ถังปฏิกรณ์ชีวภาพอากาศยกแบบแบนไร้แผ่นกั้นแบบใหม่สำหรับการเลี้ยง
Ankistrodesmus sp. และ *Scenedesmus* sp. ในระบบกลางแจ้ง

นายเอกชัย คงเกษม

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิศวกรรมศาสตรมหาบัณฑิต
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ปีการศึกษา 2556

NOVEL NON-BAFFLED FLAT PANEL AIRLIFT PHOTOBIOREACTOR FOR OUTDOOR CULTURES OF *Ankistrodesmus* sp. and *Scenedesmus* sp.

Mr. Eakkachai Khongkasem

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Engineering Program in Chemical Engineering
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Thesis Title	NOVEL NON-BAFFLED FLAT PANEL AIRLIFT PHOTOBIOREACTOR FOR OUTDOOR CULTURES OF *Ankistrodesmus* sp. and *Scenedesmus* sp.
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เอกชัย คงเกษม : ถังปฏิกรณ์ชีวภาพอากาศยกแบบแบนไร้แผ่นกั้นแบบใหม่สำหรับการเลี้ยง *Ankistrodesmus* sp. และ *Scenedesmus* sp. ในระบบกลางแจ้ง. (NOVEL NON-BAFFLED FLAT PANEL AIRLIFT PHOTOBIOREACTOR FOR OUTDOOR CULTURES OF *Ankistrodesmus* sp. and *Scenedesmus* sp.) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร.ประเสริฐ ภวสันต์, 104 หน้า.

การเลี้ยงจุลสาหร่าย *Ankistrodesmus* sp. และ *Scenedesmus* sp. ในถังปฏิกรณ์ชีวภาพอากาศยกแบบแบนไร้แผ่นกั้น (NB-FP-ARPBR) ในสภาวะกลางแจ้ง ศึกษาผลการเจริญเติบโตที่มีผลต่อการออกแบบของถังปฏิกรณ์ชีวภาพในเชิงความกว้างที่แตกต่างกัน (20, 30, 40 และ 50 ซม.) และความสูงที่แตกต่างกัน (40, 50 และ 60 ซม.) ซึ่งตัวแปรที่อาจมีผลต่อสภาวะการเลี้ยง เช่น พฤติกรรมการไหลของของไหล ความเข้มแสง อุณหภูมิ ตลอดจนการดำรงชีวิตของสาหร่ายเอง จากการทดลองพบว่า ขนาดของถังปฏิกรณ์ชีวภาพมีผลต่อการเจริญเติบโตของทั้งสองสาหร่ายทางสถิติอย่างมีนัยสำคัญ มีการเจริญเติบโตสูงสุดที่ความกว้าง 50, 40, 30, และ 20 ซม. ตามลำดับ แต่อย่างไรก็ตาม เฉพาะที่ระดับความสูง 40 ซม. *Scenedesmus* sp. มีการเจริญเติบโตสูงสุดที่ความกว้าง 50, 20, 40 และ 30 ซม. ตามลำดับ และส่วนในเชิงความสูงนั้นพบว่า มีการเจริญเติบโตสูงสุดที่ 50, 40 และ 60 ซม. ตามลำดับ นอกจากนั้นแล้วยังมีการวิเคราะห์องค์ประกอบทางชีวเคมีของสาหร่าย พบว่าไม่มีความสัมพันธ์โดยตรงระหว่างองค์ประกอบทางชีวเคมีกับขนาดของถังปฏิกรณ์ชีวภาพ จุลสาหร่ายทั้งสองมีโปรตีนเป็นองค์ประกอบหลัก (35-45%) รองลงมาเป็นไขมัน แต่ปริมาณคาร์โบไฮเดรตจะขึ้นอยู่กับปริมาณแสงที่ได้รับ การเลี้ยงสาหร่ายจะมีค่าปฏิบัติการเลี้ยงเป็นค่าต้นทุนเป็นส่วนใหญ่ (43%) ค่าไฟฟ้า (32%), ค่าอาหาร (23%) และค่าน้ำ (2%) รวมค่าใช้จ่ายทั้งสิ้น 420.20 US$ ต่อปี

ภาควิชา วิศวกรรมเคมี ลายมือชื่อนิสิต .....

สาขาวิชา วิศวกรรมเคมี ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

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EAKKACHAI KHONGKASEM: NOVEL NON-BAFFLED FLAT PANEL AIRLIFT PHOTOBIOREACTOR FOR OUTDOOR CULTURES OF *Ankistrodesmus* sp. and *Scenedesmus* sp.. ADVISOR: ASSOC. PROF. PRASERT PAVASANT, Ph.D., 104 pp.

The cultivations of both *Ankistrodesmus* sp. and *Scenedesmus* sp. were carried out in a large scale non-baffled flat plate airlift photobioreactor (NB-FP-ARPBR). This placed a focus on the design effect of the reactor using the reactors of different widths, i.e. 20, 30, 40 and 50 cm and different unaerated liquid heights i.e. 40, 50 and 60 cm, operated under Thailand climate conditions. As a whole, the configuration of the NB-FP-ARPBR had an influence on fluid flow behavior, light availability, temperature and other factors affecting the livelihood of microalgae. The size of reactor appeared to be statistically important for the growth in NB-FP-ARPBR for both *Ankistrodesmus* sp. and *Scenedesmus* sp. that grew best in NB-FP-ALPBR with the width of 50, followed by those with 40, 30 and 20 cm, respectively. However, *Scenedesmus* sp. was on exception at the unaerated liquid height of 40 cm where the growth rate was ordered from high to low as 50, 20, 40 and 30 cm, respectively. In terms of liquid height, both microalgae grew best in NB-FP-ALPBR with the height of 50, followed by those with 40 and 60 cm, respectively. There was no direct relationship between biochemical compositions with the configuration of NB-FP-ALPBR. Protein seemed to be the major composition (35-45%) whereas lipid was the minor. Carbohydrate was found to be rich when there was adequate light for the growth. The total cost was approx. 420.40 US$ per year where the major costs for the cultivation of *Ankistrodesmus* sp. and *Scenedesmus* sp. was the capital cost which contributed almost 43% of the total investment cost, followed by those with electricity (32%), nutrient (23%) and water (2%).

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

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

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CHAPTER I

Introduction

1.1 Motivations

Algae are unicellular microorganisms with high chlorophyll content and use carbon dioxide in the Photosynthesis process. Algae have attracted much interest as an alternative for several natural chemicals such as carbohydrates, proteins, vitamins, minerals, and antioxidants. Some algae can accumulate a large quantity of oil which is considered to be one of the future possible alternatives fuel source. With proper control of environment, algae can grow with a faster growth rate than other in-land plants. In addition, algae can be harvested daily; the culture did not require arable land; and they can grow anywhere (Mata *et al.*, 2010). This is why algae were considered to become potential bio-fuel in the future compared with other potential sources such as soybean, corn, oil palm and others. In addition, algae are regarded as one alternative source of food, bioactive compounds and also for their usefulness in cleaning the environment (Ugwu *et al.*, 2008). *Ankistrodesmus* sp. is a unicellular green microalga, which can accumulate the total lipid content up to 24% (Mata et al., 2010). On the other hand, *Scenedesmus* sp. effectively produces useful substances like carbohydrates, proteins and lipid (Singh *et al.*, 2011).

Airlift photobioreactors have been proven to be an effective alternative for the cultivation of microalgae (Krichnavaruk *et al.*, 2007, Kaewpintong *et al.*, 2007, Issarapayup *et al.*, 2009). This system might be due to several main advantages over the other types of reactors. For example, the operation causes lesser stress force, good mixing, well-defined fluid flow pattern, relatively high gas-liquid mass transfer rate and low cost (Krichnavaruk *et al.*, 2005). Also, this system is easy to maintain. The mixing in the airlift photobioreactor could be obtained without causing too much shear force in the liquid phase, which could inhibit the growth of the algae. In addition, it was mentioned that the well-defined circulation pattern between riser and downcomer result in a better light utilization particularly for the system with high density of algal cell (Merchuk *et al.*, 1998). Moreover, airlift was also proven to be successfully scaled up without losing much of the cultivating performance. However, typical performance of large-scale systems is significantly different from the small scale (Wongsuchoto *et al.*, 2003, Ugwu et al., 2008, Ruen-ngam *et al.*, 2008) and quite often the productivity of the alga varies inversely with the size of the reactor (Harker *et al.*, 1996, Río *et al.*, 2005, Del Río *et al.*, 2008, Kaewpintong et al.,

2007, García ‐ Malea *et al.*, 2009, García-Malea *et al.*, 2005, López *et al.*, 2006, Ranjbar *et al.*, 2008). However, flat plate airlift photobioreactor with draft tube growth has decreased in size possibly due to the inevitable non-uniformity of fluid in the large scale (Issarapayup et al., 2009). For this reason, airlift photobioreactor without draft tube will be suitable for culture of algal cell.

This study aimed to investigate the optimal growth behavior of *Ankistrodesmus* sp. and *Scenedesmus* sp. in non-baffled flat panel airlift photobioreactors (NB-FP-ALPBR) under outdoor condition using solar energy as a sole light source under Thailand climate conditions. This placed a focus on the design effect of the reactor using the reactors of different widths, i.e. 20, 30, 40 and 50 cm and different unaerated liquid heights i.e. 40, 50 and 60 cm. As a whole, the configuration of the NB-FP-ARPBR had an influence on fluid flow behavior, light availability, temperature and other factors affecting the livelihood of microalgae. The properties of the obtained microalgae also were analyzed as well as economic analysis for the cultivation.

1.2 Objectives

1.2.1 Optimize the configuration of the large scale non-baffled flat panel airlift photobioreactor, i.e. width and height of the airlift, for the growth of green algae (*Ankistrodesmus* sp. and *Scenedesmus* sp.) under outdoor condition

1.2.2 Evaluate economics of large scale NB-FP-ALPBR on the cultivation of both algae

1.3 Scopes

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

  - *Ankistrodesmus* sp. and *Scenedesmus* sp. was chosen for this study.
  - The algae were cultivated in batch cultivation.
  - The experiment was performed with non-baffled flat panel airlift photobioreactor located in Bangkok Thailand.

- The effect of the reactor width was investigated using the reactors with different width, i.e. 20, 30, 40 and 50 cm (working volume 65-220 L).
- The superficial gas velocity (U_{sg}) was fixed at 0.16 cm s^{-1}.

CHAPTER II

Backgrounds and literature review

2.1 Microalgae

The size of most microscopic algae ranges from about 3 - 100 micrometers. Microalgae can live in a wide range of environmental conditions, not only aquatic but also terrestrial. Microalgae live in saline and fresh water and convert sunlight, water and carbon dioxide to biomass. All microalgae contain protein, carbohydrate, lipid, nucleic acid, vitamin including antioxidants while the proportions are vary with the species of algae. Many microalgae species can accumulate substantial amount of compositions (Demirbas & Fatih Demirbas, 2011). Chemical compositions of the microalgae are different from species to species as show in Table 2.1**.** Utilization of these chemical composition variables, e.g. use as raw material for biodiesel or bioethanol production, or as protein supplement in food and animal feed as well as application in cosmetics and pharmaceutical industry as well.

Table 2.1 composition of green microalgae

Strain	Protein (%)	Carbohydrate (%)	Lipid (%)	Reference
Scenedesmus obliquus	50-60	10-17	12-14	(Singh et al., 2011).
Scenedesmus quadricauda	47	-	1.9	(Singh et al., 2011).
Scenedesmus dimorphus	8-18	21-52	16-40	(Singh et al., 2011).
Ankistrodesmus sp.	-	-	24-31	(Mata et al., 2010)
Ankistrodesmus TR-87	-	-	28-40	(Demirbas & Fatih Demirbas, 2011).
Chlorella vulgaris	51-58	12-17	14-22	(Singh et al., 2011).
Spirogyra sp.	6-20	33-64	11-21	(Singh et al., 2011).
Dunaliella bioculate	49	4	8	(Singh et al., 2011).

The advantages of microalgae are as followings: (Demirbas & Fatih Demirbas, 2011, Gong & Jiang, 2011, Mata et al., 2010)

- ✓ Microalgae grow rapidly and contain high content of lipids.
- ✓ Microalgae grow anywhere and live in harsh conditions.
- ✓ The culture could be used to treat agricultural wastewaters that contain excess nitrogen and phosphorus nutrients.
- ✓ Microalgae can be harvested daily.
- ✓ Microalgae produce useful by-products including long-chain polyunsaturated fatty acids, carotenoids for foodstuffs.
- ✓ The cultivation of microalgae does not require arable land.
- ✓ Microalgae can be used to fix CO_2 and reduce carbon emission via photosynthetic process.
- ✓ When used as biofuel, algae release minimal concentrations of sulfur dioxide, nitrous oxide and other contaminants.

2.1.1 *Ankistrodesmus* sp.

Ankistrodesmus sp. is a green alga under the scientific classification as detailed in Table 2.2. *Ankistrodesmus* cells are long and needle or spindle-shaped, or sometimes curved or slightly crescent-shaped depending on the growth conditions (Figure 2.1). The cells lack mucilage and may be found individually, clustered, twisted around each other, or in tufts among other algae. The parietal chloroplasts sometimes have pyrenoids. *Ankistrodesmus* is a form of asexual reproduction by autospore formation and colony fragmentation, the parent cells divides to give the new cell. Cells rarely solitary, mostly in few to many celled colonies with 4-16 cells that develop into new cells. *Ankistrodesmus* have contained essential nutrients such as protein, minerals, lipid, minerals, fiber, essential amino acids, and polyunsaturated fatty acid. The amount of crude protein and lipid of *Ankistrodesmus convoiutus* cultured in effluent mediums were higher than control. *Ankistrodesmus convoiutus* contained most of the polyunsaturated fatty acids of C18 and C20 (Habib *et al.*, 2004). Sipaúba-Tavares & Pereira, (2008) reported that *Ankistrodesmus gracilis* was used as food source for fish larvae due to its relatively high protein content. Radakovits *et al.,* (2010) reported that *Ankistrodesmus densus* secretes

polysaccharides when exposed to light during stationary phase. National institute for environmental studies (2011) reported that *Ankistrodesmus densus* TISTR 8505 found at the first time at Nakornpathom, Thailand, which appropriate cultivation conditions as temperature is 28°C and light intensity is 60 µmol photons m^{-2} s^{-1}.

Table 2.2 Scientific classification of *Ankistrodesmus* sp.

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

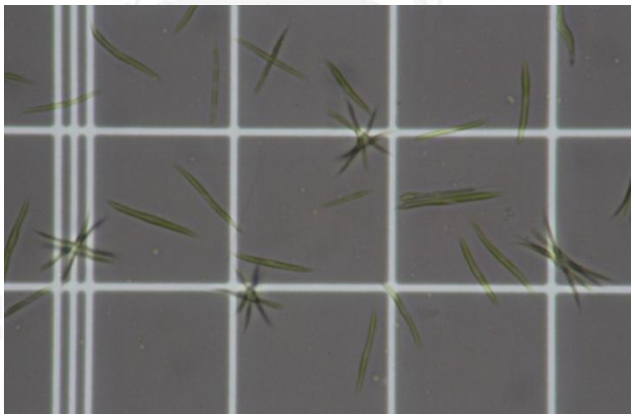


Figure 2.1 *Ankistrodesmus* sp.

2.1.2 *Scenedesmus* sp

Scenedesmus sp. is a green photoautotrophic and unicellular microalgae under the scientific classification as detailed in Table 2.3. *Scenedesmus* sp. is commonly found in the plankton of freshwater rivers, ponds, and lakes, and sometimes in brackish habitats. This is a genus of green algae whose coenobia are a flat plate of ellipsoidal to spindle-shaped cells that are arranged in a single,

alternating or double series with their long axis parallel to one another (Figure 2.2). The number of cells per coenobium is always a multiple of two, usually 4, 8, 16 or 32. The cell walls may be smooth, corrugated, granulate or spicate. Some are with marginal or lateral spines or teeth. The chloroplast in young cells is laminate that has a single pyrenoid. The chloroplast in old cells fill up the entire cavity. Growth may be dense in nutrient- rich waters but is not typically considered a nuisance. Like many other algae, *Scenedesmus* is an important primary producer and food source for higher trophic levels use as animal feed due to its relatively high protein content. 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). *Scenedesmus almeriensis* a potential candidate make lutein production for commercial scale. For outdoor cultivation, the selecting strain should grow reasonably well under normal environmental conditions. For Thailand, the culture of *Scenedesmus armatus* TISTR 8591 is quite interesting because this specie of alga is a rapid growing local species with minimum requirement for system maintenance.

Table 2.3 Scientific classification of *Scenedesmus* sp.

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

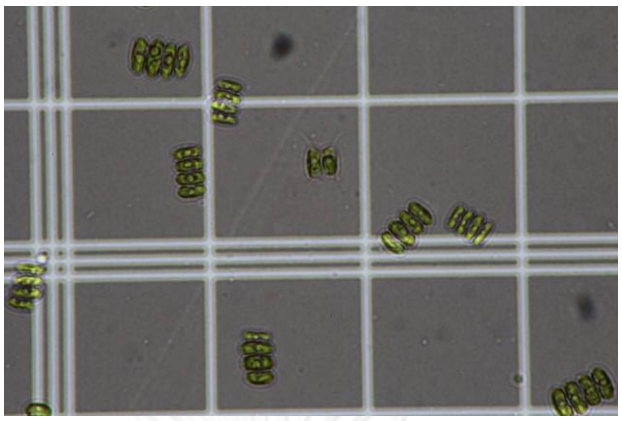


Figure 2.2 *Scenedesmus* sp.

2.2 Photosynthesis in microalgae

Photosynthesis are light energy is converted into chemical energy stored in chloroplasts. Four major classes of pigments consist of chlorophylls, carotenoids, phycobilins and phycoerythrin which absorb light in the range of 450-475 (green), 630-675 (green), 400-550 (yellow, orange) , 500-650 (blue, red) and 500-600 nm (yellow), respectively (Carvalho *et al.*, 2011). This is shown in Figure 2.3. All photosynthetic organisms (plants, certain protistans, prochlorobacteria, and cyanobacteria) have chlorophyll *a*. Accessory pigments include chlorophyll *b* (also *c*, *d*, and *e* in algae and protistans), xanthophylls, and carotenoids (such as beta-carotene) are absorb some of the energy in the green wavelength.

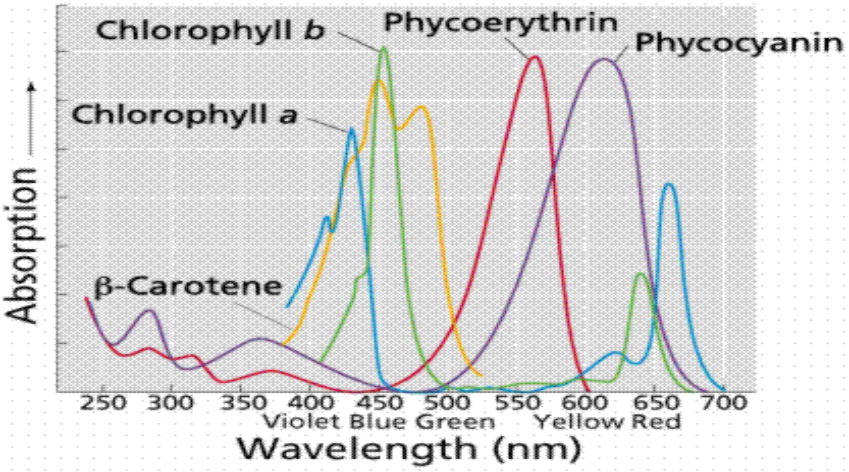


Figure 2.3 Absorption spectrum of several plant pigments

Photosynthesis is a two steps mechanism, light-dependent reaction occured in thylakoids membrane and light-independent reactions or known as the Calvin cycle or carbon dioxide fixation process occur in stoma (Figure 2.4). Photosynthesis uses light energy, carbon dioxide and water to produce end products, e.g. carbohydrate (sugar) and oxygen. The chemical equation of reaction is shown below

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

In light dependent processes, light strikes chlorophyll a in such a way as to excite electrons to a higher energy state. In a series of reactions the energy is converted into ATP and NADPH. Water is split in the process, releasing oxygen as a by-product. The ATP and NADPH are used to make C-C bonds in the light independent process (dark reactions). Photosystems are arrangements of chlorophyll and other pigments packed into thylakoids. Algae have Photosystem II plus Photosystem I. Photosystem I use chlorophyll a, in the form referred to as P700. Photosystem II uses a form of chlorophyll a known as P680. Both "active" forms of chlorophyll a function in photosynthesis due to their association with proteins in the thylakoid membrane.

In the light independent process, carbon dioxide from the captured and modified by the addition of hydrogen to form carbohydrates. The incorporation of carbon dioxide into organic compounds is known as carbon fixation. The energy for this comes from the first phase of the photosynthetic process. Living systems cannot directly utilize light energy, but can, through a complicated series of reactions, convert it into C-C bond energy that can be released by glycolysis and other metabolic processes.

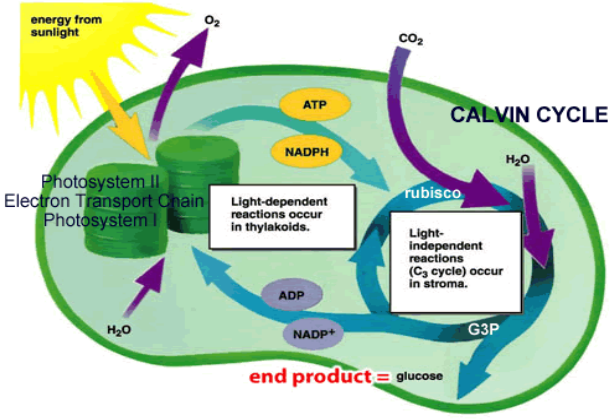


Figure 2.4 Major products of the light and dark reactions of photosynthesis

2.3 Process factors

The biochemical composition of algae is generally affected by cultivation conditions and many other factors such as nutrient composition, light intensities, pH, aeration, gas mass transfer and shear, inoculum and temperature, etc.

2.3.1 Light

Light intensity plays an important role for algae, light is the source of energy which drives photosynthetic reaction. The intensity of light, spectral quality and photoperiod should be considered. A long lighting period can increase biomass concentration including the amount of proteins, carbohydrates and lipid in cell (Fábregas *et al.*, 2002, Seyfabadi *et al.*, 2011). The light intensity must be increase at higher depth and cell concentration of algae.

2.3.2 Temperature

Temperature is also an important parameter influencing the biochemical composition of algae in outdoor culture. The temperature range for the growth of microalgae is between 15 and 32°C. Generally, the optimal temperature ranges between 22 and 30°C and are species dependent (Ong *et al.*, 2010). At temperature above 35°C, the growth rate decreases, perhaps due to inactivation of intercellular enzymes, cell damage and solubility of gases. Lower temperature results in suboptimum growth. Most large scale microbial cell are exothermic with heat generated due to growth, activity and power input by aeration.

2.3.3 pH

Generally, microalgae grow well at neutral pH but some species can grow at higher and lower pH such as *Spirulina platensis* (pH 9) (Sydney *et al.*, 2010) and *Chlorococcum littorale* (pH 5.5) (Iwasaki *et al.*, 1996), respectively. In outdoor culture, carbon dioxide is consumed by the algae and end products from light reaction such as ATP and NATPH are used as energy sources. This reduction in CO_2 resulted in a decrease in the pH level of the solution. At night however, there is no light reaction and therefore CO_2 cannot be up taken, and an increase in the solution pH can be observed. The pH is also indicative of the growth of the microalgae. A high pH

indicates that the microalgae have a high carbon dioxide uptake that may be due to microalgae proliferation.

2.5.4 Gas-liquid mass transfer and shear

Optimum cultivation of algae in any bioreactor requires proper supply and distribution of nutrients, carbon dioxide transfer as well as a means to prevent cell sedimentation. The problems encountered such as some anaerobic zones, heterogeneous mixing and low cell mass can lead to suboptimum mixing characteristics and lower growth efficiency (Scragg, 1995). The use of high air superficial velocity in most instances has resulted in high shear, more turbulent-like flow and low productivity (Acién Fernández *et al.*, 2001, Tanaka, 1981). During process scale-up, the size and the geometry of tank are altered and so one must consider and take into account these change because they also result in increased hydrostatic pressure between the top and the bottom of the tank. However, for large scale operation, such modifications have influenced the solubility of gas (Scragg, 1995, Tanaka, 1987).

2.3.5 Nutrients

- **Carbon**

Carbon supplied as inorganic substrate in form of carbon dioxide (CO_2), bicarbonate ($NaHCO_3$) and carbonate (Na_2CO_3) is most important for high rates of autotrophic production as algal biomass contains up to 50% of carbon. In water, CO_2 may occur as H_2CO_3, HCO_3^- and CO_3^{2-} depending on pH as demonstrated in Figure 2.5. The gaseous CO_2 can be produced from the bicarbonate and carbonate buffer system for photosynthesis.

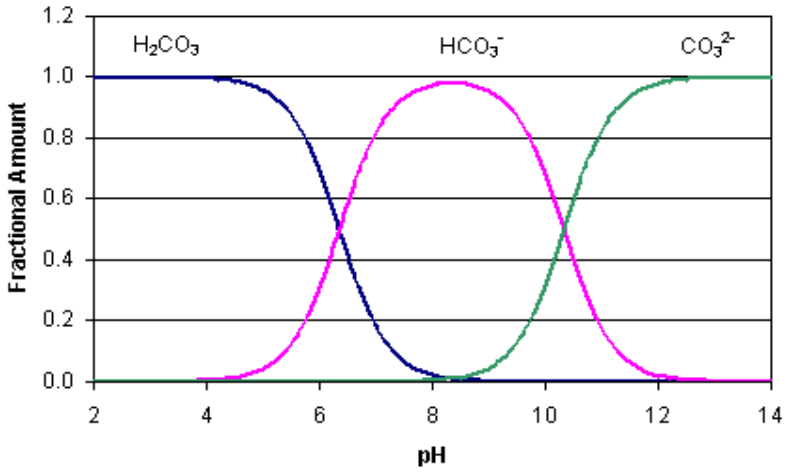


Figure 2.5 pH-dependency of $H_2CO^3/HCO^{3-}/CO_3^{2-}$ equilibrium (Steel *et al.*, 2013)

- **Nitrogen**

Nitrogen is an important nutrient associated with the primary metabolism of microalgae and this also constitutes cell protein in algal biomass. The range of nitrogen content of the biomass is between 1 and 10%. Nitrate (NO_3^-), ammonium (NH_4^+) and urea (NH_2CONH_2) are commonly used as nitrogen source for algal cultivation.

- **Phosphorus**

Phosphorus is essential for algae growth as it plays an important role for many cellular processes such as energy transfer, synthesis of nucleic acid. Although algal biomass contains less than 1%Phosphorus, it is one of the most important growth limiting factors. Most algae acquired phosphorus in inorganic forms, either as $H_2PO_4^-$ or HPO_4^{2-}.

- Other nutrients

Many inorganic elements and organic compounds can be utilized for algal nutrition. Other macronutrients are important for algal cultivation also potassium (K), sulfur (S), sodium (Na), Magnesium (Mg) iron (Fe), and calcium (Ca). In addition, many trace elements are important in enzyme reactions such as boron (B), manganese (Mn), copper (Cu), zinc (Zn), cobalt (Co) and Molybdenum (Mo). The functions of the minerals in algal cells are summarized in Table 2.4

Table 2.4 Elements required for algal growth (Graham & Wilcox, 2000)

element	examples of function and location in algal cells
N	amino acid, nucleotides, chlorophyll, phycobilins
P	ATP, DNA, phospholipids
S	some amino acids, nitrogenase, thylakoid lipids
Na	nitrate reductase
Ca	alginates, calcium carbonate, calmodulin
Mg	chlorophyll
Fe	ferrodoxin, cytochromes, nitrogenase, nitrate and nitrite reductase, catalase, glutamate synthetase
K	agar and carrageenan, osmotic regulation (ionic form), cofactor for many
Mo	enzymes
Mn	nitrate reductase, nitrogenase
Zn	oxygen-evolving complex of photosystem II
Cu	carbonic anhydrase, Cu/Zn superoxide dismutase, alcohol dehydrogenase
Co	plastocyanin, Cu/Zn superoxide dismutase, cytochrome oxidase
	vitamin B12

2.3.6 Inoculum

Minimum inoculum is essential for optimum growth of microalgae cell and product expression. In most instances, a minimum of 0.5% (wet weight/volume) serves the purpose. The reason for this minimum level is not clear, but several studies on inoculum have suggested the presence of a stimulation metabolite in spent media. Substituting spent media with fresh media have resulted in longer lag phase, lower growth rate, and heterogeneous population. Beside the inoculum, the physiological stage of inoculum is also critical for batch processes (Srinivasan *et al.*, 1996).

2.4 Batch cultures

The batch culture consists of a single inoculation of cells into a Duran bottle of fertilized water followed by a growing period of several days and finally harvesting when the algal near-maximum density. Then, algae are transferred to larger culture volumes prior to reaching the stationary phase and the larger culture volumes are then brought to a maximum density and harvested**.**

Batch culture systems are the most common culture system and widely applied for cultivation of microalgal cells because of their simplicity and flexibility, allowing to change species and to remedy defects in the system rapidly. In batch cultures, a limited amount of culture medium is given at the beginning of the culture and no further input of nutrients. The algal cell density increases with time, leading to a denser cell culture which decreases light transmission and finally limits its own growth.

The growth of microalgal cell can be classified into several different phases which also indicate the change in biomass quantity. The several different phases can be divided into 4 major phases as illustrated in Figure 2.6. The different phases of the growth curve may alter in length and slope according to the condition prevailing in the culture.

A). Lag phase: At the beginning, the algal cell culture adjusts itself to altered environmental condition. During this phase, the algal growth rate is close to zero as algae synthesize inducible enzymes whilst using stored food reserves.

B). Log phase: the cell growth rate is increasing rapidly. The rate of multiplication is almost constant, and the cell density increases as a function of time.

C). Stationary phase: the equilibrium is reached between the cell growth and death rate. This phase normally describes the maximum attainable concentration of algal biomass in a closed system.

D). Death phase: the algal cells begin to die at a more rapid rate than that of reproduction and exhaustion of nutrients or high toxic concentration.

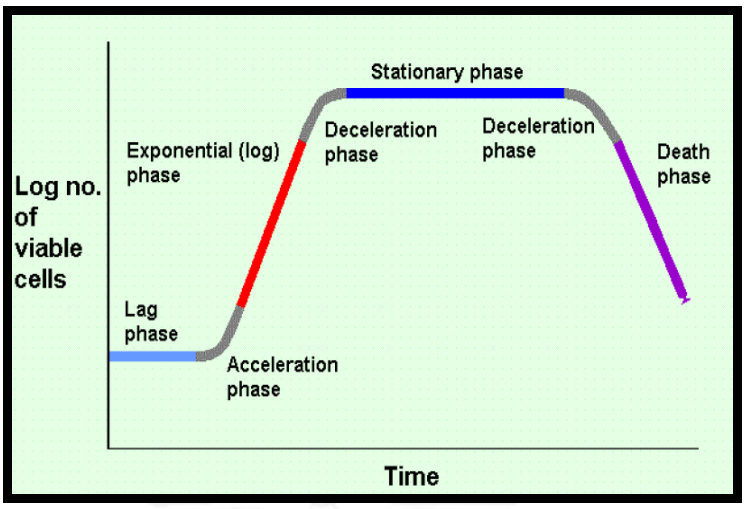


Figure 2.6 Growth phase of algal under batch culture conditions

2.5 Photobioreactors for microalgae cultivation

2.5.1 Open ponds

Cultivation of algae in open ponds has been extensively studied in the past decades (Boussiba *et al.*, 1988, Tredici *et al.*, 1991, Hase *et al.*, 2000). Nowadays, open ponds system are often used for commercial algal production, especially tank, shallow ponds, raceway ponds and circular ponds it agitated or circulated by mechanical equipments such as paddle wheel or rotating scraper to produce the circulation of water (Suh & Lee, 2003)(Figure 2.7). The major advantage of open pond systems are that they are easier to construct, cleaning up and operate than closed systems. However, this system has many disadvantages of cultivation including low cell densities resulting in a large area requirement which it is difficult to distribute nutrients and dark zone appearance, poor light utilization by the cells, case of contamination, poor batch consistency and unpredictable culture crashes caused by changes in weather, sunlight or water quality. Including increase the capital and operating costs of these systems.

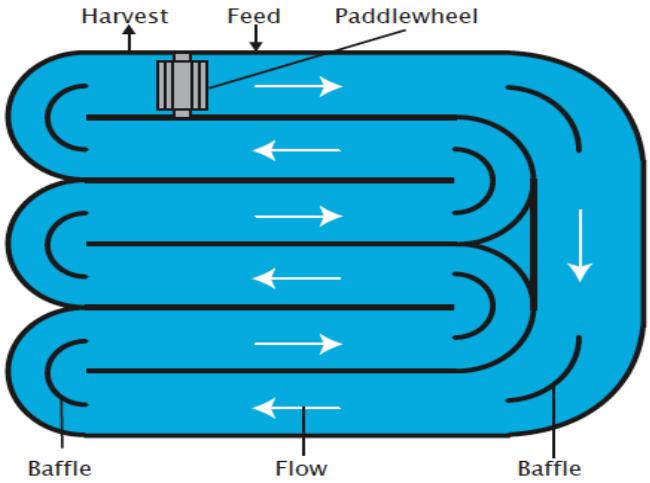


Figure 2.7 Open pond on top view (Singh & Sharma, 2012)

2.5.2 Enclosed photobioreactors

To avoid problems in open ponds systems, much attention is now focused on the closed systems which are believed to give high biomass productivity and easy to control of culture condition. There are different types of photobioreactor, where the majors are flat-plate, tubular, bubble column and airlift photobioreactor.

- **Tubular photobioreactors**

Tubular photobioreactors are designed as short radius and long cylindrical shape in order to provide a larger ratio of surface area to culture volume for effective growth. Tubular cylindrical geometry allows the system to work as solar collectors to capture sunlight for photosynthesis. The tubular type airlift is the most successful on large scale to produce algal biomass (Chisti, 2007). They can be in several forms such as horizontal/serpentine (Gudin & Chaumont, 1991, Molina *et al.*, 2001), vertical (Henrard *et al.*, 2011), near horizontal (Chini Zittelli *et al.*, 1999), conical (Watanabe & Saiki, 1997), inclined (Lee & Low, 1991, Ugwu *et al.*, 2005) photobioreactors, as present in Figure 2.8 (Chisti, 2007). However, it is difficult to control culture temperature, poor mass transfer and limited by oxygen accumulation, carbon dioxide depletion and pH variations (Eriksen, 2008).

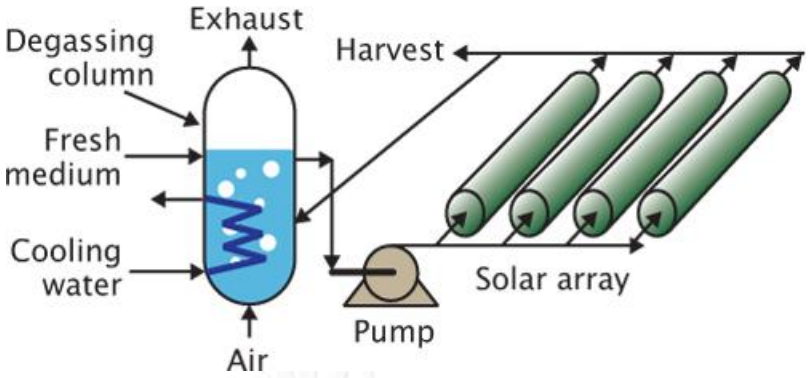


Figure 2.8 Horizontal tubular photobioreactor (Singh & Sharma, 2012)

- **Bubble column photobioreactors**

Bubble-column bioreactors are simple devices that have gained wide acceptance in gas-liquid contacting, high mass transfer and low shear stress. The introduction of gas takes place at the bottom of the column and causes a turbulent stream to enable an optimum gas exchange (Figure 2.9). However, the scale up of bubble column photobioreactors has some limitation if scaled up increasing the diameter and height of reactor effect to aeration rate are posed by considerations of shear sensitivity (Acién Fernández *et al.*, 1999) and light penetration (Sánchez Mirón *et al.*, 1999). A certain minimal aeration rate is essential so that the cells do not stagnate for long in the dimly lit interior of the reactor (Sánchez Mirón et al., 1999). At the same time, there is an upper limit on the acceptable level of turbulence, because hydrodynamic forces affect certain algal cells (Acién Fernández et al., 1999)

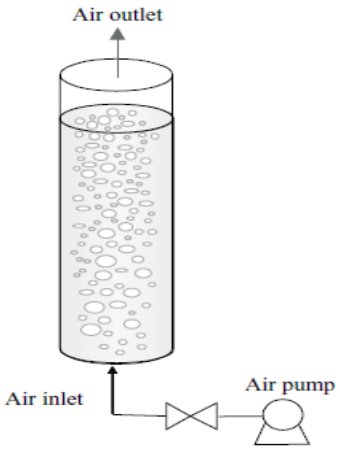


Figure 2.9 Bubble column photobioreactor (Krichnavaruk et al., 2005)

- **Flat plate photobioreactors**

Flat-plate or flat panel photobioreactors have received much attention for cultivation of photosynthetic microorganisms due to their large illumination surface area is suitable for outdoor culture (Figure 2.10) (Ugwu et al., 2008). The preparation of flat culture vessels for cultivation of algae was presented by (Ugwu et al., 2008). Subsequently, the development of outdoor flat panel reactor was examined by (Ramos de Ortega & Roux, 1986) by using thick transparent PVC materials. Afterwards, extensive works on various designs of vertical alveolar panels and flat plate reactors for mass cultivation of different algae were reported (Tredici et al., 1991, Wang *et al.*, 2013, Issarapayup et al., 2009). The flat-plate photobioreactors should be made from transparent materials for maximum utilization of solar light energy. Characteristic fluid-dynamic and mass transfer of the flat plate photobioreactors was investigated and it was concluded that the main advantages of this reactor were low power and high mass transfer capacity (Sierra *et al.*, 2008). Furthermore, they can increase volume by increase length of reactor. Nevertheless, the major disadvantage is high stress damage from aeration.

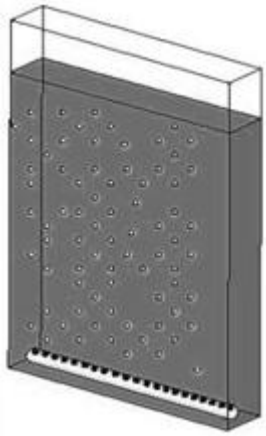


Figure 2.10 Flat-plate photobioreactors

- **Airlift photobioreactors**

Airlift photobioreactors are generally classified as pneumatic reactors without any mechanical stirring arrangements for mixing, similar to bubble column reactors. However, airlift contains a draft tube (Figure 2.11) to separate the system to aeration

and nonaeration compartments. This helps improve circulation and oxygen transfer and equalizes shear forces in the reactor. The two separated fluid zones (riser and downcomer) have different density due to the differences in gas holdup and this is the main concept of such airlift. One advantage of airlift reactors is the elimination of attrition effects generally encountered in mechanical agitated reactors. It is ideally suited for aerobic cultures since oxygen mass transfer coefficient are quite high in comparison to stirred tank reactors. Furthermore, the fluid is well mixed and becomes homogeneously distributed to the algae. Major energy requirements for airlift are electricity used to aerate the system, but without agitator. This simple design aids in the maintenance and cleaning up of the system. Our previous work by (Issarapayup et al., 2009) reported that the flat panel airlift photobioreactor (FP-ALPBR) could be scaled up simply by extending the length of the reactor without losing growth behavior.

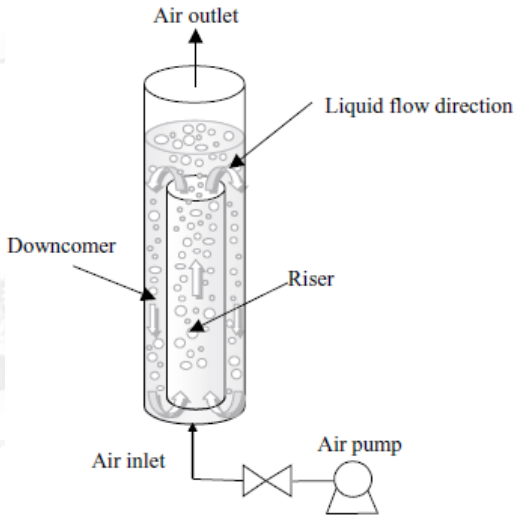


Figure 2.11 Airlift photobioreactor (Krichnavaruk *et al*., 2005)

Table 2.5 Reviews of *Ankistrodesmus sp.* cultivation

Strains	reactor	medium	volume	Temp (°C)	light intensity ($\mu E\ m^{-2}\ s^{-1}$)	pH	CO_2 (%)	time (d)	Biomass concentration ($g\ L^{-1}$)	Productivity	μ (d^{-1})	Reference
A. falcatus	pond	Basal medium	1.4 m^2			6.5-9.6				18 g m^{-2} d^{-1}		Weissman (1984)
A. braunii (ATCC12744)	flask	Bristol medium	500 mL	24	30							Burrell *et al*. (1985)
A. convolutus	flask	Bold Basal medium (BBM)	1 L	28	42	7.0-7.6			0.453		0.93	Chu *et al.* (1995)
A. falcatus	flask	liquid sterilized medium	100 mL	27	70	7.0		7				Nayak *et al*. (1996)
A. braunii (no.202.7a)	flask	BBM	50 mL	25	100	6.2		5			3.85	Pinto *et al.* (2003)
A. falcatus	airlift	BBM	3.2 L	25	250		0.29	14	1.12-1.48	13-55 mg L^{-1} d^{-1}	0.99	Griffiths *et al.* (2011)

Table 2.6 Reviews of *Scenedesmus* cultivation

Strains	reactor	medium	volume (L)	Temp (°C)	light intensity (Lux)	pH	CO_2 (%)	time (d)	Biomass concentration (gL^{-1})	Productivity	μ (d^{-1})	Reference
S. armartus	plate-parallel vessel	Bristol's medium	0.6	30	21.82		2					Tukaj et al. (2003)
S.almeriensis	bubble column	Mann and Myer's medium	2	34	120,250					0.73		Sánchez et al. (2008)
S.obliquus CNW-N	stirr tank	Detmer's medium	1	28	4,440	6	10	12	3.51	0.29	1.19	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	Ho et al. (2010b)
Scenedesmus sp.	stirr tank	BG11	0.2	25±1	11100	7.5 (in.)		16				Kim et al. (2011)
S. obliquus SJTU-3	bubble	modified BG11	0.8	25±1	13,320	7.0 (in.)	10	14	1.84±0.01	0.155± 0.004	0.037 (Max)	Tang et al. (2011)

CHAPTER III

Materials and methods

3.1 Microalgae in batch culture

Green microalgae used for this study were *Ankistrodesmus* sp. and *Scenedesmus* sp. These were obtained from Microbiological Resources Centre (MIRCEN), Thailand Institute of Science and Technology Research (TISTR). Both microalgae were cultured with BG11 medium which contains the components as shown in Table 3.1. The incubation was cultured in 250 mL flask at 25°C, light intensity at 350 Lux. for 7 days. This was then transferred to 2 L Duran bottle containing sterilized BG11 medium. The cultivation contained approximately 5x10^{5} cell mL^{-1} starter as the same initial condition for the subsequent cultivation and aerated with sterile air (filtered through 0.2 micron filter, Gelman Acrodisc 50). The batch cultivation was cultured until the stationary growth was reached (Figure 3.1).

Figure 3.1 Batch cultivation of green microalgae in 2 L Duran bottle

Table 3.1 Composition of BG11 medium (1 Liter) (Stanier *et al.*, 1971)

Stock	Compositions	Amount of chemicals
(1)	$NaNO_3$	1.5 g
(2)	$K_2HPO_4 \cdot 3H_2O$	0.040 g
(3)	$MgSO_4 \cdot 7H_2O$	0.075 g
(4)	$CaCl_2 \cdot 2H_2O$	0.036 g
(5)	Citric acid ($C_6H_8O_7$)	0.006 g
(6)	Ammonium ferric citrate ($C_6H_8O_7 \cdot n$Fe$\cdot n NH_3$)	0.006 g
(7)	$EDTANa_2$	0.001 g
(8)	Na_2CO_3	0.020 g
(9)	*Microelement stock solution*	
	H_3BO_3	2.860 mg
	$MnCl_2 \cdot 4H_2O$	1.810 mg
	$ZnSO_4 \cdot 7H_2O$	0.220 mg
	$Na_2MoO_4 \cdot 2H_2O$	0.390 mg
	$CuSO_4 \cdot 5H_2O$	0.080 mg
	$Co(NO_3)_2 \cdot 6H_2O$	0.050 mg
	pH = 7.4	

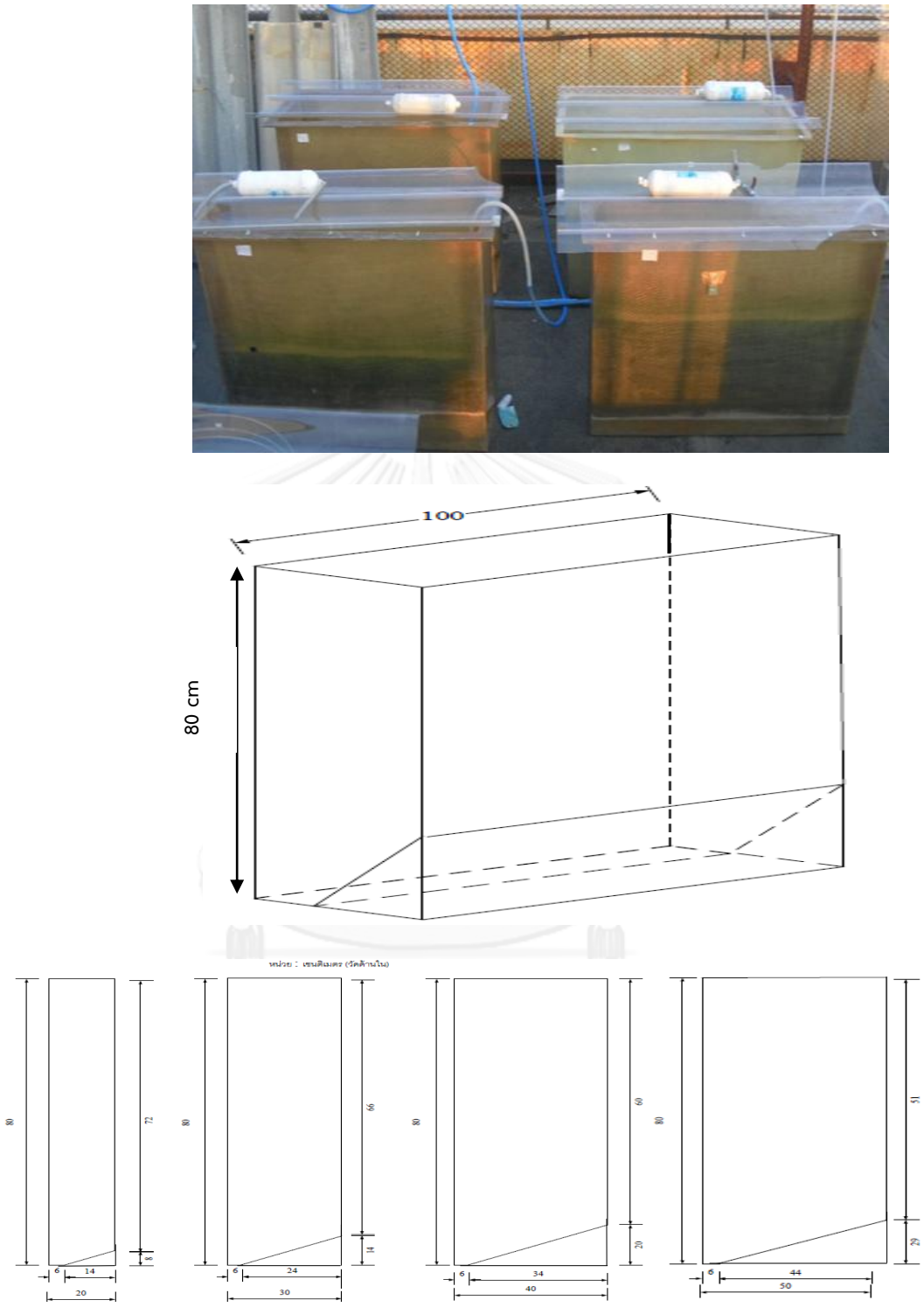


Figure 3.2 Schematic of non-baffled flat panel airlift photobioreactors (NBFPAPs)

3.2 Experimental setup

The configuration of airlift photobioreactor chosen for both green microalgae culture was non-baffled flat panel airlift photobioreactor (NB-FP-ALPBR) under outdoor condition with sunlight and atmospheric temperature. The reactor was made from fiberglass with the following dimension: 80 cm height, 100 cm length and different widths, i.e. 20, 30, 40 and 50 centimeters, where the bottom was sloped 30° (Ritcharoen, 2014, Sintharm, 2014) as illustrated in Figure 3.2. Air flow was supplied through to a long porous gas sparger (filtered through 0.2 micron filter, Gelman Acrodisc 50). The light intensity and the temperature were measured and collected by HOBO Temperature Light (3500 DP Logger UA-002-08) apparatus. The pH value was measured by portable pH meter.

3.3 Outdoor cultivation system

3.3.1 Effect of width of reactor

1. Fill freshwater into the non-baffled flat panel airlift photobioreactors

2. Check for residual chlorine with potassium iodide, the sample without chlorine is clear in color, otherwise a yellow solution is formed

3. Add nutrients and initial cell inoculum at concentration $4x10^{5}$ cell mL^{-1} for *Scenedesmus* sp. and $1x10^{6}$ cell mL^{-1} for *Ankistrodesmus* sp.

4. Supply air at the bottom part of the reactor at the flow rate of 0.16 cm s^{-1} for both algae at the dark:light period of 12:12

5. Take sample and count for cell density using Haemacytometer at once a day frequency until a stationary phase of growth is observed

6. Calculate specific growth rate using Equation 3.6 and productivity using Equation 3.8

3.3.2 Effect of height of reactor

1. Fill freshwater into the non-baffled flat panel airlift photobioreactor with width best condition from 3.3.1 at height of 40, 50 and 60 cm.

2. Check for residual chorine with potassium iodide, and if the water sample without chlorine will be clear, On the other hand a yellow solution is formed

3. Add nutrients and initial cell inoculumn at concentration $4x10^5$ cell mL^{-1} for *Scenedesmus* sp. and $1x10^6$ cell mL^{-1} for *Ankistrodesmus* sp.

4. Sparge a mixture of air flow rate 0.16 cm s^{-1} for both algae for the dark:light period 12:12

5. Take sample and count for cell density using Haemacytometer at once a day frequency till stationary phase of growth

3.3.3 Evaluate economic

1. Analyse only the system with the best operating condition from Sections 3.3.1 and 3.3.2 for both microalgae

2. Calculate operating cost based on the production of 10 kilograms of dry cell, generated in one year (based on 300 operating days per annum)

3.4 Analyses

3.4.1 Determination of cell concentration

3.4.1.1 Cell count with Haemacytometer

A haemacytometer was used to measure cell densities (cell should be more than 10^4 cell mL^{-1}). Haemacytometer consists of two chambers, each with a volume of 0.1 mm^3, containing a marked counting grid of 1 mm^2 in area. The cell concentration can be determined as follows:

1. Clean the counting chamber and cover glass

2. Fill the sample into counting chamber, the volume of sample for analyzed is about 15 microliters

3. Cover the chamber with cover glass, avoid of bubbles and the cells stop drifting around the chamber

4. Check grid under the microscope (x40 objective) and count the cell in 25 medium square on the grid (per 1 large square)

5. Calculate the average cell count and multiply by the conversion factor (10^4) using equation 3.1

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

where

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

n = cell number was calculation from haemacytometer.

3.4.1.2 Dry weight

Dry weight is an estimate of a cell dry weight when all water has been removed, meaning the weight of the sample, excluding the weight of the water in the sample.

1. Collect 40 mL samples

2. Dry the Whatman GF/C filter paper with 1.6 µm pore size membrane and 50 mm of diameter in an oven at 80°C overnight or until weight is constant

3. Weight the filter paper

4. Filter 40 mL of the microalgae through the Whatman GF/C filter paper

5. Place the paper filter in an oven at 80°C overnight or until weight is constant

6. Cool down the paper filter at room temperature in desiccator

7. Weight the filter paper

8. Calculate cell dry weight using Equation 3.2

$$\text{Algal dry weight } \left(mg\ L^{-1}\right) = \frac{Wt_A - Wt_B}{V} \times 1000 \quad (3.2)$$

where

Wt_A is weight of filter paper and algae (mg)

Wt_B is weight of filter paper (mg)

V is volume of culture (mL)

3.4.2 Determination of medium composition

3.4.2.1 Determination of Nitrogen

Nitrogen concentration was analyzed by UV-Visible spectrophotometer at wavelength of 220 and 275 nm following the steps below.

1. Collect the sample (approximately 10 mL)

2. Filter the sample through the Whatman GF/C filter paper diameter 25 mm and 1.6 µm pore size membrane

3. Measure the concentration of Nitrogen by UV-Visible spectrophotometer at wavelength of 220 and 275 nm, dilute the sample if the optical density is greater than 1.0. Nitrate is calculated from

$$\text{Nitrate}\left(NO_3\text{-ppm}\right) = \frac{\left(Abs_{220nm} - Abs_{275nm}\right) \times A}{B} \quad (3.3)$$

where

A is concentration of nitrate in the standard curve (N-ppm)

B is the absorbance of the standard curve (220-275 nm).

3.4.2.2 Determination of other element

The amount of other element of BG 11 such as B, Ca, Co, Cu, Fe, K, Mg, Mn, Mo, P and Zn were analyzed by ICP-OES (700 series Inductively Couple Plasma-Optical Emission Spectrometer, Agilent technologies) as follow steps.

1. Collect the sample (approximately 10 mL)

2. Filter the sample through the Whatman GF/C filter paper (diameter 25 mm and 1.6 µm pore size membrane)

3. Prepare the standard solutions from standard mixture

4. Measure the concentration of elements by using ICP-OES

3.4.2 Determination of cell composition

3.4.2.1 Determination of total lipid

1. Weigh the dried algae 1 gram into the thimble and record them, put it into the soxhlet extractor

2. Weigh the round bottom flask and record them, fill Chloroform 120 mL and methanol 60 mL as mixed solvent, after that heat over until colorless

3. Take a the round bottom flask to the evaporator until all solvent is removed, leave it to cool in desiccator for 2 hours and then record the weight of the sample with the flask

3.4.2.2 Determination of protein

The crude protein content is obtained by multiplying the amount of nitrogen content by the factor of 4.44 (González López *et al.*, 2010). The nitrogen content was analyzed by CHNS/O Analyzer (Perkin Elmer, PE2400 Series II) from Scientific and Technological Research Equipment Centre. The crude protein content, w_p, is calculated as a percentage by mass, using the following equation:

$$w_p = w_N \times F \tag{3.4}$$

where

w_N = the nitrogen content of the sample, expressed as a percentage by mass

F = the factor to convert Kjeldahl nitrogen to protein, F = 4.44

3.4.2.3 Determination of carbohydrate

Calculate protein, lipid, ash, and moisture contents in the dry cell, then calculate percentage of total carbohydrate in the dry cell by subtracting the sum of percentages of protein, fat, moisture and ash from 100, as the following equation:

$$\text{Total carbohydrates [\%]} = 100 - \{(\text{protein} + \text{fat} + \text{moisture} + \text{ash}) [\%]\} \tag{3.5}$$

3.4.2.4 Determination of moisture

1. Keep crucible dried in an oven at 100^0C for 2 hours and then put it in desiccators for 2 hours after that record them.

2. Weight the dried algae 1 gram into the crucible and record, calcined at 100°C for 2 hours and then put it in desiccators for 2 hours after that record them.

3.4.2.5 Determination of ash

1. Keep crucible dried in an oven at 800^0C for 2 hours and then put it in a desiccators for 2 hours after that record them.

2. Weight the dried algae 1 gram into the crucible and record, calcined at 750°C for 2 hours and then put it in desiccators for 2 hours after that record them.

3.5 Calculations

3.5.1 Determination of specific growth rate

The specific growth rate can be calculated from Equation 3.6 as follows

$$\mu = \frac{\ln x_2 - \ln x_1}{t_2 - t_1} \qquad (3.6)$$

where

μ is the specific growth rate (d^{-1})

x_1 is the initial cell concentration (cell d^{-1})

x_2 is the final cell concentration(cell d^{-1})

t_1 is the initial time (d) of exponential growth phase

t_2 is the final time (d) of exponential growth phase

3.5.2 Determination of doubling time

The doubling time is the period of time required for a quantity to double in growth rate. When the relative growth rate (not the absolute growth rate) is constant, the quantity undergoes exponential growth and has a constant doubling time or period, which can be calculated directly from the growth rate.

$$t_d = \frac{\ln 2}{\mu} = \frac{0.693}{\mu} \quad (3.7)$$

where

t_d is the doubling time (day)

μ is the specific growth rate (d^{-1})

3.5.3 Determination of productivity

The productivity of the microalgae was calculated from Equation 3.8 as follows:

$$P = \frac{X_2 - X_1}{t_2 - t_1} \times V \times 1000 \quad (3.8)$$

where

P is productivity (cells d^{-1})

X_1 is cells concentration at t_1 (cells mL^{-1})

X_2 is cells concentration at t_2 (cells mL^{-1})

t_1 is first sampling time (d)

t_2 is second sampling time (d)

V is harvest volume (L)

3.5.4 Determination of specific productivity

The specific productivity for the cultivation of the microalgae in the NB-FP-LPBR can be calculated from Equation 3.9

$$SP = \frac{P}{V \times 86{,}400} \tag{3.9}$$

where

SP is specific productivity (cells L^{-1} s^{-1})

P is productivity (cells d^{-1})

V is harvest volume (L)

3.5.5 Determination of light energy

1. Measure and record light intensity (Lux) by HOBO Temperature Light meter (3500 DP Logger UA-002-08)

2. Multiply the recorded value with 0.0015 to convert the unit from Lux to Walt m^{-2}

3. Plot curve between Walt m^{-2} versus hour

4. Calculate area under the curve, and multiply by 3.6×10^{-3} to obtain the light energy in the unit of MJ m^{-2}

3.6 Statistical analysis

The experimental results were evaluated by comparison of the growth curves and analysis of variance (ANOVA) of the kinetic parameters, significance being tested by the Tukey test at $p \leq 0.05$.

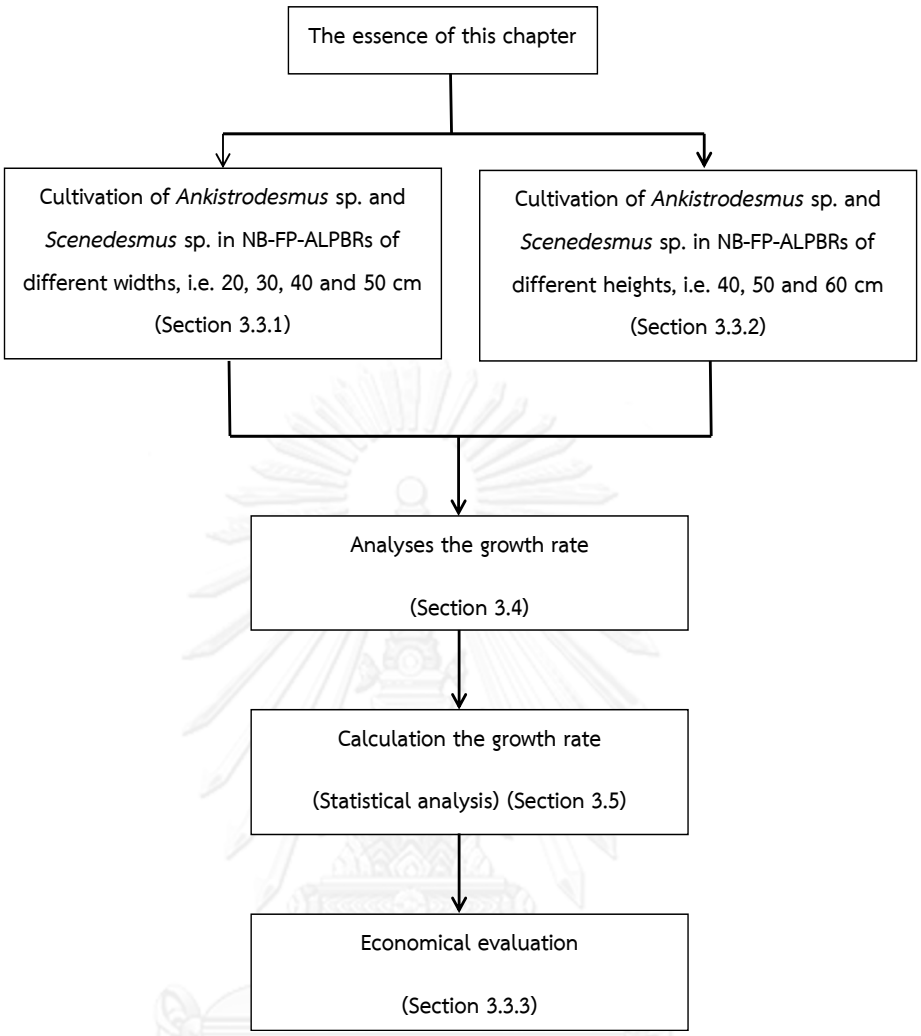


Figure 3.3 The flow chart in this study

CHAPTER IV

RESULTS AND DISCUSSION

The cultivations of *Ankistrodesmus* sp. and *Scenedesmus* sp. were investigated in a large scale non-baffled flat plate airlift photobioreactor (NB-FP-ARPBR). This placed a focus on the design effect of the reactor using the reactors of different widths, i.e. 20, 30, 40 and 50 cm and different unaerated liquid heights i.e. 40, 50 and 60 cm. As a whole, the configuration of the NB-FP-ARPBR had an influence on fluid flow behavior, light availability, temperature and other factors affecting the livelihood of microalgae. The properties of the obtained microalgae also were analyzed as well as economical analysis for the cultivation. In the following discussion, each of the conditions is discussed in detail.

4.1. Effect of width on cultivations of *Ankistrodesmus* sp. and *Scenedesmus* sp. in NB-FP-ALPBR

The growths of *Ankistrodesmus* sp. in NB-FP-ALPBR of various unaerated liquid heights are shown in Figures 4.1 - 4.2 and Appendix A. The growths were typically started with a 1 day lag phase, after which *Ankistrodesmus* sp. entered its exponential growth period at approx. 5-7 days. To examine the effect of reactor width, the discussion is only limited to the cultivation of 40 cm unaerated liquid height. It is noted that similar growth pattern curve was obtained from the reactors with other unaerated liquid heights.

The cultures of *Ankistrodesmus* sp. grew best in NB-FP-ALPBR with the width of 50, followed by those with 40, 30 and 20 cm, respectively. For this culture, the dry weight consistently followed the cell density. The experiment was repeated two times (1^{st} and 2^{nd} Runs in Fig. 4.1 - 4.2 and Appendix A, but at a different time period in a year. Hence, depending on the environmental conditions, different growth patterns might be obtained from the two different Runs. However, the relative effects of reactor widths from the two Runs did not differ from each other, indicating that the effect of reactor width did not change with environmental conditions.

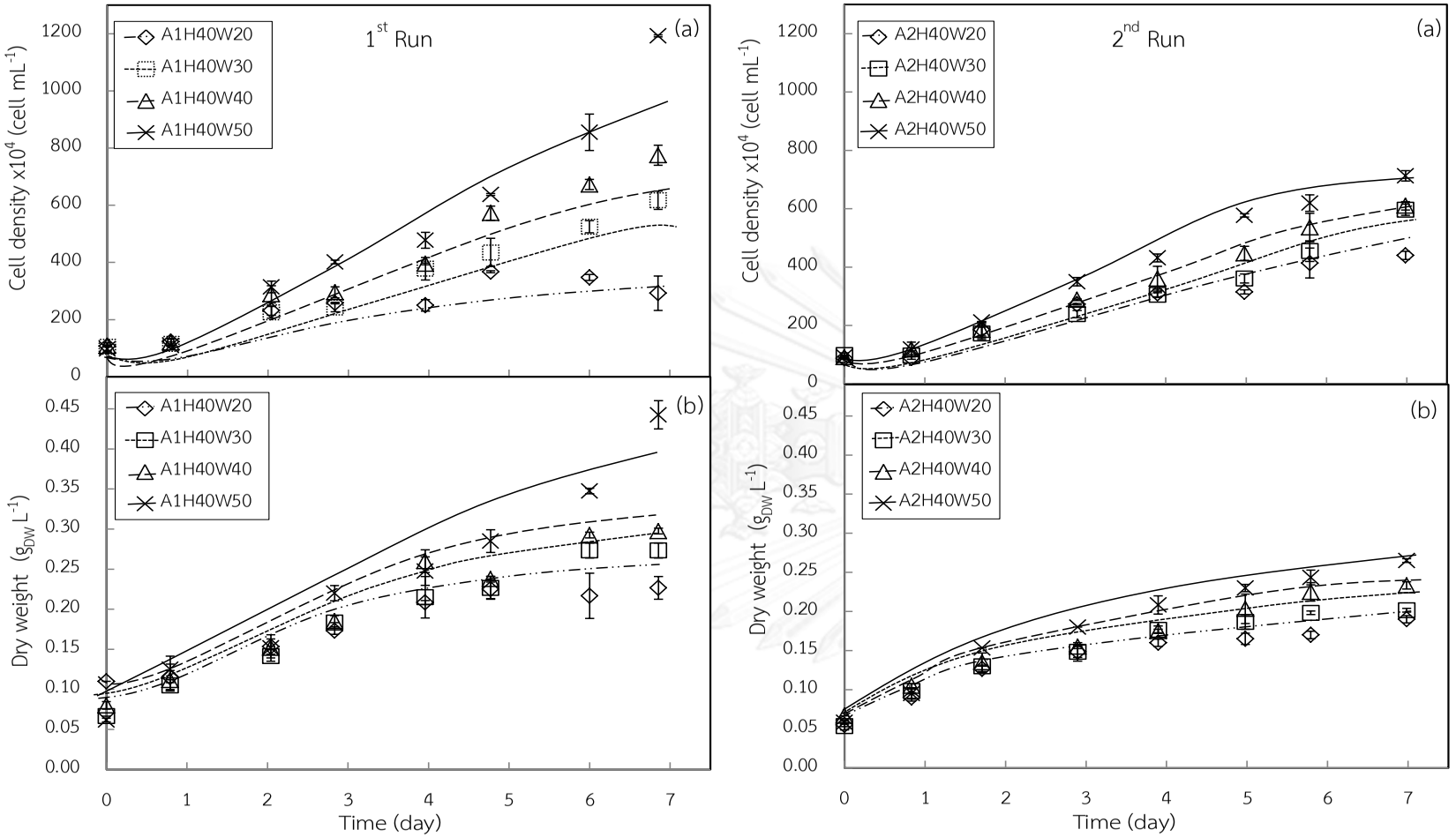


Figure 4.1 Comparison between growth behavior of *Ankistrodesmus* sp. in NB-FP-ALPBR with various widths at 40 cm unaerated liquid height :(a) Cell density (cell mL^{-1}); and (b) Dry weight (g_{DW} L^{-1})

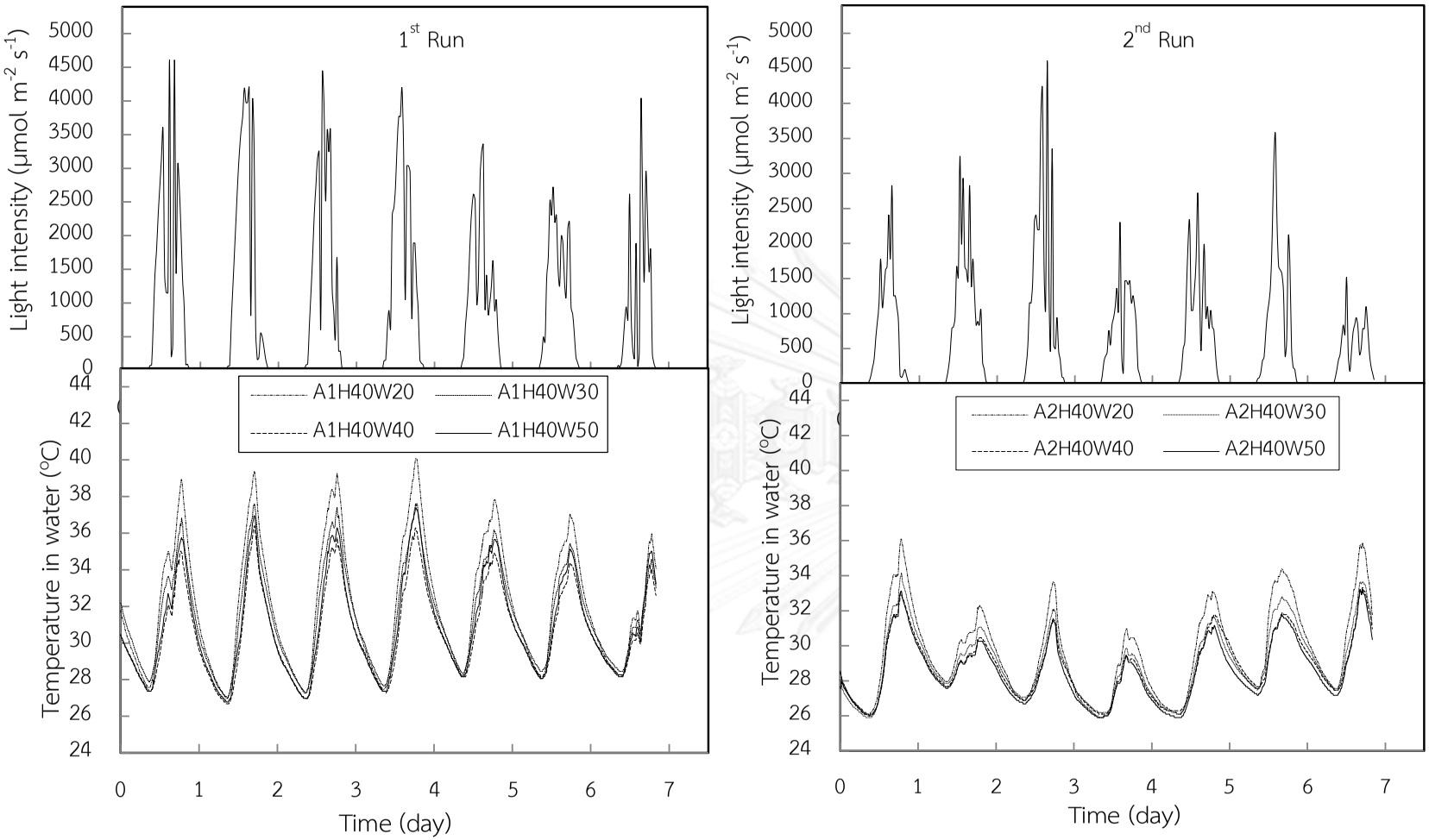


Figure 4.2 Time courses of temperatures and light intensities during the growth of *Ankistrodesmus* sp. in NB-FP-ALPBR with various widths at 40 cm unaerated liquid height

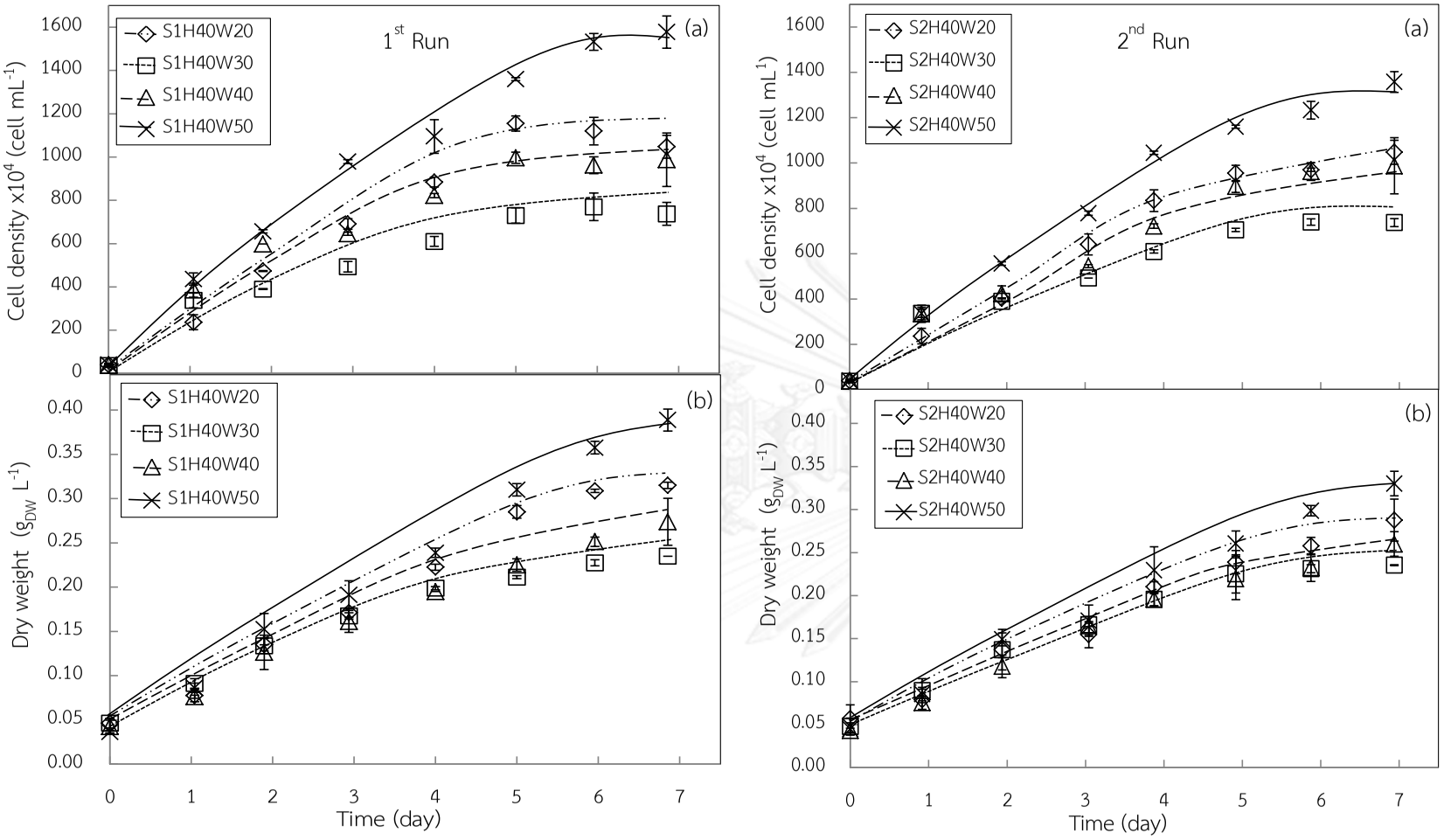


Figure 4.3 Comparison between growth behavior of *Scenedesmus* sp. in NB-FP-ALPBR with various widths at 40 cm unaerated liquid height :(a) Cell density (cell mL^{-1}); and (b) Dry weight (g_{DW} L^{-1})

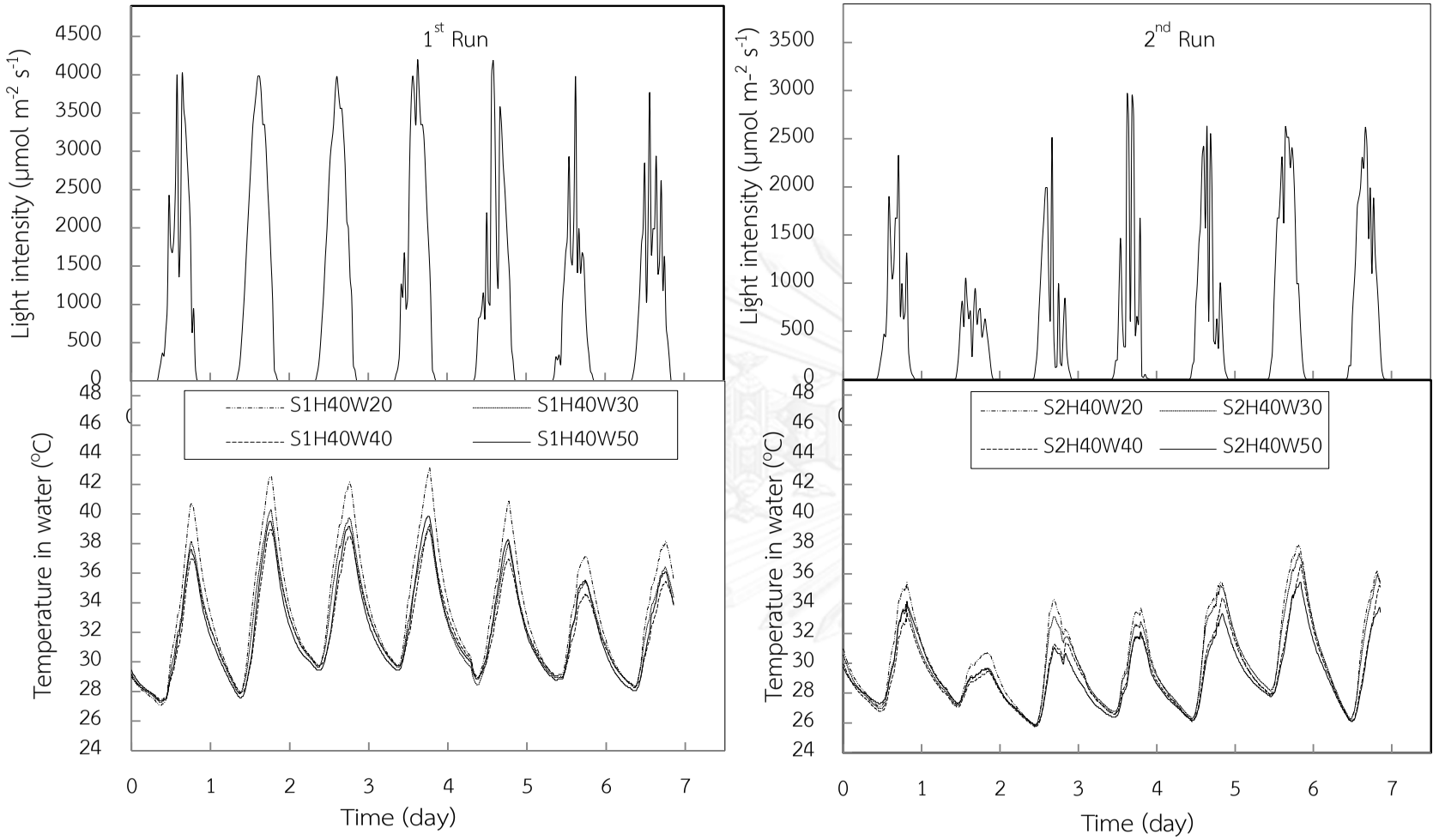


Figure 4.4 Time courses of temperatures and light intensities during the growth of *Scenedesmus* sp. in NB-FP-ALPBR with various widths at 40 cm unaerated liquid height

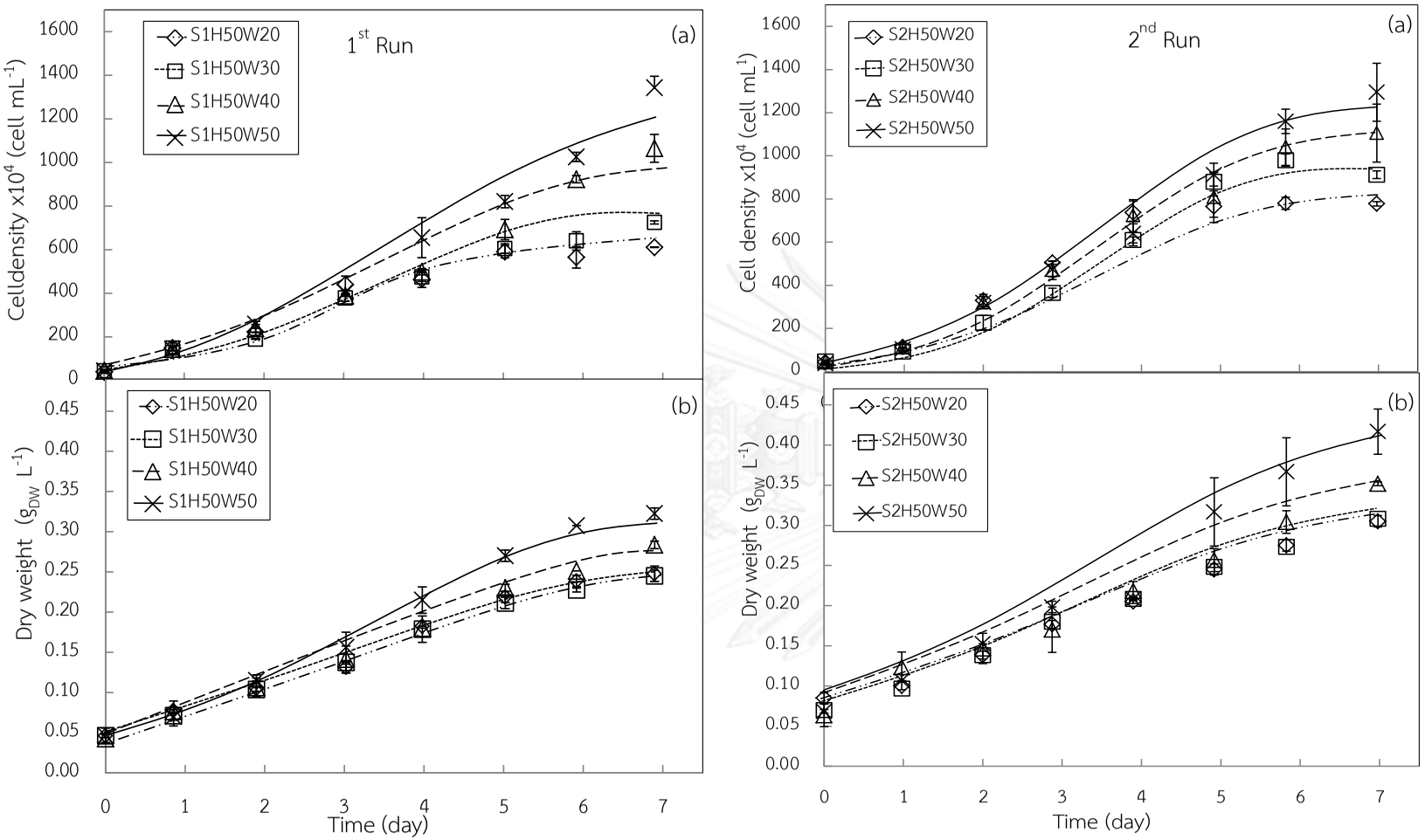


Figure 4.5 Comparison between growth behavior of *Scenedesmus* sp. in NB-FP-ALPBR with various widths at 40 cm unaerated liquid height :(a) Cell density (cell mL^{-1}); and (b) Dry weight (g_{DW} L^{-1})

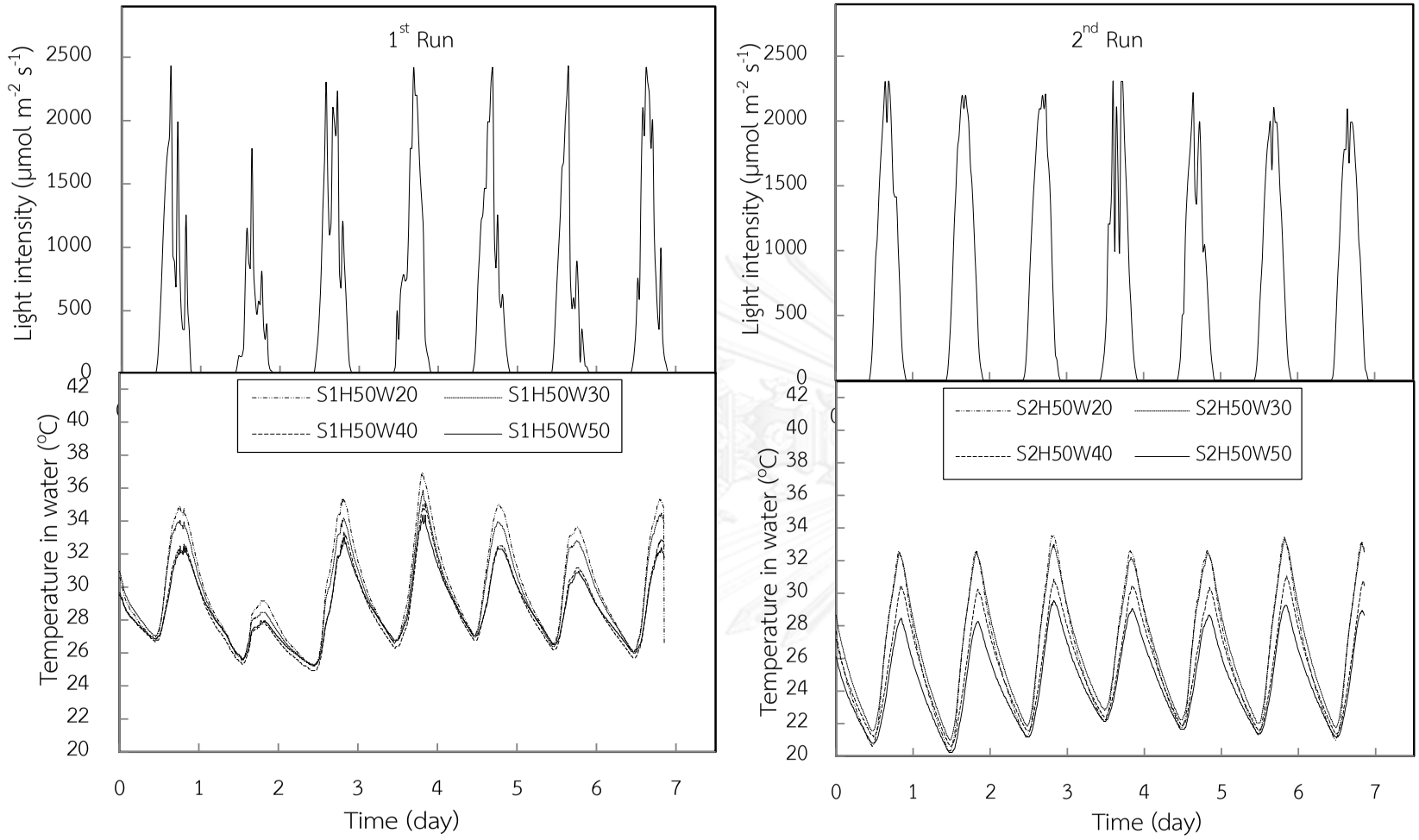


Figure 4.6 Time courses of temperatures and light intensities during the growth of *Scenedesmus* sp. in NB-FP-ALPBR with various widths at 50 cm unaerated liquid height

Table 4.1 Comparison between cell concentration, dry weight, specific growth rate, productivity, specific productivity and doubling time in a non-baffled flat plate airlift photobioreactor (NB-FB-ALPBR) in the various widths at fixed height of culture was maintained at 40, 50 and 60 centimeters for *Ankistrodesmus* sp.

	Maximum cell density $x10^6$ (cell mL^{-1})		Maximum dry weight ($g_{DW}L^{-1}$)		Specific growth rate (μ; day^{-1})		Productivity $x10^7$ (cells day^{-1})		Specific productivity (cells L^{-1} s^{-1})		Doubling time (day)	
Condition	1	2	1	2	1	2	1	2	1	2	1	2
AH40W20	3.68^{a}	4.40^{a}	0.23^{a}	0.19^{a}	0.14^{a}	0.23^{a}	0.18^{a}	0.44^{a}	0.31^{a}	0.58^{a}	4.94^{a}	3.00^{a}
AH40W30	6.18^{b}	5.97^{b}	0.27^{ab}	0.20^{a}	0.26^{b}	0.26^{ab}	0.74^{b}	0.94^{b}	0.86^{b}	0.82^{b}	2.70^{b}	2.70^{b}
AH40W40	7.75^{c}	6.10^{b}	0.30^{b}	0.23^{c}	0.29^{bc}	0.27^{ab}	1.19^{c}	1.20^{c}	1.17^{c}	0.88^{bc}	2.41^{b}	2.55^{b}
AH40W50	11.96^{d}	7.13^{d}	0.44^{d}	0.27^{d}	0.37^{c}	0.29^{b}	1.89^{d}	1.50^{d}	1.86^{d}	1.00^{c}	1.90^{c}	2.38^{c}
AH50W20	5.94^{a}	5.10^{a}	0.20^{a}	0.22^{a}	0.25^{a}	0.25^{a}	0.58^{a}	0.54^{a}	0.76^{a}	0.70^{a}	2.81^{a}	2.77^{a}
AH50W30	6.79^{b}	6.80^{b}	0.22^{a}	0.22^{a}	0.27^{a}	0.30^{ab}	1.06^{b}	1.13^{b}	0.93^{ac}	0.99^{b}	2.59^{b}	2.35^{b}
AH50W40	8.71^{c}	8.25^{c}	0.25^{a}	0.26^{c}	0.30^{c}	0.32^{b}	1.75^{c}	1.73^{c}	1.17^{c}	1.19^{b}	2.32^{c}	2.16^{b}
AH50W50	10.00^{d}	10.20^{d}	0.34^{b}	0.30^{d}	0.33^{d}	0.34^{b}	2.02^{c}	2.26^{d}	1.29^{c}	1.48^{d}	2.10^{d}	2.06^{b}
AH60W20	3.01^{a}	3.83^{a}	0.15^{a}	0.16^{a}	0.16^{a}	0.19^{a}	0.32^{a}	0.45^{a}	0.33^{a}	0.46^{a}	4.42^{a}	3.74^{a}
AH60W30	3.30^{a}	4.20^{a}	0.16^{ab}	0.18^{ab}	0.16^{a}	0.19^{a}	0.50^{a}	0.73^{b}	0.35^{a}	0.51^{a}	4.39^{a}	3.66^{a}
AH60W40	4.75^{c}	5.48^{c}	0.18^{b}	0.20^{ab}	0.20^{c}	0.24^{ac}	1.00^{c}	1.31^{c}	0.53^{c}	0.70^{c}	3.43^{c}	2.93^{c}
AH60W50	5.10^{c}	6.05^{c}	0.21^{d}	0.23^{b}	0.22^{c}	0.26^{c}	1.30^{d}	1.62^{d}	0.65^{c}	0.86^{c}	3.09^{c}	2.70^{c}

* Mean in columns with the different alphabets were statistically different at the significant level of 0.05 when compared by Tukey's test

*Remark: Light energy

A1H40 = 38.086 MJ m^{-2} A2H40 = 25.872 MJ m^{-2}

A1H50 = 24.439 MJ m^{-2} A2H50 = 32.619 MJ m^{-2}

A1H50 = 21.852 MJ m^{-2} A2H50 = 32.204 MJ m^{-2}

Similar findings were obtained from the culture of *Scenedesmus* sp. as Illustrated in Figures 4.3 - 4.6 and Appendix A. However, the growth at unaerated liquid height of 40 cm exhibited a slightly different pattern where the growth at unaerated liquid height of 40 cm could be ordered from high to low as 50, 20, 40 and 30 cm, respectively.

4.1.1 *Ankistrodesmus* sp. culture

Ankistrodesmus sp. was cultivated in various airlift reactors with different widths and heights. Only the system with the unaerated liquid height of 40 cm is discussed here as the cultures at 50 and 60 cm unaerated liquid height gave results with similar trends and the same discussion can be applied.

Table 4.1 demonstrates that the reactor width statistically affected the growth performance ($p \leq 0.05$). The highest growth of *Ankistrodesmus* sp. was observed in the 50 cm width airlift. Several possible reasons might be responsible for this as described in the following items:

A. Fluid flow direction

The fluid flow directions in NB-FB-ALPBRs at different heights are illustrated in Figures 4.7, 4.8 and 4.9. It was shown that the reactor with larger width had a larger downcomer cross sectional area (Ad) and induced a clearer recirculation pattern. Wongsuchoto *et al*., (2003) reported that airlifts with larger riser had a high tendency to create a non-ideal circulation where internal fluid circulation might take place which resulted in a less effective recirculation pattern. Also, recirculation pattern might be enough to stabilize the temperature for microalgae. Therefore this suggested that a better fluid flow occurred in the culture system with larger width when compared with the smaller airlifts.

B. Gas holdups ($\varepsilon_d, \varepsilon_r$)

To promote a better circulation of the fluid within the airlift system, it was recommended that there should exist a larger difference between downcomer and riser gas holdups or hydrostatic pressure difference (ε_r - ε_d) (Rujiruttanakul & Pavasant, 2011). Data from Sintharm, (2014) (shown in Table 4.2) as quoted in the tabulation below shows that the airlift with larger width had a larger gas holdup

difference than the smaller ones. Noted that there was no data available for the reactor with 50 cm width, but the trend could be extrapolated from such dataset.

Table 4.2 hydrostatic pressure difference **(ε_r - ε_d)**between downcomer and riser

	Height (cm)		
Width (cm)	40	50	60
20	0.023	0.015	0.023
30	0.027	0.016	0.017
40	0.030	0.029	0.030

This suggested that NB-FP-ALPBR with 50 cm width induced a better fluid flow than the other reactors.

C. Light intensity

The experiment was repeated twice at the same height but with different light intensity. For example, the highest of growths in the first and second Runs in A1H40W50 and A2H40W50 were 11.96 x 10^6 and 7.13 x 10^6 cell mL^{-1}. This discrepancy could be because the two Runs were exposed to the different light intensity (38.086 and 25.872 MJ m^{-2} for the 1^{st} and 2^{nd} Runs, respectively). The area per volume of the 50 cm of width was greater than those of the other three sizes of width, this allowed a better light utilization for the growth of the microalgae. Light is the sole source of energy which drives photosynthesis for microalgae, and as a result, the maximum growth increased with more energy from sun light.

D. temperature

Most algae can grow well in their suitable temperature range. A much too high temperature could easily inhibit the algal growth perhaps due to inactivation of intercellular enzyme, cell damage and solubility of gases. For *Ankistrodesmus* sp., the growth ceased at temperature higher than 35^{o}C. Therefore any condition, either reactor configuration or environmental condition, that led to this high temperature in

the system would have a negative effect on the growth, for instance the system with small width such as 20 cm in a very hot day where the liquid volume might not be enough to stabilize the temperature.

To sum up, when compared the growth of the culture of *Ankistrodesmus* sp. in NB-FB-ALPBR with different widths, statistics indicated that the width of 50 cm provided the highest values of cell density, dry weight, specific growth rate, productivity and specific productivity and the lowest values of doubling time.

Table 4.3 Comparison between cell concentration, dry weight, specific growth rate, productivity, specific productivity and doubling time in a non-baffled flat plate airlift photobioreactor (NB-FB-ALPBR) in the various widths at fixed height of culture was maintained at 40, 50 and 60 centimeters for *Scenedesmus* sp.

	Maximum cell density $x10^6$ (cell mL^{-1})		Maximum Dry weight ($g_{DW}L^{-1}$)		Specific growth rate (μ; day^{-1})		Productivity $x10^7$ (cells day^{-1})		Specific productivity (cells L^{-1} s^{-1})		Doubling time (day)	
Condition	1	2	1	2	1	2	1	2	1	2	1	2
SH40W20	11.55^{a}	10.48^{a}	0.32^{a}	0.29^{acd}	0.48^{a}	0.47^{a}	0.99^{a}	0.98^{a}	1.70^{a}	1.68^{a}	1.45^{a}	1.47^{a}
SH40W30	7.70^{b}	7.40^{b}	0.24^{b}	0.24^{ab}	0.44^{b}	0.43^{b}	1.02^{a}	1.01^{a}	1.18^{b}	1.17^{b}	1.59^{b}	1.61^{b}
SH40W40	9.97^{a}	9.88^{b}	0.27^{ab}	0.26^{bc}	0.48^{a}	0.47^{a}	1.70^{c}	1.67^{c}	1.46^{ab}	1.44^{ab}	1.46^{a}	1.48^{a}
SH40W50	15.78^{d}	13.58^{d}	0.39^{d}	0.33^{d}	0.53^{a}	0.50^{a}	2.65^{d}	2.24^{d}	2.51^{d}	2.15^{d}	1.30^{d}	1.37^{d}
SH50W20	6.11^{a}	7.80^{a}	0.25^{a}	0.31^{a}	0.40^{a}	0.41^{a}	1.30^{a}	1.34^{a}	0.96^{a}	1.22^{a}	1.74^{a}	1.68^{a}
SH50W30	7.25^{a}	9.80^{ab}	0.25^{a}	0.31^{a}	0.41^{a}	0.43^{a}	1.10^{b}	1.39^{a}	1.15^{a}	1.41^{a}	1.70^{a}	1.63^{a}
SH50W40	10.65^{c}	11.05^{ab}	0.28^{c}	0.35^{c}	0.46^{b}	0.46^{a}	1.60^{c}	2.01^{c}	1.71^{c}	1.76^{c}	1.50^{c}	1.51^{ac}
SH50W50	13.43^{d}	12.95^{b}	0.32^{d}	0.42^{d}	0.50^{b}	0.51^{d}	3.18^{d}	3.05^{d}	2.24^{d}	1.92^{c}	1.39^{c}	1.36^{c}
SH60W20	5.38^{a}	5.73^{a}	0.18^{a}	0.18^{a}	0.38^{a}	0.39^{a}	0.82^{a}	0.88^{a}	0.84^{a}	0.90^{a}	1.82^{a}	1.76^{a}
SH60W30	5.50^{a}	5.98^{a}	0.18^{a}	0.19^{a}	0.37^{a}	0.37^{a}	1.31^{b}	1.32^{b}	0.92^{b}	0.93^{a}	1.85^{a}	1.89^{b}
SH60W40	6.22^{c}	6.63^{c}	0.21^{c}	0.21^{c}	0.40^{c}	0.42^{c}	1.73^{c}	1.76^{c}	0.99^{c}	1.01^{c}	1.73^{c}	1.65^{c}
SH60W50	7.16^{d}	7.68^{d}	0.24^{d}	0.22^{c}	0.41^{c}	0.46^{d}	2.17^{d}	2.40^{d}	1.15^{d}	1.24^{d}	1.69^{c}	1.51^{d}

* Mean in columns with the different alphabets were statistically different at the significant level of 0.05 when compared by Tukey's test

*Remark:Light energy

S1H40 = 42.348 MJ m^{-2} S2H40 = 21.352 MJ m^{-2}

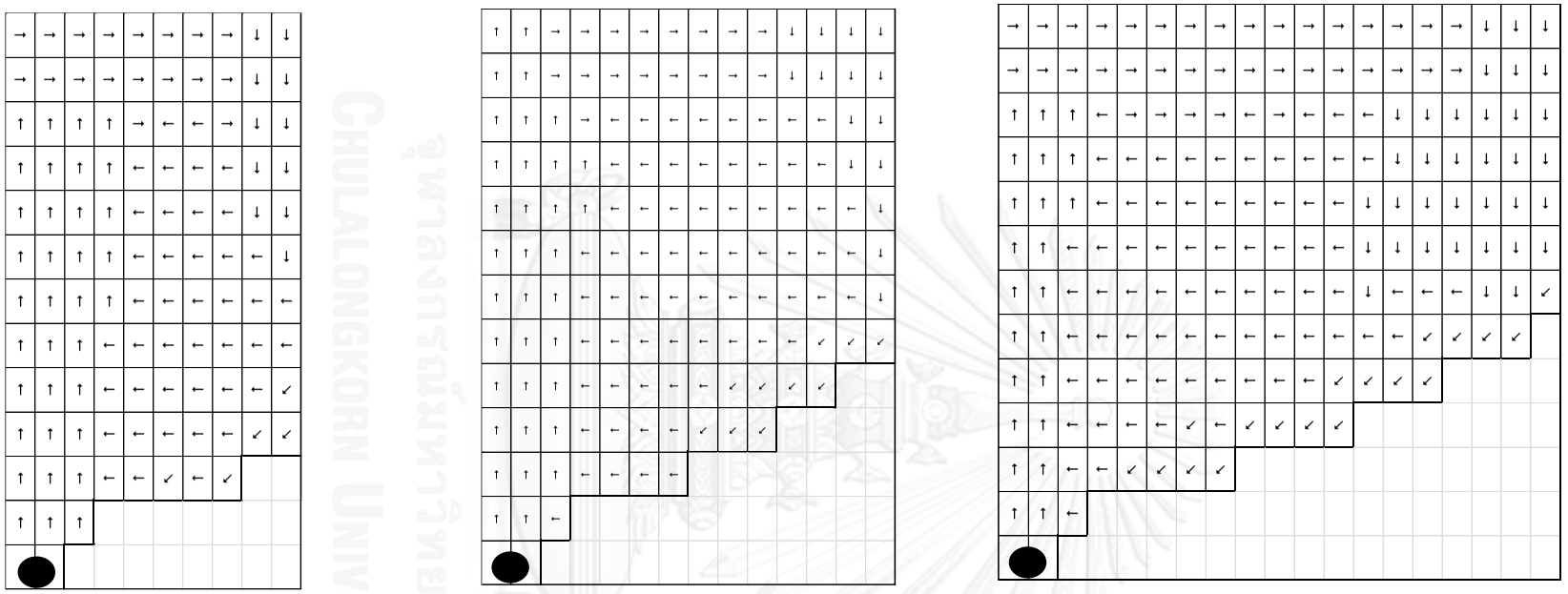
S1H50 = 19.993 MJ m^{-2} S2H50 = 25.568 MJ m^{-2}

S1H50 = 41.099 MJ m^{-2} S2H50 = 31.147 MJ m^{-2}

4.1.*2 Scenedesmus sp.* culture

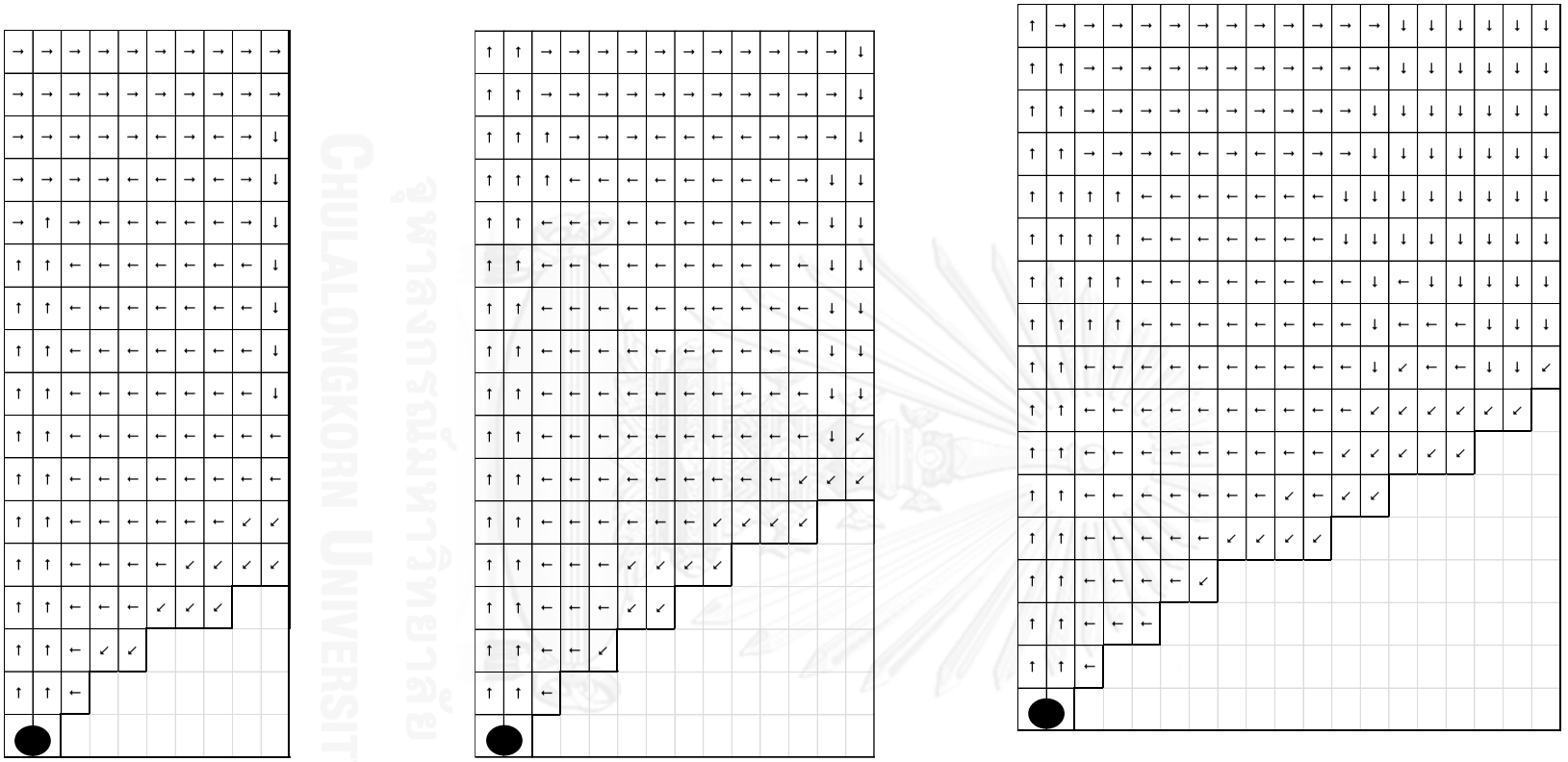
Similar to the discussion above, this section examines the effect of reactor width on the growth of *Scenedesmus* sp. and the results with statistical evaluation are displayed in Table 4.3. The growth of *Scenedesmus* sp. in NB-FB-ALPBR followed the same trend with that of *Ankistrodesmus* sp. where the growth was best in the reactor with the width of 50 cm followed in order from high to low by those with the widths of 40, 30, and 20. This was on exception at the unaerated liquid height of 40 cm where the growth rate was ordered from high to low as 50, 20, 40 and 30 cm, respectively. The reason for this is still unclear but it was possible that the temperature of the culture might play a significant role here. The reactor with 20 cm width and 40 cm unaerated liquid height possessed the highest temperature of all reactors employed in this work, and our unreported data (Ritcharoen, 2014) suggested that this culture might favor such condition for their growth.

To sum up, the culture of *Scenedesmus* sp. in NB-FB-ALPBR in the different of width indicated that 50 cm of width at unaerated liquid height 40, 50 and 60 cm the highest values of cell density, dry weight, specific growth rate, productivity and specific productivity and the lowest values of doubling time statistically significant ($p \leq 0.05$)



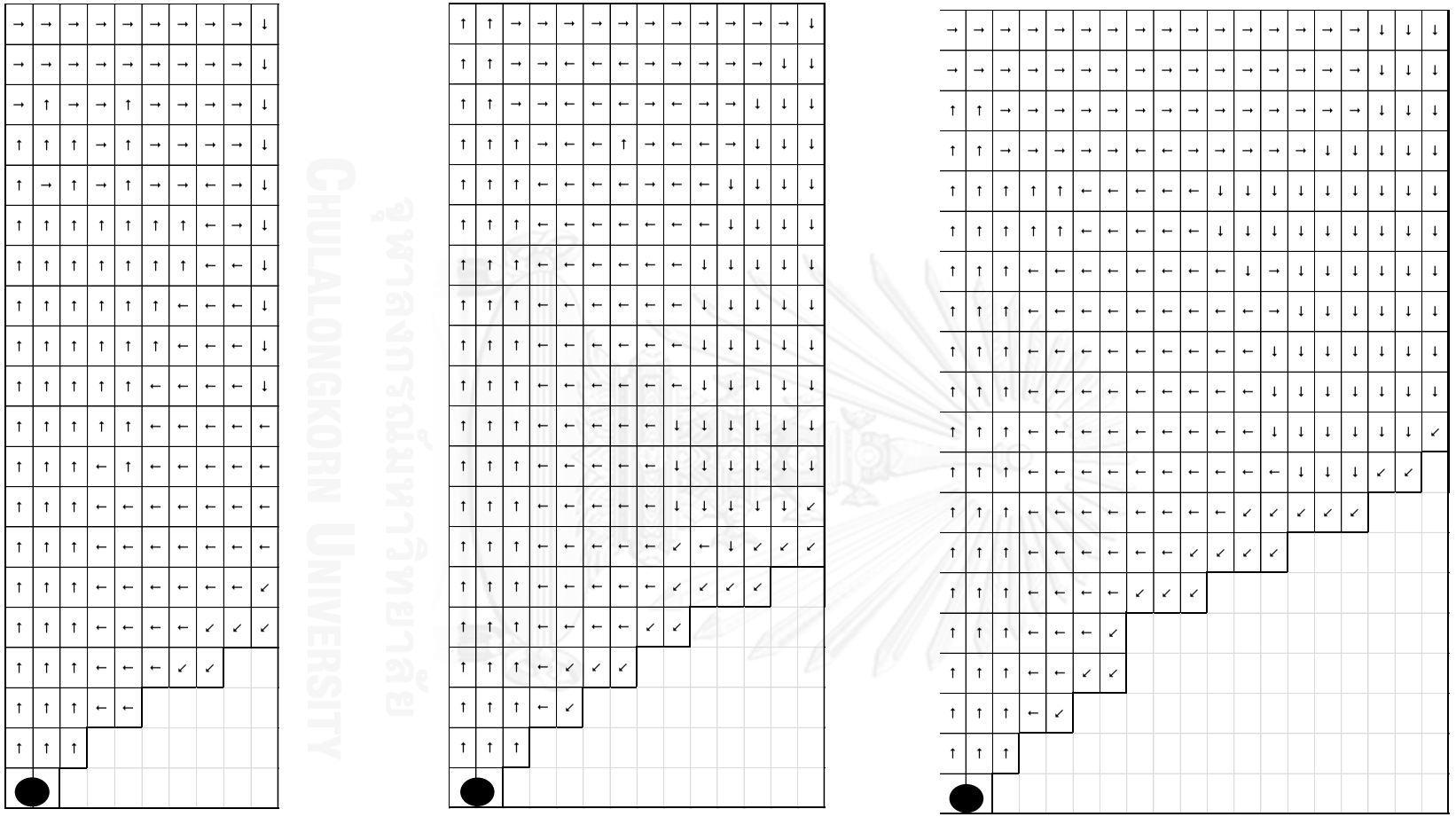
a. b c

Figure 4.7 Fluid flow direction in NB-FP-ALPBR with various widths at 40 cm unaerated liquid height :(a) 20 cm of width ;(b) 30 cm of width ;(c) 40 cm of width (Sintharm, 2014)



a. b c

Figure 4.8 Fluid flow direction in NB-FP-ALPBR with various widths at 50 cm unaerated liquid height :(a) 20 cm of width ;(b) 30 cm of width ;(c) 40 cm of width (Sintharm, 2014)



a. b c

Figure 4.9 Fluid flow direction in NB-FP-ALPBR with various widths at 60 cm unaerated liquid height :(a) 20 cm of width ;(b) 30 cm of width ;(c) 40 cm of width (Sintharm, 2014)

4.2 Effect of an unaerated liquid height on cultivations of *Ankistrodesmus* sp. and *Scenedesmus* sp. in a NB-FB-ALPBR

Section 4.1 demonstrates that both microalgae grew best in the system with the width of 50 cm. Hence, the discussion in this section will only be confined to the 50 cm width airlift. Figures 4.10 - 4.13 illustrate that, among the unaerated liquid height investigated in this work, i.e. 40, 50 and 60 cm, NB-FP-ALPBR with the liquid height of 50 cm exhibited the best growth for both cultures (11.51×10^6 (1^{st} Run) and 9.18×10^6 (2^{nd} Run) cell mL^{-1} for *Ankistrodesmus* sp. and 14.08×10^6 (1^{st} Run) and 13.45×10^6 (2^{nd} Run) cell mL^{-1} for *Scenedesmus* sp.), followed by those with the heights of 40 and 60 cm. In fact, the growth at the height of 50 cm was only slightly higher than that at the height of 40 cm where the culture started with a 1 day lag phase and reached the stationary phase within 6-7 days. On the other hand, the growth at the height of 60 cm was relatively poor where the cells entered its stationary phase within only 3 - 4 days. The results of cell density were consistent well with cell density and dry weight indicating the uniformity of cell texture.

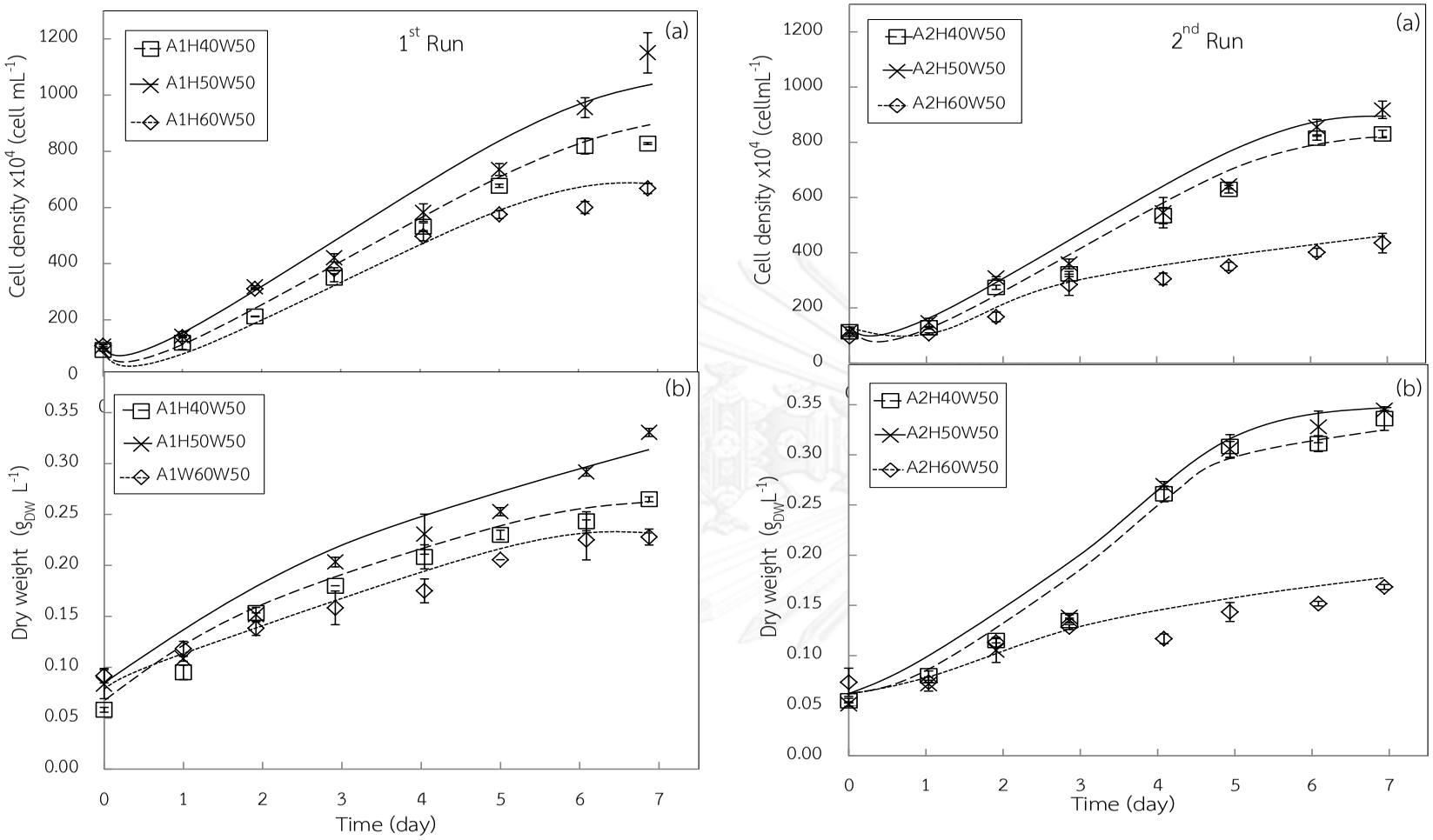


Figure 4.10 Comparison between growth behavior of *Ankistrodesmus* sp. in NB-FP-ARPBR with various heights and fixed width of 50 cm: (a) Cell density (cell mL^{-1}); and (b) Dry weight (g_{DW} L^{-1})

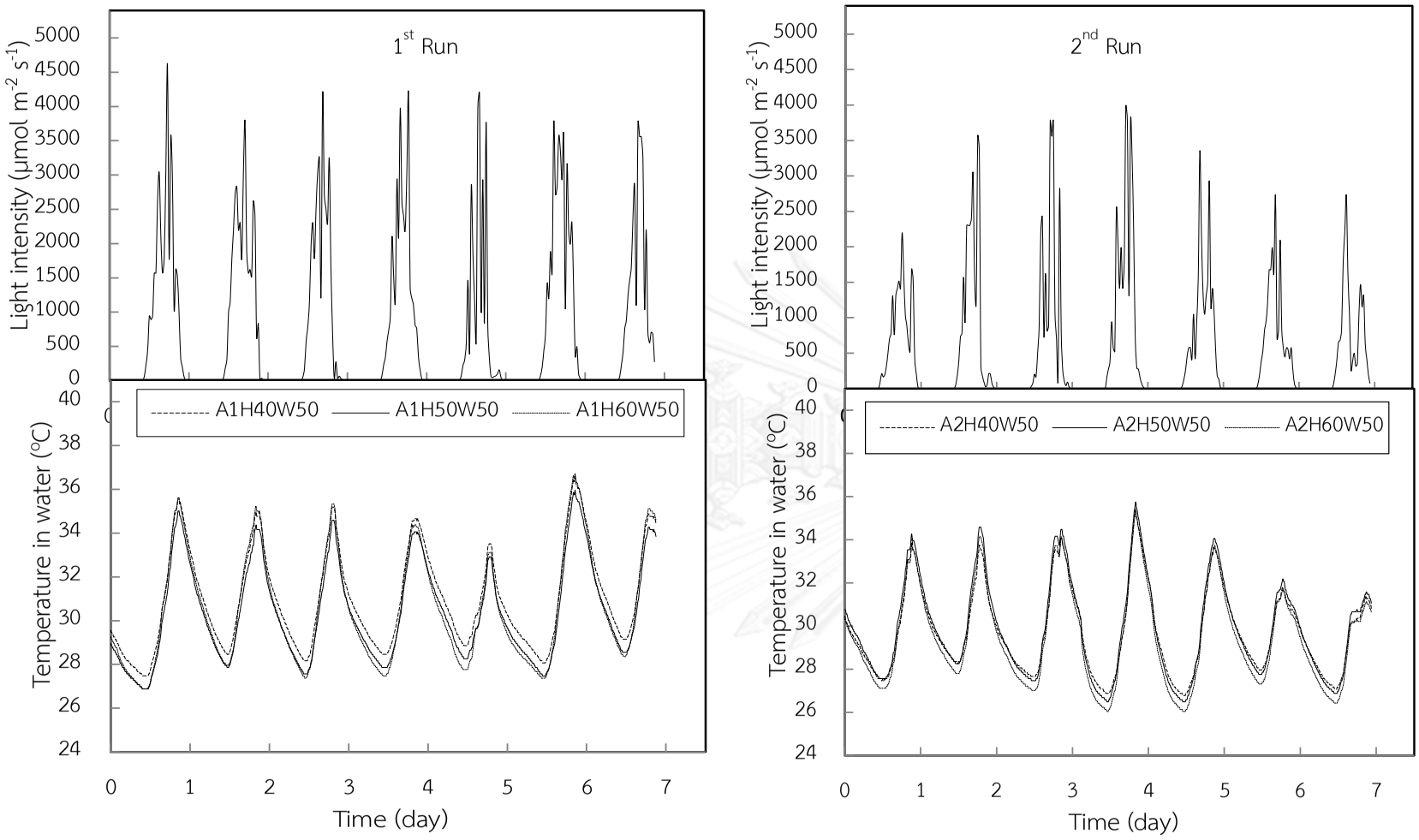


Figure 4.11 Time courses of temperatures and light intensities during the growth of *Ankistrodesmus* sp. in NB-FP-ALPBR with various with heights and fixed width of 50 cm

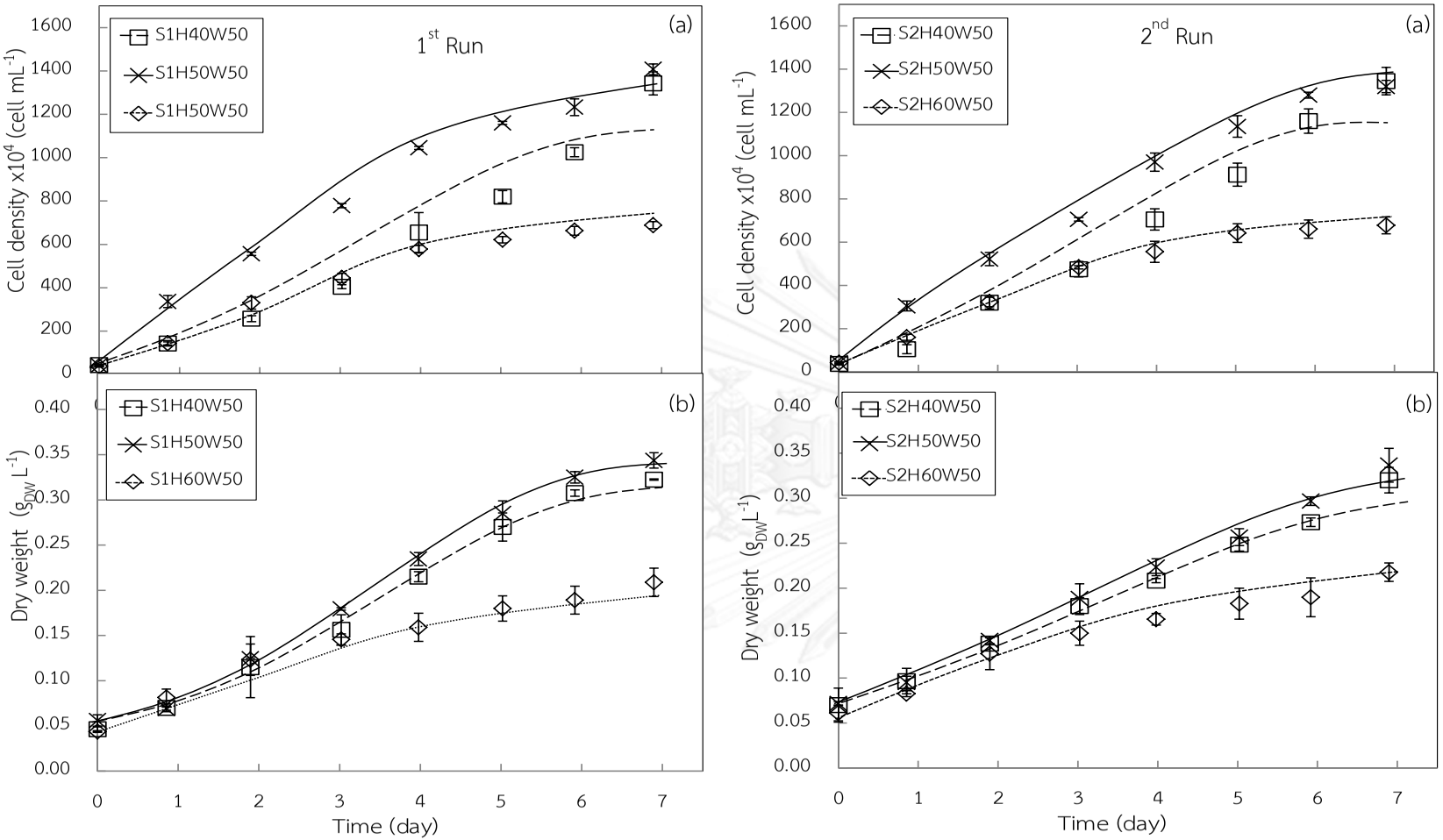


Figure 4.12 Comparison between growth behavior of *Scenedesmus* sp. in NB-FP-ARPBR with various heights and fixed width of 50 cm: (a) Cell concentration (cell mL^{-1}); and (b) Dry weight ($g_{DW}\,L^{-1}$)

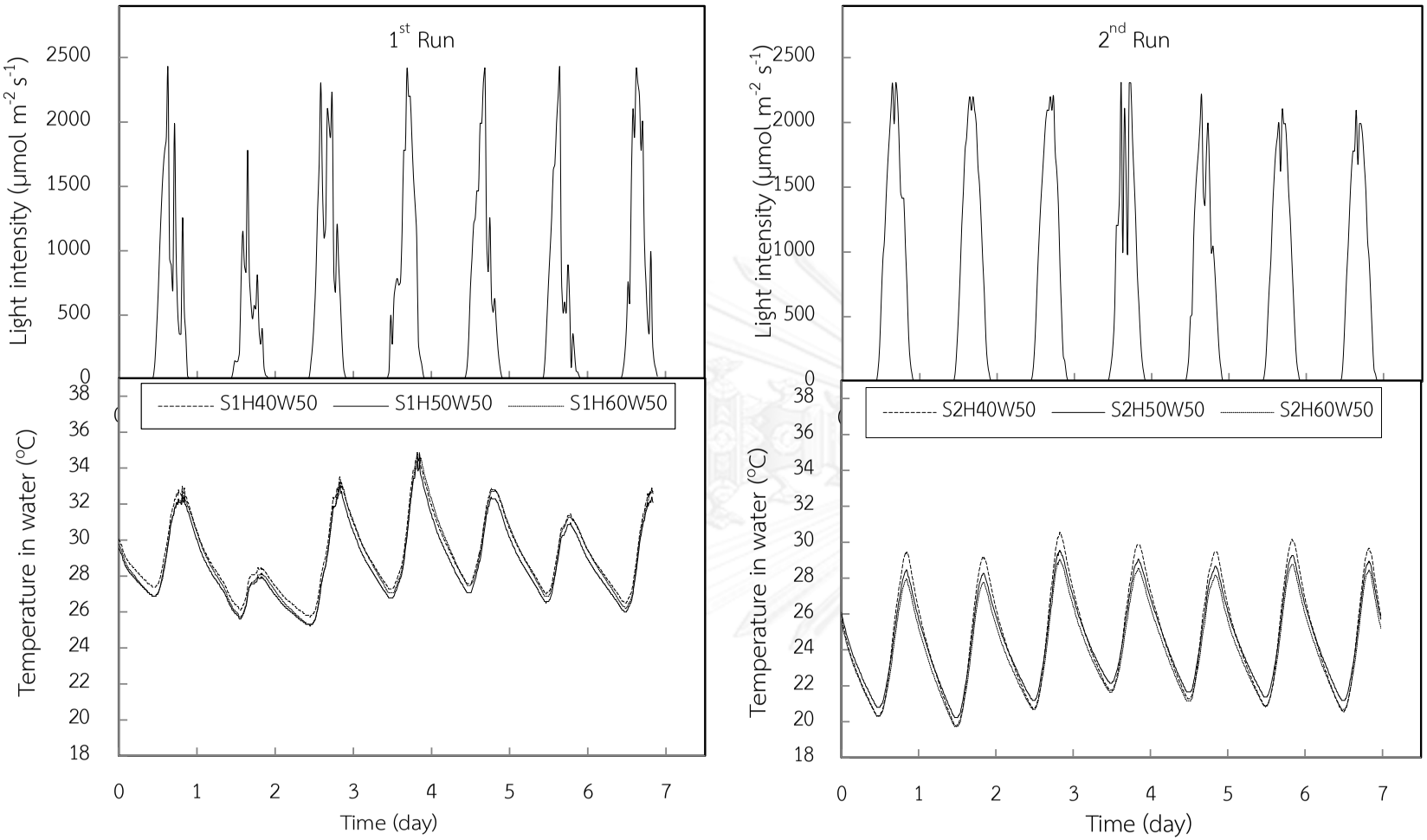


Figure 4.13 Time courses of temperatures and light intensities during the growth of *Scenedesmus* sp. in NB-FP-ALPBR with various with heights and fixed width of 50 cm

Table 4.4 Comparison between cell concentration, dry weight, specific growth rate, productivity, specific productivity and doubling time in a non-baffled flat plate airlift photobioreactor in the various heights at width of culture was maintained at 50 centimeters for *Ankistrodesmus* sp. and *Scenedesmus* sp.

	Maximum cell density x10^6 (cell ml^{-1})		Maximum dry weight ($g_{DW}L^{-1}$)		Specific growth rate (μ; day^{-1})		Productivity x10^7 (cells day^{-1})		Specific productivity (cells L^{-1} s^{-1})		Doubling time (day)	
Condition	1	2	1	2	1	2	1	2	1	2	1	2
AH40W50	8.28^a	8.30^a	0.27^a	0.33^a	0.31^a	0.29^a	1.24^a	1.22^a	1.22^a	1.20^a	2.21^a	2.42^a
AH50W50	11.51^b	9.18^b	0.33^b	0.34^a	0.35^b	0.30^a	2.57^b	1.96^b	1.76^b	1.31^b	2.00^b	2.31^a
AH60W50	6.68^c	4.35^c	0.23^c	0.17^c	0.27^c	0.22^c	1.82^c	1.09^a	0.97^c	0.51^c	2.61^c	3.20^c
SH40W50	13.43^a	13.20^a	0.32^a	0.32^a	0.50^a	0.50^a	2.34^a	2.19^a	2.18^a	2.15^a	1.39^a	1.39^a
SH50W50	14.08^a	13.45^a	0.34^a	0.34^a	0.51^a	0.52^a	3.18^b	3.21^b	2.28^a	2.20^a	1.36^a	1.33^a
SH60W50	6.89^b	6.78^b	0.21^b	0.22^b	0.42^b	0.40^b	2.11^c	2.04^a	1.12^b	2.02^b	1.64^b	1.73^b

* Mean in columns with the different alphabets were statistically different at the significant level of 0.05 when compared by Tukey's test

*Remark: Light energy

A1W50 = 36.371 MJ m^{-2} A2W50 = 25.131 MJ m^{-2}

A1W50 = 19.993 MJ m^{-2} A2W50 = 25.568 MJ m^{-2}

The growth rate of both *Ankistrodesmus* sp. and *Scenedesmus* sp. at different unaerated liquid heights, i.e. 40, 50 and 60 cm are given in Table 4.4. For *Ankistrodesmus* sp., this unaerated liquid height seemed to have significant effect on cell growth where the height of 50 cm provided the best growth. Nevertheless, the growths rate of *Scenedesmus* sp. at unaerated liquid heights of 40 and 50 cm were not statistically significant ($p \leq 0.05$) and both were better than the growth at 60 cm. As the reactor with higher liquid height has a greater volume than the reactor at lower height, and therefore if they give the same growth performance, it is better to select the system with a greater volume as it gives a larger productivity. The height below 40 cm was not considered here as there were adequate research indicating that, for the culture even in normal outdoor ponds, effective growth could be obtained even for the liquid height of 40 cm (Sutherland *et al.*, 2014). The

differences in the growth performance of each reactor could be described in the following discussions:

A. Light intensity

One of the main effects for the cultivation of the both microalgae in large-scale systems was the light intensity. In particular, for the system with the same width, when cells grew high in density, poor light penetration became a serious problem that retarded the effective growth of the both microalgae. Although the NB-FP-ALPBR was made from translucent fiber, the penetration of light through the wall of this fiber was quite small, and measurement showed that about 70-80% of light was filtered out. Therefore most light came from the top surface. Light intensities measured at the bottom of the airlift at different unaerated liquid heights were compared in Figure 4.14. The light energy (from 7 days of cultivation) at the bottom of NB-FP-ARPBR with 40 cm of unaerated liquid height (1.188 MJ m^{-2}) was almost twofold of that with 60 cm of unaerated liquid height (0.582 MJ m^{-2}). Hence, it became quite obvious that the performance of the system with 60 cm of unaerated liquid height was lower than others. The light energy measured at the bottom of the airlift with 50 cm unaerated liquid height was 0.774 MJ m^{-2} which was also less than that at 40 cm. However, the growth rate from such system (50 cm unaerated liquid height) was slightly higher than that at 40 cm. This could be due to the fact that cells were already in a light saturation zone (at 50 cm) and increasing light intensity above this level no longer affected the algal growth. Note that light-saturation region for *Scenedesus* sp. was reported at 8.428 - 25.285 MJ m^{-2} (ShihHsin *et al.*, 2012) and 18.729-74.918 μmol m^{-2} s^{-1} for *Ankistrodesmus* sp. (Kessler *et al.*, 1957)

B. Temperture

The temperatures in the systems compared in this section were only slightly different from each other. However, the differences in light intensity and liquid volume could have an effect on the temperature buffering capacity of the system. In other words, high light intensity and low liquid volume could raise the temperature of the system. However, this difference of only 2 - 4^{o}C as observed here was not enough to have significant effect on the system performance.

C. Gas and liquid transfer

Increasing the unaerate liquid height provided a longer riser section and also a longer contract time between liquid and gas, promoting high liquid velocity through the momentum and energy transfers. However, Sintharm, (2014) stated that, in airlift with configuration as employed in this work, the unaerated liquid height in the range of 40-60 cm did not have significant differences in the gas and liquid mass transfer. However, a faster liquid velocity might exert a larger shear force on the algal cell which might adversely affect the growth as perhaps seen in the system with 60 cm liquid height.

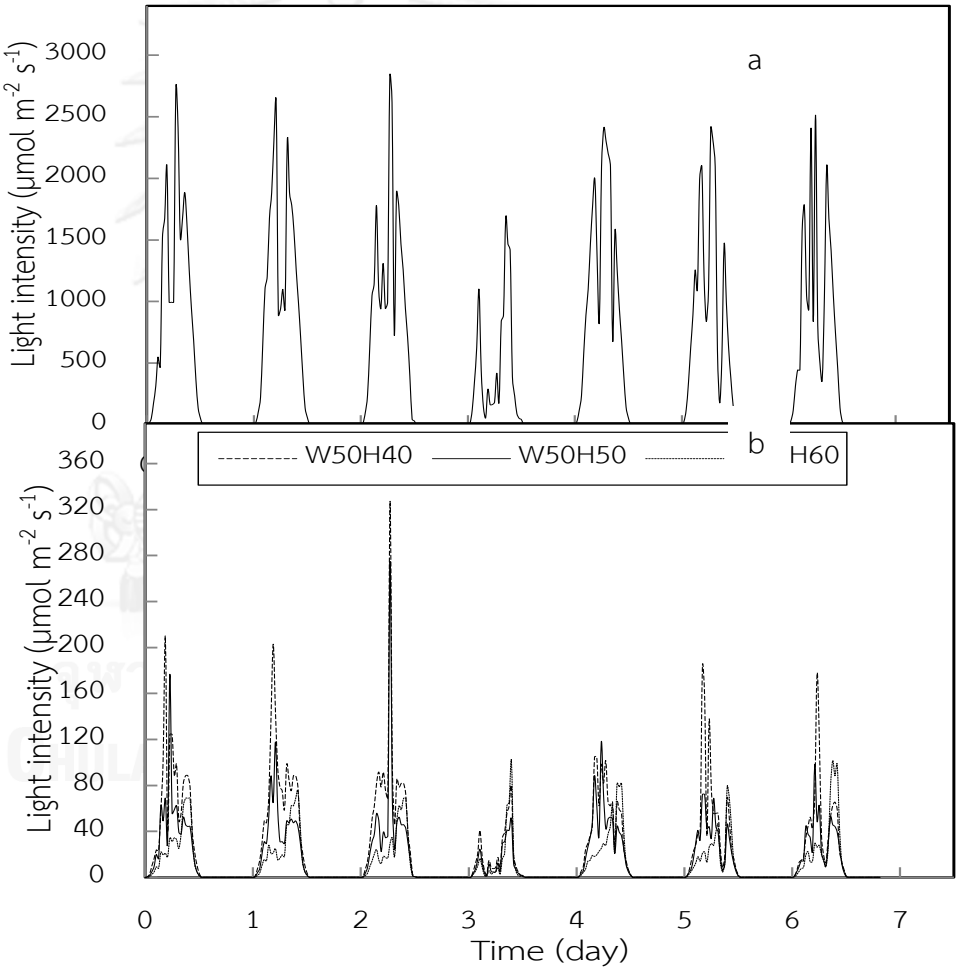


Figure 4.14 Time courses of light intensities in NB-FP-ALPBR with various with heights and fixed width of 50 cm: (a) light intensities at reactor surface; and (b) light intensities at the bottom of reactor

4.3 Influence of reactor configuration on biochemical compositions of the microalgae

4.3.1 Effect of reactor width

The biochemical composition of *Ankistrodesmus* sp. and *Scenedesmus* sp. in NB-FP-ALPBR of various unaerated liquid widths are shown in Figures 4.15 - 4.20**.** In fact, the biochemical content of microalgae is influenced by the culture conditions such as temperature, nutrient concentration, pH, CO_2 level, irradiation, etc. (Converti *et al.*, 2009, Li *et al.*, 2008, Xin *et al.*, 2011). Changing reactor configuration was anticipated to affect these parameters and also the composition of microalgae.

For *Ankistrodesmus* sp., at 40 cm unaerated liquid heights, *Ankistrodesmus* sp. had protein as the main chemical component (range 32.08-35.16, 38.79-41.55 %dry weight for the 1st and 2nd Runs, respectively), carbohydrate (range 34.51-40.36, 27.60-31.04 %dry weight for the 1st and 2nd Runs, respectively) and lipid (range 27.08-31.76, 27.41-31.63 %dry weight for the 1st and 2nd Runs, respectively). At this height, the cultivation was repeated twice but at different time of the year. The results turned out to be different, one with high carbohydrate and the other with high protein. This discrepancy could be because the two Runs were exposed to the different light intensity, high light intensity (also high temperature) from Run 1 gave the microalgae with high carbohydrate, and low light intensity (and low temperature) from Run 2 gave high protein. Carvalho *et al.*, (2009) demonstrated that an increase in light intensity could slightly increase the accumulation of carbohydrate which agreed with the finding here. The drop in protein content of microalgae culture was also reported for the culture in the upper extreme temperature range (Tomaselli *et al.*, 1988, De Oliveira *et al.*, 1999) as this was associated with the breakdown of protein structure and interface with enzyme regulation (Pirt, 1975) and disruption of cell metabolism (Richmond, 1986). However, when considered the effect of reactor width on the biochemical composition in the algal cell, no significant results could be drawn suggesting that the effect of width in the range of 20 – 50 cm was not visible. The differences in the productivity at different widths were due to the different growth rates which was quite high at the width of 50 cm.

At 50 cm unaerated liquid heights, the performance of the system became more stable and the difference between the biochemical compositions from the different Runs became small. This was because the system held a larger quantity of water which could stabilize the temperature of the culture more effectively and

yield a more stable behavior. Small deviation might be observed from the reactor with various width particularly from the 1st Run where reactors with small width seemed to give a slightly higher level of protein but more carbohydrate was found from reactor with large width. This could be the effect of temperature which was high when the width of the reactor was small, and as the width increase, the overshoot in temperature was not found and therefore a higher carbohydrate was observed. This was not apparent in the 2nd Run as the irradiation was relatively high and the temperature was generally high in this condition in all cases.

At 60 cm unaerated liquid heights, the system performed differently as this high liquid level decreased the level of light penetration as discussed earlier. In this condition, carbohydrate accumulation was small whereas the lipid content was enhanced. However, the effect of reactor width was not seen at all at this liquid level.

For *Scenedesmus* sp., Figures 4.18 - 4.20 demonstrate that the effect of reactor width (in the range employed here in this work) was very small and could not be concluded. The differences in the productivity of the various biochemical components were due primarily to the differences in the biomass productivity which was discussed earlier.

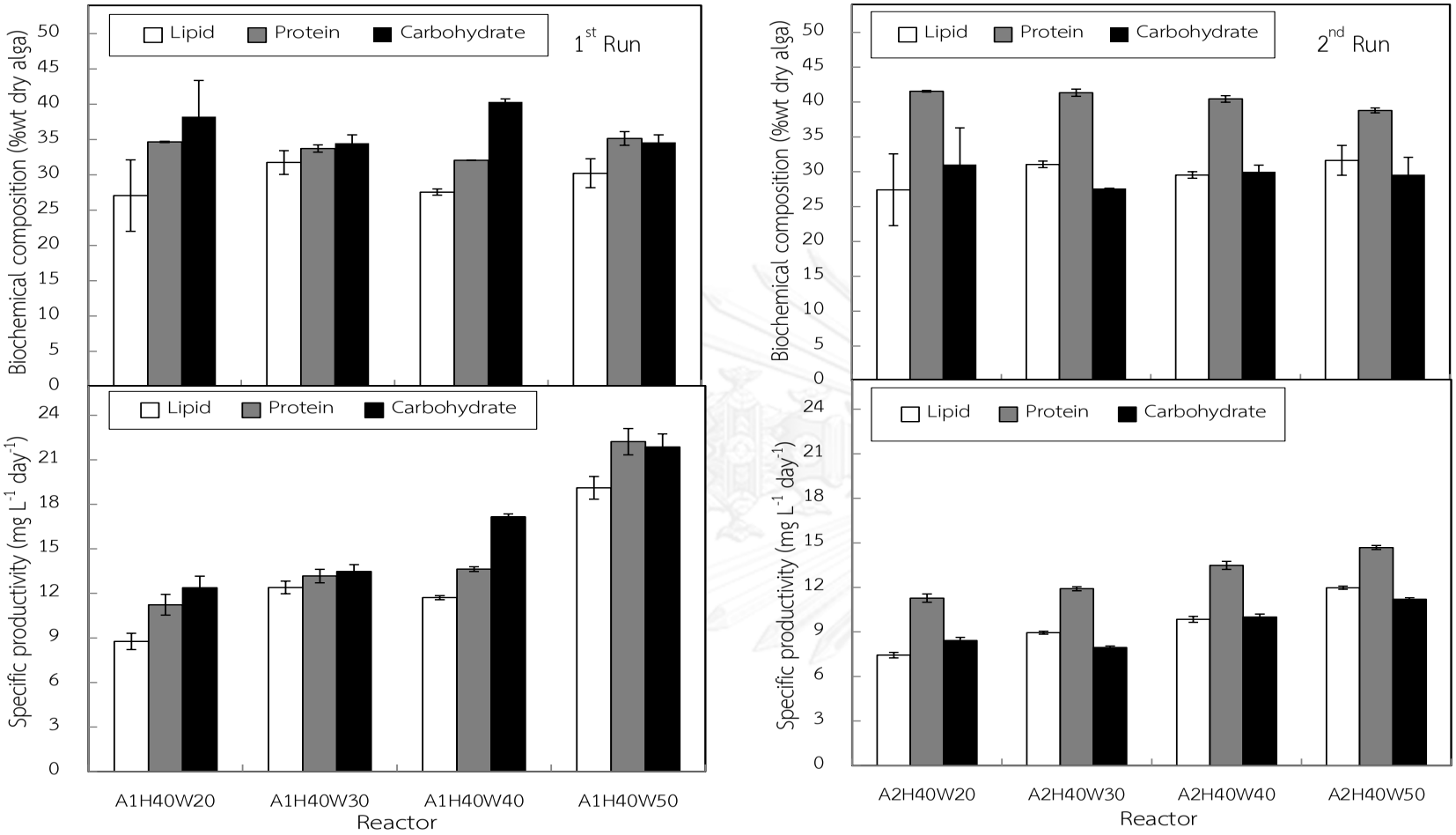


Figure 4.15 Comparison between protein, carbohydrate and lipid in dried *Ankistrodesmus* sp. in NB-FP-ALPBR with various widths at 40 cm unaerated liquid height

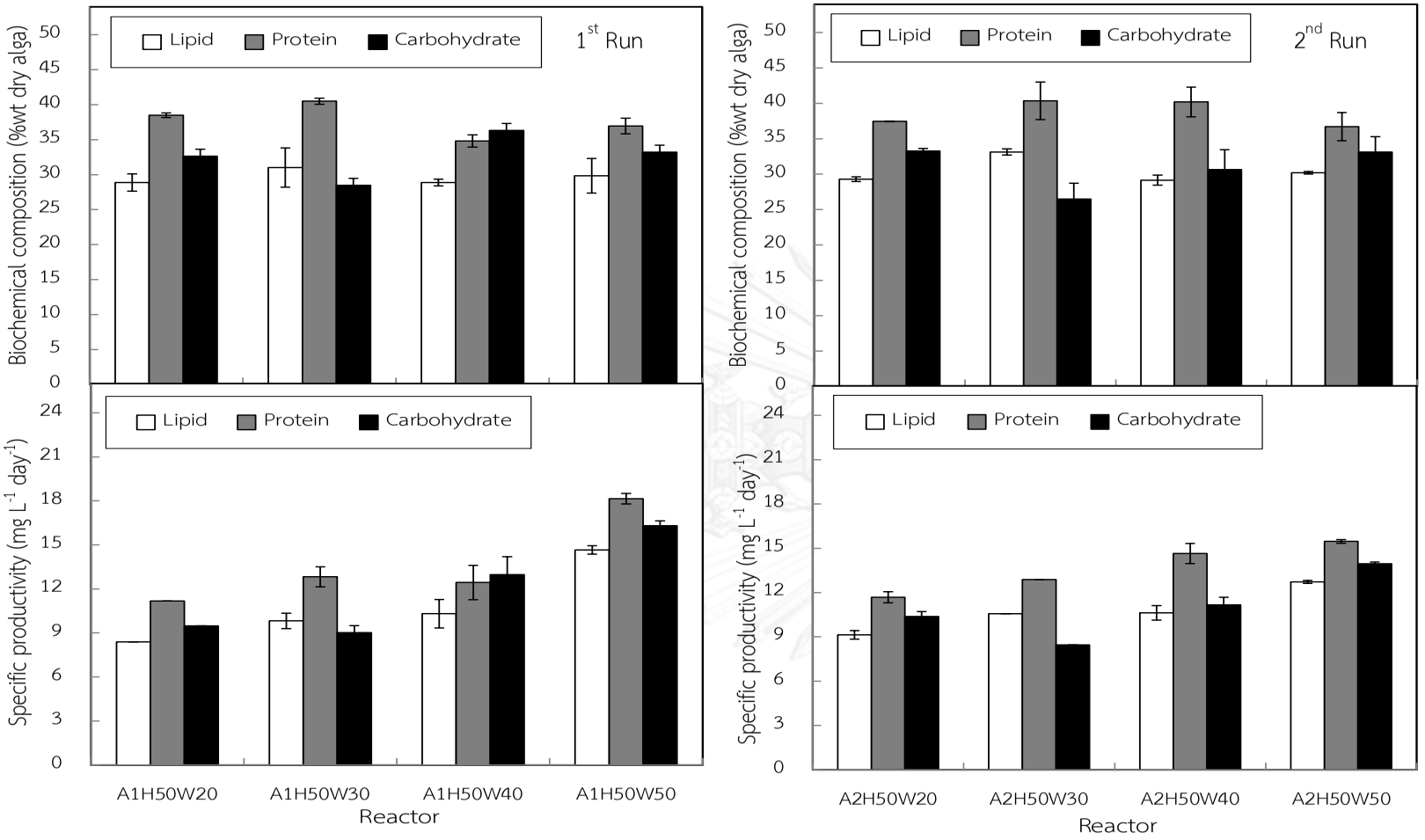


Figure 4.16 Comparison between protein, carbohydrate and lipid in dried *Ankistrodesmus* sp. in NB-FP-ALPBR with various widths at 50 cm unaerated liquid height

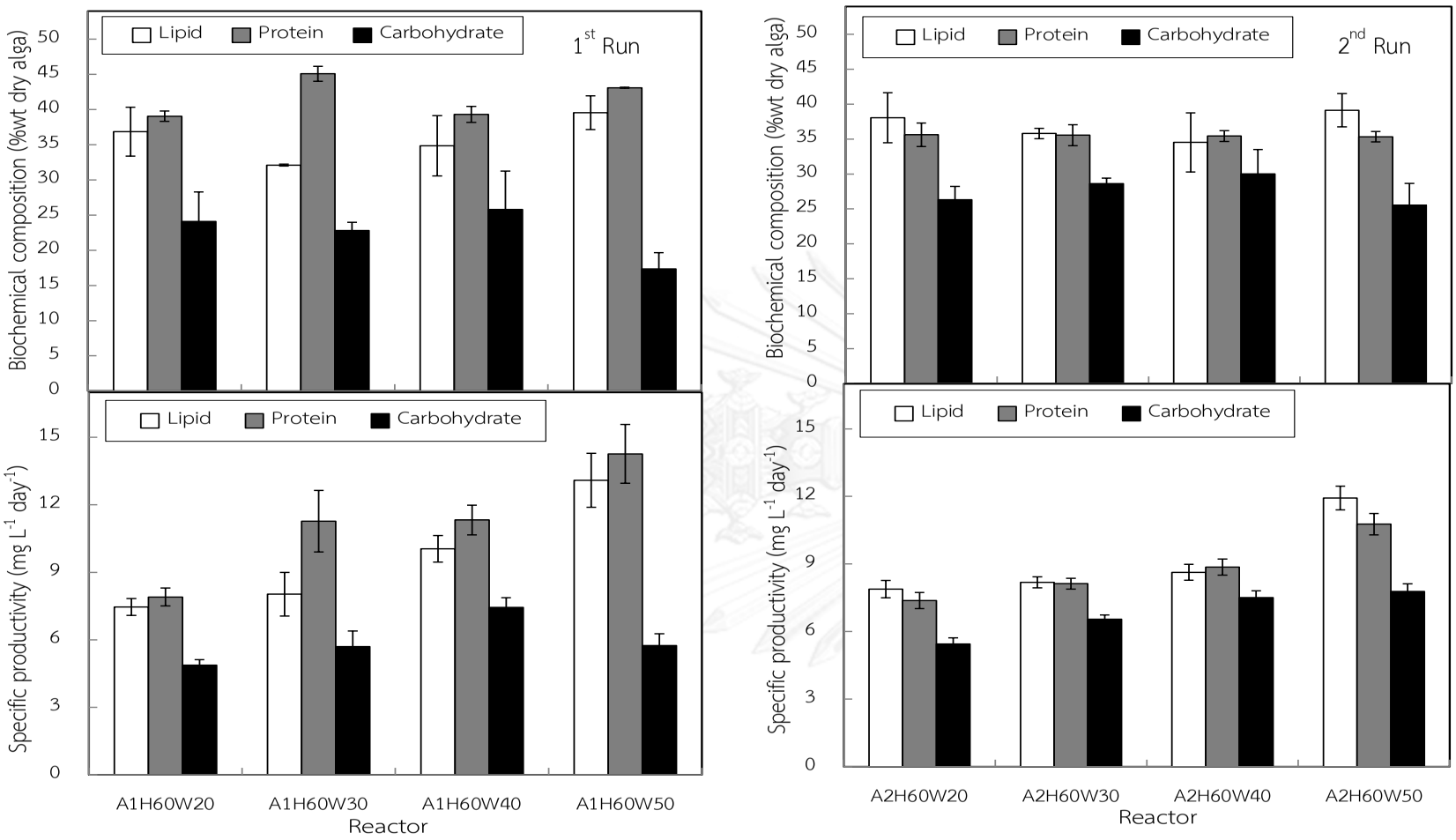


Figure 4.17 Comparison between protein, carbohydrate and lipid in dried *Ankistrodesmus* sp. in NB-FP-ALPBR with various widths at 60 cm unaerated liquid height

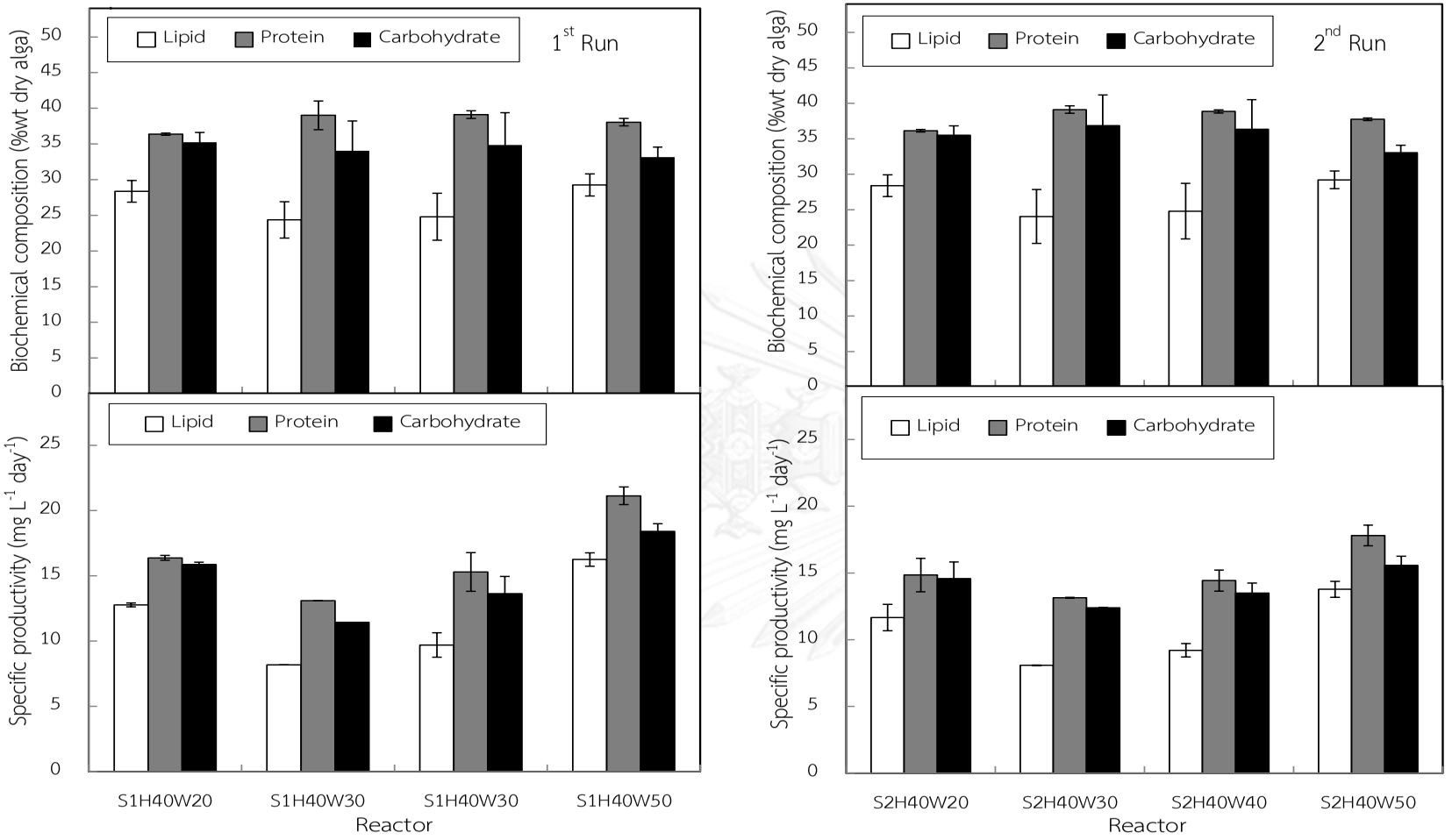


Figure 4.18 Comparison between protein, carbohydrate and lipid in dried *Scenedesmus* sp. Iin NB-FP-ALPBR with various widths at 40 cm unaerated liquid height

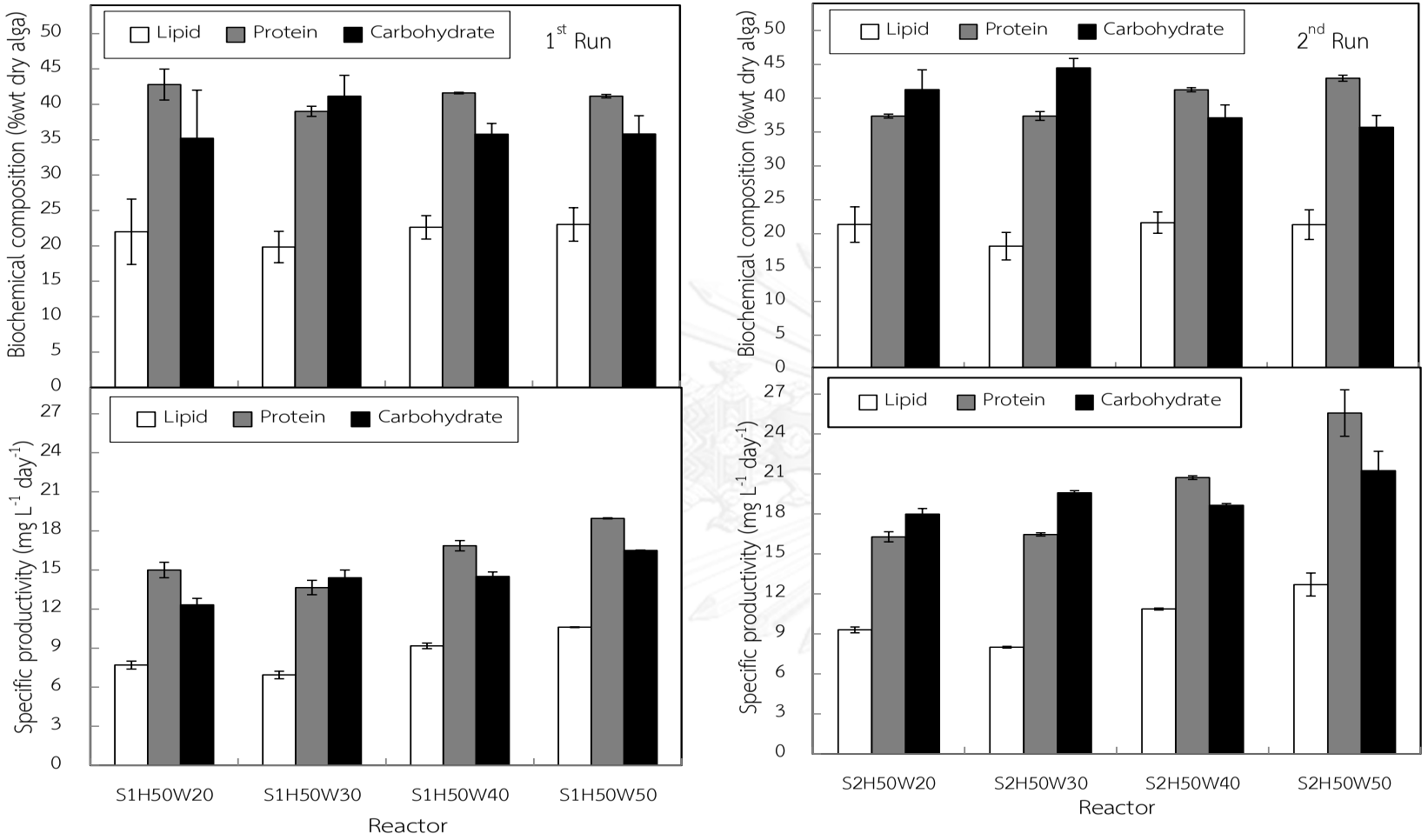


Figure 4.19 Comparison between protein, carbohydrate and lipid in dried *Scenedesmus* sp. Iin NB-FP-ALPBR with various widths at 50 cm unaerated liquid height

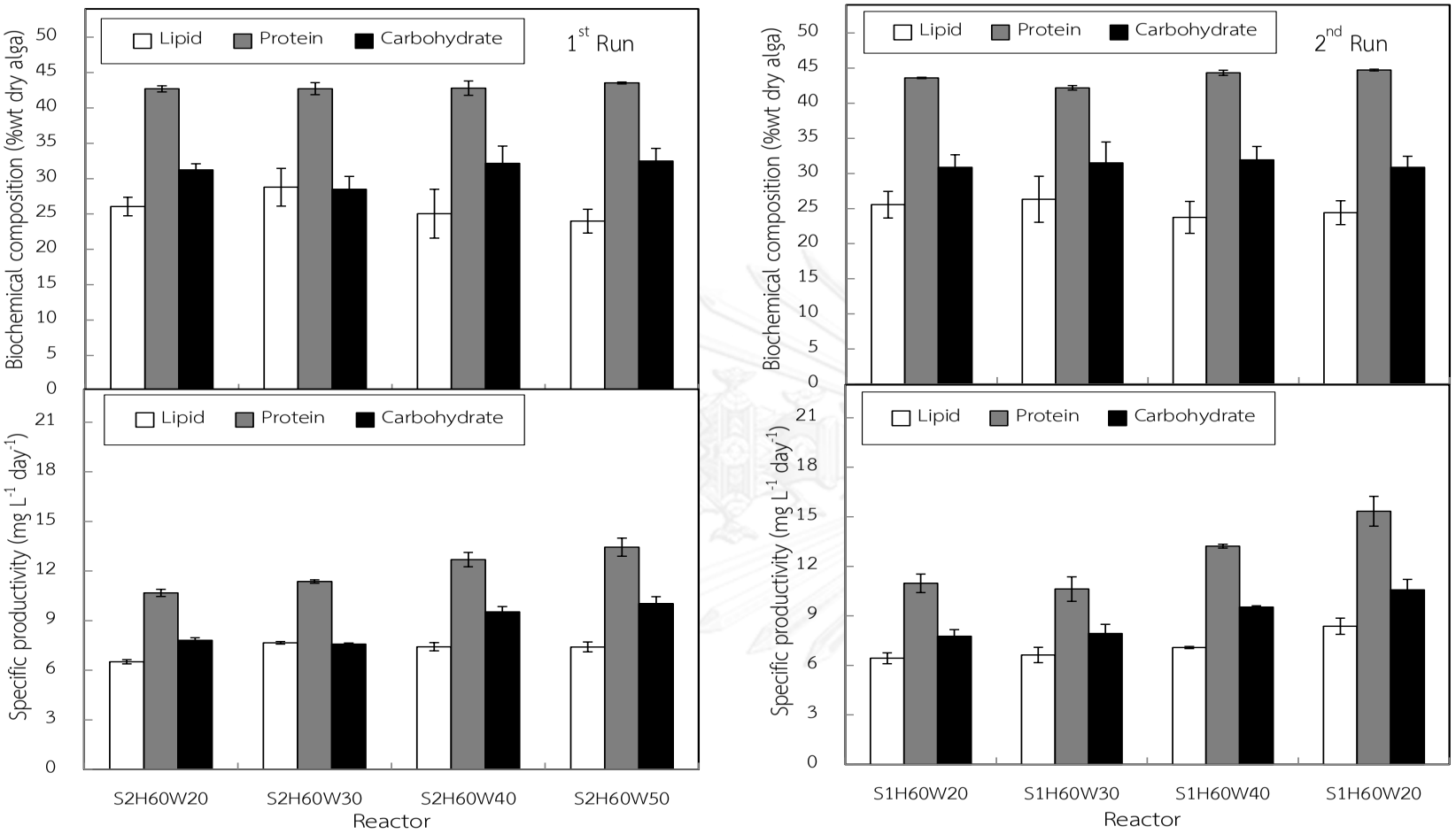


Figure 4.20 Comparison between protein, carbohydrate and lipid in dried *Scenedesmus* sp. Iin NB-FP-ALPBR with various widths at 60 cm unaerated liquid height

4.3.1 Effect of reactor height

Light plays central role in microalgae, providing the photon energy required in photosynthesis reaction to convert dissolved inorganic nutrient into organic molecule. The efficiency of light energy supply thus becomes one of the major limiting factors for outdoor or large-scale microalgae cultivation. Thereby, the configuration of microalgae cultivation systems should be designed to provide uniform and sufficient irradiance to microalga cells. Design with different light intensity could lead to differences in culture temperature which resulted in a variation in biochemical composition of the algal biomass. Generally, design with high light intensity would be associated with the operation with high temperature, and a high level of carbohydrate could be expected. On the other hand, if the temperature could be kept low, cells would accumulate more of protein or lipid rather than carbohydrate (Cuhel & Lean, 1987, Scott, 1980)

For both microalgae, Figures 4.21 - 4.22 demonstrate that the height of the system increased, the system was then operated with higher amount of liquid which could then maintain the level of temperature at low level. Therefore a low accumulation of carbohydrate was generally obtained as can be seen from the airlift with 60 cm unaerated liquid height. At low liquid height, either protein or lipid was accumulated. For *Ankistrodesmus* sp, more protein was found in such condition, whereas a larger lipid was accumulated for *Scenedesmus* sp. Having mentioned so, protein was still among the major biochemical components in *Scenedesmus* sp. culture regardless of the growth condition.

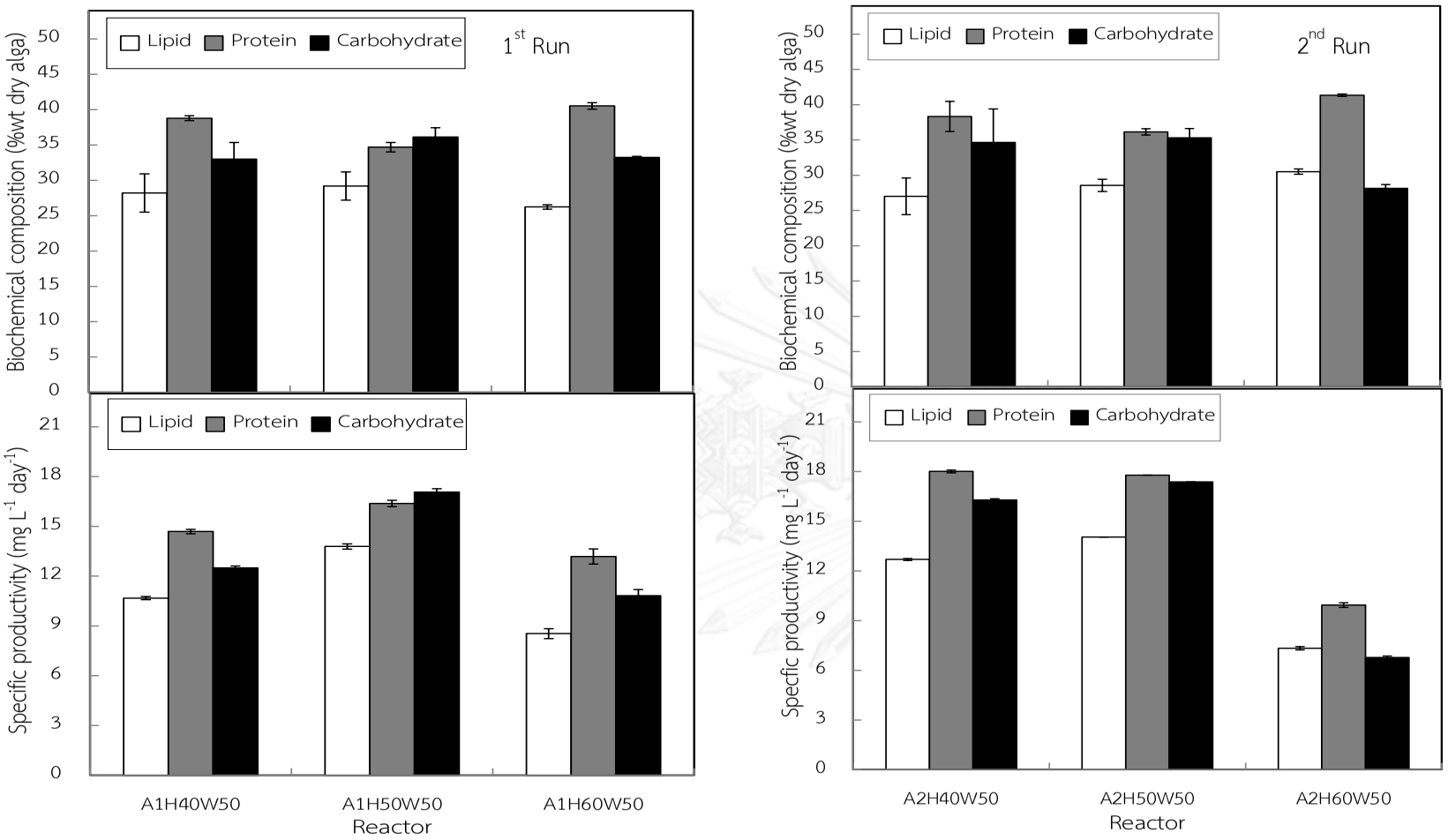


Figure 4.21 Comparison between protein, carbohydrate and lipid in dried *Ankistrodesmus* sp. in NB-FP-ALPBR with various heights and fixed width of 50 cm

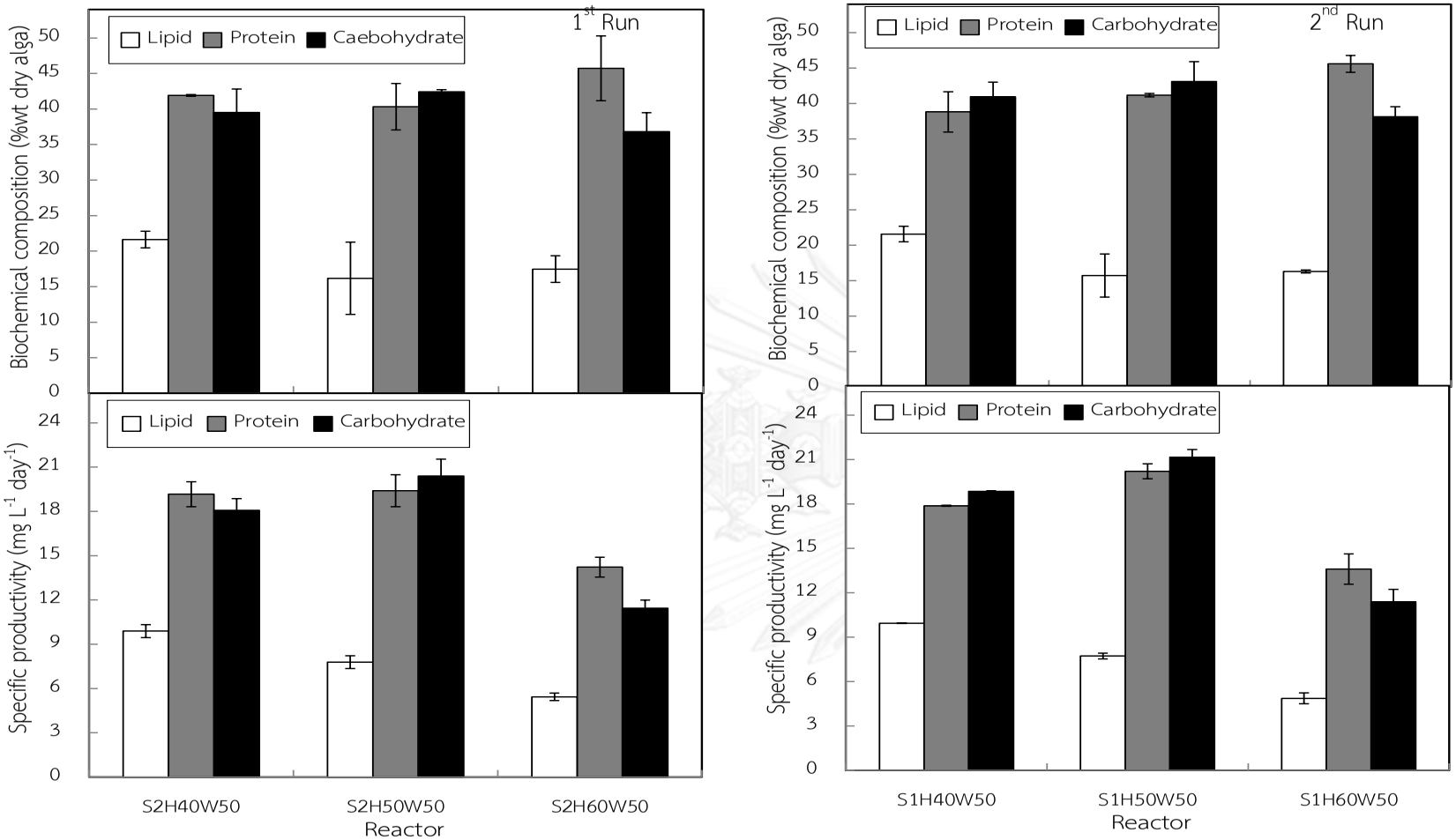


Figure 4.22 Comparison between protein, carbohydrate and lipid in dried *Scenedesmus* sp. in NB-FP-ALPBR with various heights and fixed width of 50 cm

4.4 Economic analysis for outdoor cultures of *Ankistrodesmus* sp. and *Scenedesmus* sp. in NB-FP-ALPBR

In this section, the economics of the outdoor cultivations of *Ankistrodesmus* sp. and *Scenedesmus* sp. in NB-FP-ALPBR as discussed in this work were evaluated. It is noted that the analysis was only performed to the system with the best operating condition, i.e. with the unaerated liquid height of 50 cm and the column width of 50 cm (the same for both cultures). The major factors determining the total production costs are identified and strategies are discussed to reduce those costs.

The results of the analysis are shown in Table 4.5. This analysis was based on 10 kilograms of dry cell which was generated in one year (based on 300 operating days per annum), and the calculations were performed using the local utility costs in Thailand in 2014. The life-span of the reactor, pump, sparger was assumed to be 5 years. The total cost was approx. 420.40 US$ $year^{-1}$ (42.04 US$ kg^{-1}) where the major costs for the cultivation of *Ankistrodesmus* sp. and *Scenedesmus* sp. was the capital cost which contributed almost 43% of the total investment cost, followed by those with electricity (32%), nutrient (23%) and water (2%) (illustrated in Fig. 4.23). (1 US$ = 32.30 Thai Baht (rate at 8th April 2014))

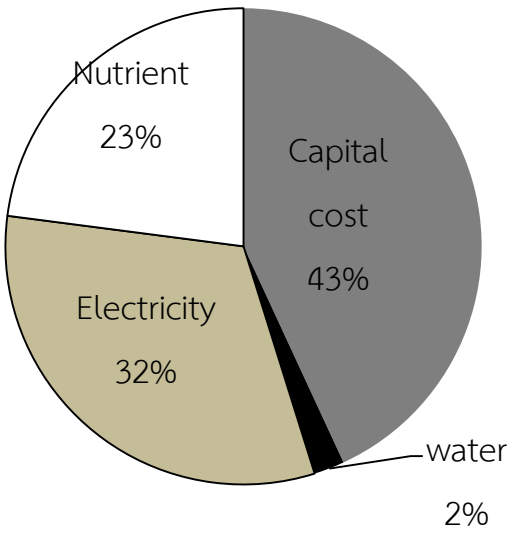


Figure 4.23 Major costs for the cultures of *Ankistrodesmus* sp. and *Scenedesmus* sp.

Table 4.5 Economical analysis for the cultivation of *Ankistrodesmus* sp. and *Scenedesmus* sp. in NB-FP-ALPBR

Detials	Symbol	NB-FP-ALPBR
Effective volume (m^3 $batch^{-1}$)	[A]	0.169
Cultivation period time (day $batch^{-1}$)	[B]	7
Number of cycle (batch $year^{-1}$)	[C=300/B]	42.86
Dry weight cell (g_{DW} L^{-1})	[D]	0.34
Productivity (g_{DW} $year^{-1}$)	[E=D x 1000 x A x 300/B]	2494.76
Target productivity (g_{DW} $year^{-1}$)	[F]	10000
Reactor requirements (unit)	[G=F/E]	4.0
Electricity requirements		
Number of air pumps (unit)	[H]	1
Power of air compressor (W)	[I]	33.33
Electricity consumption (KWh $year^{-1}$)	[J=(I/1000) x 300 x 24 x H]	961.92
Electrical cost, 0.1393 US$ KWh^{-1} (US$ $year^{-1}$)	[K=0.1393 x J]	134.02
Nutrient requirements		
Nutrient charge, 0.0033 US$ L^{-1} (US$ $year^{-1}$)	[L=A x G x 1000 x 0.0033 x C]	96.47
Water requirements		
Nutrient solution (m^3 $year^{-1}$)	[M=A x G x C]	29.03
Water charge, 0.3 US$ m^{-3} (US$ $year^{-1}$)	[N=M x 0.3]	8.71
Instruments		
Reactor cost, 123.85 US$ $unit^{-1}$ (US$ $year^{-1}$)	[O=(123.85 x G)/5]	99.28
Pump cost, 46.44 US$ $unit^{-1}$ (US$ $year^{-1}$)	[P=(46.44 x G)/5]	37.23
Sparger cost, 55.73 US$ $unit^{-1}$ (US$ $year^{-1}$)	[Q=(55.73 x G)/5]	44.68
Total capital cost (US$ $year^{-1}$)	[R=O+P+Q]	181.19
Total operating cost (US$ $year^{-1}$)	[S=K+L+N]	239.20
Total cost (US$ $year^{-1}$)	[T=R+S]	420.40

CHAPTER V

Conclusions and Recommendation

5.1 Conclusions

The culture of *Ankistrodesmus* sp. and *Scenedesmus* sp. in the non-baffled flat plate airlift photobioreactor (NB-FP-ALPBR) was shown to be possible. Main findings obtained from this work can be summarized as follows:

1. The size of reactor appeared to be statistically important for the growth in NB-FP-ARPBR for both *Ankistrodesmus* sp. and *Scenedesmus* sp.

2. The cultures of *Ankistrodesmus* sp. grew best in NB-FP-ALPBR with the width of 50 (0.44 g_{DW} L^{-1}), followed by those with 40 (0.30 g_{DW} L^{-1}), 30 (0.27 g_{DW} L^{-1}) and 20 cm (0.23 g_{DW} L^{-1}), respectively.

3. The cultures of *Scenedesmus* sp. grew best in NB-FP-ALPBR with the width of 50 (0.42 g_{DW} L^{-1}), followed by those with 40 (0.35 g_{DW} L^{-1}), 30 (0.31 g_{DW} L^{-1}) and 20 cm (0.31 g_{DW} L^{-1}), respectively. However, this was on exception at the unaerated liquid height of 40 cm where the growth rate was ordered from high to low as 50 (0.39 g_{DW} L^{-1}), 20 (0.32 g_{DW} L^{-1}), 40 (0.27 g_{DW} L^{-1}) and 30 cm (0.24 g_{DW} L^{-1}), respectively.

4. The cultures of both microalgae grew best in NB-FP-ALPBR with the height of 50, followed by those with 40 and 60 cm, respectively. (0.34, 0.33 and 0.27 g_{DW} L^{-1} for *Ankistrodesmus* sp. and 0.34, 0.32 and 0.22 g_{DW} L^{-1} for *Scenedesmus* sp.)

5. There was no direct relationship between biochemical composition with the configuration of NB-FP-ALPBR. However, the system with high liquid height would be subject to a slightly lower light penetration, especially through the top surface of the system.

6. For both microalgae, protein seemed to be the major composition (35-45%) whereas lipid was the minor. Carbohydrate was found to be rich when there was adequate light for the growth particularly for *Ankistrodesmus* sp; a similar trend (but less clear) was found for *Scenedesmus* sp.

7. The total investment cost was approx. 420.40 US$ $year^{-1}$ (42.04 US$ kg^{-1}) where the major costs for the cultivation of *Ankistrodesmus* sp. and *Scenedesmus* sp. was the instruments cost which contributed almost 43% of the total investment cost, followed by those with electricity (32%), nutrient (23 %) and water (2 %).

5.2 Contributions

This work has achieved a steady and successful culture of *Ankistrodesmus* sp. and *Scenedesmus* sp. in flat plate airlift photobioreactor without draft tube under Thailand climate conditions. The results suggest that there exist cofactors or various induction design parameters, e.g. width and height, which could affect the growth of *Ankistrodesmus* sp. and *Scenedesmus* sp. The maximum height of the reactor employed in this work is limited at 50 cm as it could practically allow a more effective control of light intensity. Nevertheless, the scale up would be feasible achieved simply by expansion the length of NB-FP-ALPBR. In addition, NB-FP-ALPBR could be installed, operated, and maintained with ease. In other words, the results from this work help fulfill the culture of the production of both microalgae.

5.3 Recommendations

This cultural system still suffered from the height energy consumption especially for the electricity requirements, amount of water and high cost of nutrients, which rendered the system not economically attractive. To reduce the production cost, any options regarding the use of alternative or renewable energy could be considered such as biogas from some specific wastewater (Hadiyanto & Hendroko, 2014). However, although this could effectively reduce the electricity cost, this does not have anything to do with the culture cultivation efficiency. The use of alternative nutrients could also be employed to ensure the cheapest, but still effective, nutrient recipe. There are reports of success in using wastewater from some specific industry such as coal seam gas industry (Hamawand *et al.*, 2014), pharmaceutical industry (Yu *et al.*, 2014) which might help reduce the nutrient costs. However, how well the microalgae could grow in such nutrients or how to effectively use such wastewater as alternative nutrient still needs to be examined. The addition of CO_2 for the growth of the algae might also give a beneficial outcome for the economics of the culture as long as CO_2 can be freely obtained. The use of CO_2 has been investigated and reported quite extensively (Talec *et al.*, 2013, Li *et al.*, 2013, Arbib *et al.*, 2013, Nakanishi *et al.*, 2014). The reuse of nutrients or the adjustment of the medium composition could also give better economical outcome. (Rodrakhee, 2014) reported that the reuse of nutrient could effectively reduce the nutrient cost for

Ankistrodesmus sp. whereas (Kim *et al.*, 2011) reported the success in the use of medium for *Scenedesmus* sp. Such cost reduction options will need to be further investigated.

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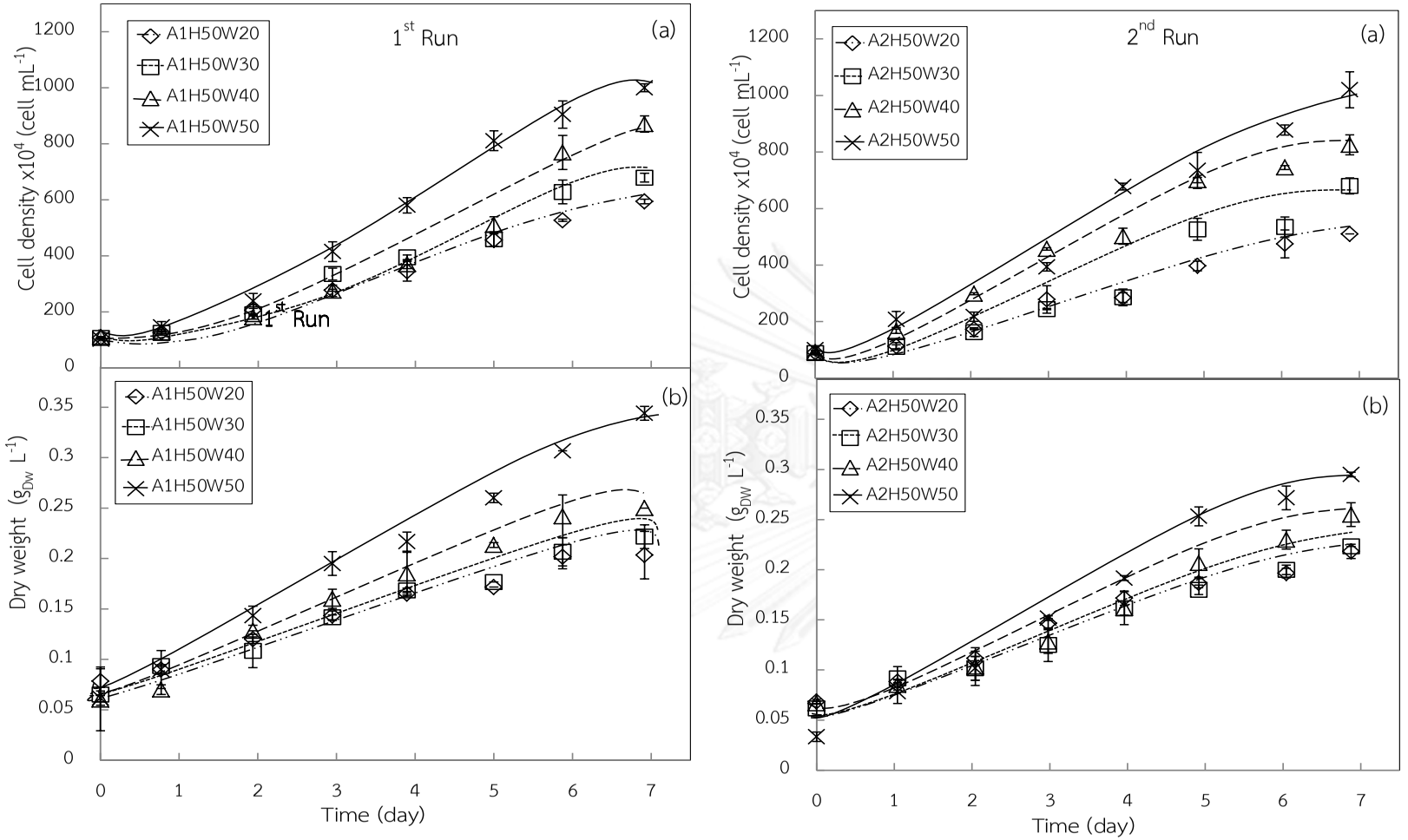
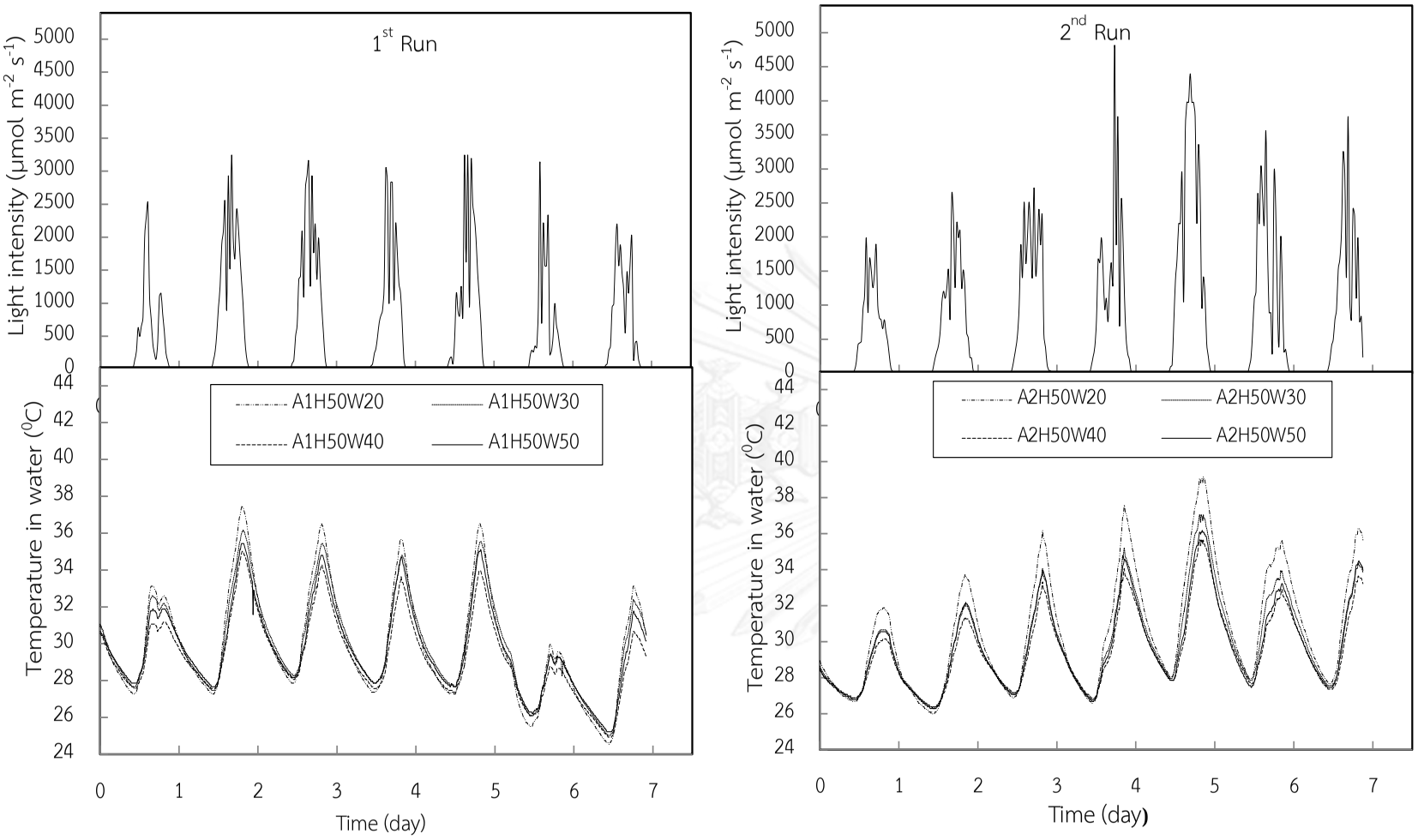
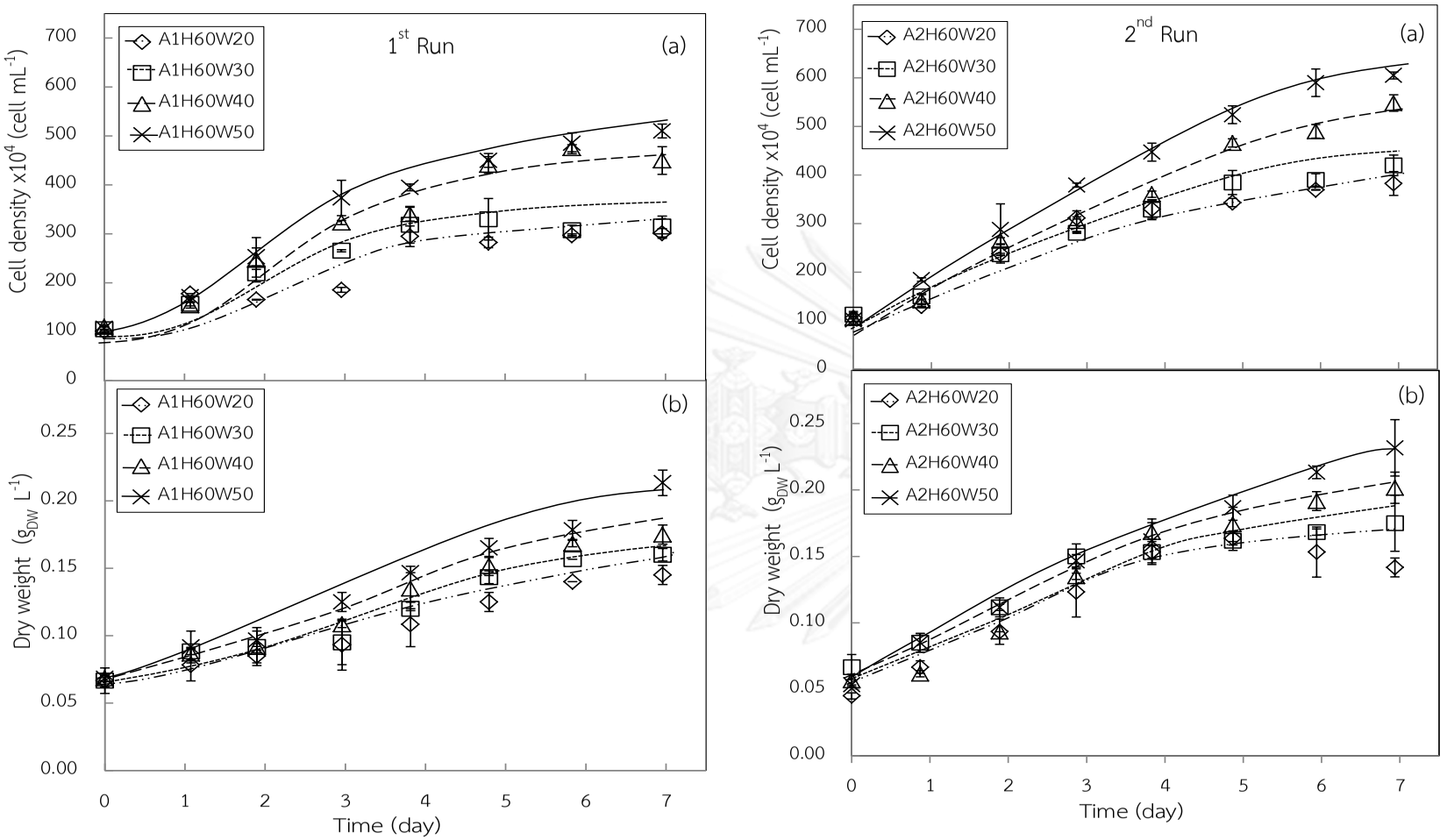
APPENDIX



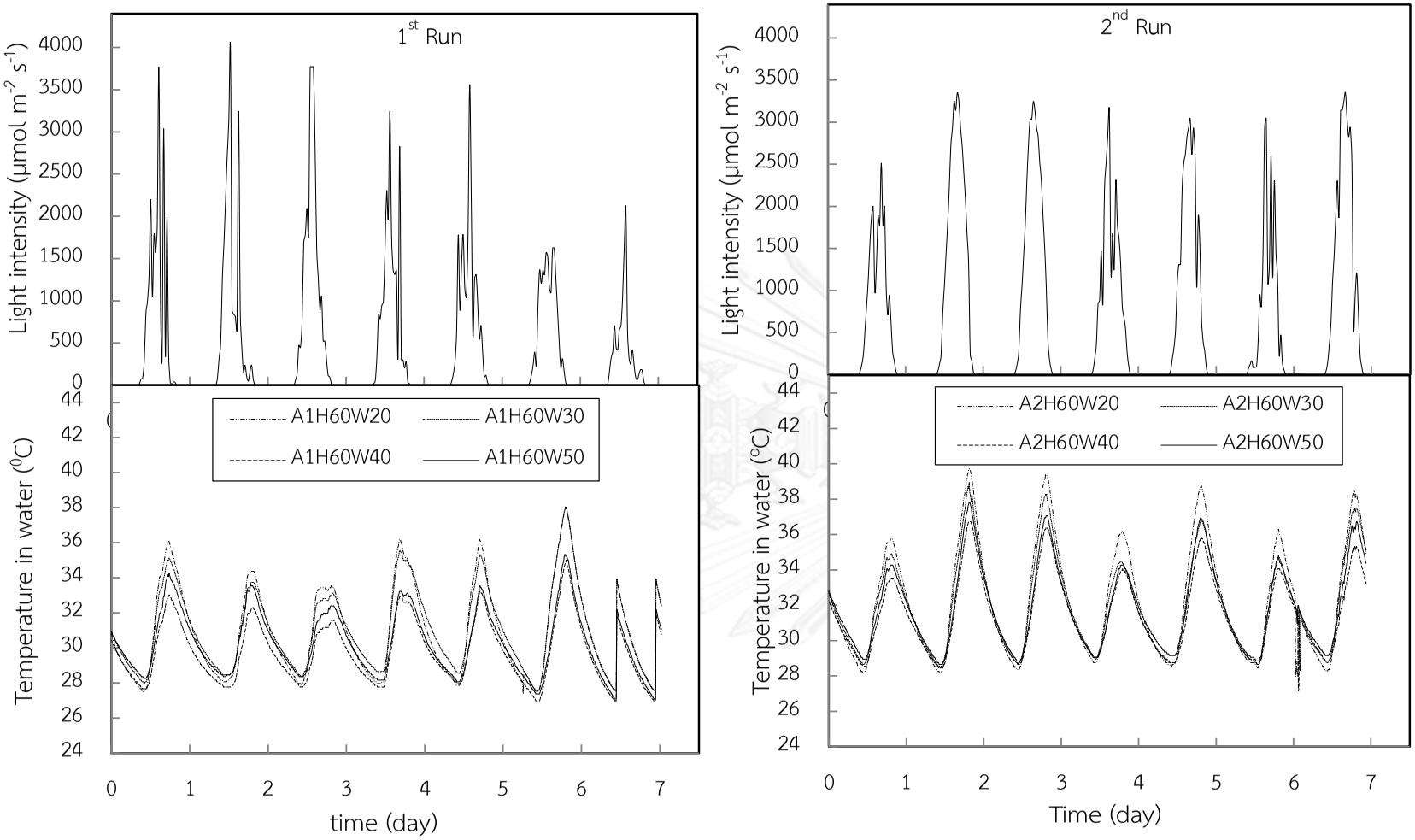
Appendix A-1 Comparison between growth behavior of *Ankistrodesmus* sp. in NB-FP-ALPBR with various widths at 50 cm unaerated liquid height :(a) Cell density (cell mL^{-1}); and (b) Dry weight (g_{DW} L^{-1})



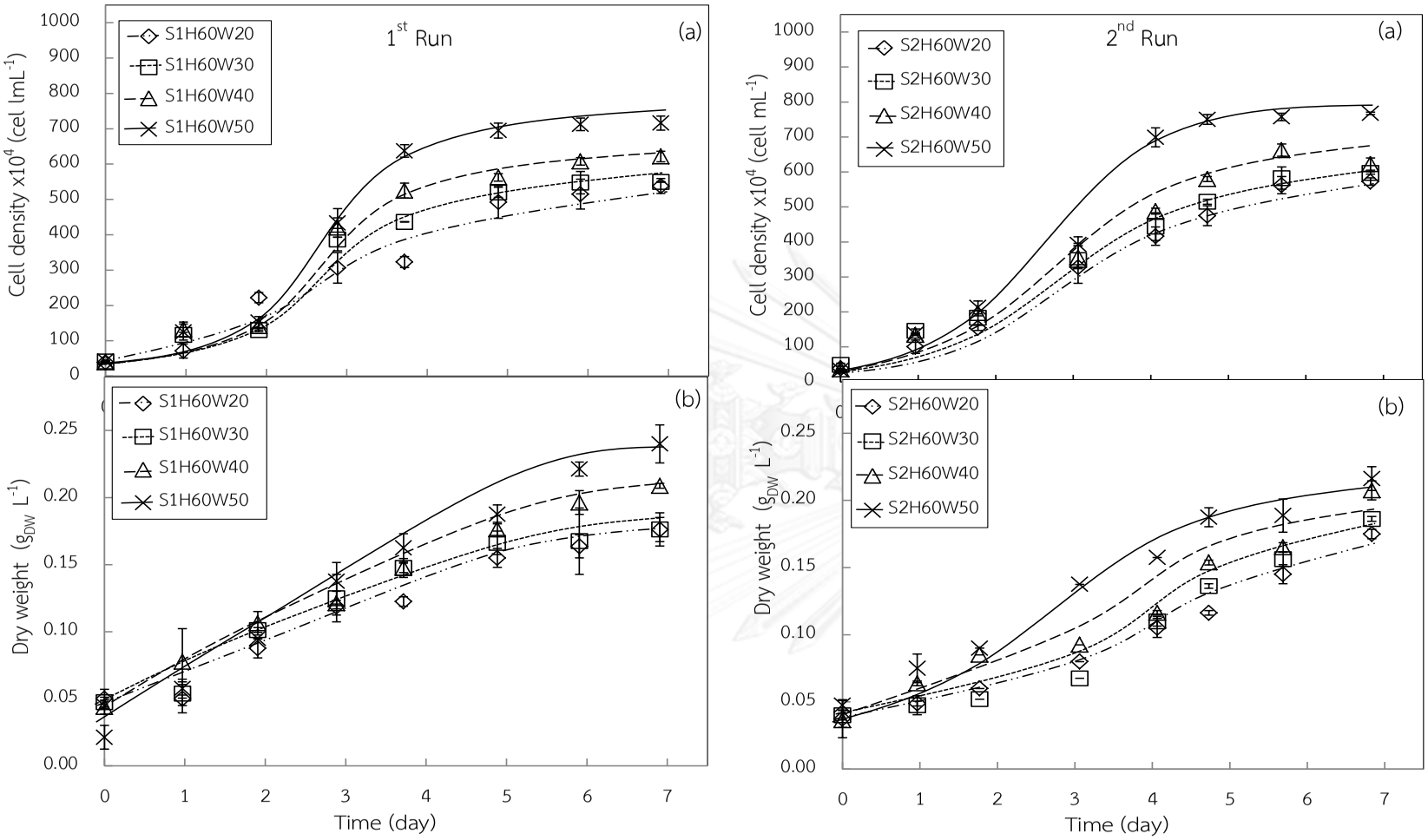
Appendix A-2 Time courses of temperatures and light intensities during the growth of *Ankistrodesmus* sp. in NB-FP-ALPBR with various widths at 50 cm unaerated liquid height



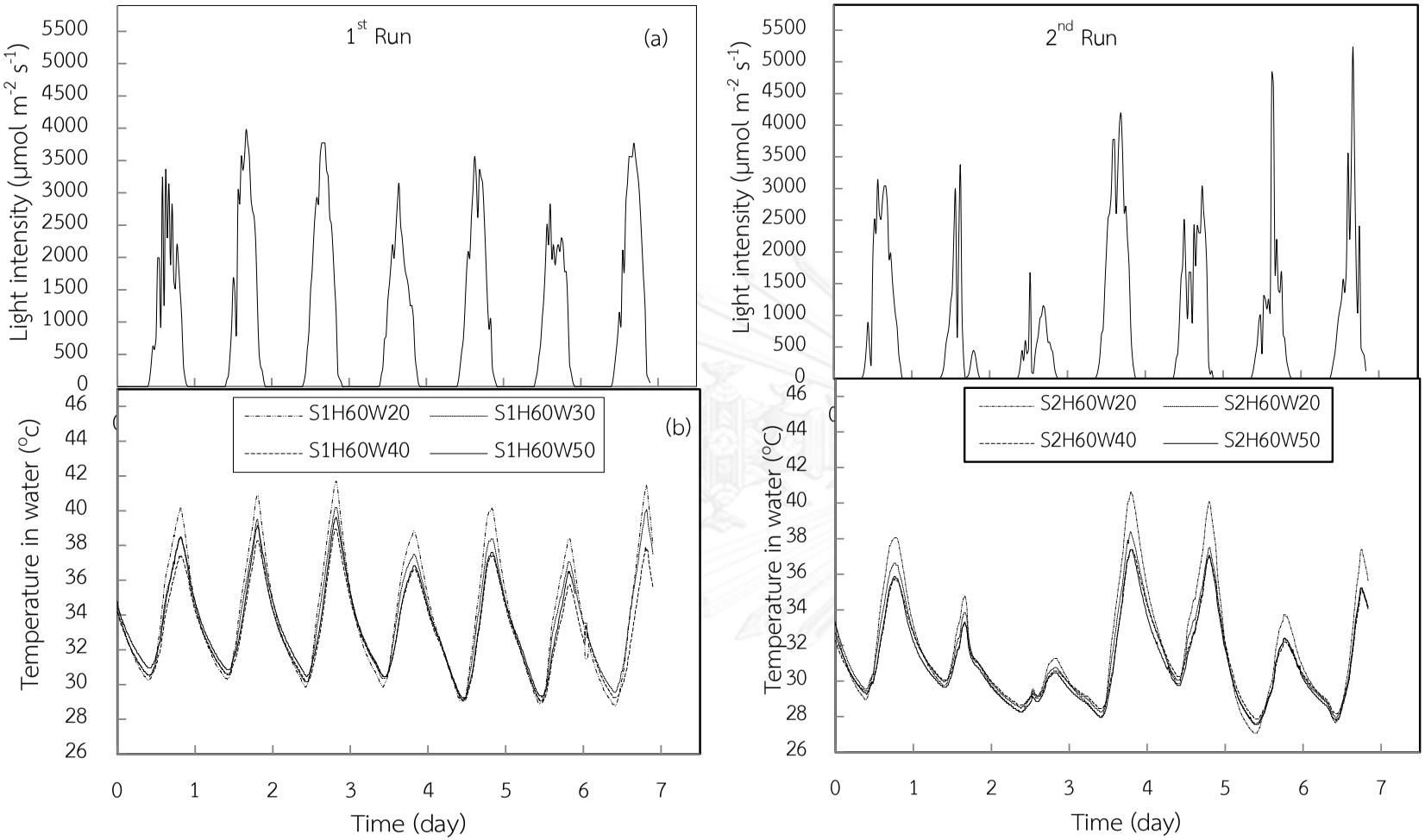
Appendix A-3 Comparison between growth behavior of *Ankistrodesmus* sp. in NB-FP-ALPBR with various widths at 60 cm unaerated liquid height :(a) Cell density (cell mL^{-1}); and (b) Dry weight (g_{DW} L^{-1})



Appendix A-4 Time courses of temperatures and light intensities during the growth of *Ankistrodesmus* sp. in NB-FP-ALPBR with various widths at 60 cm unaerated liquid height



Appendix A-5 Comparison between growth behavior of *Scenedesmus* sp. in NB-FP-ALPBR with various widths at 60 cm unaerated liquid height :(a) Cell density (cell mL^{-1}); and (b) Dry weight (g_{DW} L^{-1})



Appendix A-6 Time courses of temperatures and light intensities during the growth of *Scenedesmus* sp. in NB-FP-ALPBR with various widths at 60 cm unaerated liquid height

Appendix B-1 Specific consumption rates of nutrients and trace elements for *Ankistrodesmus* sp. in NB-FP-ALPBRs with various widths at fixed height ($mg_{elements}/mg_{cell}$)

	B x10^5	Ca x10^3	Co x10^6	Cu x10^6	Fe x10^6	K x10^3	Mg x10^3	Mn x10^5	Mo x10^5	N x10^3	P x10^3	Zn x10^3
A1H40W20	10.19	15.97	5.29	44.12	7.94	3.90	7.19	9.04	14.51	130.89	7.48	0.13
A1H40W30	9.99	10.98	2.93	5.85	16.83	3.58	16.02	23.93	10.98	132.09	5.14	0.06
A1H40W40	46.79	11.19	9.08	15.80	22.52	3.07	9.82	17.08	3.93	123.13	5.65	0.15
A1H40W50	24.77	55.74	22.37	33.90	15.82	14.38	12.34	14.76	11.77	132.93	6.59	0.09
A2H40W20	47.79	8.68	22.63	22.63	34.21	9.00	16.21	5.26	2.63	116.94	9.50	0.11
A2H40W30	29.90	7.14	23.80	23.8	46.12	5.54	19.29	7.69	17.80	103.98	16.46	0.17
A2H40W40	16.89	2.36	19.71	19.71	11.14	2.72	13.03	3.64	7.33	111.11	12.51	0.12
A2H40W50	33.55	11.02	38.11	38.11	13.21	2.79	12.91	2.83	11.92	101.33	5.27	0.14
A1H50W20	68.11	46.87	39.34	75.74	49.18	71.78	23.73	22.82	19.03	87.39	12.66	8.07
A1H50W30	49.44	39.79	43.76	67.22	12.18	71.19	18.53	29.57	22.11	93.20	5.69	6.72
A1H50W40	52.28	15.64	22.40	49.20	20.00	84.79	12.15	17.64	20.32	91.23	3.38	7.23
A1H50W50	60.49	24.08	30.83	40.72	14.25	95.25	18.41	12.33	18.28	87.29	4.36	7.14
A2H50W20	65.68	22.95	7.79	49.47	13.28	304.73	12.26	26.61	9.02	56.49	11.93	0.11
A2H50W30	54.45	25.93	29.20	62.24	6.27	372.99	55.40	30.22	60.36	90.23	5.04	0.31
A2H50W40	33.92	18.23	30.59	55.29	2.75	133.84	56.65	21.80	50.75	67.56	6.17	0.23
A2H50W50	8.92	13.53	23.73	41.68	13.56	110.47	46.01	28.58	41.59	77.44	6.91	0.21
A1H60W20	29.79	11.17	22.07	53.10	48.97	330.34	14.48	0.76	10.14	151.05	7.75	0.27
A1H60W30	26.56	13.50	32.81	50.00	20.63	290.63	4.25	0.69	7.38	183.64	9.14	0.33
A1H60W40	82.74	12.74	8.57	44.00	41.14	216.00	15.26	0.63	11.83	141.14	9.70	0.26
A1H60W50	47.72	9.56	9.84	46.88	26.86	109.69	9.05	0.84	10.97	104.68	4.31	0.20
A2H60W20	100.54	27.88	18.35	72.71	16.22	162.21	44.96	8.05	12.99	128.51	10.76	0.26
A2H60W30	111.09	31.43	25.71	81.71	5.17	108.86	43.15	21.14	19.75	132.39	9.54	0.21
A2H60W40	94.76	18.05	5.95	56.03	4.05	104.93	16.61	35.76	9.72	135.30	5.01	0.16
A2H60W50	73.99	30.26	24.17	38.68	9.97	90.78	19.47	29.4	14.55	128.49	5.44	0.46

Appendix B-2 Specific consumption rates of nutrients and trace elements for *Scenedesmus* sp. in NB-FP-ALPBRs with various widths at fixed height ($mg_{elements}/mg_{cell}$)

	B x10^5	Ca x10^3	Co x10^6	Cu x10^6	Fe x10^6	K x10^3	Mg x10^3	Mn x10^5	Mo x10^5	N x10^3	P x10^3	Zn x10^3
S1H40W20	3.30	7.33	11.43	52.06	71.43	2.60	17.65	23.05	5.52	87.53	9.26	0.15
S1H40W30	76.64	13.70	17.87	56.60	88.94	3.69	24.85	32.85	9.02	110.458	10.60	0.17
S1H40W40	36.89	9.06	15.34	23.38	84.02	2.77	23.91	30.54	7.12	119.30	11.36	0.14
S1H40W50	35.37	5.20	5.40	29.58	55.79	3.96	14.12	20.60	5.14	107.22	9.19	0.10
S2H40W20	35.70	11.09	10.43	43.35	10.78	3.09	14.70	49.85	5.91	34.98	4.32	4.40
S2H40W30	23.75	16.36	12.32	46.94	9.77	3.02	8.41	41.29	6.67	84.98	4.57	2.49
S2H40W40	23.81	21.85	18.85	74.00	12.27	2.90	17.73	41.04	7.23	76.99	4.42	5.90
S2H40W50	13.27	9.91	5.45	36.78	5.39	3.11	12.62	19.09	8.15	65.09	6.43	8.03
S1H50W20	54.52	12.79	4.44	13.92	95.24	76.19	5.25	52.46	8.03	76.90	6.61	0.13
S1H50W30	59.10	7.35	6.94	30.20	129.18	71.51	9.14	34.49	4.45	98.29	10.83	0.16
S1H50W40	112.32	11.95	20.37	17.62	86.34	72.00	16.50	57.8	20.41	34.34	9.64	0.17
S1H50W50	33.15	8.37	13.33	26.98	78.14	76.78	7.50	47.44	9.46	67.45	7.79	0.20
S2H50W20	42.20	13.15	40.98	17.64	61.97	62.95	6.36	59.28	15.61	99.48	6.19	0.11
S2H50W30	25.72	6.65	25.62	26.92	60.32	127.14	3.79	75.15	8.96	109.23	5.20	0.18
S2H50W40	36.61	6.68	24.45	20.67	39.81	50.05	5.89	65.00	5.08	86.90	6.53	0.09
S2H50W50	35.55	5.62	11.76	30.24	62.83	32.88	3.86	50.42	4.39	111.23	3.56	0.14
S1H60W20	39.80	22.20	13.06	59.62	28.96	157.73	13.63	58.48	5.90	78.09	12.24	0.27
S1H60W30	19.57	17.36	7.38	49.93	28.26	128.40	6.24	64.79	1.19	67.35	16.58	0.11
S1H60W40	50.49	16.86	3.83	40.24	24.14	136.72	4.60	45.56	0.81	65.98	8.83	0.06
S1H60W50	33.08	9.13	6.67	40.50	21.13	119.00	4.83	42.00	2.83	71.09	8.98	0.11
S2H60W20	87.77	16.91	14.29	45.71	23.43	65.37	18.46	58.23	8.11	34.98	10.06	0.13
S2H60W30	32.23	11.01	19.87	46.71	23.62	82.41	5.96	56.86	6.23	29.89	15.12	0.18
S2H60W40	97.83	21.25	14.94	41.45	23.13	70.98	9.29	54.65	3.57	28.84	12.84	0.19
S2H60W50	65.62	7.31	11.56	44.39	19.56	78.28	8.69	54.43	4.30	31.87	10.53	0.14

Appendix B-3 Specific consumption rates of nutrients and trace elements for *Ankistrodesmus* sp. in NB-FP-ALPBRs with various heights at fixed width ($mg_{elements}/mg_{cell}$)

	B x10^5	Ca x10^3	Co x10^6	Cu x10^6	Fe x10^6	K x10^3	Mg x10^3	Mn x10^5	Mo x10^5	N x10^3	P x10^3	Zn x10^3
A1H40W50	40.11	4.89	5.98	18.97	50.38	47.34	5.20	71.04	5.29	130.22	11.59	0.21
A1H50W50	45.35	4.05	4.24	16.94	46.29	49.01	4.45	50.22	9.17	129.66	8.16	0.13
A1H60W50	43.81	5.40	7.48	23.27	68.05	45.66	6.76	77.66	3.47	158.48	14.27	0.12
A2H40W50	28.95	5.65	6.55	10.12	28.86	24.69	3.66	14.58	3.60	24.46	8.26	0.12
A2H50W50	22.65	3.69	4.94	14.23	24.97	51.68	1.34	16.58	2.50	31.91	7.65	0.06
A2H60W50	27.87	4.19	4.11	12.44	25.94	34.97	2.19	14.96	3.98	29.11	7.39	0.18

Appendix B-4 Specific consumption rates of nutrients and trace elements for *Scenedesmus* sp. in NB-FP-ALPBRs with various heights at fixed width ($mg_{elements}/mg_{cell}$)

	B x10^5	Ca x10^3	Co x10^6	Cu x10^6	Fe x10^6	K x10^3	Mg x10^3	Mn x10^5	Mo x10^5	N x10^3	P x10^3	Zn x10^3
S1H40W50	23.11	6.48	5.93	69.39	91.98	30.19	3.97	74.64	6.34	87.09	5.35	0.34
S1H50W50	22.70	4.48	3.87	80.46	99.35	29.10	1.16	81.57	2.97	77.40	2.37	0.13
S1H40W50	23.93	10.77	10.53	78.21	94.54	29.20	5.70	93.87	7.75	91.59	14.24	0.49
S2H40W50	31.78	10.63	7.50	24.72	37.19	79.69	3.06	2.64	1.38	67.69	8.47	0.10
S2H50W50	15.62	6.95	14.55	44.55	51.03	31.78	1.81	6.23	3.89	51.59	4.41	0.39
S2H60W50	22.89	11.39	12.49	31.39	43.29	43.44	2.94	1.97	0.99	56.33	6.33	0.11

Appendix C-1 Measurement of nitrogen concentration by spectrophotometer (Strickland, and Parsons, 1972)

Blank

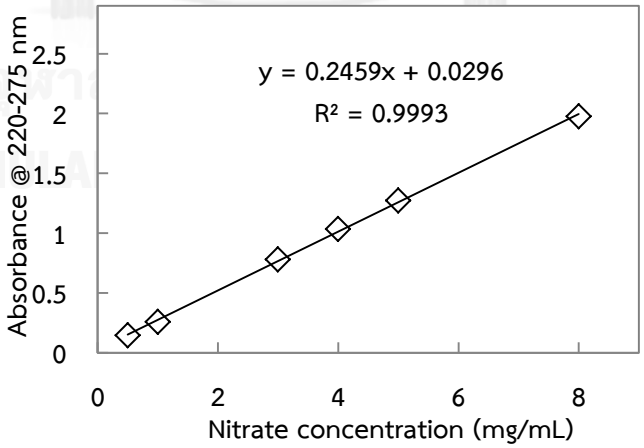
1 mL of distilled water is measured by spectrophotometer at wavelength of 220 and 275 nm and blank is set to zero.

Calibration

1. KNO_3 stock solution 100 mg NO_3-N/L is prepared by dissolved 0.7128 g of KNO_3 in 1000 mL of distilled water and keep with 1 mL of chloroform in dark glass.
2. KNO_3 stock is diluted using distilled water as 0.15, 0.25, 0.75, 1.0, 1.25, and 2.0 mg NO_3-N/L.
3. The solution is measured by spectrophotometer at wavelength of 220 and 275 nm.

Procedure

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



Appendix C-1 Standard of nitrogen concentration by spectrophotometer

Appendix D Hydrodynamic method (Sintharm, 2014)

D-1 Riser and downcomer cross section area

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 (with cone bottom) airlift and rectangular cylinder for the flat panel airlift.

For circular column with cone bottom airlift, the riser cross section area is

$$A_r = \pi R_r^2 \qquad D\text{-}1$$

R_r was obtained from the experiment, and the downcomer cross sectional area (A_d) can be calculate from

$$A_d = Area\ of\ column - Area\ of\ riser \qquad D\text{-}2$$

For flat panel airlift; the riser cross section area is

$$A_r = W_r \times L \qquad D\text{-}3$$

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

$$W_d = width\ of\ reactor - W_r \qquad D\text{-}4$$

and the downcomer cross section area is

$$A_d = W_d \times L \qquad D\text{-}5$$

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.

D-2 Determination of virtual riser and downcomer cross-section area

As the airlift had no solid partition to indicate the riser and downcomer area, the sizes of riser and downcomer were variable. It was the purpose of this section to propose a method employed to determine the 'virtual' cross sectional area of riser and downcomer. This boundary between riser and downcomer was important for the calculation of hydrodynamics and mass transfer in the system. The liquid flow directions were observed by directions of light rope as follows:

- Turn on the air at a desired flow rate to the water-filled airlift contactors, wait until the system operates at steady state (no further changed in fluid movement)
- Inject pole with a light rope and analyze the characteristics of the light rope directions. If the rope flow up show that, this point is defined in riser zone if the rope flow down, this point is downcomer
- Repeat experiments at various height
- Estimate the distances of riser and calculate area of riser of each type of contactor with Equations *D-1* and *D-3*
- Calculate downcomer area by Equations *D-2* and *D-5*

D-3 Overall gas holdup

The overall gas holdup could be calculated from the volume expansion which was measured from the difference between aerated and unaerated liquid heights. The definition of gas holdup is

$$\varepsilon = \frac{V_G}{V_G+V_L} \qquad D\text{-}6$$

The volume of gas could not be measured directly, so that we defined V_A (volume in system when aeration) as the total volume of gas phase plus volume of liquid phase. Then

$$\varepsilon = \frac{V_A-V_L}{V_A} \qquad D\text{-}7$$

$$\varepsilon = 1 - \frac{V_L}{V_A} \qquad D\text{-}8$$

$$\varepsilon = 1 - \frac{h_u A}{h_A A} \qquad D\text{-}9$$

$$\varepsilon = 1 - \frac{h_u}{h_A} \qquad D\text{-}10$$

where ε = overall gas holdup

h = liquid height (cm)

V = volume (cm^3)

subscript L = liquid

G = gas

A = aerated

u = unaerated.

VITA

Mister Eakkachai Khongkasem was born on 28th January, 1988 in Bangkok. He finished his secondary course from Pakchong School in March, 2007. After that, he studied in the major of Chemical Engineering in Faculty of Engineering at Srinakharinwirote University and achieved his Bachelor's degree in March, 2011. He continued his further study for Master's degree in Chemical Engineering at Chulalongkorn University. He participated in the Environmental Chemical Engineering and Safety Research Laboratory. He finally achieved his Master's degree in 2014.