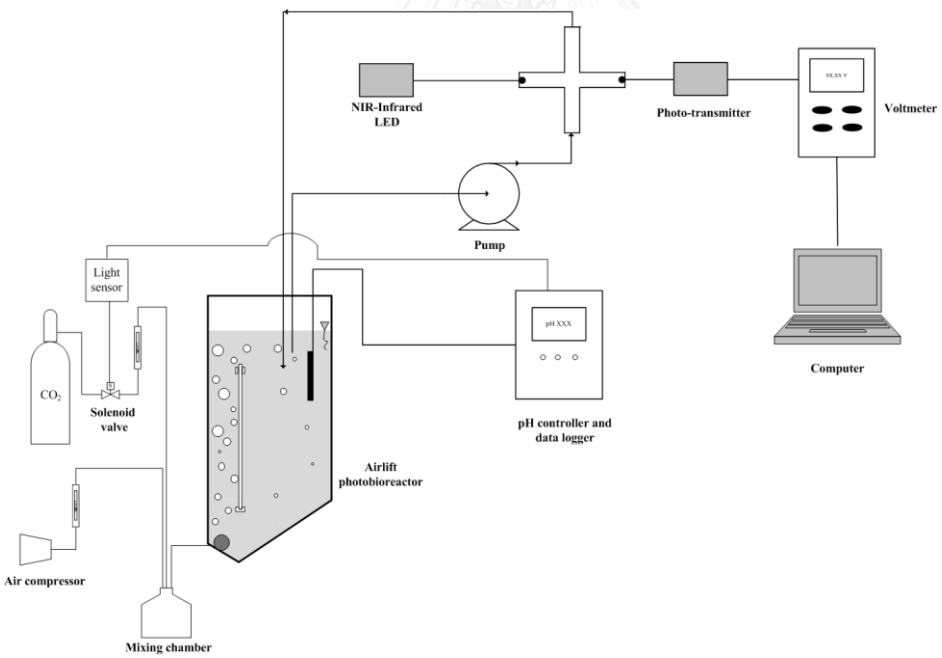

Figure3.7 Experiment setup with non-invasive turbidity cell measurement device

3.6 Determination of virtual riser and downcomer cross-section area in NB-CAP and NB-FPAP

Sintharm (2013) proposed a method to determine the 'virtual' cross sectional area of riser and downcomer of NB-FPAP and NB-CAP by observing directions of light rope. The experimental setup is displayed in Figure 3.8. Air was supplied through rotameter to a porous gas sparger at a desired flow rate and a light rope bar was put into the water at varying positions. If the rope flow up show that, this point is defined in riser zone if the rope flow down, this point is downcomer.

The estimate of the riser and downcomer cross section area depended on the size of riser obtained from experiment. The shape of riser was assumed to be ring cylinder for the circle column NB-CAPs and rectangular cylinder for NB-FPAPs.

For circular column with NB-CAPs, the riser cross section area (A_r) and downcomer cross sectional area (A_d) are

$$A_r = \pi R_r^2 \tag{3.1}$$

$$A_d = \text{Area of column} - \text{Area of riser} \tag{3.2}$$

where R_r was obtained from the experiment.

For NB-FPAPs, the riser cross section area (A_r) and downcomer cross sectional area (A_d) are

$$A_r = W_r x\ L \tag{3.3}$$

$$A_d = W_d x\ L \tag{3.4}$$

where the width of riser (W_r) was known from the experiment and the width of downcomer (W_d) is estimated from:

$$W_d = \text{width of NB-FPAP} - W_r - W_{rd} - W_{dr} \tag{3.5}$$

where

A = cross section area (cm^2)

R = radius (cm)

W = width of section (cm)

L = length of contactor (cm)

and the subscript

r = riser

d = downcomer

rd = rd cross flow (→)

dr = dr cross flow (←)

3.7 Determination of downcomer liquid velocity in NB-CAP and NB-FPAP

The liquid velocity in downcomer was estimated by the color tracer injection method where the time in which the tracer used to move at fixed vertical distances was used for the calculation:

$$v_d = \frac{L_d}{t_d} \tag{3.6}$$

where

v = downcomer liquid velocity ($cm\ s^{-1}$)

L = fixed vertical distances in downcomer (cm)

t = time for the tracer used for moving in downcomer (s)

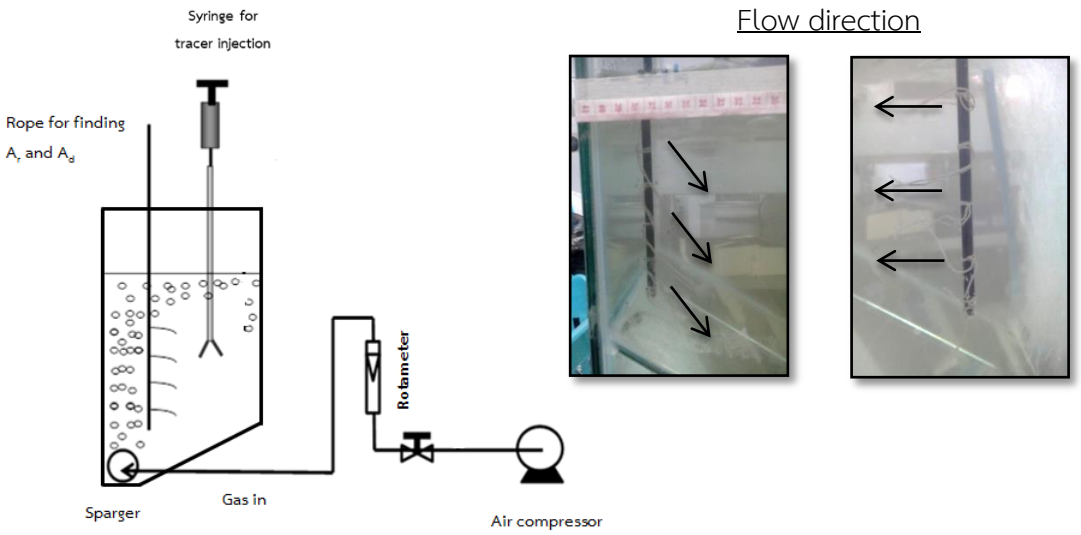

Figure3.8 Experimental setup of finding A_d, A_r and downcomer liquid velocity

3.8 Analyses and Calculations

3.8.1 Determination of cell concentration

The cell concentration can be determined using a normal blood cell counting slide, Haemacytometer. The depth of the counting grid and the medium area are 0.1 mm and 0.04 mm^2, respectively. The cell concentration can be determined as follows:

1. Clean the counting slide and cover glass
2. Fill the slide with sample
3. Cover the slide with cover glass, avoid of bubbles
4. Count the cell in 25 medium squares on the grid (per 1 large square)
5. Calculate the cells number, using Equation 3.7:

$$N = \frac{1}{4} x \frac{n}{25} x 10^6 \qquad (3.7)$$

where

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

n = cell number was calculated from haemacytometer

3.8.2 Determination of specific growth rate

The specific growth rate for batch cultivation is obtained from the slope of plot between the natural logarithm of biomass concentration during the exponential phase and cultivation time as follows:

$$\mu = \frac{\ln(X_2) - \ln(X_1)}{t_2 - t_1} \tag{3.8}$$

where

μ = specific growth rate (d^{-1})

X_1 = biomass concentration at t_1 (g L^{-1})

X_2 = biomass concentration at t_2 (g L^{-1})

t_1 = first sampling time (d)

t_2 = second sampling time (d)

3.8.3 Determination of specific biomass productivity

The specific biomass productivity is calculated from the overall growth period, which represents the average growth of the culture as follows:

$$SP = \frac{X_f - X_i}{t_f - t_i} \tag{3.9}$$

where

SP = specific biomass productivity (g $L^{-1} d^{-1}$)

X_i = initial biomass concentration (g L^{-1})

X_f = final biomass concentration (g L^{-1})

t_i = initial time cultivation (d)

t_f = final time cultivation (d)

3.8.4 Determination of carbon dioxide fixation

Carbon dioxide fixation is calculated from the relationship between the carbon content of the cells and the specific biomass productivity as follows:

$$R_{CO_2} = C_C * SP * \frac{MW_{CO_2}}{MW_C} \quad (3.10)$$

and, carbon dioxide utilization efficiency can be calculated from

$$\%CO_2 \ untilization \ efficiency = \frac{R_{CO_2}}{CO_2 \ loading} * 100 \quad (3.11)$$

where

R_{CO2} = carbon dioxide fixation (g L^{-1} day^{-1})

Cc = percent of carbon in biomass (%)

MW_{CO2} = molecular weight of CO_2

MW_C = molecular weight of Carbon

3.8.5 Determination of nitrate concentration

The remaining nitrate concentration (mg NO_3-N L^{-1}) in the medium after the removal of algal cells was measured daily with Ultraviolet spectrophometric screening method (Greenberg et al., 1992) using spectrophotometer (Agilent Cary 60 UV-Vis Spectrophotometer, Australia) at wavelength of 220 and 275 nm. The methods are shown in Appendix.

Specific nitrogen uptake was calculated from:

$$N_{uptake} = \frac{N_i - N_f}{X_f - X_i} \quad (3.12)$$

where

N_{uptake} = the nitrogen uptake (mg N g cell^{-1})

N_i = the initial nitrate concentration in medium (mg NO_3-N L^{-1})

N_f = the final nitrate concentration in medium (mg NO_3-N L^{-1})

3.8.6 Biochemical compositions analysis

The carbon content including nitrogen and hydrogen of *Scenedesmus armatus* was measured using CHNS/O Analyzer (Perkin Elmer PE2400 Series II). Lipid was extracted by chloroform and methanol (2:1 by volume) with soxhlet apparatus. Fatty acids contents were carried out by AOAC 996.06 method (AOAC, 2010) with GC analysis (6890 Algilent). Protein was determined by multiply nitrogen content (from the ultimate test) with the conversion factor of 4.44 (López et al., 2010). The moisture content in biomass was determined by heating at temperature at 105 ºC for 2 h and then ash content was measured at 750^{o}C for 6 h (ASTM D 1762-84). Carbohydrate was calculated from the remaining weight after the subtraction of lipid, protein, ash and moisture.

3.8.7 Determination of sugar content

Biomass (0.5 g) was hydrolyzed with 0.75% H_2SO_4 (25 mL). Suspension was heated at 180 ^{o}C for 180 min (Phuklang, 2013). Sugar content was measured by HPLC analysis (Shimadzu, LC-20AD, Japan) and compared with standard solutions. HPLC was operated with a Bio-Rad Aminex HPX-87H column (7.8 mm I.D. x 300 mm Length) and a refractive index detector (Shimadzu, RID-10A, Japan) at temperature of 45^{o}C and 0.6 mL min^{-1} flow of 5 mM sulfuric acid.

3.8.8 Light unit conversion (Thimijan and Heins, 1983)

- Cool-white fluorescent

[µmol m^{-2} s^{-1}] = [Lux] / 74

[W m^{-2}] = [µmol m^{-2} s^{-1}] /4.59

- Sunlight

[µmol m^{-2} s^{-1}] = [Lux] / 54

[W m^{-2}] = [µmol m^{-2} s^{-1}] /4.57

3.8.9 Determination of the average daily medium temperature and surface energy

The average daily medium temperature was calculated from the average of the sum of the maximum and minimum temperatures as follows:

$$T_m = \frac{T_{max} + T_{min}}{2} \quad (3.13)$$

and the daily average surface energy of one square metre (MJ $m^{-2}d^{-1}$) was obtained from the integration over time of solar irradiance as follows:

$$I_{w,m} = \int_0^{24} I_w dt \quad (3.14)$$

where T_m is the average medium temperature (^{O}C), T_{max} the maximum medium temperature (^{O}C), T_{min} the minimum medium temperature (^{O}C), $I_{w,m}$ the daily average surface energy (MJ $m^{-2}d^{-1}$) and I_w the solar irradiance (w m^{-2}).

3.8.10 Unit of aeration

The unit of aeration in this work was reported in both aeration rate (vvm) and superficial gas velocity, u_{sg} (cm s^{-1}) as follows:

- vvm (gas volume per liquid volume per minute)

[vvm] = Air flow rate (L min^{-1})/ Volume of liquid (L)

- u_{sg}

[cm s^{-1}] = Air flow rate (L min^{-1}) *1000/area of riser (cm^2)/60

3.9 Statistical analysis

All results were reported as mean values ± SD of triplicate tests. Each of effect was compared using one-way ANOVA at level of $p<0.05$ followed by Tukey's test.

Chapter IV
Growth characteristics of indoor and outdoor cultures of *Scenedesmus armatus*

Scenedesmus armatus biomass contains high protein and carbohydrate contents that are suitable for aquatic fishery and bio-ethanol fuel industries. Feasibility of the system scale up needs to be evaluated based on indoor and outdoor growth characteristics. This includes the information on growth rate, biochemical compositions, and seasonal variations on outdoor cultures, all of which are discussed in this chapter.

4.1 Effect of light intensity, temperature and aeration rate on biomass production

S. armatus was cultivated under controlled light intensity and temperature chamber with 2L bubble column made of clear glass bottles, for 7 days (Figurer 4.1). The temperature, light intensity and superficial gas velocity were examined in the range of 28-40°C, 10-40 kLux and 0.5-1.5 cm s^{-1}, respectively with initial inoculum cell concentration of $10x10^{4}$ cells mL^{-1} (~0.03 g L^{-1}). Final cell concentration, specific biomass productivity and specific growth rate obtained from all culture conditions are presented in Table 4.1.

The average final cell concentration, specific biomass productivity and specific growth rate increased significantly with temperature from 28 to 30-35°C. Cell died immediately from the thermal effect at temperature above 40°C. Optimal cultivation temperature for *Scenedesmus* species such as *Scenedesmus obtusiusculus* was reported at 35°C (Cabello et al., 2015).

The average final biomass concentration and specific biomass productivity decreased clearly when light intensity increased from 10 to 40 kLux whereas specific growth rates did not significantly differ from each other. Increasing of light intensity above 10 kLux could be photoinhibiting. Gris et al. (2014) suggested that the

maximum growth rate of *Scenedesmus obliquus* occurred at 150 μmole m^{-2} s^{-1} (11,100 Lux) whereas cell concentration and specific growth rate decreased with increasing light intensity of 200-1,000 μmole m^{-2} s^{-1} due to reach saturation point of photosynthesis.

Biomass production increased when superficial gas velocity (u_{sg}) increased from 0.5 to 1.0 cm s^{-1} and dropped significantly with increasing u_{sg} to 1.5 cm s^{-1}. This might be due to bubble shading where a large swam of bubbles could obstruct light penetration and prevent effective light utilization of the cell culture.

Figure4.1 *S. armatus* cultivation in temperature and light intensity controlled chamber

Table4.1 Final biomass concentration, specific biomass productivity and specific growth rate of *S. armatus* indoor cultivation for 7 days

Parameter	u_{sg} (cm s^{-1})	Light intensity (kLux)	Temperature (°C)	Final biomass concentration (g L^{-1})	specific biomass productivity (mg $L^{-1}d^{-1}$)	Specific growth rate (d^{-1})
Temperature						
	1	10	28	0.42±0.05[b]	57.9±8.1[b]	0.32±0.02[b]
	1	10	30	0.70±0.08[a]	97.7±10.7[a]	0.45±0.05[b]
	1	10	35	0.75±0.07[a]	106±6.6[a]	0.63±0.07[a]
	1	10	40	dead	-	-
Light intensity						
	1	10	35	0.75±0.07[a]	106±6.6[a]	0.63±0.07[a]
	1	20	35	0.48±0.05[b]	66.9±4.8[b]	0.69±0.05[a]
	1	30	35	0.49±0.05[b]	68.5±1.0[b]	0.61±0.07[a]
	1	40	35	0.46±0.04[b]	64.4±3.5[b]	0.59±0.04[a]
u_{sg}						
	0.5	10	35	0.63±0.03[a]	87.8±4.0[b]	0.58±0.04[a,b]
	1	10	35	0.75±0.07[a]	106±6.6[a]	0.63±0.07[a]
	1.5	10	35	0.54±0.09[a]	75.6±2.0[b]	0.44±0.02[b]

a, b are the significant level of differences (for each parameter) of the results obtained from ANOVA and Tukey Method ($p<0.05$)

All these results suggested that the optimum of temperature, light intensity and u_{sg} were 35°C, 10 kLux and 1 cm s^{-1}, respectively, to provide the highest *S. armatus* biomass production. Biochemical compositions of *S. armatus* under optimal condition are illustrated in Table 4.2 compared with other species. Due to the relatively high biomass productivity and also biochemical values, the productivities of the various nutrients are relatively high. It is the aim of this section to investigate whether the cultivation technique could further improve the productivity of such biomass and these nutrients and the results are illustrated in the following subsections.

Table4.2 Biomass productivity of *Scenedesmus* culture and its biochemical contents

Species	Light intensity (Lux)	Temperature (°C)	Incubation time (d)	Volume (L)	specific biomass productivity (mg $L^{-1}d^{-1}$)	Lipid content (%w/w)	Protein content (%w/w)	Carbohydrate content (%w/w)	Reference (Year)
S.cenedesmus obtusiusculus	23,680	35	6.7	2	970	14.2	52.4	21.3	Cabello et al. (2015)
Scenedesmus obliquus SA1	6,000	25±1	28	5	18.54±0.11	20.83±2.3	10.22±1.76	37.09±1.48	Basu *et al.* (2014)
Scenedesmus obliquus SJTU-3	13,320	25±1	14	0.8	60.7	15.15	-	-	Tang et al. (2011)
S. armatus	**10,000**	**35±0.5**	**7**	**2**	**106±6.8**	**21.7±1.0**	**32.9±2.6**	**45.4±1.6**	**This work**

4.2 Indoor cultivation

Flat panel airlift photobioreactors (FPAP) was selected for a large scale cultivation of *S. armatus* due to easy setup, maintenance, and scale up. The 17 L FPAP cultivation was conducted with the conditions as reported in Section 4.1, i.e. u_{sg} 1 cm s^{-1} and surface light intensity 10 kLux. Temperature was not controlled, but the indoor temperature (air conditioned) only varied within a small range of 28-30°C (Figure 4.2). Figure 4.3 illustrates growth characteristics of *S. armatus* where biomass rose two-fold in one day, after that, cells entered the exponential phase until Day 6 where the stationary phase was reached. The average final biomass concentration, specific biomass productivity and specific growth rate were 0.59±0.03 g L^{-1}, 70.4±9.6 mg $L^{-1}d^{-1}$ and 0.21±0.01 d^{-1}, respectively. The growth characteristics under this condition were quite similar to those with controlled temperature at in the range of 28-30°C as reported in Section 4.1, however, with a lower specific growth. This was probably due to the higher initial cell density that was employed in the culture. This was inevitable as the culture was not properly sterilized as a higher initial cell density was required to minimize the contamination. However, this high initial cell density (~ 0.1 g L^{-1}) could lead to the self-shading effect where a dense cell blocked the light

penetration. All biochemical contents were also similar to those obtained from smaller scale cultivations (2 L), i.e. lipid, protein and carbohydrate contents were 22.1±0.3, 32.0±0.5 and 45.9±0.8%w/w, respectively. These results confirmed that *S. armatus* could grow reasonably well in a larger cultivation system, and that the configuration of FPAP was effective for the growth of such alga.

In the next section, the outdoor cultivation of microalgae was investigated to see if this FPAP system could be further extended to a large-scale system that could reduce the operating cost and electricity consumption. The effect of environmental uncertainties would be examined.

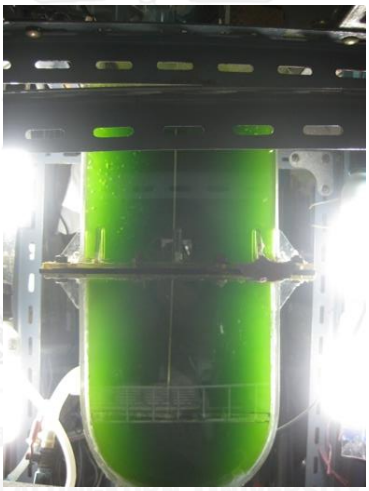

Figure4.2 *S. armatus* indoor cultultivation in 17 L of FPAP

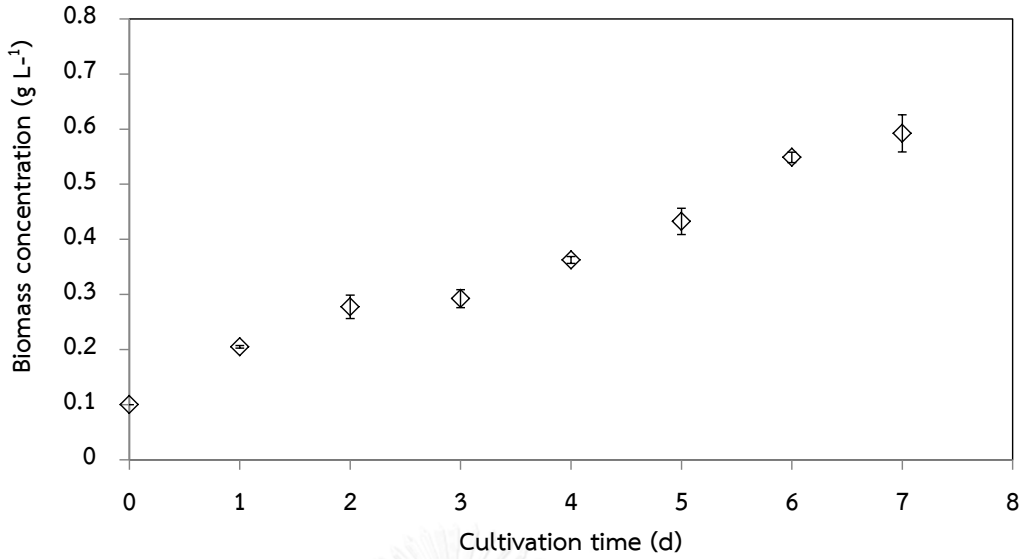

Figure4.3 Growth characteristics of *S. armatus* from indoor cultivation with 17 L FPAP

4.3 Large scale outdoor cultivation

4.3.1 Flat panel airlift photobioreactors (FPAP)

Issarapayup et al. (2009) proved that the extending of length of flat panel airlift photobioreactor did not affect the growth behavior and hydrodynamic properties of the FPAP system. This led to a special design in this work where FPAPs were expanded in length from 20 cm to 120 cm to obtain the volume as required (the total working volume of 100L in this case). The aeration rate or aerating superficial velocity, u_{sg}, was examined again in order to evaluate the effect of aeration in the large scale reactor (Figure 4.4). Light intensity and medium temperature profiles of three experiments are illustrated in Figure 4.5. It is noted that in one of the experiments, the weather condition was disturbed by rainy and clouds throughout the cultivation period (Round dot) with an average maximum light intensity of only 105,840 Lux and the average maximum/minimum medium temperature of 32/26°C, whereas all other experiments were conducted with the average maximum light intensity and average maximum/minimum medium temperature of 189,000 Lux and 41/29°C, respectively. The fluctuating weather condition could cause high variety in growth characteristics. Figure 4.6 illustrates the growth under various u_{sg}, where the

initial biomass concentration was 0.03 g L^{-1}. Cells gradually grew and became constant at Day 7. The results reveal that growth rates at u_{sg} of 0.35 cm s^{-1} were similar to that at u_{sg} of 0.52 cm s^{-1} where the specific biomass productivity was around 41.7±6.6 to 48.3±8.4 mg $L^{-1}d^{-1}$. However, the growth rate decreased significantly when u_{sg} decreased to 0.18 cm s^{-1} where the specific biomass productivity went down to 25.6±6.6 mg $L^{-1}d^{-1}$. At this condition, cell precipitation and surface adhesion were observed. Therefore, aeration with u_{sg} at 0.35 cm s^{-1} was concluded to be most suitable that could induce adequate cell circulation.

Figure4.4 *S. armatus* outdoor cultivation in 100 L of FPAP

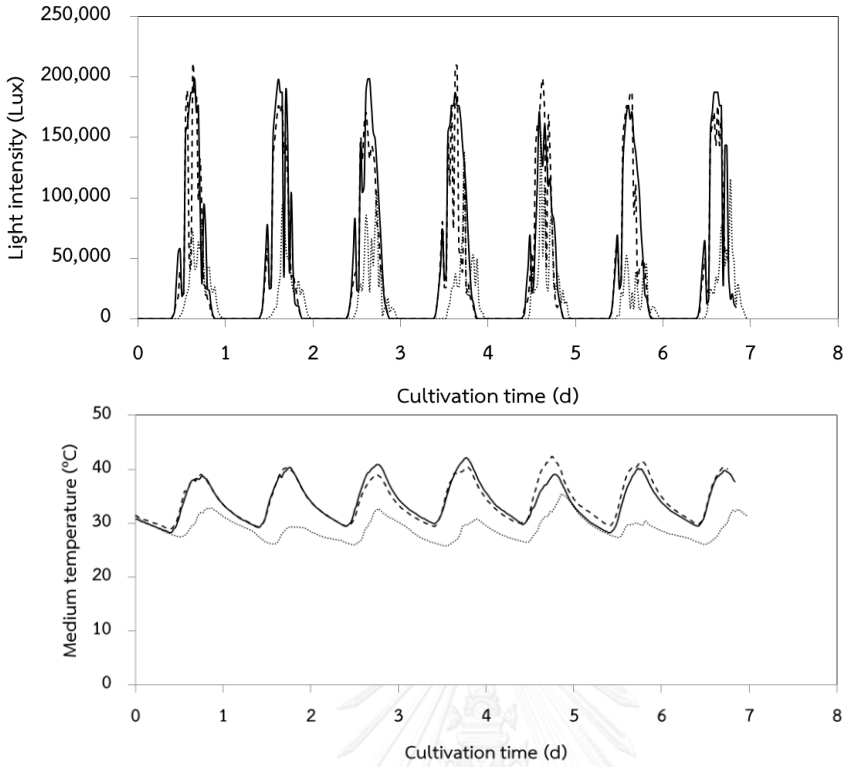

Figure4.5 Light intensity and medium temperature profiles of three outdoor experiments in FPAP

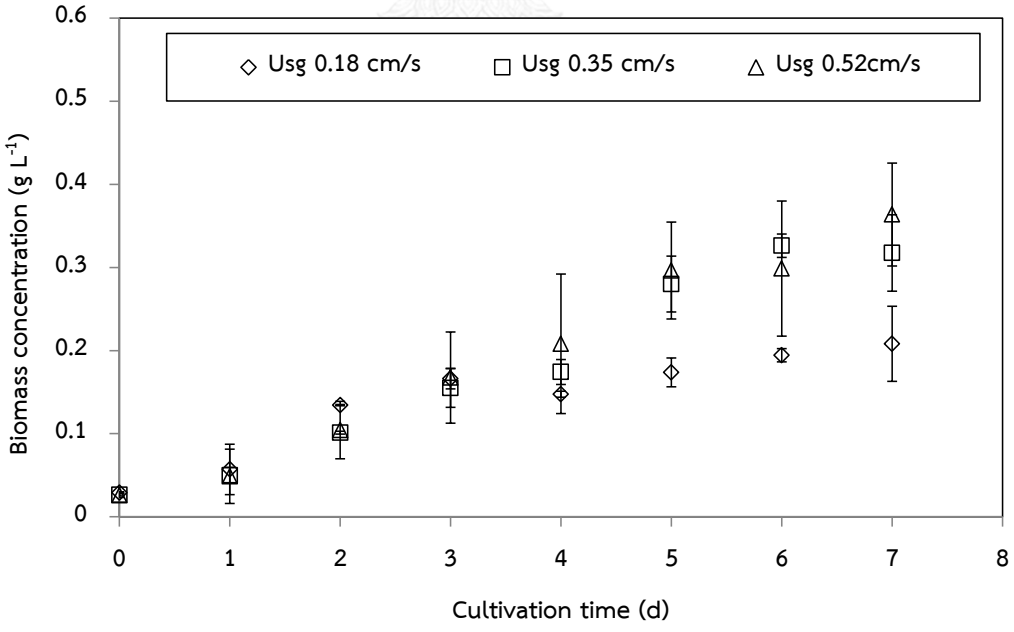

Figure4.6 Growth characteristic of *S. armutus* under outdoor cultivation in FPAP with various u_{sg}

In addition, biochemical contents in biomass under outdoor cultivation were not different from those obtained from the small scale indoor cultivation (Figure 4.7). This could be due to that fact that the medium temperature of outdoor culture was similar to the indoor temperature (30-35°C). Although the outdoor culture was exposed to high light intensity, this took place for a short period of time, and this was compensated by the 24 hour constant indoor temperature. The outdoor culture, however, suffered from the low productivity which might be due to the varying environmental conditions. Similar finding on poor outdoor growth is also reported by Wang et al. (2014) who compared the growth, fatty acid and protein contents of *Chlorella ellipsoidea* from indoor to outdoor cultures, and found that growth rate and biochemical contents under large scale outdoor cultivation were lower than small scale indoor cultivation due to lower temperature, poor irradiance and short photoperiod.

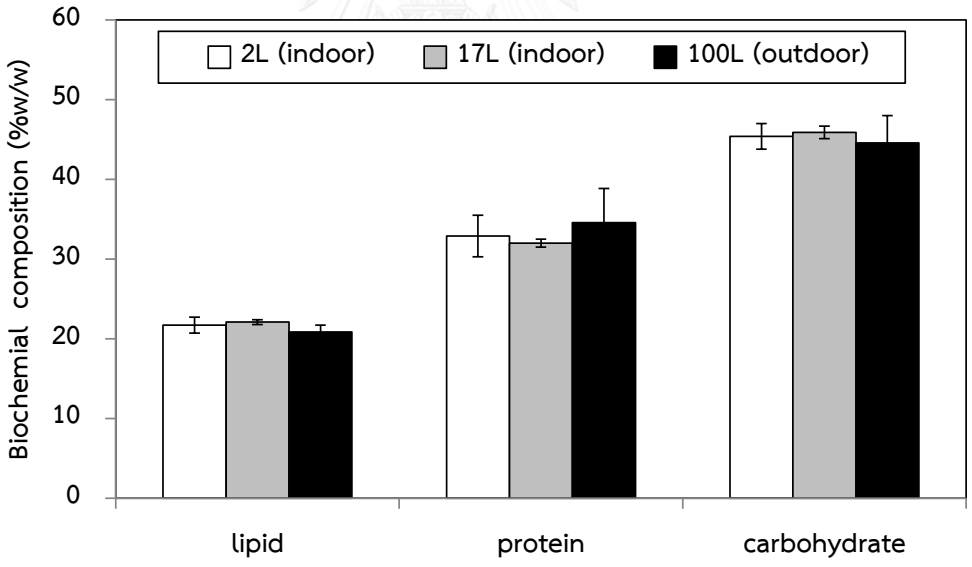

Figure4.7 Biochemical compositions with various cultured scales

4.3.1 Effect of seasons on *S. armatus* growth characteristic

Due to uncontrolled the environment of outdoor condition, Thailand has official three seasons, i.e. summer (mid- February to mid- May), rainy season (mid- May to mid-October) and winter (mid- October to mid-February) (http://www.tmd.go.th). In this section, the effect of seasonal change was studied on growth of *S. armatus* and biochemical contents.

Figure 4.8 illustrates light intensity and medium temperature profiles where, in summer (Figure 4.8 A-B), the maximum light intensity and medium temperature were approximately 216,000 Lux and 38-42^{o}C and the minimum medium temperature was 29-31^{o}C. In rainy season (Figure 4.8 C-D), the maximum light intensity could be as high as 216,000 Lux but mostly in the range of 54,000-81,000 Lux. Due to cloud shades and rain, the maximum and minimum medium temperatures were in the range of 34-39^{o}C and 28-29^{o}C. In winter (Figure 4.8 E-F), the maximum light intensity was around 162,000 Lux where the maximum and minimum medium temperatures were 34-37^{o}C and 25-29^{o}C. Daily average surface energy and medium temperature are depicted in Table 4.3. In general, summer provided the highest average surface energy and medium temperature while rainy season and winter exhibited similar weather conditions.

Figure 4.9 displays the growth rates of *S. armatus* with batch culture cultivated through the three seasons where the initial biomass density was set at a higher level, 0.08 g L^{-1}, in order to shorten the cultivation time. Similar growth characteristics were obtained from the cultivations in rainy season and winter where the microalga spent one day of lag phase and reached stationary phase within the fifth day. The growth performance in the rainy season was slightly better than in winter where the final biomass concentration, specific growth rate and specific biomass productivity in the rainy season were 0.29±0.03 g L^{-1}, 0.29±0.07 d^{-1} and 45.1±10.8 mg $L^{-1}d^{-1}$, and in winter, 0.27±0.03 g L^{-1}, 0.28±0.04 d^{-1} and 39.2±8.5 mg $L^{-1}d^{-}$ $^{\mu 1}$. In summer, cells seem to grow better with very short lag phase and a faster growth rate where the final biomass concentration, specific growth rate and specific biomass productivity were 0.37±0.04 g L^{-1}, 0.29±0.02 d^{-1} and 59.3±6.7 mg $L^{-1}d^{-1}$.

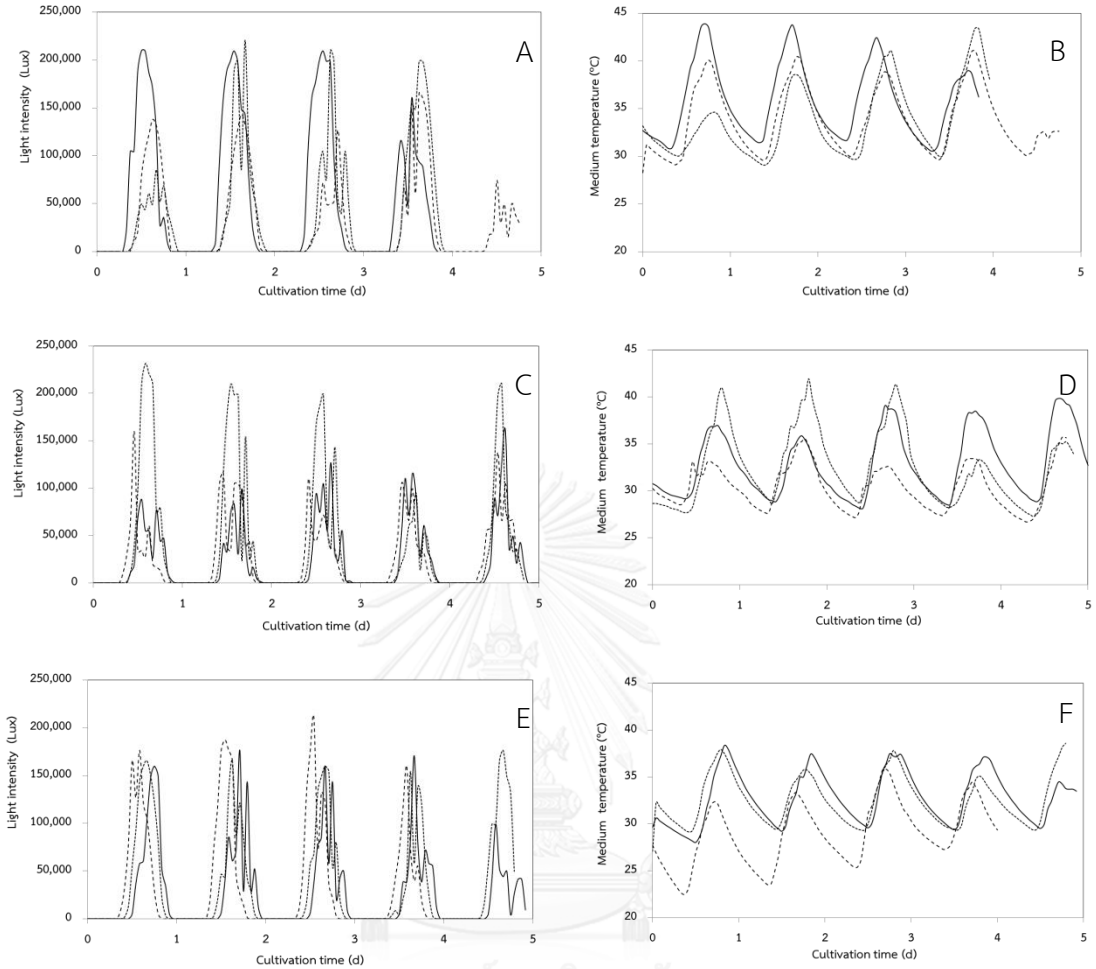

Figure4.8 Light intensity and medium temperature profiles: Summer (A-B); Rainy season (C-D); Winter (E-F) (triplicate experiment)

Table4.3 Average daily surface energy imposed on the FPAPs and the associate medium temperature during the cultivation in various seasons

Season	Surface energy ($MJ\ m^{-2}d^{-1}$)	Medium temperature (^{o}C)
Summer	25.2±7.8	35.0±2.4
Rainy season	17.7±6.1	32.6±1.7
Winter	18.8±4.1	31.8±2.1

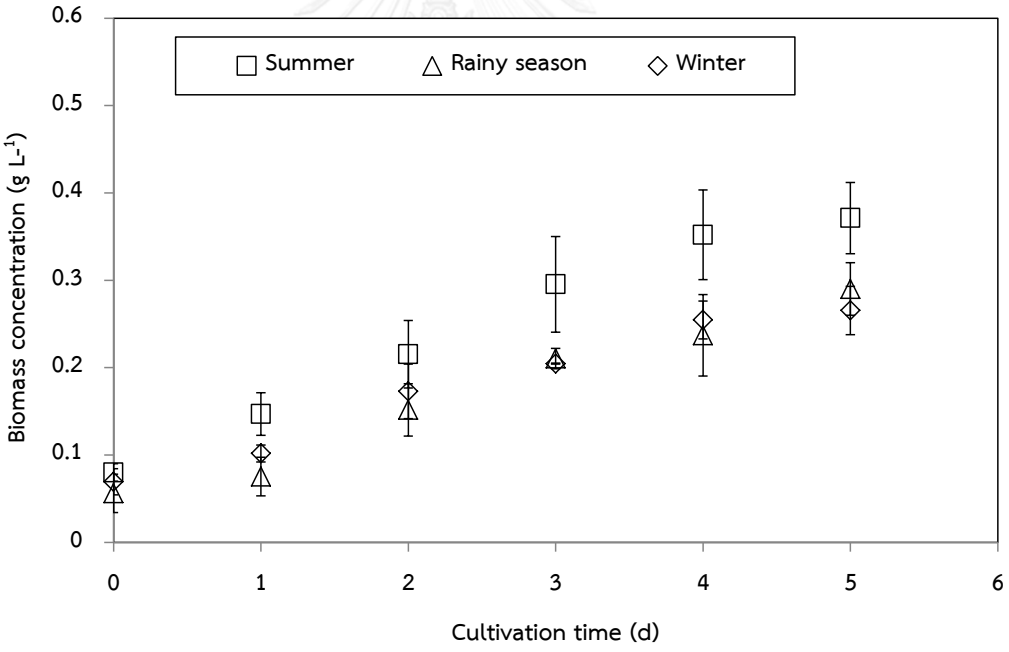

Figure4.9 Growth characteristics of *S. armatus* from outdoor cultivation

4.3.2 Typical biochemical compositions

Figure 4.10 illustrates biochemical compositions, i.e. lipid, protein and carbohydrate, in the dry cell of *S. armatus* obtained from the growth at various seasons. The average lipid, protein and carbohydrate were 19.8-22.9%, 36.4-37.8% and 39.6-42.5% w/w where the average specific biochemical productivities are reported in Table 4.4, i.e. lipid 11-14 mg $L^{-1}d^{-1}$, protein 19-21 mg $L^{-1}d^{-1}$ and carbohydrate 20-23 mg $L^{-1}d^{-1}$. These results suggest that both biochemical contents and productivities did not statistically vary with changes in ambient conditions examined in this work, unlike previous reports where microalgal biochemical components varied significantly with seasons (Carlozzi and Sacchi, 2001, Olofsson et al., 2012). This was possibly because the local annual changes in weather condition as summarized in Table 4.3 were not adequate to cause notable changes in the cell culture characteristics.

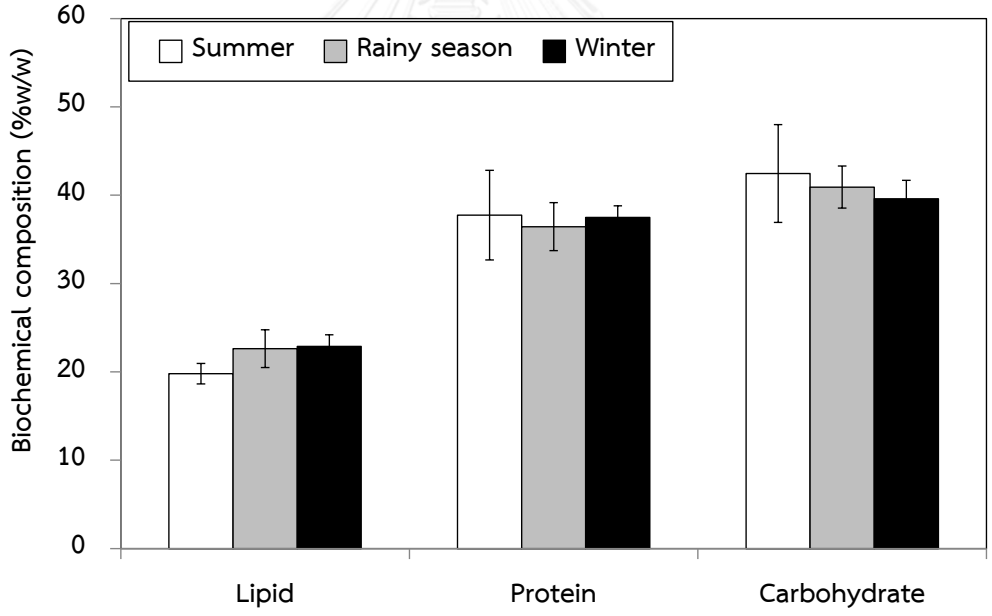

Figure4.10 Biochemical compositions in *S.armatus*

Table4.4 Specific biomass and biochemical productivities from *S.armatus* cultivated in this work

Season	Biomass ($mg\ L^{-1}d^{-1}$)	Lipid ($mg\ L^{-1}d^{-1}$)	Protein ($mg\ L^{-1}d^{-1}$)	Carbohydrate ($mg\ L^{-1}d^{-1}$)
Summer	59.3±6.7[a]	11.1±2.2[a]	21.1±4.9[a]	23.4±2.5[a]
Rainy season	45.1±10.8[a]	11.5±2.1[a]	19.0±6.0[a]	21.1±5.8[a]
Winter	39.2±8.5[a]	13.9±6.0[a]	19.5±8.9[a]	20.1±7.5[a]

a is the insignificant level of differences (for each parameter) of the results obtained from ANOVA and Tukey Method ($p>0.05$)

4.4 Concluding remarks

S. armatus was proven to be able to grow in large scale outdoor systems at all tropical seasons which indicated that cells could tolerate high light intensity and relatively high temperature. The resulting biomass productivity was not as good as the indoor culture but the outdoor system benefits from the lower operating costs and the economy of scale. Table 4.5 illustrates the total cost of the production of 1 kg dry biomass. The total costs of FPAP (100 L) outdoor cultivation were around 215.77 \$US kg^{-1} (6,904 THB kg^{-1}) whereas the small scale indoor cultivation cost about 867-1,064 \$US kg^{-1} (27,744 - 34,048 THB kg^{-1}). The major cost of indoor cultivation was electricity cost (lighting and air compressor) which was around 90% of the overall cost, whereas 80% of the outdoor cost was from nutrient. In the next chapter, the enhancement of biomass production by providing additional carbon sources such as CO_2 is investigated.

Table4.5 Costs estimation for cultivation of *S. armatus* in indoor and outdoor (basis 1kg dry biomass per batch)

Character	Cost ($ kg^{-1})		
	2 L (indoor)	**17 L (indoor)**	**100 L (outdoor)**
Operating cost			
Nutrient charge	52.92 (5.38%)	67.27 (8.56%)	124.02 (82.87%)
Lighting	756.00 (77.00%)	678.36 (86.00%)	0 (0%)
Air compressor	175.0 (17.78%)	39.26 (5.00%)	26.61 (16.44%)
Tap water	0.44 (0.04%)	0.56 (0.07%)	1.03 (0.69%)
Total operating cost	**984.35 (100%)**	**785.45 (100%)**	**149.66**
Fixed cost*			
Reactor	19.98 (25.08%)	59.75 (73.26%)	63.68 (96.32%)
Compressor	17.31 (21.74%)	3.88 (4.76%)	2.43 (3.68%)
Lamp	39.95 (50.17%)	17.93 (21.98%)	0 (0%)
Temperature controller	2.40 (3.01%)	0 (0%)	0 (0%)
Total fixed cost	**79.64 (100%)**	**81.56 (100%)**	**66.11 (100%)**
Total cost	**1,064.00**	**867.01**	**215.77**

Remark: current Thailand rates: 1$ = 32 THB.

* assume life time of 10 years for glass reactor and compressor, 5 years for acrylic reactor and temperature control system, 1 year for lamp

4.5 Application of FPAP for outdoor cultivation of other algal species

Chaetoceros gracilis is one of the most popular diatoms in Thailand and is used in feeding shrimp larvae. Semi-continuous cultures were selected to examine the effect of initial cell concentration. This experiment was conducted in the 100L FPAPs of *C. gracilis* in the modified F/2 medium at u_{sg} 3 cm s^{-1} under the summer condition as specified in Figure 4.11. The maximum cell concentration was found to increase with an increase in initial cell concentration as illustrated in Figure 4.12. The maximum cell concentration of 4.5×10^6 cells mL^{-1} was obtained during the first round of cultivation with an initial cell concentration of 0.1×10^6 cells mL^{-1}. This was equivalent to a specific productivity of 3.76×10^4 cells mL^{-1} h^{-1} (2.93×10^9 cells h^{-1}) at 117 h. Then the culture was partially harvested at the seventh day and replenished with fresh medium in the predefined volume such that the initial cell concentration for the second round became 1×10^6 cells mL^{-1}. In this consecutive batch, the maximum cell concentration increased to 7.1×10^6 cells mL^{-1} after 96 h of cultivation, with the specific productivity of 6.50×10^4 cells $mL^{-1}h^{-1}$ (5.59×10^9 cells h^{-1}). The culture was thereafter harvested in a similar technique with the same initial cell concentration for the next batch. The third batch could be harvested after three days at the same maximum cell concentration (7.2×10^6 cells mL^{-1}), but with a much higher specific productivity of 8.86×10^4 cells $mL^{-1}h^{-1}$ (8.86×10^9 cells h^{-1}). These results lead to the conclusion that the culture grew better if started with higher cell density as the low density culture could be subjected to light inhibition during the initial stage. This result corresponded well to the reported cultivation of *Arthrospira platensis* (Carlozzi, 2003) and *Tetraselmis chuii* (López-Elías et al., 2011).

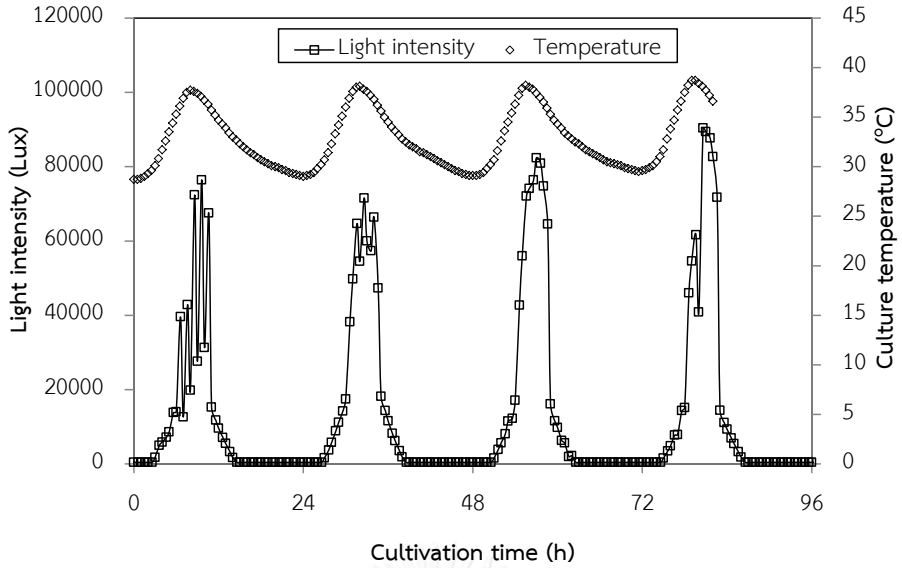

Figure4.11 Profile of light intensity and temperature

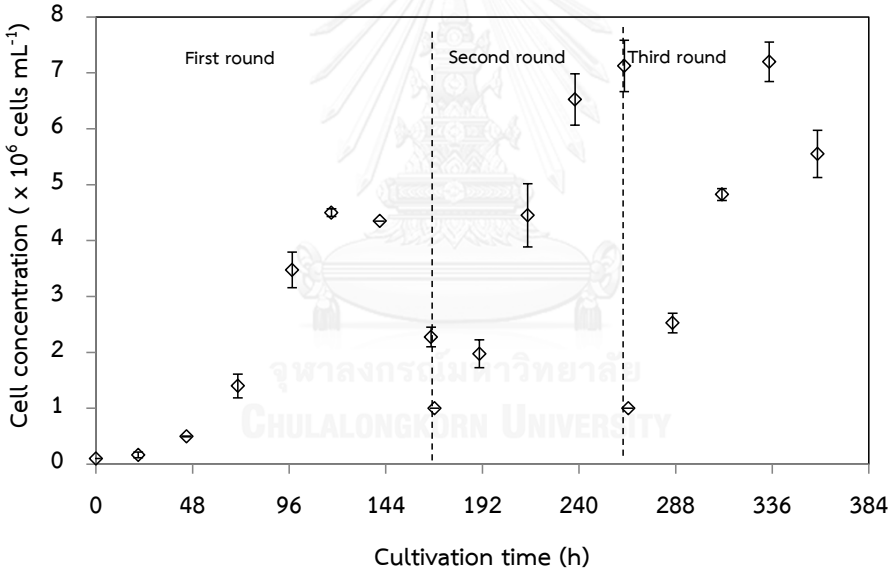

Figure4.12 Growth of *C. gracilis* in semi-continuous 100 L FPAP

Chapter V

Effect of carbon dioxide and pH on growth rate of *S. armatus*

This chapter presented how CO_2 and pH could affect the growth of *S. armatus* with 100 L of FPAPs. The algal growth was tested by varying CO_2 in the concentration range of 2 to 15% by volume which was equivalent to CO_2 loading rate of 0.44 to 3.32 g L^{-1} h^{-1}. The pH was controlled to examine its influence on growth when compared with the cultivation without pH control.

5.1 Effect of CO_2

5.1.1 Growth characteristics

S. armatus cultivations with CO_2 feeding were cultivated for 4 days with an average daily light energy and medium temperature of 23.8±6.1 MJ m^{-2} and of 34.5±1.4 ^{o}C. It was observed that, in average, *S.armatus* enters its stationary phase after the fourth day of the operation and therefore the results displayed in this work were extracted from the first four days of the cultivation. Figure 5.1 illustrates inorganic species in aqueous as a function of pH. The equilibrium inorganic carbon follows eq. (5.1) where CO_2 and bicarbonate (HCO_3^-) forms could be up-taken into cells (Markou *et al.*, 2014).

$$CO_{2(aq)}+H_2O \overset{pKa_1=6.3}{\leftrightarrow} H_2CO_3^* \overset{pKa_2=10.3}{\leftrightarrow} HCO_3^-+H^+ \leftrightarrow CO_3^{2-}+2H^+ \qquad (5.1)$$

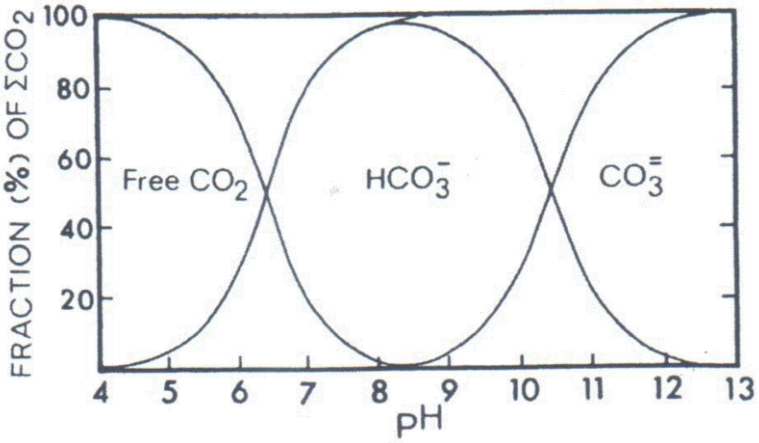

Figure5.1 Fractions of inorganic carbon in aqueous (Wetzel, 2001)

Figure 5.2 shows daily pH profiles of cultures with various CO_2 concentrations. pH value of the culture without CO_2 feeding (around 0.03% by vol. CO_2) increased steadily from 8.4-8.5 to 10-10.5 during 6:00 AM to 3:00 PM. This is due to the generation of OH^- group from the equilibrium and uptake of HCO_3^- and CO_2 ($HCO_3^- \overset{CA}{\leftrightarrow} CO_2+OH)^-$ where HCO_3^- was enzymatically converted to CO_2 (via carbonic anhydrase, CA) (Markou et al., 2014). pH remained constant after that which corresponded to the period where the growth slowed down, and started to decrease as sunset due to the release of CO_2 from cell respiration. A drop in pH was observed when CO_2 was supplied to system where pH decreased from 7.3-7.5 to 6.40 with an increase in CO_2 concentration from 2 to 15% vol. of the air supply (only during the daytime, 12h duration). This is because the extra CO_2 could not be totally consumed from cell growth and therefore the remaining CO_2 caused the drop in the pH level. In this case, the supply of CO_2 was ceased after 6.00 PM where pH bounced back to 8.5-8.6 due to the stripping of CO_2 to maintain the equilibrium within the system (Valdés et al., 2012). It is noted that, cells release CO_2 during the night time from respiration activity, however, pH value during this time period remained reasonably unchanged at around 8.5-8.6 both without CO_2 and with enriched CO_2. This was due to chemical buffering capacity of the inorganic carbons bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) according to the following equations (Wetzel, 2001):

$$HCO_3^- + H_2O \leftrightarrow H_2CO_3 + OH^- \quad (5.2)$$

$$CO_3^{2-} + H_2O \leftrightarrow HCO_3^- + OH^- \quad (5.3)$$

Figure 5.3 illustrates an example of diurnal variation profiles (extracted from Day 2 of the operation) in the medium culture both with and without CO_2 supply to demonstrate how environmental parameters such as light intensity, temperature and pH influenced the algal growth. Cells grew only slightly during 6:00 to 9:00 AM where light intensity was still low. During this period, light intensity only slowly increased from 10,300 to 71,600 Lux, whereas the temperature went up slightly from 29.1 to 30.3°C. The highest growth rate occurred during 9:00 to 3:00 PM where light intensity reached a more proper range and increased up to 240,000 Lux. During this period,

the medium temperature increased from 30.3 to 38.6°C and the biomass concentration increased from 0.13 to 0.20 g L^{-1} with an actual specific growth rate of 0.0757 h^{-1}. The pH value was observed to rise from 9.14 to as high as 11.05 (without CO_2 feeding). When pH is more than pKa_2 (10.33), carbonate (CO_3^{2-}) predominates which cannot be utilized by cells. A better growth was obtained when the culture was aerated with air plus 2% by vol. CO_2 where the biomass reached up to 0.24 g L^{-1} with an actual growth rate of 0.0839 h^{-1}. Due to the supply of CO_2, the pH value of the culture could be maintained in the range of 7.3 to 7.5 in which HCO_3^- predominates. Biomass concentration gently declined during the night time due to the effect of night loss where percentage of night biomass loss was 20-30%. Figure 5.4 demonstrates the cultivation at a longer period of time (4 days) where similar results were obtained, i.e. the growth rates of *S. armatus* with 2% CO_2 enriched air substantially increased when compared with those without CO_2. This corresponds to the average final biomass concentration of 0.41±0.04 g L^{-1} (2% CO_2) and 0.32±0.01 g L^{-1} without CO_2. The difference in cell growth became visible more clearly when the growth rate was further converted to biomass productivity.

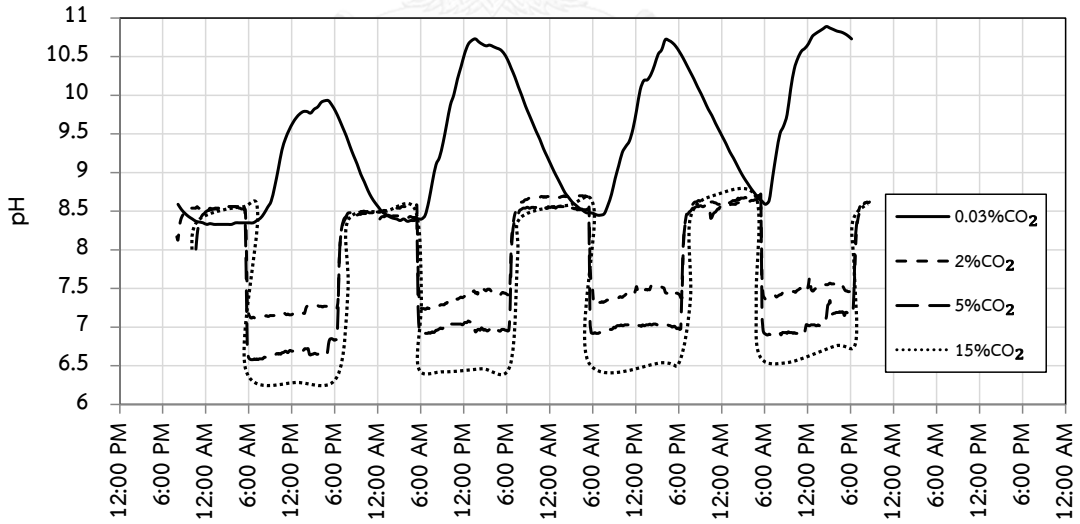

Figure5.2 pH profiles of *S. armatus* cultivated under outdoor condition: CO_2 supply only during the daytime for 12 h with average light energy and medium temperature of 23.8±6.1 MJ $m^{-2}d^{-1}$ and of 34.5±1.4°C

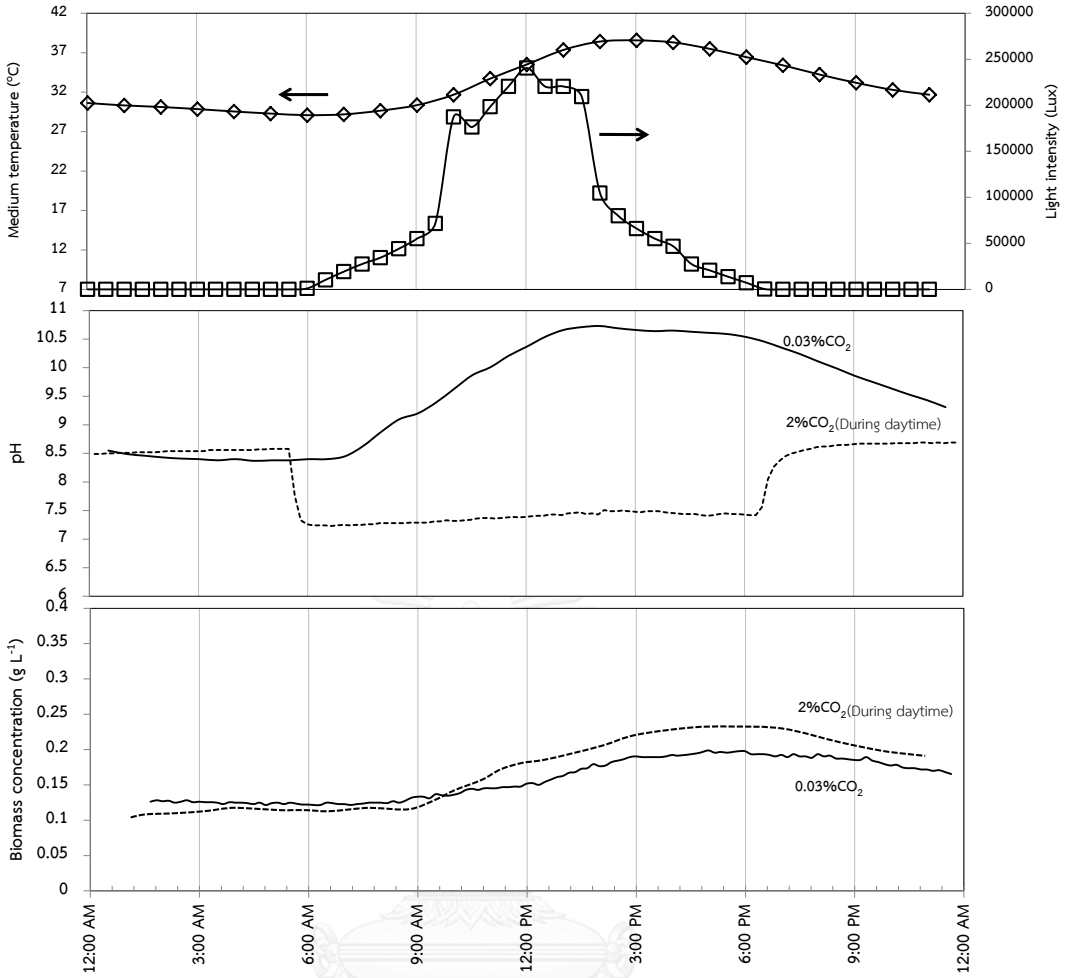

Figure5.3 Diurnal variation profiles in medium culture: (A) Light intensity and medium temperature; (B) pH; (C) biomass concentration

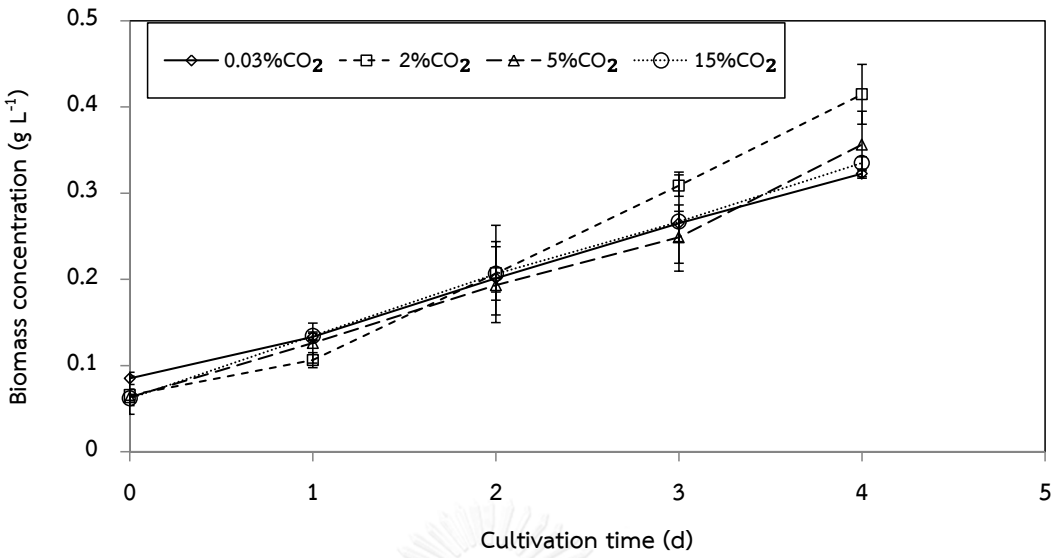

Figure5.4 Growth characteristics of *S. armatus* with various CO_2 concentrations

Figure 5.5 illustrates that the average productivities increased from 65.8 (without adding CO_2) to 91.3 mg $L^{-1}d^{-1}$ (with 2% CO_2) with an observed growth rate of 0.0124 to 0.0169 h^{-1}, accordingly. Above 2% CO_2, the average final biomass concentration and the observed specific growth rate slightly declined to 0.356±0.039 g L^{-1} , 0.0143 h^{-1} (at 5% CO_2) and 0.335±0.007 g L^{-1}, 0.0129 h^{-1} (at 15% CO_2), and the average final biomass productivity dropped to 78.0 and 74.5 mg $L^{-1}d^{-1}$, respectively. At this elevated CO_2 supply level, the uptake rate for CO_2 could not match the supply resulting in a dramatic drop in pH and this could be the main reason for the slight decline in the growth. However, the results still indicated that *S. armatus* could grow reasonably well regardless of CO_2 level and pH condition. A report from Thielmann et al. (1990) who stated that *Scenedesmus* could take inorganic carbon via two mechanisms, i.e. dissolved CO_2 at pH 5 to 8, and bicarbonate (HCO_3^-) accumulation at pH 7 to 11 (See Figure 5.2) could well describe this observation as the dissolved CO_2 might take place at low pH (high CO_2 supply) whereas bicarbonate accumulation prevailed at low CO_2 supply. The proposed model for inorganic carbon transport and accumulation in *Scenedesmus obliquus* can be expressed as illustrated in Figure 5.6. CO_2 is being taken up via two mechanisms, i.e. passive diffusion across

membrane, and active transport inside cells. On the other hand, HCO_3^- can be only taken up by active transport (ATPase) and is converted to CO_2 using carbonic anhydrase (CA) enzyme.

Note that the observed specific growth rate was lower than the actual specific growth rate as this accounted for the cell death and maintenance during the night time where photosynthesis did not take place. The observed specific growth rate was determined from the average daily biomass concentration.

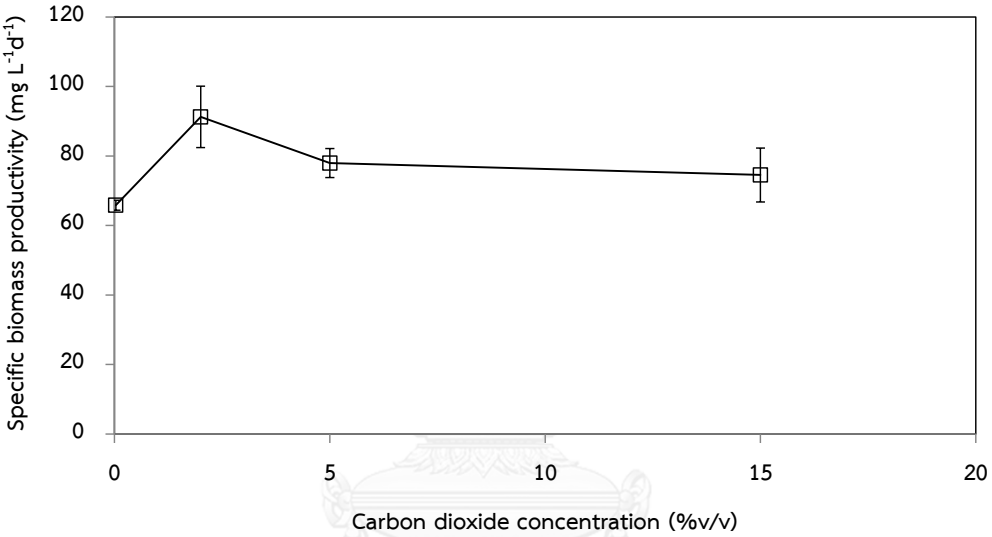

Figure5.5 Specific biomass productivity of *S. armatus* from outdoor cultivation

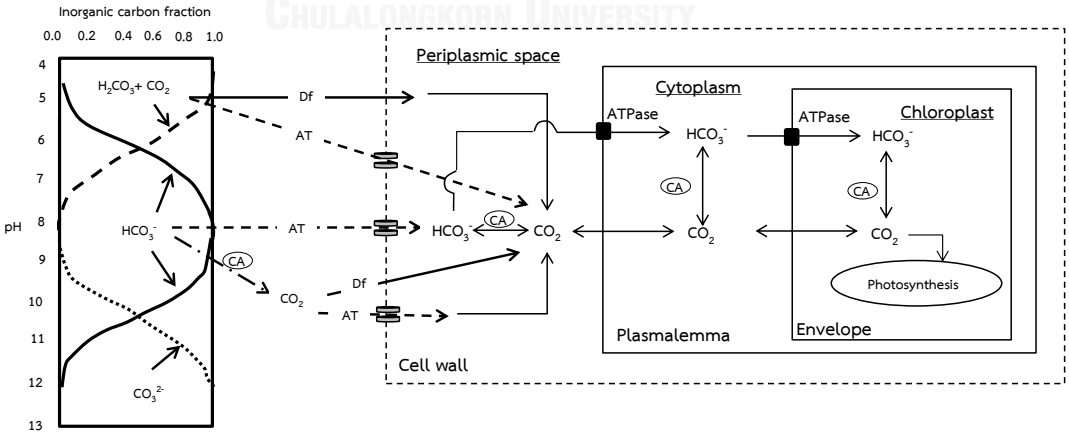

Figure5.6 Schematic model for inorganic carbon transport and accumulation in Scenedesmus obliquus: Diffusion (Df); Active Transport (AT); Carbonic Anhydrase (CA); ribulose bisphosphate carboxylase/oxygenase (Rubisco); transporter (▬, ■ ATPase) (Markou et al., 2014, Thielmann et al., 1990)

5.1.2 CO_2 fixation rate

CO_2 is fixed inside cell through the first step of the Calvin cycle where CO_2 is catalyzed by Ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco). Therefore the growth rate should theoretically depend on the rate of CO_2 fixation. In a typical air fed culture, the average carbon content in dry cell and CO_2 fixation rate were 39.0% and 101 mg CO_2 $L^{-1}d^{-1}$ (Table 5.1). This average carbon content went up significantly to 49.3% when *S. armatus* was aerated with 2%CO_2 enriched air. The average CO_2 fixation rate increased significantly to 165 mg CO_2 $L^{-1}d^{-1}$ when *S. armatus* was cultivated with 2 %CO_2 enriched air, however, at 5-15%, the average carbon content and CO_2 fixation rate dropped to 45.6-48.5% and 124-129 mg CO_2 $L^{-1}d^{-1}$, respectively. This was due to the limited CO_2 uptake capacity of the alga, and therefore the extra CO_2 just escaped the reactor at the top surface resulting in a low CO_2 utilization efficiency with increasing of CO_2 concentration. Moreover, there was a drastic drop in the pH value at this condition (see Figure 5.2) which could lead to an observed drop in cell growth.

Table 5.2 illustrates biomass productivity and CO_2 fixation rate of *S. armatus* compared with other *Scenedesmus* species. The results illustrate that the outdoor culture provided a mostly similar level of biomass production and CO_2 utilization with those indoor cultures. The outdoor cultivation is therefore considered an economical investment due to a reduced cost for illumination and a larger scale culture was also considered economically feasible.

Table5.1 Carbon content and carbon fixation rate (Daytime CO_2 supply only for the first 4 days)

CO_2 (%v/v)	%C in biomass	CO_2 fixation rate (mg CO_2 $L^{-1}d^{-1}$)	%CO_2 utilization efficiency
0.03	39.0±3.1	101±13	63.4±8.1
2	49.3± 0.4	165±15	1.55±0.14
5	48.5 ±0.9	129±18	0.48±0.07
15	45.6 ±4.1	124±2	0.16±0.01

Table5.2 Specific biomass productivity and CO_2 fixation rate from *Scenedesmus*

Species	Condition	CO_2 (%)	Specific biomass productivity (mg L^{-1} d^{-1})	CO_2 fixation rate (mg CO_2 $L^{-1}d^{-1}$)	Reference
S. obliquus	Indoor	12	76.0	-	Ferriols et al. (2013)
S. obliquus CNW-N	Indoor	20	201	390	Sydney et al. (2010)
S. obliquus As-6-1	Indoor	20	151	290	Sydney et al. (2010)
S. dimorphus	Indoor	15	80.0	174	Izumo et al. (2007)
Scenedesms sp. IMMTCC-6	Indoor	10	71.7	135	Nayak et al. (2013)
S. obliquus SA1	Indoor	35	51.9	97.7	Basu et al. (2014)
S. obtusus XJ-15	Outdoor	1	86.5	170	Xia et al. (2013)
S. armatus	**Outdoor**	**2**	**91.3**	**165**	**This work**

5.1.3 Biochemical compositions

Figure 5.7 illustrates the total biochemical compositions with various CO_2 concentrations. Lipid and protein increased with CO_2 feeding. On the other hand, carbohydrate decreased with increasing CO_2 concentrations.

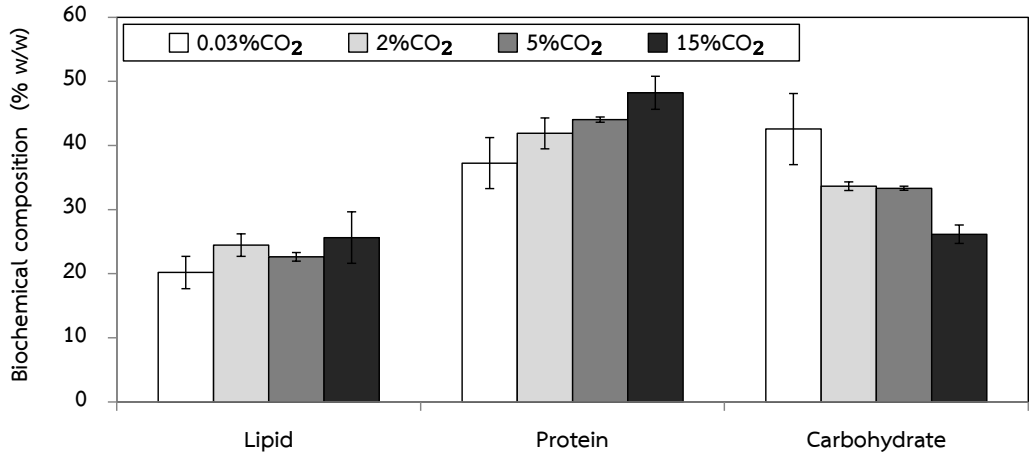

Figure5.7 Biochemical composition in *S.armatus* cultivated in the system with CO_2 enriched air

Lipid composition slightly increased from 20.2 %w/w without CO_2 to 22.6-25.6 %w/w when CO_2 concentration increased from 2 to 15 %. This indicates that the additional carbon from CO_2 could be converted to long chain carbon lipid. This agreed with the previous finding of Tang et al. (2011) who reported that total lipid content of *Scenedesmus obliquus* increased from 15.15 to 24.4% w/w with increasing of CO_2 concentration from 0.03 to 50%. Figure 5.8 shows the free fatty acid profiles in dry biomass obtained from the cultures with 2% CO_2 and 0.03% CO_2 supply concentrations. Both unsaturated and saturated fatty acids increased significantly from 1.31% to 2.43% and 0.71% to 2.01% of dry algal weight when additional CO_2 was given. Among the various species of fatty acids, Palmitic acid (C16:0), Oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3) were observed to increase remarkably. These results agree with the finding of Muradyan et al., 2004 who reported that C16:0 was enhanced when *Dunaliella salina* was cultivated under high CO_2 concentration and the poly unsaturated fatty acid contents of *Scenedesmus obliquus* tended to increase with increasing of CO_2 (Tang et al., 2011). The reason for this enhancement in polyunsaturated fatty acids still could not be drawn from this work, but it is likely that CO_2 facilitates the dehydrogenation of the fatty acids within the cells leading to fatty acids with a larger number of double bonds and longer

chain. However, the average lipid productivity only increased when CO_2 was enriched up to 2% (from 13.05 to 22.24 mg $L^{-1}d^{-1}$) (Table 5.3) after which lipid productivity decreased to 17.94 (5% CO_2) and 19.26 mg $L^{-1}d^{-1}$ (15% CO_2). This was mainly due to the reduction in the biomass productivity at high CO_2 throughput.

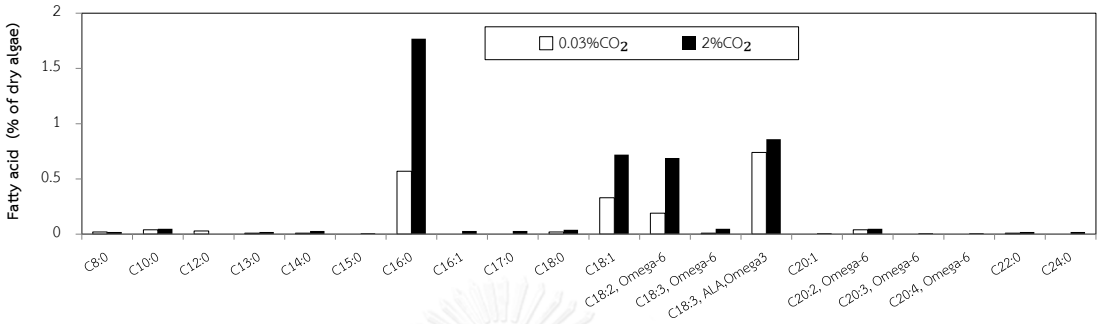

Figure5.8 Fatty acid contents in dry biomass withoutCO_2 (0.03%) and with 2%CO_2 enriched air

Protein composition also changed with the level of CO_2 supply, i.e. protein contents were 37.2 %w/w (without CO_2) and 48.2 %w/w (with 15%CO_2 enriched air) (see Table 5.3). This corresponded to a reduced C:N ratio in the biomass as illustrated in Figure 5.9. Similar to the case of lipid, however, the average protein productivity only increased with CO_2 supply up to 2% (where protein productivity went up to 38.34 mg $L^{-1}d^{-1}$) after which the poor biomass productivity significantly lowered the protein productivity. The increase in protein productivity was observed to be consistent with the average nitrogen uptakes from medium which directly varied with increasing CO_2 concentrations from 82.5 to 107.7 mg N g $cell^{-1}$ where about 92-99% of total protein was produced from this nitrogen source. This result agreed with that of Ferriols et al. (2013) who illustrated that nitrogen uptake increased with increasing CO_2 enriched air. Past reports for many other algal species like *Dunaliella tertiolecta*, *Botryococcus branunii*, *Chlorella vulgaris* and *Spirulina platensis* stated that approximate 56-91% of protein productions were converted from nitrogen uptake (Sydney et al., 2010).

Carbohydrate dramatically decreased with increasing CO_2 concentrations whereas average carbohydrate decreased from 42.6 % without CO_2 to 26.2-33.7

%w/w with 2 and 15 %CO_2 enriched air. The average carbohydrate productivity consequently decreased significantly from 27.93 (without CO_2) to 19.43 mg $L^{-1}d^{-1}$ with 15 %CO_2 enriched air. This could be due to the carbon dioxide concentrating mechanism (CCM) which is basically the fixation mechanism for CO_2 to biomass, and this CCM generally takes place more effectively at low CO_2 concentration. Hence, a low CO_2 system typically leads to a higher accumulation of carbohydrate (Izumo et al., 2007, Markou et al., 2012, Thyssen et al., 2001). At higher CO_2 concentration as a consequence, carbon was preferentially stored in other forms, e.g. protein or lipid, as observed in the findings stated previously.

Table5.3 Biochemical productivity from *S.armatus* cultivated in this work

CO_2 (%v/v)	Lipid (mg L^{-1} d^{-1})	Protein (mg L^{-1} d^{-1})	Carbohydrate (mg L^{-1} d^{-1})
0.03	13.05±2.87	24.86±3.41	27.93±3.45
2	22.24±0.57	38.34±5.90	30.67±2.37
5	17.94±1.21	34.18±1.56	25.85±1.42
15	19.26±4.99	35.84±1.80	19.43±0.95

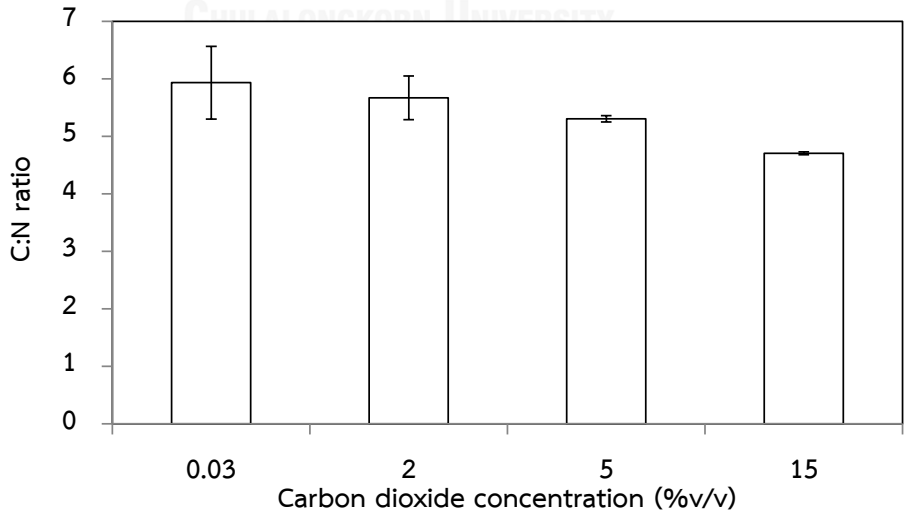

Figure5.9 C:N ratio in biomass of *S. armatus* as a function of CO_2 concentration in the provided aeration

In order to confirm that carbohydrate or polysaccharide contents decreased with increasing of CO_2, biomass was hydrolyzed with sulfuric acid to obtain sugar contents (glucose and xylose). Total sugar contents exhibited similar tend with total carbohydrate, i.e. sugar contents decreased with increasing of CO_2 concentration. In other words, the total sugar contents (glucose+xylose) decreased from 30.4% to 18.1% of cell dry weight with increasing CO_2 from 0.03% to 5%CO_2 (Figure 5.10). Glucose decreased significantly from 29.8% to 10.3% of dry weight whereas xylose greatly increased from 0.6 % to 7.8 % of dry weight. It is noted that the total sugar content was around 12-16% lower than the total carbohydrate which could be due to loss of content during acid hydrolysis.

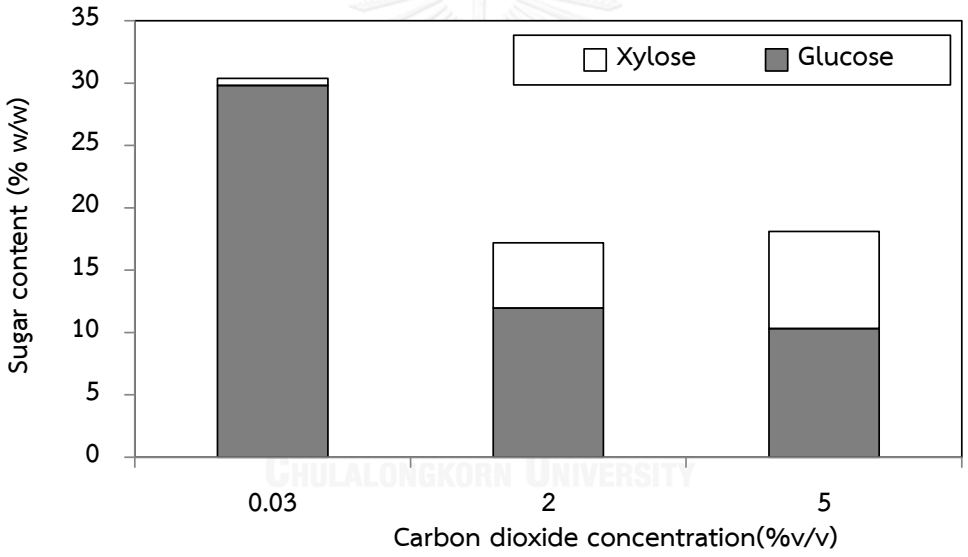

Figure5.10 sugar content with various cultivated CO_2 concentrations

5.2 Effect of pH control system with CO_2

pH of medium is important for inorganic carbon soluble form (Figure 5.1). pH should be adjusted less than pKa_2 (~10.3) to obtain CO_2 and HCO_3^- that cell can utilize. From results in Section 5.1, CO_2 concentration 2% by vol. was the optimum for carbon fixation. Therefore pure CO_2 was supplied at the flow rate of 400 mL min^{-1} (2%CO_2 by vol.) to control pH at the set point (pH_{sp}) of 7±0.2 and 7.7±0.2.

5.2.1 Growth rate

The experiment was carried out with the average light energy and medium temperature of 19.3±1 MJ $m^{-2}d^{-1}$ and 33.9 ±1^{o}C. Figure 5.11 illustrates an example of daily pH profiles of the medium with pH control compared with that without pH control. CO_2 was regularly supplied during the day time to replenish the CO_2 consumption by the microalgae for photosynthesis. After sunset, CO_2 was not consumed and reached the set point, and generally there was no need for CO_2 at the night time. It is noted that pH remained at the set point during the night due to the properties of the bicarbonate buffer.

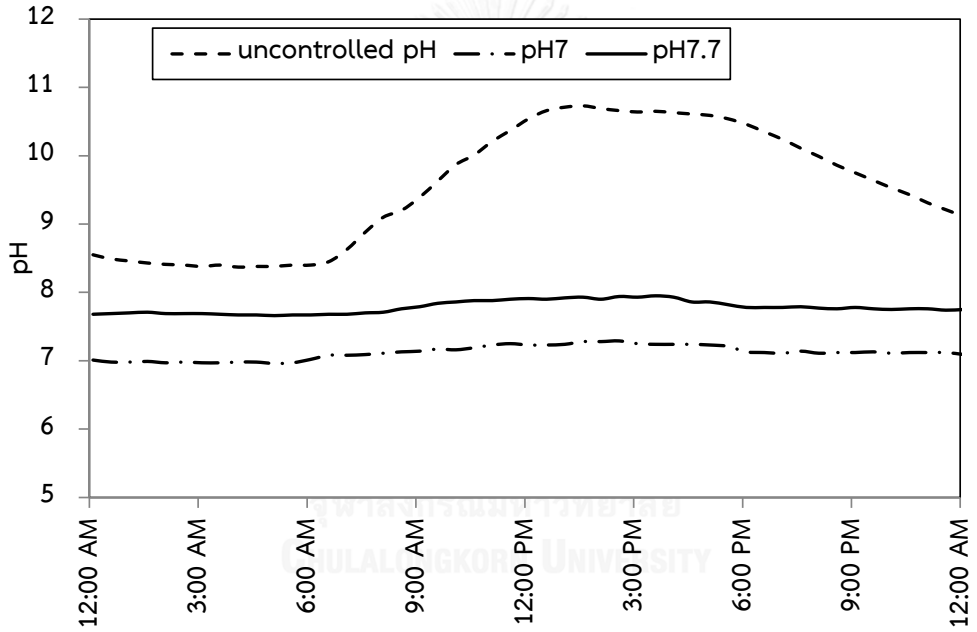

Figure5.11 pH profiles of *S. armatus* cultivated under outdoor condition: CO_2 supply to control pH set point value (7and 7.7)

Figure 5.12 illustrates the growth rate of *S. armatus*. During the first two days, the growth in the uncontrolled pH condition was higher than controlled pH conditions which were due to the acclimatization of the cells to the new environments (where CO_2 was intermittently supplied). After that growth rate under pH control system was higher than uncontrolled pH. This is because, in this pH control system, the pH was maintained between 7-7.7 where the inorganic carbon dissolved in the medium was in the form of CO_2 and HCO_3^-, both of which could be uptaken directly by the alga. On the other hand, the pH of the uncontrolled system raised to more than 10.3 during the day. At this pH range, the dissolved inorganic carbon is in carbonate form (CO_3^{2-}) which could not be utilized by the alga. This was reflected in the results where the medium with pH7 provided the highest average final biomass concentration of 0.37±0.01 g L^{-1} followed by that at pH 7.7 (0.34±0.01 g L^{-1}) and uncontrolled pH (0.30±0.02 g L^{-1}), respectively. The biomass productivities obtained from the system with pH7 and 7.7 were quite similar at 75.73±1.98 and 75.48±1.25 mg $L^{-1}d^{-1}$ whereas the uncontrolled pH condition provided the lowest productivity of 62.77±1.92 mg $L^{-1}d^{-1}$. In addition, the average carbon content in biomass and CO_2 fixation rate under controlled pH7 and 7.7 were higher than uncontrolled pH (see Table 5.4). Therefore, it was concluded at this point that the highest growth and carbon utilization were achieved by controlling the pH of the medium in the range of 7-7.7.

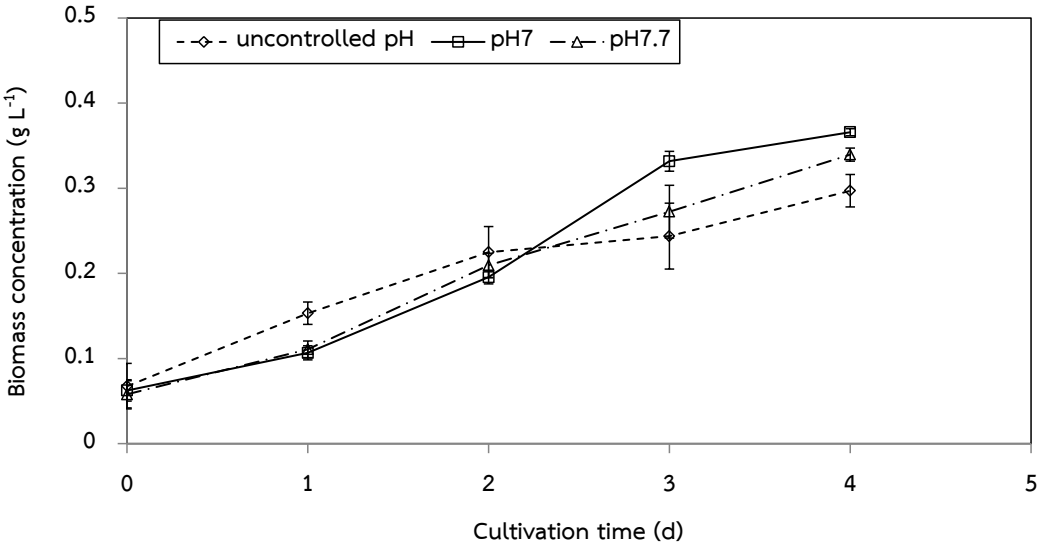

Figure5.12 Growth characteristics of *S. armatus* with controlled pH

5.2.2 Biochemical compositions with controlled pH

Biochemical compositions in biomass from the controlled pH condition were similar to those with the day time supply CO_2 only during daytime (Section 5.1.3) where lipid and protein increased but carbohydrate decreased (Figure 5.13). However, the productivities of such nutrients differed slightly from the concentration, where the productivity of lipid and protein increased significantly when pH was controlled, but the productivity of carbohydrate did not vary substantially due to the effect of biomass productivity (see results in Table 5.4). These results indicate that alkaline medium (uncontrolled pH) provided high carbohydrate whereas protein and lipid increased with pH was shifted to neutral. There was also a relationship between nitrogen uptake and pH level, i.e. more nitrogen was uptaken at neutral condition (86.12 mg N g cell^{-1}) compared with the basic condition obtained when the pH of the medium was not controlled (73.7 mg N g cell^{-1}). However, it is important to note that the biochemical compositions of the algal biomass depend significantly also on algal species. For example, the highest biomass composition, carbohydrate and protein contents of *Chlorella ellipsoidea* were obtained at pH10, pH9 and pH4, respectively, whereas biomass concentration, lipid, protein and carbohydrate contents of *Dunaliella bardawil* could be enhanced when microalgae were cultivated at pH7.5 (Khalil et al., 2010) and pH 7 for biomass concentration and lipid production of *Chlorella pyrenoidosa* (Han et al., 2013).

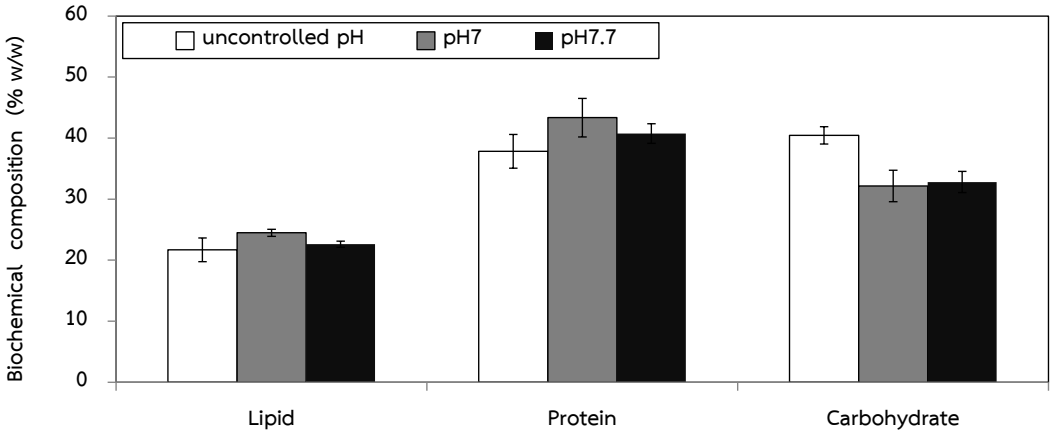

Figure5.13 Biochemical compositions with uncontrolled and controlled pH

Table5.4 Carbon content, carbon fixation rate, biomass and biochemical productivities from *S.armatus* cultivated with controlled pH

Condition	% C	CO_2 fixation rate (mg CO_2 $L^{-1}d^{-1}$)	Biomass productivity (mg $L^{-1}d^{-1}$)	Lipid productivity (mg L^{-1} d^{-1})	Protein productivity (mg L^{-1} d^{-1})	Carbohydrate productivity (mg L^{-1} d^{-1})
Uncontrolled pH	46.9±0.7	108±5	62.8±1.9	12.4±0.1	25.1±2.3	25.2±0.5
pH7	50.8±0.7	141±2	75.7±2.0	18.6±0.9	32.8±1.5	24.4±2.6
pH7.7	50.9±0.4	140±1	75.5±1.3	17.1±0.1	30.7±0.7	24.7±1

5.3 Concluding remarks

S. armatus was proven to be able to grow with a wide pH range of 6.4 to 11. CO_2 was used in two functions namely, CO_2 feeding during daytime and controlled pH. *S. armatus* cultivated with 2% CO_2 enriched air in flat panel airlift photobioreactor (FPAP) provided the highest the average of biomass productivity, CO_2 fixation rate and all biochemical productivities. Controlled pH between 7-7.7 provided similar tend with uncontrolled pH by CO_2 feeding during daytime.

Chapter VI

Effect of non-baffle airlift configurations on *S. armatus* growth

Typical airlift photobioreactors are equipped with draft tubes (for cylindrical geometry) or separator plates (for flat panel) to separate riser from downcomer areas. However, the need to have this separator leads to some operational disadvantages such as installation difficulty, cleaning, and maintenance. This work proposed a novel large scale airlift system without the separator called "Non-baffled airlift photobioreactor". This system was designed such that a pattern cyclic movement of fluid can be naturally induced without having to install physical separators. In this chapter, the two basic typical reactor configurations, i.e. cylindrical cone and flat panel, are selected as the model non-baffled airlift systems (as non-baffled cone airlift photobioreactors (NB-CAPs) and non-baffled flat panel airlift photobioreactors (NB-FPAPs)), and the growth of *Scenedesmus* in such non-baffled systems was examined.

6.1 Non-baffled cone airlift photobioreactors (NB-CAPs)

Non-baffled cone airlift photobioreactors (NB-CAPs) have a cylindrical shape with cone bottom as shown in Figure 6.1. The cylinder has the diameter of 100 cm and the height of 80 cm. The bottom cone inclined at the angle of 30, 45 and 53° with the x-plane, which is the design to minimize cell sedimentation. This conical design is beneficial for cell harvest particularly when the algal cells could be self-precipitated when stop aerating. The experiment was set out to investigate the effects of aeration rate and unaerated medium height on *S. armatus* cell growth.

(A) (B) (C)

Figure6.1 Experiments: (A) flow pattern; (B) constant volume of 100 L; (C) constant unaerated medium height of 75 cm

6.1.1 Effect of aeration rate

Air was supplied through porous sparger at the center of cone bottom which acted as a riser. A large quantity of bubbles disengaged at the liquid surface whilst some small bubbles re-entered and moving downward on the side surface of reactor which resembled a downcomer area (Figure 6.1). The air flow rate in the range of 0.1-0.3 vvm was investigated for the cultivation of *S.armatus* in a 100 L NB-CAPs. The maximum surface light intensity and maximum/minimum medium temperature were around 920-1500 µmole $m^{-2}s^{-1}$ and 31/27°C in cloudy and rainy days whereas these could be as high as 3200 -4800 µmole $m^{-2}s^{-1}$ and 38/27 °C, respectively in sunny days. Daily average surface energy and medium temperature were 12.9±5.4 MJ m^{-2} and 31.7±1.8°C Figure 6.2 illustrates growth rate with various angle of the cone bottom affected the growth. All of three angles bottom NB-CAPs (30, 45 and 45°) provided similar growth which could be that the ratio between the illuminated surface area and working volume (S/V) was in a similar range (Table 6.1). The inoculum started with the initial biomass of 0.03 g L^{-1}, and it took 1 day of lag phase before entering the exponential phase for another 6-7 days. The average final biomass concentration

and specific biomass productivity were found to vary with aeration rate as demonstrated in Table 6.2. The average final biomass concentration increased from 0.35±0.02 to 0.49±0.02 g L^{-1} when air flow rate increased from 0.1 to 0.2 vvm, this was due to an increasing liquid velocity that prevented cell precipitation. In contrast, the final concentration decreased to 0.44±0.01 g L^{-1} with increasing air flow rate to 0.3 vvm which could be due to the excessive shear stress induced at higher aeration rate that was damaging to the cell (Camacho et al., 2000). The highest average specific biomass productivities were in the range of 67.5-71.8 mg $L^{-1}d^{-1}$ with the highest occurred at 0.2 vvm of aeration (1-1.5 fold greater than those at 0.1 and 0.3 vvm). The results in this experiment indicate that the growth of *S. armatus* did not depend significantly with the changing of slope of the cone bottom, but rather, depended on the aeration rate where the optimum aeration rate was 0.2 vvm.

Liquid velocity in downcome ($v_{L,d}$) was highest in 53° NB-CAP followed by 45° and 30° NB-CAPs, respectively (Figure 6.3A) whereas $v_{L,d}$ was inverse with downcomer cross sectional area (A_d) (Figure 6.3B). A_d was independent of aeration rate, however, riser cross sectional area (A_r) increased obviously with increasing aeration rate from 0.1 to 0.2vvm and reached a constant level when aeration rate reached 0.3 vvm (Figure 6.3C). Due to an increasing amount of gas in the system, bubbles distributed more evenly in radial direction leading to a larger A_r. The highest A_r occurred in 30° NB-CAP followed by 45° and 53°, respectively. In other words, the ratio between A_d and A_r (A_d/A_r) became the highest in the 53° NB-CAP and was the lowest in the 30° NB-CAP. A_d/A_r decreased with increasing aeration rate (Figure 6.3D) and this seems to have negative influence on cell growth due to the bubble shading effect. For this case, the most suitable range of A_d/A_r for the growth of *S. armatus* in NB-CAPs of all configurations was in the range of 10-20 and $v_{L,d}$ of 5-15 cm s^{-1} with aeration rate of 0.2 vvm.

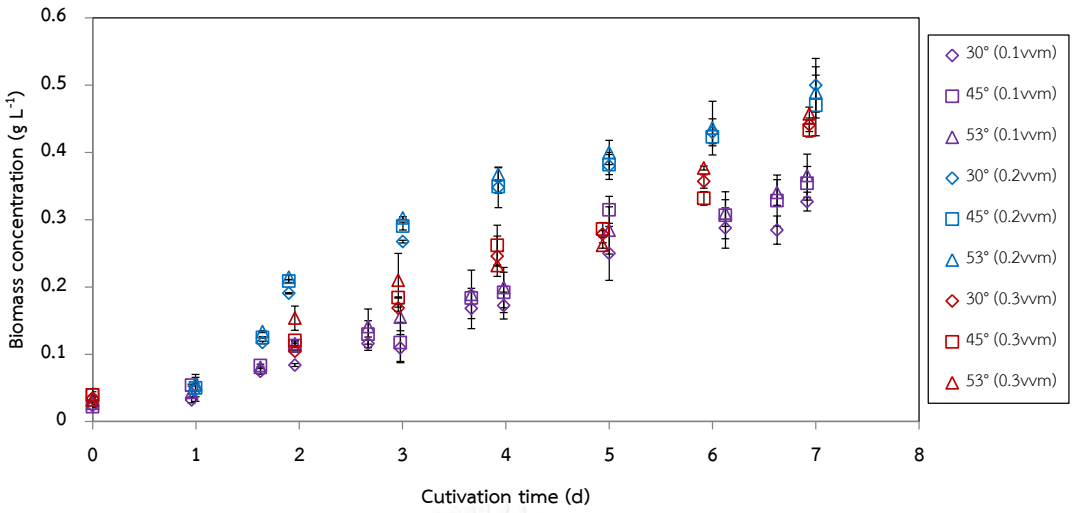

Figure6.2 Growth rate of *S. armatus* in 100 L NB-CAPs

Table6.1 Illuminated surface area per volume (S/V), liquid velocity in downcomer ($v_{L,d}$), A_d/A_r at aeration rate of 0.2 vvm with different unaerated medium height

Parameter	30°NB-CAP		45°NB-CAP		53°NB-CAP	
	H32 cm	H75cm	H46cm	H75cm	H56cm	H75cm
S/V (m^{-1})	17.7	7.3	15.9	8.3	14.5	9.9
$v_{L,d}$ (cm s^{-1})	8.6	19.0	11.6	14.1	16.6	17.6
A_d/A_r	9.8	1.2	13.4	6.5	20.8	15.9

Table6.2 Specific biomass productivity with various aeration rate and unaerated medium height

Bottom angle (°)	Constant volume 100 L (mg $L^{-1}d^{-1}$) (30°H32cm, 45°H46cm and 53°H56cm)			Constant medium height (mg $L^{-1}d^{-1}$) (H 75 cm)
	0.1 vvm	0.2 vvm	0.3 vvm	0.2 vvm
30	43.9±1.1[a]	71.8±4.1[b]	58.7±3.0[c]	32.8±2.9[d]
45	48.0±2.5[a]	67.5±4.6[b]	56.8±2.2[c]	34.0±2.2[d]
53	49.0±3.1[a]	70.2±3.9[b]	61.4±1.0[c]	38.9±3.3[d]

a, b, c, d are the significant level of differences of the results obtained from ANOVA and Tukey Method ($p<0.05$)

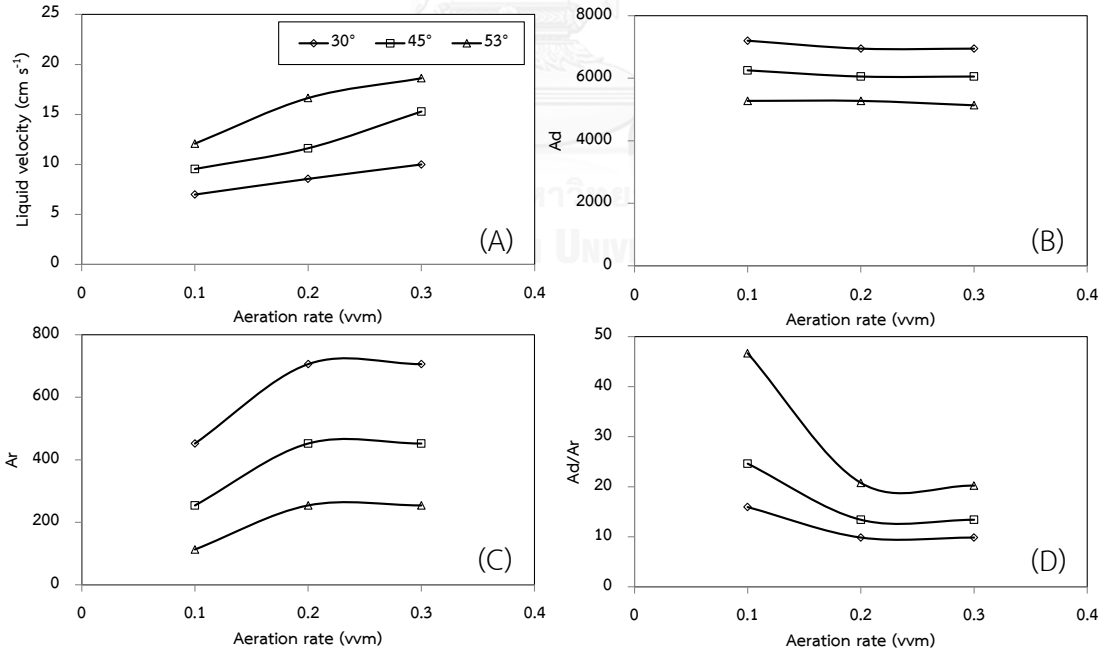

Figure6.3 Liquid velocity in downcomer (A); downcomer cross sectional area, A_d (B); riser cross sectional area, A_r (C); ratio A_d/A_r (D) with various aeration rate at 100 L of 30°, 45° and 53° NB-CAPs

6.1.2 Effect of medium height

To examine the effect of medium height, the experiments were carried out with a constant aeration rate of 0.2 vvm in all NB-CAPs. The average final biomass concentration (Figure 6.4, filled symbol) and average specific biomass productivity (Table 6.2) were 0.27±0.03 to 0.32±0.04 g L^{-1} and 32.8±2.9 to 38.9±3.3 mg $L^{-1}d^{-1}$, respectively. When compared with the results from the system with lower unaerated medium height (Figure 6.4, transparent symbol), it was found that the growth rate obtained from the system with higher unaerated medium height was only half of those obtained from the system with lower unaerated medium height. This could be due to several reasons. First, light could penetrate better into the system with lower liquid height especially when there was a growth of algae inside the system as described by Wang et al. (2015). Second, S/V decreased with increasing of unaerated height meaning that there was less light for the growth when the liquid level was high (Table 6.1). Third, Ad/Ar decreased with liquid height (Table 6.1) which could reduce the light utilization efficiency, as in a typical airlift system, there would exist a large swarm of bubbles in riser where cells could not utilize the light as effectively as in the downcomer (Kaewpintong et al., 2007). And finally, cells might die with higher distance of riser because cell could be captured by bubble and broke at the liquid surface (Camacho et al., 2000).

Even though the specific biomass productivity was low with higher unaerated medium height, a large scale operation was still preferable to enlarge the productivity. Therefore, the 30° NB-CAP was suggested for *S. armatus* outdoor cultivation as this configuration provided the largest working volume with the same liquid height when compared with the systems with other cone angles.

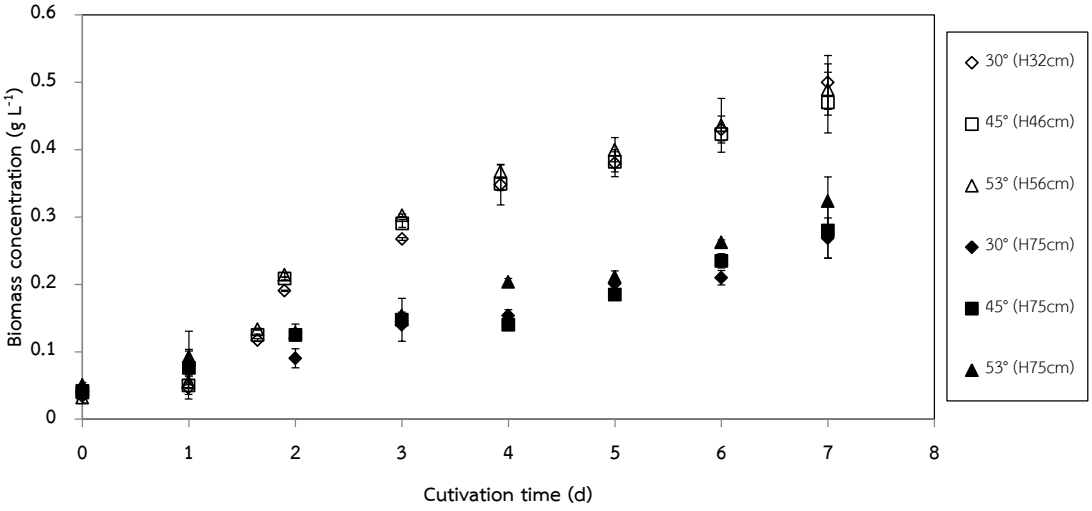

Figure6.4 growth rate of *S. armatus* with various unaerated medium height (H)

6.2 Non-baffle flat panel airlift photobioreactor (NB-FPAPs)

Non-baffle flat panel airlift photobioreactors (NB-FPAPs) were designed for easy operation particularly for large scale system where the installation of separator could exert some operational difficulty. In this case, air was supplied through a porous sparger located near the wall along the length of NB-FPAP. This enables the liquid to move up near one wall (as a riser) and moves down near the opposite wall (as downcomer) (Figure 6.5). The example of the flow-direction profile of the fluid in this reactor is illustrated in Figure 6.6. The optimum aeration rate for *S. armatus* was reported at 0.2 vvm by Rodrakhee (2013) which agreed well with that for NB-CAP as aforementioned.

To investigate the performance of NB-FPAP, the reactors of various widths and liquid heights were employed followed the method suggested by Khongkasem (2013), i.e. the width of the reactor (W) varied from 20, 30, 40 and 50 cm, whereas the unaerated medium height (H) varied from 40, 50 and 60 cm. NB-FPAPs were made from fiber glass which can reduce light intensity about 50% of transparent material (Figure 6.5). This material was chosen due primarily to economical and ease of fabrication/maintenance reasons. However, it was proven that, for some certain algal

species like *Haematococcus pluvialis*, the light shading could help enhance the productivity (Poonkum et al., 2015). Although this might not be the case for *S. armatus*, this reactor configuration still poses a distinct advantage due to its simplicity and ease of operation, and it can be employed to examine the effect of other operating parameters. Figure 6.7 illustrates the specific biomass productivity in this system. The reactor width particularly from 20 to 40 cm did not seem to pose notable effect on the specific biomass productivity and the growth did not vary significantly with reactor width. However, at 50 cm reactor width, the growth seemed to be enhanced significantly. On the other hand, the unaerated medium height clearly showed negative influence as a decrease in productivity was observed with an increasing medium height from 40 to 60cm. The highest average specific biomass productivities were around 34.2-45.5 mg $L^{-1}d^{-1}$ with 40 cm medium height of W20, W30 and W50, except at 40 cm (W40), the highest specific biomass productivity was around 38 mg $L^{-1}d^{-1}$ at 50 cm medium height. These values significantly decreased to 20-28 mg $L^{-1}d^{-1}$ when medium height increased to 60 cm of all reactors (W20 to W50). Several possible reasons might explain these results as follows:

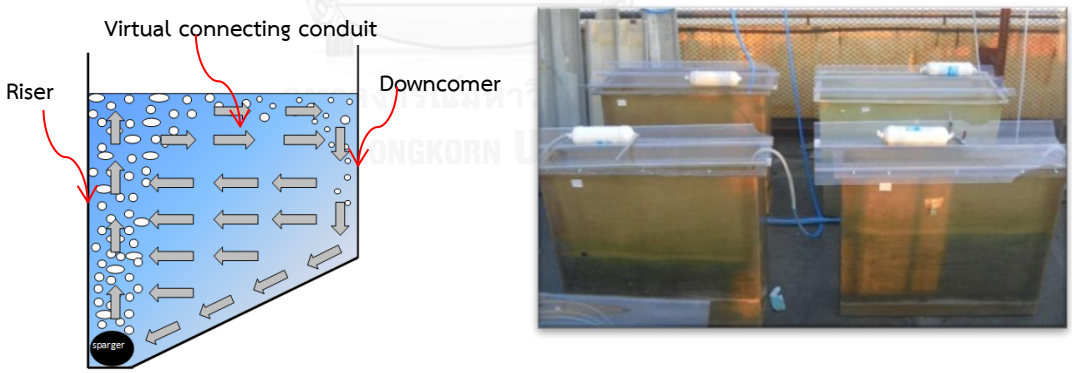

Figure6.5 Flow direction in NB-FPAP and experimental setup

w / h	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48	50
3	↑	→	→	→	→	→	→	→	→	→	→	→	→	→	→	↓	↓
6	↑	↑	→	→	→	→	→	→	→	→	→	→	→	→	↓	↓	↓
9	↑	↑	↑	→	→	→	→	→	→	→	→	→	→	↓	↓	↓	↓
12	↑	↑	↑	→	→	→	→	→	→	→	→	↓	↓	↓	↓	↓	↓
15	↑	↑	↑	↑	←	←	←	←	→	←	↓	↓	↓	↓	↓	↓	↓
18	↑	↑	↑	↑	←	←	←	←	↓	↓	↓	↓	↓	↓	↓	↓	↓
21	↑	↑	↑	←	←	←	←	←	↓	↓	↓	↓	↓	↓	↓	↓	↓
24	↑	↑	↑	←	←	←	←	←	←	↓	↓	↓	↓	↓	↓	↓	↓
27	↑	↑	↑	←	←	←	←	←	←	←	↓	↓	↓	←	↓	↓	↓
30	↑	↑	↑	←	←	←	←	←	←	←	←	↓	←	←	↓	↓	↓
33	↑	↑	↑	←	←	←	←	←	←	←	←	←	←	←	↓	↓	↓
36	↑	↑	↑	←	←	←	←	←	←	←	←	←	←	←	↓	↓	↓
39	↑	↑	↑	←	←	←	←	←	←	←	←	←	←	←	↓	↓	↓
42	↑	↑	↑	←	←	←	←	←	←	←	←	←	←	←	↓		
45	↑	↑	↑	←	←	←	←	←	←	←	←	←	←				
48	↑	↑	↑	←	←	←	←	←	←	←	←						
50	↑	↑	↑	←	←	←	←	←	←								

Figure6.6 Flow directions diagram in NB-FPAP at width of contactor 50 cm with unaerated liquid height 50 cm

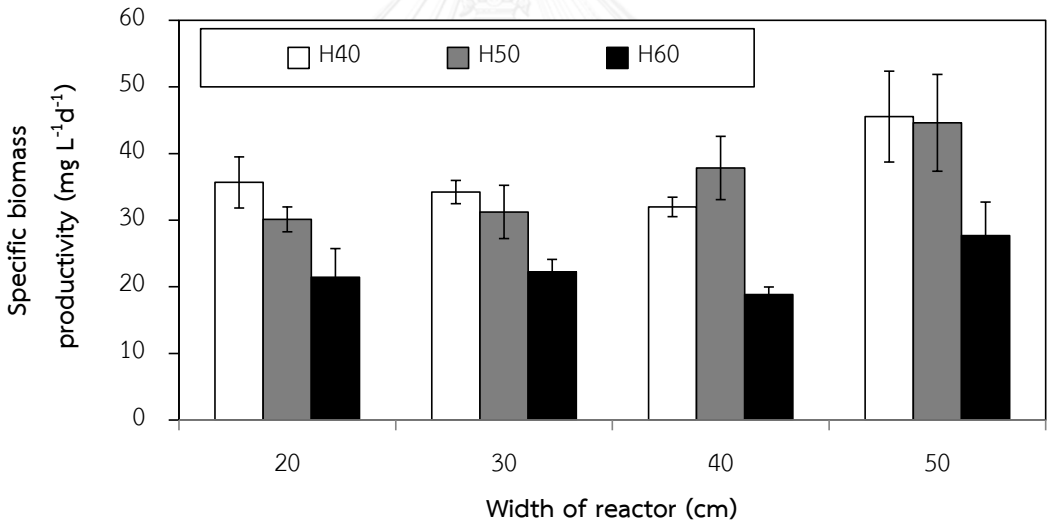

Figure6.7 Specific biomass productivity of *S. armatus* with various widths of NB-FPAPs (W) and unaerated medium heights (H)

An analysis of the specific surface area (S/V) (Figure 6.8) showed that the reactor of different width exhibited a large range of specific surface area, where S/V did not markedly change for the widths of 40 and 50 cm. This indicates that S/V did not exert significant effect on cell growth and the reason for a different growth characters at 50 cm must be due to some other factor.

Figure 6.9 displays the results from the analysis of A_d/A_r as reported by Sintharm (2013). This demonstrates that the airlift with different width/liquid height exhibited different flow pattern characteristics. At 20 cm reactor width, the width of the reactor was not large enough to induce the natural separation between riser and downcomer (as visually observed). A_d/A_r of approximately one indicates that the downcomer and riser areas were approximately in the same range, the behavior similar to that of bubble columns. This unclear flow pattern did allow cell precipitation which negatively influenced cell growth. Increasing reactor width enhanced the cyclic flow pattern in the system as observed from the large deviation in A_d/A_r. The results in Figure 6.7 demonstrate that the width of 50 cm and 40 cm liquid height provided the best growth behavior. Figure 6.9 shows that at this condition, A_d/A_r was close to unity meaning that riser and downcomer areas were almost equal. All other configurations would lead to a higher downcomer area. Unfortunately, this finding in A_d/A_r did not support the finding of better cell growth. However, it was observed from Figure 6.7 that cell growth seemed to be best when the reactor width was fixed at 50 cm regardless of the liquid height. It could mean that at this width, which was the maximum width employed in this experiment, the reactor obtained the highest sunlight from the liquid top surface (as it had the largest top surface) and this induced the growth in a much greater extent than other factors.

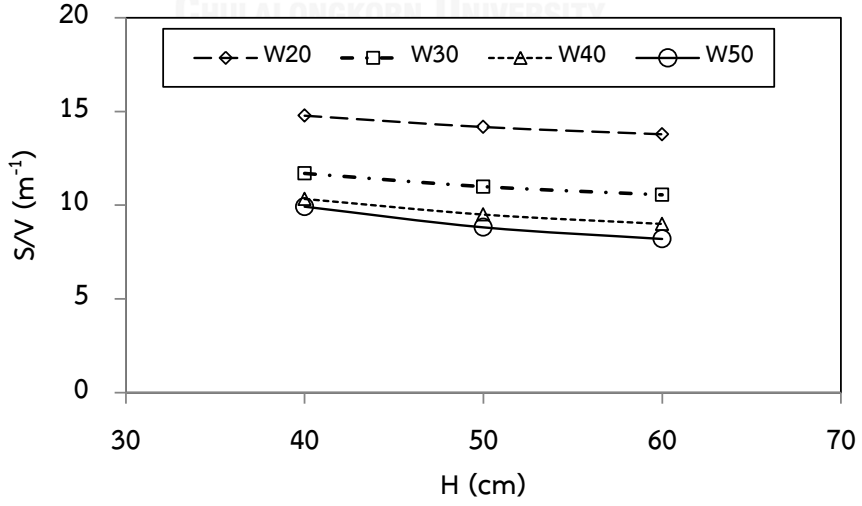

Figure6.8 Surface area per volume ratios (S/V) with various unaerated medium height (H) and width of reactor (W)

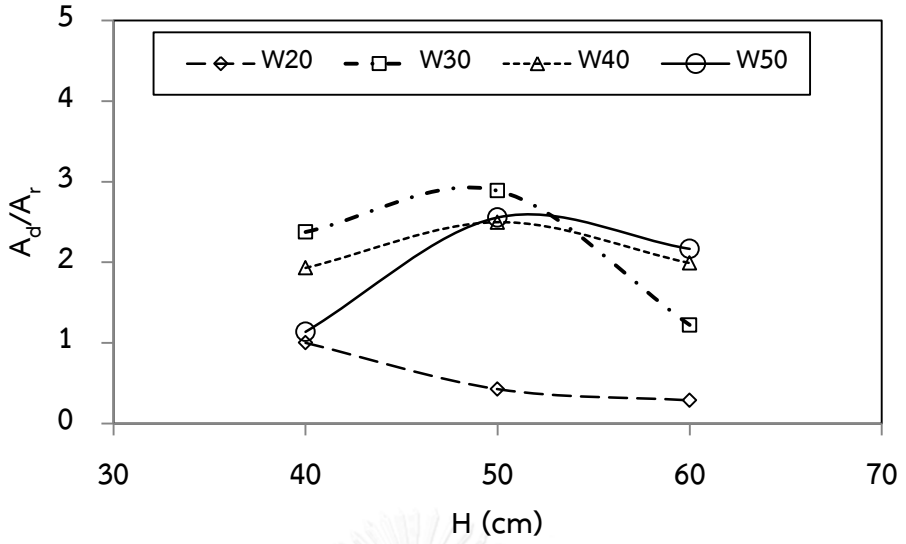

Figure6.9 A_d/A_r ratios with various unaerated medium height (H) and width of reactor (W)

6.3 Concluding remarks

Large scale of NB-CAPs and NB-FPAPs were effective for *S. armatus* cultivation. Growth of *S. armatus* was independent with the slope of the cone bottom NB-CAPs (30°, 45° and 53°). The widths of NB-FPAPs in the range of experiment (20 to 50 cm) were insignificant on the specific biomass productivity. It is noted that the width of 50 cm seemed to provide better growth due to the highest top surface for light exposure. While medium height did have significantly on growth both of all configurations. The optimum medium height of NB-FPAPs was 40-50 cm and NB-CAPs was 32 cm, 46 cm and 56 cm for 30°, 45°, 53° NB-CAPs, respectively.

Chapter VII

Conclusions and Contributions

7.1 Conclusions

Major findings obtained from this work can be summarized as follows:

1. The optimum of temperature, light intensity and u_{sg} in 2 L glass bubble column for *Scenedesmus armatus* indoor cultivation were 35^{O}C, 10 kLux and 1 cm s^{-1}, respectively. The biomass production and biochemical compositions from this condition for 7 days cultivation are summarized below:

Parameter	Value	Unit
Final biomass concentration	0.75±0.07	g L^{-1}
Specific biomass productivity	106±6.6	mg $L^{-1}d^{-1}$
specific growth rate	0.63±0.07	d^{-1}
Lipid	21.7±1.0	%w/w
Protein	32.9± 2.6	%w/w
Carbohydrate	45.4±1.6	%w/w

2. Flat panel airlift photobioreactors (FPAP) was employed for a large scale cultivation where sizing of FPAP was 17 L for indoor and scaled up to 100 L for outdoor. The specific biomass productivity decreased from 70.4±9.6 mg $L^{-1}d^{-1}$ in the 17L to 41.7±6.6 mg $L^{-1}d^{-1}$ in the 100 L systems. The size did not have significant influence on biochemical contents of the biomass.

3. The optimal condition of superficial gas velocity for 100 L FPAP outdoor culture was 0.35 cm s^{-1} in batch bode. The highest actual growth rate occurred at around 9:00 AM to 3:00 PM. The alga seemed to be able to grow under a wide pH range of 6.4 to 11.

4. S*. armatus* could well grow in summer when compared with the growth in rainy season and in winter. However, both specific biomass productivities and biochemical contents did not statistically change with seasons ($p>0.05$) as follows:

Season	Surface energy ($MJ\ m^{-2}d^{-1}$)	Medium temperature (^{o}C)	Biomass productivity ($mg\ L^{-1}d^{-1}$)	Lipid content (%w/w)	Protein content (%w/w)	Carbohydrate content (%w/w)
Summer	25.2±7.8	35.0±2.4	59.3±6.7[a]	19.8±1.2[a]	37.8±5.1[a]	42.5±5.5[a]
Rainy season	17.7±6.1	32.6±1.7	45.1±10.8[a]	22.6±2.1[a]	36.4±2.7[a]	40.9±2.4[a]
Winter	18.8±4.1	31.8±2.1	39.2±8.5[a]	22.9±1.3[a]	37.5±1.3[a]	39.6±2.1[a]

5. Growth of *S. armatus* was tested with varying CO_2 concentration in the range of 2 to 15% by volume of aeration rate with 100 L FPAP at daily light energy and medium temperature of 23.8±6.1 MJ m^{-2} and of 34.5±1.4 ^{o}C for 4 days. The aeration with 2% enriched CO_2 provided the highest average specific biomass productivity of 91.3 mg $L^{-1}d^{-1}$, where the CO_2 fixation rate was 165±15 mg CO_2 $L^{-1}d^{-1}$. Lipid and protein contents increased with CO_2 supply, but carbohydrate decreased where specific biochemical productivities at 2% CO_2 were 22.24±0.57 mg lipid $L^{-1}d^{-1}$, 38.34±5.90 mg protein $L^{-1}d^{-1}$ and 30.67±2.37 mg carbohydrate $L^{-1}d^{-1}$.

6. The experiments with pH control at 7-7.7 by adding 2% CO_2 under the average light energy and medium temperature of 19.3±1 MJ $m^{-2}d^{-1}$ and 33.9 ±1^{o}C for 4 days yielded the highest biomass production and carbon utilization of 75.5-75.7 mg $L^{-1}d^{-1}$ and 140-141 mg CO_2 $L^{-1}d^{-1}$. Similar to the case with CO_2 supplement, the experiment with pH control resulted in a higher accumulation of lipid and protein, but a lower carbohydrate content.

7. The optimal aeration rate for *S. armatus* in Non-baffled cone airlift photobioreactors (NB-CAPs) was 0.2 vvm. The cultures grew under the average light energy and medium temperature of 12.9±5.4 MJ $m^{-2}d^{-1}$ and 31.7±1.8^{o}C. Growth was independent of the slope of the cone bottom NB-CAPs (30°, 45° and 53°). Specific biomass productivity was in the range of 32.8±2.9 to 38.9±3.3 mg $L^{-1}d^{-1}$ at medium

height 75 cm. The 30° NB-CAP was suggested for *S. armatus* outdoor cultivation due to the highest biomass productivity with the largest working volume.

8. *S. armatus* was cultivated using Non-baffled flat panel airlift photobioreactors (NB-FPAPs) made from fiberglass with various the widths of the reactor from 20, 30, 40 and 50 cm and the unaerated medium heights from 40, 50 and 60 cm. The optimal medium height of NB-FPAPs was 40-50 cm whereas the width of 50 cm seems to provide better growth due to the highest top surface for light exposure. The specific biomass productivities were around 44.6-45.5 mg $L^{-1}d^{-1}$.

9. The advantages and disadvantages of all reactor configurations for microalgal cultivation as follows:

Reactor	Advantages	Disadvantages
FPAP	• certain flow direction • easy to scale up	• prone to Dead zone • difficult to install and maintain
NB-CAP	• less area • easy to install and maintain	• difficult to scale up
NB-FPAP	• easy to scale up • easy install and maintain	• limit of medium height

7.2 Contributions

This research shows that *Scenedesmus armatus* is a potential green alga for large-scale outdoor cultivation system. The alga could grow well with a very small problem of contamination in varying tropical environmental conditions with a simple reactor configuration. This means it can be cultivated economically in a large scale culture without the need for a complicating sterilizing system. This allows a large scale culture with lower capital investment and lower maintenance costs. It can also be used to effectively fix CO_2 as a means to capture the greenhouse gas. The alga also contains reasonable amounts of nutrients which can be further used in various

applications especially food additives. This can be important considering the problem of food security in the present increasing global population. A relatively high lipid content also suggests that the algal biomass can also be used as a raw material for biofuel.

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APPENDIX

Measurement of nitrogen concentration by spectrophotometer

(Greenberg et al., 1992)

Blank

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

Calibration

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.

KNO_3 stock is diluted using distilled water as 0.5, 1.0, 2.0, 2.5, 3.0, and 4.0 mg NO_3-N/L.

The solution is measured by spectrophotometer at wavelength of 220 and 275 nm.

The correlation between Absorbance and nitrate concentration is

$$\textbf{Absorbance} = 0.25142^{*} [\text{Nitrate-N concentration}],\ R^2 = 0.9950$$

where Absorbance = Absorbance at 220nm-Absorbance at 275nm (Agilent Cary 60 UV-Vis Spectrophotometer)

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.

and blank is set to zero.

VITA

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