ถังปฏิกรณ์ชีวภาพอากาศยกแบบแบนเพื่อการเพาะเลี้ยงเซลล์เวเจทเตทีฟของสาหร่ายเซลล์เดียว *Haematococcus pluvialis*

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิศวกรรมศาสตรมหาบัณฑิต
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FLAT PANEL AIRLIFT PHOTOBIOREACTOR FOR CULTIVATION OF VEGETATIVE CELLS OF MICROALGA *Haematococcus pluvialis*

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A Thesis Submitted in Partial Fulfillment of the Requirements
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กีรติ อิสระพายัพ : ถังปฏิกรณ์ชีวภาพอากาศยกแบบแบนเพื่อการเพาะเลี้ยงเซลล์เวเจทเตทีฟของสาหร่ายเซลล์เดียว *Haematococcus pluvialis*. (FLAT PANEL AIRLIFT PHOTO-BIOREACTOR FOR CULTIVATION OF VEGETATIVE CELLS OF MICROALGA *Haematococcus pluvialis*) อ. ที่ปรึกษา: รศ.ดร. ประเสริฐ ภวสันต์, อ.ที่ปรึกษาร่วม: ดร. สรวิศ เผ่าทองศุข, 77 หน้า.

งานวิจัยนี้ทำการศึกษาการเพาะเลี้ยงสาหร่าย *Haematococcus pluvialis* NEIS-144 ในถังปฏิกรณ์ชีวภาพเชิงแสงแบบอากาศยกสองระบบและทำการเปรียบเทียบ ระบบแรกเพาะเลี้ยงเซลล์สาหร่ายในถังปฏิกรณ์ชีวภาพเชิงแสงแบบอากาศยกชนิดท่อในกลวง ประกอบด้วยขนาด 3 ลิตร และ 17 ลิตร ได้ค่าอัตราการเจริญเติบโตจำเพาะเท่ากับ 0.45 ต่อวัน และค่าการผลิตเท่ากับ $87x10^4$ เซลล์ต่อมิลลิลิตรต่อวัน ในถัง 3 ลิตร และได้ค่าอัตราการเจริญเติบโตจำเพาะเท่ากับ 0.38 ต่อวัน และค่าการผลิตเท่ากับ $6.2x10^4$ เซลล์ต่อมิลลิลิตรต่อวัน ในถัง 17 ลิตร ส่วนระบบที่สองเป็นระบบที่ออกแบบมาเพื่อสะดวกต่อการขยายขนาด โดยทำการเพาะเลี้ยงเซลล์สาหร่ายในถังปฏิกรณ์ชีวภาพเชิงแสงแบบอากาศยกชนิดท่อในแบนขนาด 17 ลิตร ได้ความเข้มข้นของเซลล์สูงที่สุดในงานวิจัยนี้คือ $41x10^4$ เซลล์ต่อมิลลิลิตร และค่าอัตราการเจริญเติบโตจำเพาะเท่ากับ 0.52 ต่อวัน ภายใต้สภาวะการเพาะเลี้ยง ดังนี้ อาหารสูตรปรับปรุง F1 ค่าอัตราส่วนของพื้นที่ของไหลไหลลงต่อพื้นที่ของไหลไหลขึ้น (A_d/A_r) เท่ากับ 0.4 ควบคุมพีเอชเท่ากับ 7 ความเข้มแสง 20 ไมโครโมลโฟตอนต่อตารางเมตรต่อวินาที ให้อากาศผสมก๊าซคาร์บอนไดออกไซด์ 1% ด้วยอัตราเร็ว 0.4 เซนติเมตรต่อวินาที ตามลำดับ การเพาะเลี้ยงสาหร่าย *H. pluvialis* แบบกึ่งต่อเนื่องโดยเก็บเกี่ยวผลผลิตทุก ๆ 3 วัน ในถังระบบ 17 ลิตร และภายใน 7 วัน ในถัง 3 ลิตร โดยมีค่าการผลิตเท่ากับ $90x10^4$, $70x10^4$ และ $77x10^4$ เซลล์ต่อมิลลิลิตรต่อวัน ในถังปฏิกรณ์ชีวภาพเชิงแสงแบบอากาศยกชนิดท่อในกลวงขนาด 3 ลิตร และ 17 ลิตร และถังปฏิกรณ์ชีวภาพเชิงแสงแบบอากาศยกชนิดท่อในแบน 17 ลิตร ตามลำดับ การเพาะเลี้ยงเซลล์สาหร่าย *H. pluvialis* ในถังปฏิกรณ์ชีวภาพเชิงแสงแบบอากาศยกชนิดท่อในแบน 90 ลิตร ถือว่าเป็นระบบที่ใหญ่ที่สุดเท่าที่ได้มีการรายงานในอดีต ได้ความเข้มข้นของเซลล์ ในงานวิจัยนี้คือ $40x10^4$ เซลล์ต่อมิลลิลิตร และค่าอัตราการเจริญเติบโตจำเพาะเท่ากับ 0.39 ต่อวัน ภายใต้สภาวะการเพาะเลี้ยงเช่นเดียวกับที่กล่าวมา และจากการวิเคราะห์ค่าความคุ้มทุนทางเศรษฐศาสตร์ ระบบ 90 ลิตร นี้ถือว่ามีความประหยัดมากที่สุด ($5.26x10^2$ บาท) เมื่อเทียบกับงานเพาะเลี้ยงในถังปฏิกรณ์ชีวภาพแบบต่างๆ ที่เคยมีรายงานมาในอดีต

ลายมือชื่อนิสิต...กีรติ อิสระพายัพ...

สาขาวิชา วิศวกรรมเคมี..... ลายมือชื่ออาจารย์ที่ปรึกษา.....

ปีการศึกษา 2550..... ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

4870225221 : MAJOR CHEMICAL ENGINEERING
KEY WORD: PHOTOBIOREACTOR / BIOREACTOR / MICRO ALGA
KERATI ISSARAPAYUP: FLAT PANEL AIRLIFT PHOTOBIOREACTOR FOR CULTIVATION OF VEGETATIVE CELLS OF MICROALGA *Haematococcus pluvialis*. THESIS ADVISOR: ASSOC. PROF. PRASERT PAVASANT, Ph.D., THESIS CO-ADVISOR: SORAWIT POWTONGSOOK, Ph.D., 77 pp.

A comparison between the cultivation of *Haematococcus pluvialis* NEIS-144 in airlift systems with different configurations was carried out. The 3L cylindrical airlift photobioreactor clearly outperformed the other two. However, the upscale of 3L to 17L cylindrical airlift photobioreactor with similar design resulted in a drastically drop in the growth performance where the specific growth rate and the productivity decreased from 0.45 d^{-1} and $87x10^4$ cell mL^{-1} d^{-1} in the 3L to 0.38 d^{-1} and $62x10^4$ cell mL^{-1} d^{-1} in the 17L. The flat panel airlift photobioreactor (FP-ALPBR) was proposed as alternative cultivation system where the upscale was simplified just by increasing the length of the reactor. Without a thorough investigation on the optimal design and operating conditions, the airlift was capable of giving reasonable growth character. The performance of the system was still quite satisfactorily where the attainable maximum cell density of 41 $x10^4$ cell mL^{-1} and specific growth of 0.52 d^{-1} were achieved. The optimal conditions for the cultivation of *H. pluvialis* in the FP-ALPBR were obtained with the modified F1 medium where: pH = 7, light illuminated at 20 μmol photon m^{-2} s^{-1} (with normal fluorescence lamps), and aeration with superficial velocity of 0.4 cm s^{-1}, A_d/A_r = 0.4. The semi-continuous culture was successfully implemented where the harvest could be periodically performed. For the 3L system, cell density increased up to the level obtained in the previous cycle within 7 days period, and for these two 17L systems, each cycle required only about 3-4 days. The productivity of semi-continuous culture in 3L, 17L cylindrical ALPBR and 17L FP-ALPBR were $9x10^7$, $7x10^7$ and $7.7x10^7$ cell L^{-1} d^{-1}, respectively. The, thus far, largest scale, 90L, FP-ALPBR could be achieved the maximum cell density of $40x10^4$ cell mL^{-1} and the specific growth rate of 0.39 d^{-1} which was found to be most attractive as it required relatively low operating cost at approx. $5.26x10^2$ THB for the cultivation of such alga.

Department Chemical Engineering Student's signature เกรติ อิสระพยุพ

Field of study Chemical Engineering Advisor's signature

Academic year 2007 Co-adviser's signature

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CHAPTER I

INTRODUCTION

1.1 Motivations

Microalgae are potential sources for various kinds of commercially valuable chemicals such as lipids, pigments, antioxidants, etc. One of the most recent processes is the production of astaxanthin (3,3' - dihydroxy- β - β - carotene - 4,4' - dione) from a green alga, *Haematococcus pluvialis,* which, under proper conditions, is able to produce astaxanthin at as high as 1.5-3.0% by weight (Lorenz and Cysewski, 2000). *H. pluvialis* has recently received increasing interest as one of the best producers of astaxanthin (Guerin et al., 2003). Astaxanthin is a red ketocarotenoid which attracts a great commercial interest due primarily to its versatile applications and high production costs (approximately $2,500-3,000 per kg). Since astaxanthin holds superior antioxidant activity than other carotenoids, it is used as potential stimulator for cancer prevention, immune response enhancement. In addition, astaxanthin is also used as pigment in feeds for farmed fish, a vitamin source for the poultry industry, and as a food additive in food industry (Lorenz and Cysewski, 2000; Guerin et al., 2003).

At the present time, astaxanthin can be manufactured by two ways. The first and most common current technique is the chemical synthesis which is naturally a complicated process leading to a higher product price. Moreover, the product might not be as effective as that produced naturally. The second and more preferable technique is the natural product which is generally achieved through the bio-synthesis in certain microorganisms such as some yeasts and microalgae (Kobayashi et al., 1991; Gong and Chen, 1998). Among all of the astaxanthin accumulated microorganisms, *H. pluvialis* has been reported to accumulate the richest amount of astaxanthin (Kobayashi et al., 1991; Harker et al., 1996a,b; Gong and Chen, 1998). The accumulation of astaxanthin in *H. pluvialis* cells is related to the physiology of the cells. Under optimal growth conditions, astaxanthin is completely absent from the cells. Upon exposure of the cells to growth-limiting conditions that are typically a result of depletion of nutrient, high light intensity and high salt concentration, it changes to cysts while the astaxanthin

content increases simultaneously (Droop, 1954; Fan et al., 1994; Harker et al., 1996b; Fabregas et al., 1998; Orosa et al., 2005).

Generally, the culture of microalgae can be performed in either open and closed systems. Open systems are the simplest and oldest from culturing ponds with advantages of low costs of construction and operation. However, the drawbacks of these systems are the ease of contamination and difficulty in control, especially for light irradiation and temperature (Richmond et al., 1993). *H. pluvialis* is susceptible to easy contamination, slow growth and preference for low growth temperature (Harker et al., 1996a), therefore outdoor cultivation has not been made possible in practice. This leads to a need in developing closed bioreactor culture systems (stirred tank, bubble columns and airlift bioreactor) for such microorganism. Airlift bioreactor is one of the alternatives for the cell cultivation (Merchuk et al., 1996) especially in bioprocesses (Blenke, 1979; and Orazem and Erickson, 1979). This is because it has many advantages over other types of bioreactors such as good mixing, simple construction, well defined fluid flow pattern and high mass transfer rate. In addition, the mixing in airlift bioreactor exerts distinctively less shear force than the mechanical ones such as stirred tanks.

Airlift photobioreactor has been employed in several studies for the production of astaxanthin from *H. pluvialis* (Harker et al., 1996a; Chen et al., 1997; Zhang et al., 1999; Fabregas et al., 2001). Surprisingly, none of these studies did emphasize the aspect of achieving high cell density of *H. pluvialis*. The highest maximum cell density was reported by Hata et al. (2001) who achieved the maximum cell density of 100×10^4 cell mL^{-1} in a 500 mL flask. Furthermore, the highest productivity (1.5×10^5 cell mL^{-1} d^{-1}) and the highest specific growth rate (0.57 d^{-1}) were obtained from Kobayashi et al. (1997) who operated the cultivation in 200 mL under mixotrophic condition. In practice, the large-scale production must be carried out in a bioreactor and some work had looked through the possibility in cultivating *Haematococcus pluvialis* in the airlift photobioreactor. For instance, Kaewpintong et al. (2006) successfully achieved the cultivation of vegetative cells of *H. pluvialis*, but only in the small scale autotrophic 3 L airlift photobioreactor with a maximum cell density of 77×10^4 cell mL^{-1} and with the maximum specific growth rate of 0.45 day^{-1}. In a larger scale system, only 25×10^4 cell mL^{-1} with the specific growth rate of 0.13 day^{-1} (in autotrophic 30 L airlift photobioreactor) was achieved from Harker et al. (1996a). Recently, Ranjbar et al.

(2007) claimed to achieve the cultivation with the highest cell density (above 50 x 10^5 cell mL^{-1}) in the autotrophic 1.6 L bubble column photobioreactor with fed-batch addition of nutrients. However, the specific growth rate obtained from this large scale system was still low at only 0.22 day^{-1}.

The scale up of bioreactor requires the whole knowledge of Chemical Engineering as the performance of large scale systems is, most of the time, significantly different from the small scale systems. For the airlift system, one main problem for the upscale is the pressure of the liquid induced from the height of the system. In addition, Wongsuchoto (2003) found that the inherited large riser often destroys the flow pattern in the riser itself as it allowed local backflow of the liquid which reduced the liquid circulation rate and also the gas-liquid mass transfer rate. Thus far, very few reports are available regarding the design of large scale airlift systems particularly with the main purposes of obtaining suitable environmental conditions for the growth of specific strains like *Haematococcus pluvialis*.

This work specifically aimed to develop the flat panel in which the width of column is fixed. The upscale of such system was achieved through the increase in the length of the reactor. With this configuration (fixed width and height), the behavior of the system was expected to only change with the length of the column. The success of this work allows an easy and simple way of upscaling the airlift photobioreactor.

1.2 Objectives

1.2.1 To determine the optimal growth condition in the flat panel airlift photobioreactors. These conditions include (i) the ratio between the downcomer and riser cross sectional area (A_d/A_r), (ii) superficial gas velocity. This is performed in the 17L airlift system.

1.2.2 To compare the cultivation of vegetative cells of *H. pluvialis* in 17L and 90L, flat panel airlift photobioreactor.

1.3 Scopes of this work

1.3.1 The flat panel airlift bioreactors employed in this work were with a volume of 19L (20 cm deep, 50 cm high, 20 cm long, working volume 17L) and 95 L (20 cm deep, 50 cm high, 100 cm long, working volume 90L).

1.3.2 The ratio between the downcomer and riser cross sectional area (A_d/A_r) were varied from 0.1 - 3.0.

1.3.3 The superficial gas velocity was approximately controlled at 0.4 $cm.s^{-1}$ during the growth stage as proposed by Kaewpintong, 2006. However, the effect of superficial velocity was re-confirmed by varying this parameter in the range from 0-2 $cm.s^{-1}$.

1.3.4 The light intensities were approximately controlled at 20µmol $photon.m^{-2}.s^{-1}$ during the growth stage (as proposed by Kaewpintong, 2006).

CHAPTER II

BACKGROUNDS AND LITERATURE REVIEW

2.1 Airlift Photobioreactor

Airlift photobioreactors can be classified into two major types: internal loop and external loop, as shown in Figure 2.1. The internal loop airlift photobioreactor is the cylindrical column with a concentric cylindrical tube or plate which creates channels required for the circulation (Figure 2.1a). In external loop airlift photobioreactors, riser and downcomer are two separate columns (Figure 2.1b) which are connected by two connecting tubes. These connecting tubes support liquid flow between riser and downcomer.

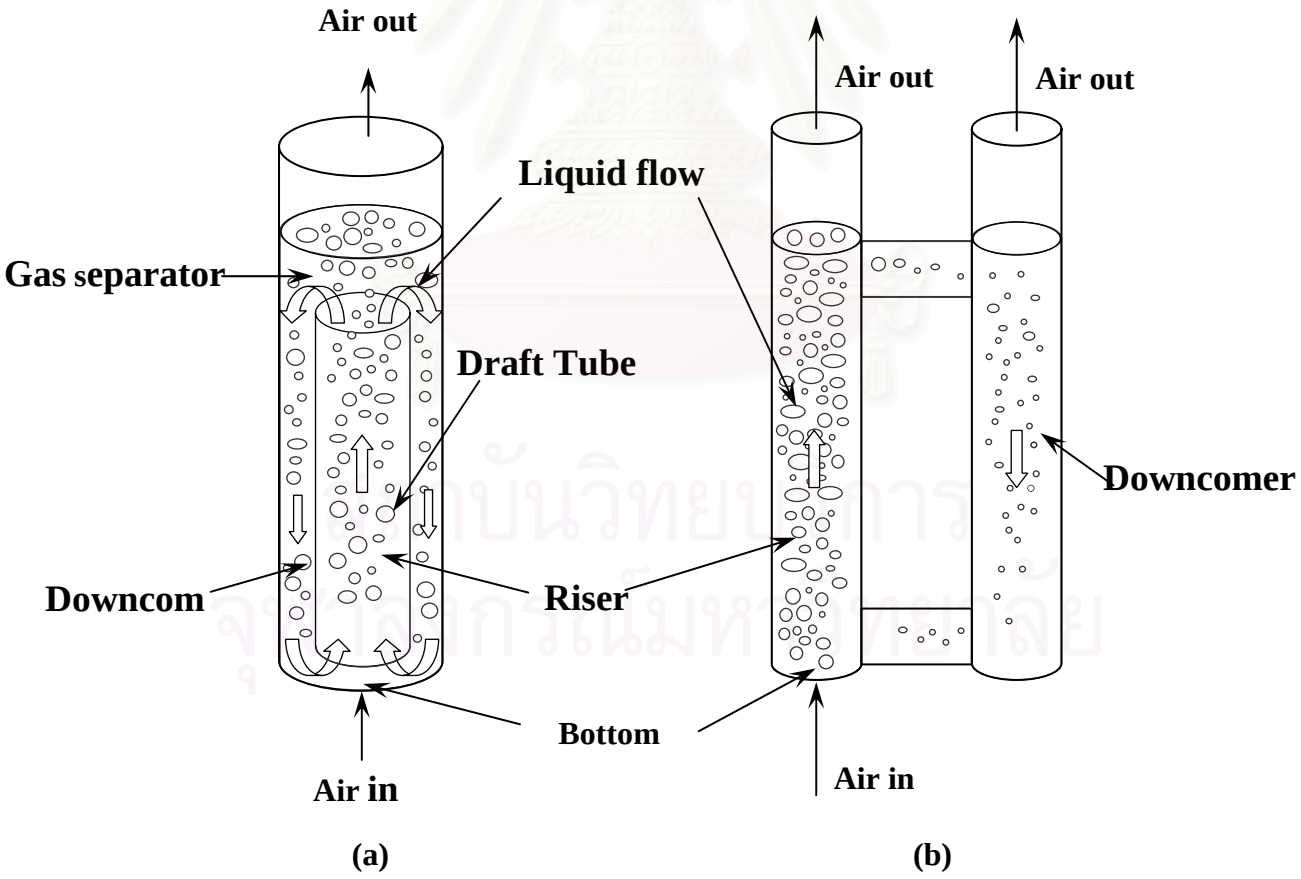


Figure 2.1 Classification of airlift photobioreactor: (a) internal loop (b) external loop

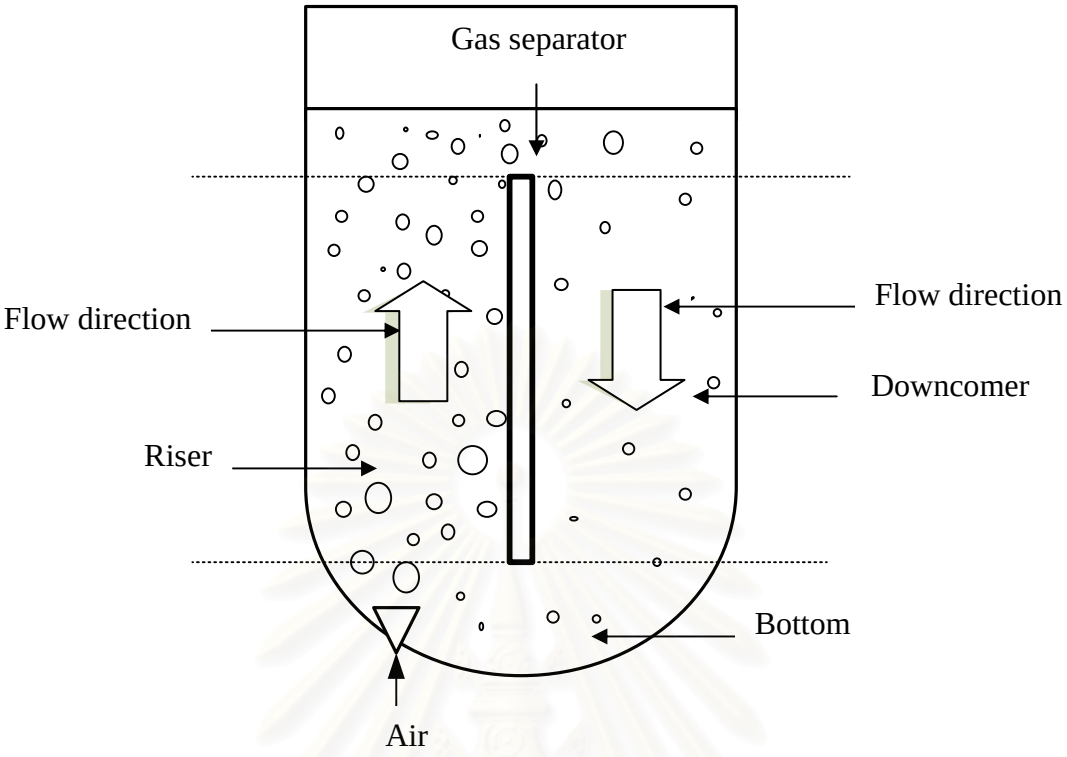


Figure 2.2 Various sections of the airlift system

1. Riser is the section through which gas is supplied, and the upward flow of liquid is induced.
2. Gas separator is the section where the flow pattern is highly turbulent. In this section, a large portion of gas disperses out at the liquid surface and creates heavier fluid.
3. Downcomer is the section that allows the heavier fluid from the gas separator section and some remaining gas bubbles to flow downwards.
4. The final bottom section is provided to allow the liquid to re-enter the riser together with the supplied gas.

Two factors govern the liquid flow pattern in the system: (i) density or the hydrostatic pressure difference between the riser and downcomer due to the difference in local gas holdups; and (ii) the energy transfer from the gas bubbles from the air compressor.

2.2 *Haematococcus pluvialis*

2.2.1 **Biological information**

Haematococcus pluvialis is a freshwater unicellular alga. *H. pluvialis* reproduces asexually by division from a single cell into and/or four motile cells (Droop, 1995). The taxonomy of *H. pluvialis* is as follows (Smith, 1950). :

Division : Chlorophyta
Class : Chlorophyceae
Order : Volvocales
Family : Chlamydomondaceae
Genus : *Haematococcus*
Species : *Haematococcus pluvialis*

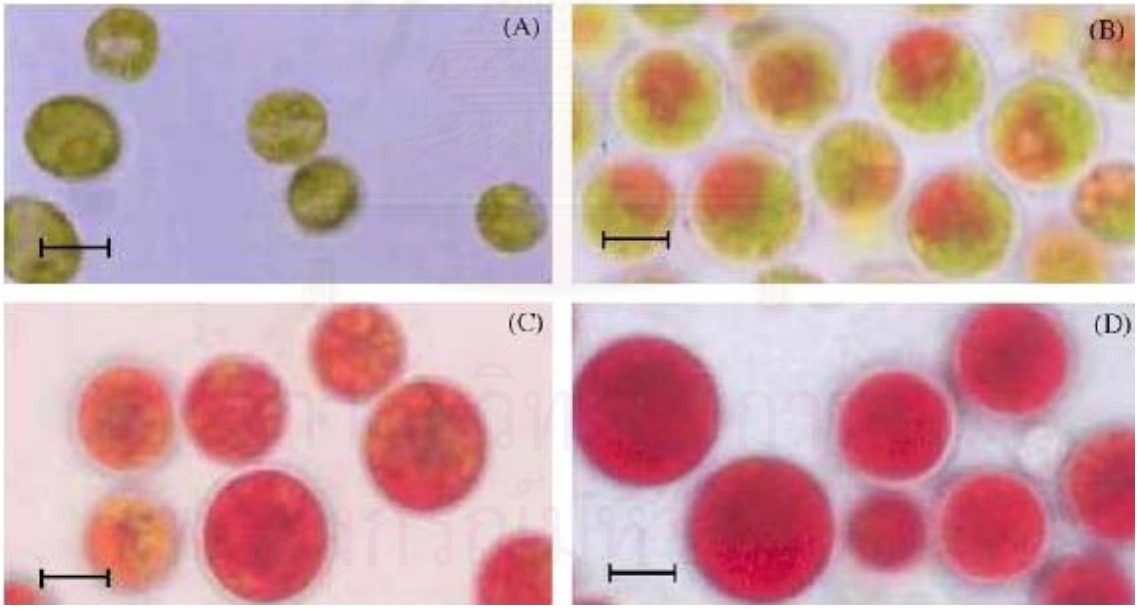


Figure 2.3 *H. pluvialis* cells in different stages of growth in autotrophic medium.
(a) vegetative motile cells (The morphology has been flagellated)
(b) initiation of astaxanthin accumulation
(c) cysts **(**The morphology has not been flagellated)
(d) complete accumulation of astaxanthin

(Kamath et al., 2005)

2.2.2 **Growth stages of *H. pluvialis***

The growth stages of *H. pluvialis* can be classified into four stages as described below:

❖ **Vegetative motile cells (Figure 2.3 a)**

Under suitable growth conditions, most cells remain in green vegetative forms capable of moving with two flagella. The cells produce chlorophylls a and b and primary carotenoids, especially β - carotene and lutein (Rockette, 1970). Astaxanthin has not been generated in this stage. The shape of these cells is typically spherical or ellipsoid with a diameter of approximately 10-20 µm. The cells are enclosed with cell walls that the within consists of golgi apparatus, chloroplast, polysaccharidic envelope, pyrenoid, and fragility zone (Gudin and Chaumont, 1991).

❖ **Initiation of astaxanthin accumulation (Figure 2.3 b)**

As the cells are in unfavorable conditions such as depletion of essential elements, e.g. carbon, nitrogen, phosphorous, light induction, etc., astaxanthin is partially created within cytoplasm of the cells. Moreover, some of the cells lose flagella and larger size of a diameter.

❖ **Cysts (Figure 2.3 c)**

Under such conditions vegetative motile green cells enter a process of encystment. The formation of astaxanthin-replete aplanospores begins which is still spherical in shape. However, they lose flagella and generating an extremely resistant cell wall that cannot be easily digested (Mendes-Pinto et al., 2001; Hagen and Braune., 2002). The diameter of cells increases dramatically from 10-20 µm to 40-50 µm. Besides, they produce secondary carotenoids such as echinenone, canthaxanthin and astaxanthin while the chlorophyll and primary carotenoids are decreasing (Droop, 1954; Lee et al., 1991). Growth rate of *H. pluvialis* in this state decreases whereas an abundance of astaxanthin in almost cells.

❖ **Complete accumulation of astaxanthin (Figure 2.3 d)**

At this stage, immature cysts transform into mature, immotile cysts. Fully cysts contain up to 5% dry weight astaxanthin (Renstrom et al., 1981; Bubrick, 1991).

2.3 Astaxanthin

Astaxanthin is one of the most important carotenoids in marine organisms, being responsible for the pigmentation of skin and flesh of fish, mainly salmonids (Lorenz and Cysewski, 2000), and also influences survival and growth of fish larvae, even though the exact mechanism is not know (Christiansen and Torrissen., 1997).

2.3.1 **Chemical properties of astaxanthin**

Astaxanthin (3,3'- dihydroxy - β - β - carotene - 4,4' - dione) is an oxycarotenoid with a molecular formula $C_{40}H_{52}O_4$ and a molecular weight of 596.86. It can be dissolved at room temperature in non-polar solvents such as dimethyl-sulfoxide and acetone. There are two different pathways for astaxanthin biosynthesis. The first one starts with the oxidation of β-carotenoid of β-carotene with echinenone, canthaxanthin and adonirubin taking part as intermediates. The second one starts with β-carotene hydroxylation with β-cryptoxanthin, zeaxanthin and adonixanthin as intermediates (Schoefs et al., 2001) (as shown in Figure 2.4). The astaxanthin molecule has two asymmetric carbons located at the 3 and 3′ positions of benzenoid rings on either end of molecule with three configurations as shown in Figure 2.5.

2.3.2 **Sources of astaxanthin**

There are two major sources of astaxanthin; chemical (synthetic) and biological (natural) sources.

2.3.2.1 Synthetic source

Synthetic astaxanthin is presently the principle source in aquaculture, more than 95% of this market consumes synthetically astaxanthin used in feed to produce coloration (Lorenz and Cysewski, 2000). However, this synthetic astaxanthin is expensive, unnaturally configurational and was considered lower quality when compared with that obtained from natural sources (Parker, 1992).

2.3.2.2 Natural sources

A. Crustaceans byproduct

Astaxanthin is isolated from shell on the back of a crustacean which has been used as natural pigment sources for trout and salmon. This process is very expensive, and the final product contains relatively low contents of astaxanthin and high levels of water, ash and other nutrients, which cause several practical problems in feed formation that limits their usefulness in animal feed (Bubrick, 1991).

B. Yeast

The yeast *Xanthophyllomyces dendrorhous* (*formerly Phaffia rhodozyma*) is one of the best microbial sources of astaxanthin (Johnson and An, 1991). It contents of astaxanthin in wide strain is only 200 to 300 µg/g yeast (0.02-0.03%). The content of this compound depends on strain and method of culture (John and An, 1999). However, the use of the yeast as a source of astaxanthin for aquaculture is limited since the cell wall is difficult to be digested by some microorganisms (Van Nieuwerburgh et al., 2005). Only free astaxanthin is found in the yeast which have lower antioxidant avtivity than esterify forms (Choi et al., 2003).

C. Algae

Astaxanthin can be produced from other strains of algae such as *Ankistrodesmus brauii* , *Chlorella sp.*. For all that, the quantility of astaxanthin generated from these microorganisms are relatively low and not suitable for mass production. The green algae, *H. pluvialis* has received much attention due to its capacity to accumulation high amount of astaxanthin (Vonshak, 1990).

D. Other microorganisms

Some bacteria such as *Brevibacterium sp.* and *Mycobacterium lacticola* and fugi *Peniophora (Hymenomycetes)* are also reported to be able to accumulate astaxanthin (Borowitzka et al., 1989). Carotenoid level of this microorganism is low

and growth is slow (Droop, 1985). Moreover, Copepods, which are tiny, shrimp-like crustaceans that swim in seas, lakes, and ponds, are the main component of the natural diet of fish larvae as well as the main source of astaxanthin in the marine web chain. Copepods can synthesize astaxanthin (Andersson et al., 2003) and the amount of astaxanthin present in the copepods is not dependent on the phytoplankton ingested (Van Nieuwerburgh et al., 2005). Some examples of sources of astaxanthin produced by microorganisms are shown in Table 2.1.

2.4 Production of astaxanthin by *H. pluvialis*

The culture requirements for astaxanthin accumulation are different from those for cell growth. Hence, the commercial processes for astaxanthin production must be separated from the cultivation step, and the overall production of astaxanthin from *Haematococcus pluvialis* is often called a two-stage process (Boussiba et al., 2000; Lorenz and Cysewski, 2000; Olaizola, 2000). The first stage is conducted by maintaining the environmental conditions to achieve high *H. pluvialis* growth rates in the vegetative form. The cells at the stationary growth phase are transferred to the second reddening stage and the astaxanthin accumulation in the cells is then induced by environmental stresses, including light intensity, nutrient levels, metal ions, oxidative stress, salt stress, temperature, and pH (Zhang et al., 1999). The details are described in Tables 2.2 and 2.3.

2.5 Culture systems

The large scale algal cultivation can be made both in open and closed systems, however, a common problem encountered in the large scale system is the ease of contamination from other unwanted species, controlling difficulties, and relatively lower reliability than the laboratory scale. Many factors should be considered in selecting the suitable system. A summary advantages and disadvantages of culture system are demonstrated in Table 2.9.

2.5.1 **Open system**

A very common commercial system for the large scale cultivation of microalgae is the opened air system, i.e. tanks, shallow ponds, circular ponds and raceway ponds. Often, the system consists of mechanical equipments such as an agitator or paddle wheel to produce the circulation of water (Lee, 1997). The performance of such system depends strongly on climate conditions and temperature. A few achievements in the cultivation of astaxanthin in open systems were reported. This included the work of Harker et al. (1996a) who studied the cultivation of *H. pluvialis* in 250 L open raceway pond and found that the culture was subject to high level of cyanobacteria contamination and predation by protozoa which resulted in poor algae growth. In addition, the cultivation of *H. pluvialis* in 25,000 L raceway pond was experimented by Olaizala (2000) who achieved the cell density 24 x 10^4 cell mL^{-1}. This level was still far lower than those obtained in the closed system as elucidated in the next section. It is worth noting that, for the slow growth microorganism like *H.pluvialis*, the contamination by bacteria, fungi and other faster growing algae, as well as protozoan predators could be crucial as this could eliminate 90% of the algal biomass within 72 h (Spencer, 1989).

2.5.2 **Closed system**

Advanced methods have been introduced for a large scale culture of microalgae, not only in opened systems, but also in closed systems e.g., stirred tank, bubble columns and airlift bioreactor (Borowitzka, 1996; Chaumont, 1993 and Tredici and Materassi, 1992). The systems were designed for cultivation of the microorganism in specified environment suitable for the growth of each species to prevent the growth of the contaminants. Growth conditions, such as temperature, could be easily regulated to achieve the maximum productivity (Lee, 2001; Lee and Richmond, 1998; Vonshak, 1997 and Richmond, 1996). For algal cultivation, the systems were designed to increase the opportunity of light available to cell, to obtain the monoculture and to reduce the light path (Lee, 1986). A summary advantages and disadvantages of each type bioreactors are given in Table 2.10.

2.6 Bioreactor systems for *H. pluvialis*

The key factors for the efficiency of the process are the continuous production of green vegetative cells and the application of a separate induction process under conditions that could avoid cell death. Airlift bioreactor has been employed in several studies for the production of astaxanthin from *H. pluvialis* (Harker et al., 1996b; Chen et al., 1997; Zhang et al., 1999; Fabregas et al., 2001). However, their studies did not focus on the aspect of achieving high cell density of *H. Pluvialis*, and most of the works could only achieve a low level of cell density (small scale cultivation) in the range of 0.7 x 10^5 to 10 x 10^5 cell mL^{-1}, and the productivity of cell was shown in the range of 0.09 x 10^5 to 1.5 x 10^5 cell mL^{-1} d^{-1}. Our recent works included those of Kaewpintong et al., (2006) who successfully achieved the cultivation of vegetative cells of *H. pluvialis*, but still only in the small scale autotrophic 3 L airlift bioreactor with a maximum cell density of 77 x 10^4 cells mL^{-1} and with the maximum specific growth rate of 0.45 day^{-1}. This specific growth rate was only second to that achieved by Tjahjono et al., (1994) (0.55 d^{-1}) who performed the cultivation in 200 mL flask. A few achievement was reported for a large scale system, such as the autotropic 30L airlift bioreactor investigated by Harker et al., (1996a) who obtained only a low cell concentration of 25 x 10^4 cells mL^{-1} with the specific growth rate of 0.13 day^{-1}. Recently, Ranjbar et al., (2007) reported the achievement of the cultivation with the highest cell density of above 50 x 10^5 cell mL^{-1} from an autotrophic 1.6 L bubble column photobioreactor. Although the attained specific growth rate obtained from this work was only 0.22 day^{-1}, this work presents a potential of further development of such large scale cultivation for *Haematococcus pluvialis*.

Turning to the astaxanthin production stage, this was often achieved under some stress conditions such as salt stress, elevated temperature, and high light intensity. Most work reported the productivity of astaxanthin in a range of 0.02 to 9.6 mg L^{-1} d^{-1}. However, the highest productivity of astaxanthin was obtained from the work of Suh et al. (2006) who could achieve by far 16 mg L^{-1} d^{-1}. Note that this system was performed in 11 L a novel double layered photobioreactor.

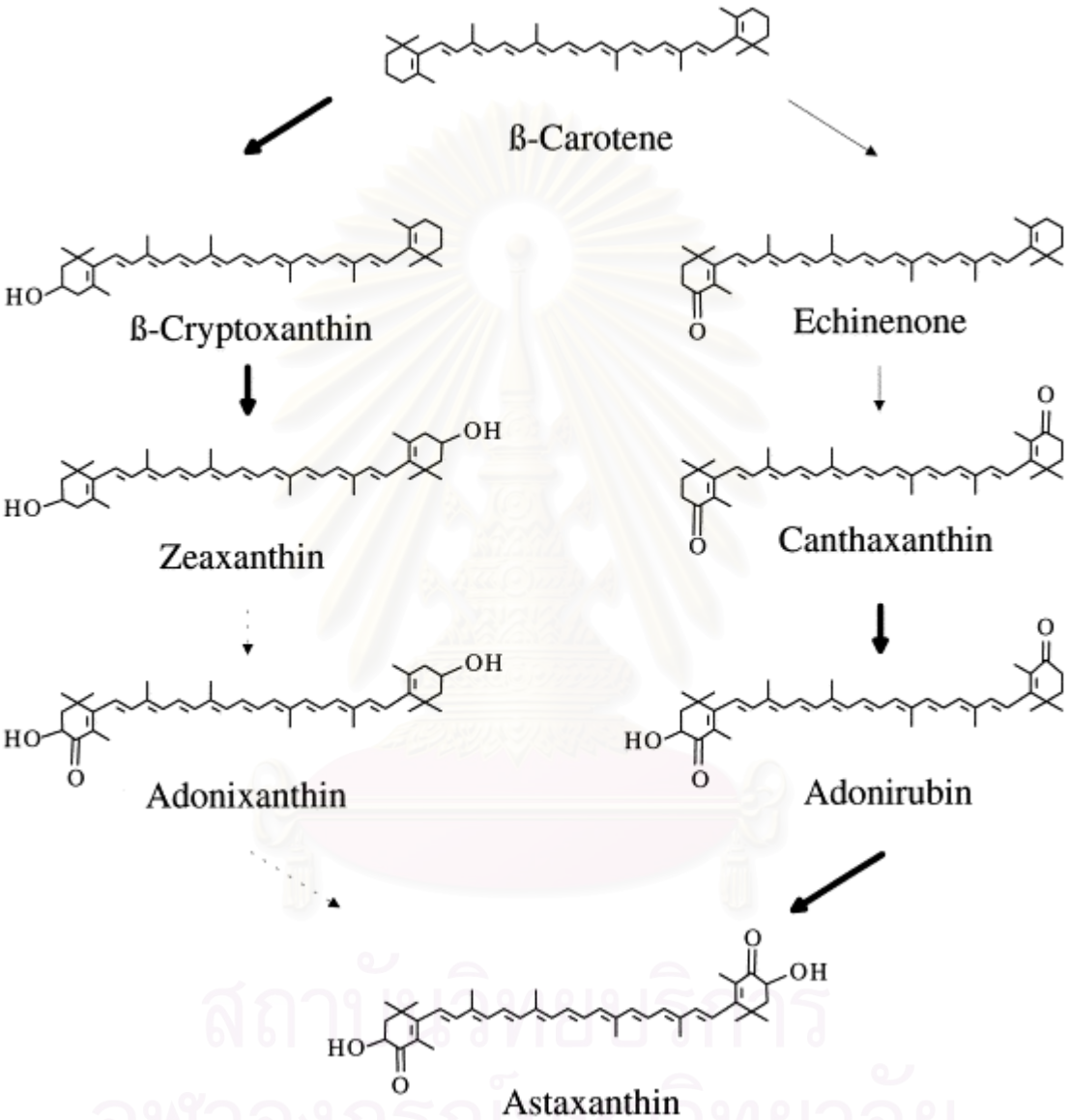


Figure 2.4 Pathway for astaxanthin biosynthesis on *H. pluvialis*
(Misawa N. and Shimada H.,1998)

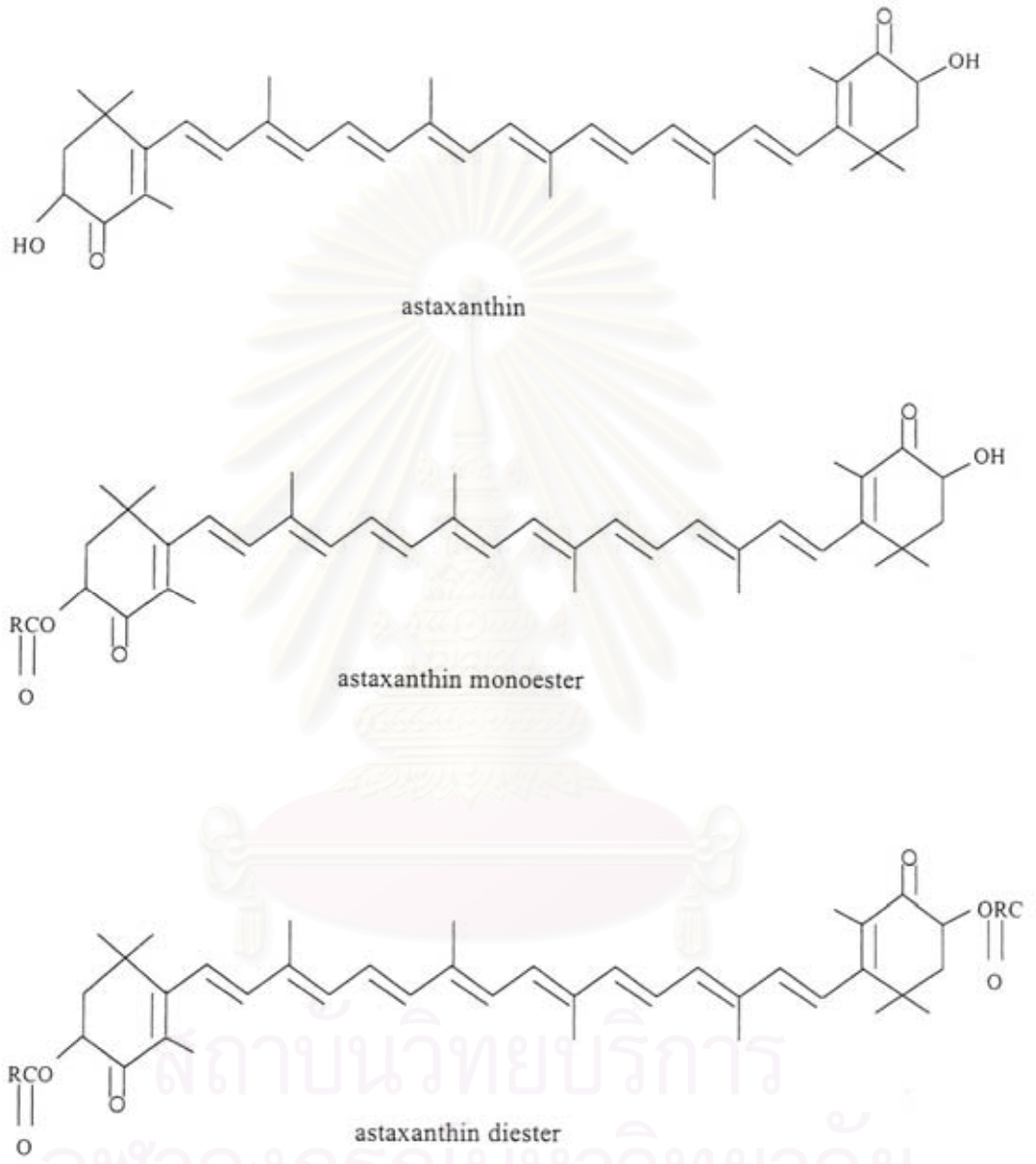


Figure 2.5 Molecular structure of astaxanthin

Table 2.2 Environmental growth factors for green vegetative cells of *H. pluvialis*

Factors	Ranges
Light	2-24 Klux
Temperature	15-28 ^{0}C
pH	6 - 8
Nutrients	
(1) Macronutrients	
• Carbon	high quantities
• Nitrogen	
• Phosphorous	
(2) Micronutrients	
• Iron	low quantities (µg/L)
• Boron	
• Manganese	
• Copper	
• Vitamins	

Remark: Summary of the operation conditions for the production of vegetative cells is given in Table 2.4-2.6.

Table 2.3 Reported suitable environmental factors for astaxanthin accumulation in *H. pluvialis*

Factors	Ranges
Light	2 - 360 Klux
Temperature	18 - 30 ^{0}C
pH	6.5 - 8
Salt stress	0.2 - 1% Sodium chloride

Remark: Summary of the operation conditions for the production of astaxanthin is given in Table 2.7-2.8

Table 2.1 Biological sources of astaxanthin (Simpson et al., 1981; Borowitzka, 1989; Harker et al., 1996)

Sources	Astaxanthin ($\mu g\ g^{-1}$ of cell)	Astaxanthin (% dry cell)	Reference
Bacteria			
Brevibacterium sp.	30		Simson et al., 1981
Mycobacterium lacticola	30		Simson et al., 1981
Holobacterium salinarium	265		Simson et al., 1981
Yeast			
Phaffia rhodozyma	200-300	0.02 - 0.03	Borowizka et al., 1989
Mold			
Peniophora (Hymenomycetes)	< 50		Borowizka et al., 1989
Algae			
Ankistrodesmus brauii *Euglena sp.* *Chlorella sp.* *Chlamydomonas nivalis* *Neospongiococcum sp.* *Neochloris wimmeri* *Spongiochloris typica* *Dunaliella salina*	< 50		Harker et al., 1996a,b
Haematococcus pluvialis	7,000-55,000	1.5 - 3	Lorenz and Cysewski, 2000

Table 2.4 Summary of the operation conditions (autotrophic) for production of vegetative cells

References	Reactor	Volume	Medium	Condition	Modes of operation						Maximum cells density ($x10^5$ cell mL^{-1})	Productivity of cells ($x10^5$ cell ml^{-1} day^{-1})	Specific growth rate (d^{-1})
					Light intensity (KLux)	Light source	pH	Temp. (^oC)	Aeration rate	Agitation rate (rpm)			
Ranjbar et al., 2007	Bubble column photobio reactor	1.6 L	Standard inorganic medium	autotrophic CO_2	1 - 2.4	fluorescent lamps	8	20	0.067 - 0.1 cm s-1		50 - 60	4.8	0.22
Kaewpintong et al., 2006	Airlift photobio reactor	3 L	Modified F 1 (Added Vit. B12)	autotrophic 1%CO_2	1	fluorescent lights	7	27	0.4 cm s^{-1}		7.7	0.55	0.45
Suh et al., 2006	A novel double-layered photobio reactor	11 L	Bold's Basal medium (BBM)	autotrophic 5%CO_2	2 - 3	white fluorescent lamps	6.5	25	100 mL min^{-1}		5.5	0.62	0.30
Dominguez - Bocanegra et al., 2004	Flask	1000 mL	Bold's Basal medium (BBM)	autotrophic 1.5%CO_2	9	white fluorescent		28	100 mL min^{-1}		3.5	0.48	0.36

Table 2.4 (cont.) Summary of the operation conditions (autotrophic) for production of vegetative cells

References	Reactor	Volume	Medium	Condition	Modes of operation						Maximum cells density ($x10^5$ cell mL^{-1})	Productivity of cells ($x10^5$ cell ml^{-1} day^{-1})	Specific growth rate (d^{-1})
					Light intensity (KLux)	Light source	pH	Temp. (^oC)	Aeration rate	Agitation rate (rpm)			
Fabregas et al., 2001	Tube	70 mL	OHM medium	autotrophic (batch) CO_2	2	white fluorescent lamps	7.2 - 7.8	25	15 L hr^{-1}		6.25	0.45	0.13
Fabregas et al., 2000	Tube	70 mL	OHM medium	autotrophic 5%CO_2	2	fluorescent lamps	< 8	25	250 mL min^{-1}		3.8	0.76	0.27
Tripathi et al., 1999	Flask		Bold's Basal medium (BBM)	autotrophic	1.5			25			1.5	0.15	0.04
Fabregas et al., 1998	Mini-reactor	70 mL	F1 medium	autotrophic (batch) CO_2	2	fluorescent lamps	7.2 - 7.8	25	15 L hr^{-1}		6	0.43	0.1
Grunewald et al., 1997	Flask	100 mL	Bold's Basal medium (BBM)	autotrophic (batch)	7.5	white lights		20			2.9	0.85	0.49
Harker et al., 1996a	Airlift photobio reactor	30 L	Basal medium } Added NaCl	autotrophic (batch)	2.5	fluorescent lamps			90-180 L hr-1		2.5	0.12	0.13

Table 2.5 Summary of the operation conditions (heterotrophic) for production of vegetative cells

References	Reactor	Volume	Medium	Condition	Modes of operation						Maximum cells density ($x10^5$ cell mL^{-1})	Productivity of cells ($x10^5$ cell ml^{-1} day^{-1})	Specific growth rate (d^{-1})
					Light intensity (KLux)	Light source	pH	Temp. (^oC)	Aeration rate	Agitation rate (rpm)			
Hata et al., 2001	Flask	500 mL	Basal medium	Hetero-trophic (fed batch)			8	25		40	10	1.12	0.29
Tripathi et al., 1999	Flask		KM1 medium	Hetero-trophic	1.5			25			4.35	0.87	0.29
Chen et al., 1997	Stirred tank	3.7 L		Hetero-trophic (batch)			7	25	100 L hr^{-1}	400	0.7	0.09	0.32

Table 2.6 Summary of the operation conditions (mixotrophic) for production of vegetative cells

References	Reactor	Volume	Medium	Condition	Modes of operation						Maximum cells density ($x10^5$ cell mL^{-1})	Productivity of cells ($x10^5$ cell ml^{-1} day^{-1})	Specific growth rate (d^{-1})
					Light intensity (KLux)	Light source	pH	Temp. (^oC)	Aeration rate	Agitation rate (rpm)			
Kobayashi et al., 1997	Flask	200 mL	Basal medium	Mixotrophic	1.5	fluorescent lamps	6.8	20			7	1.5	0.57
Chen et al., 1997	Stirred tank	3.7 L		Mixotrophic (batch)	8.5	fluorescent lamps	7	25	100 L hr^{-1}	400	1.45	0.23	0.44
Chumpolkulwong et al., 1997			Basal medium + Added sodium acetate	Mixotrophic	7			20			5.5	1.17	0.34
Tjahjono et al., 1994	Flask	200 mL	Basal medium + Added 45 mM MSA + 450mM Fe^{2+}	Mixotrophic	8.6	white fluorescent lamps	6.8	20			5.22	1.4	0.55

Table 2.7 Summary of the operation conditions (autotrophic) for production of astaxanthin

References	Reactor	Volume	Medium	Condition	Modes of operation						Productivity of astaxanthin (mg L^{-1} day^{-1})
					Light intensity (KLux)	Light source	pH	Temp. (^{o}C)	Aeration rate	Agitation rate (rpm)	
Ranjbar et al., 2007	Bubble column photobio reactor	1.6 L	Standard inorganic medium	autotrophic CO_2	2.4	fluorescent lamps	8	20	0.23 cm s-1		14.4
Suh et al., 2006	A novel double-layered photobio reactor	11 L	Bold's Basal medium	autotrophic 5%CO_2	39	white fluorescent lamps	6.5	25	100 mL min^{-1}		16
Lopez et al., 2006	Tubular photobio reactor (outdoor)	55 L	Inorganic medium free of acetate	autotrophic CO_2	2-100	day light	8	20	500 mL min^{-1}		4.4
	Bubble column (outdoor)	55 L		autotrophic CO_2	2-100	day light	8	20	500 mL min^{-1}		0.12

Table 2.7 (cont.) Summary of the operation conditions (autotrophic) for production of astaxanthin

References	Reactor	Volume	Medium	Condition	Modes of operation						Productivity of astaxanthin (mg L^{-1} day^{-1})
					Light intensity (KLux)	Light source	pH	Temp. (^{o}C)	Aeration rate	Agitation rate (rpm)	
Kim et al., 2006	Bubble column	2 L	Bold's Basal medium	autotrophic 5%CO_2	87	flashing light	6.5	23	100 mL min^{-1}		Improved the astaxanthin yield
Orosa et al., 2005	Mini - reactor	400 mL	Bold's Basal medium + Added 0.15 g/L $NaNO_3$	autotrophic	3.5	day light (12 h)	7	18			Improved the astaxanthin yield
Dominguez - Bocanegra et al., 2004	Flask	1000 mL	BAR medium + Added 1 g/L sodium acetate	autotrophic 1.5%CO_2	18	day light (12 h)		28	no aeration		0.33
Tripathi et al., 2002	Flask	100 mL	KM1 medium	autotrophic	1.5			25			0.82
Hata et al., 2001	Flask	500 mL	Basal medium + Added 10 mM sodium acetate	autotrophic	47.5		8	30		100	5.14

Table 2.8 Summary of the operation conditions (mixotrophic) for production of astaxanthin

References	Reactor	Volume	Medium		Condition	Modes of operation						Productivity of astaxanthin (mg L^{-1} day^{-1})
						Light intensity (KLux)	Light source	pH	Temp. (^oC)	Aeration rate	Agitation rate (rpm)	
Sarada et al., 2002	Flask		Basal medium	Added $NaNO_3$	Mixotrophic	1.5			25			0.25
Fabregas et al., 2001	Tube	70 mL	OHM medium		Mixotrophic (batch) CO_2	12	white fluorescent lamps	7.8	25	250 mL min^{-1}		3.27
Sarada et al., 2001	Flask		Basal medium	Added NaCl + sodium acetate	Mixotrophic	1.5			25			0.68

Table 2.8 (cont.) Summary of the operation conditions (mixotrophic) for production of astaxanthin

References	Reactor	Volume	Medium	Condition	Modes of operation						Productivity of astaxanthin (mg L^{-1} day^{-1})
					Light intensity (KLux)	Light source	pH	Temp. (^{o}C)	Aeration rate	Agitation rate (rpm)	
Zhang et al., 1999	Stirred tank	3.7 L	Hong Kong } Added sodium acetate	Mixotrophic (fed batch)			7	30	100 L/hr	350	3.22
	Stirred tank	3.7 L	Hong Kong	Mixotrophic (batch)			7	30	100 L/hr	350	2.67
	Stirred tank	3.7 L	Hong Kong	Mixotrophic (fed batch)	360		7	30	100 L/hr	350	4.16
Kobayashi et al., 1998	Flask	200 mL	Modified medium	Mixotrophic	4.5	fluorescent lamps	7	20			1.67

Table 2.9 Comparison between open and closed systems.

Open system	Closed system
▪ Poor mixing	▪ Uniform mixing
▪ Poor light utilization	▪ Excellent light utilization
▪ Unable for temperature control	▪ Excellent temperature control
▪ Low gas transfer	▪ High gas transfer
▪ Low hydrodynamic stress on algal cell	▪ Low-high hydrodynamic stress on algal cell
▪ Difficult species control	▪ Easy species control
▪ Unable to operate in continuous mode	▪ Able to operate in continuous mode

Table 2.10 Comparison of properties of different large scale algal culture systems

Reactor type	Mixing	Light utilization	Temperature	Gas transfer	Hydrodynamic	Species control	Scale up
unstirred shallow ponds	very poor	poor	none	poor	very low	difficult	very difficult
tank	poor	very poor	none	poor	very low	difficult	very difficult
circular stirred ponds	fair	fair good	none	poor	low	difficult	very difficult
stirred tank reactor	largely uniform	fair good	excellent	low - high	high	easy	difficult
airlift reactor	generally uniform	good	excellent	high	low	easy	difficult
tubular reactor	uniform	excellent	excellent	low - high	low - high	easy	easy
flat plate reactor	uniform	excellent	excellent	high	low - high	easy	difficult

CHAPTER III

EXPERIMENTS

3.1 Experimental setup

3.1.1 Setup of the flat panel airlift phorobioreactor

The column and draft tube were made of clear acrylic plastic with the thickness of 5 mm and 2 mm, respectively. The bioreactor was 20 cm wide, 50 cm high and 20 cm long. This made the volume of 17 L. For the larger volume (90 L) is 20 cm wide, 50 cm high and 100 cm long. Table 3.1 shows geometric details of such reactor. The ratio between the downcomer and riser cross section area (A_d/A_r) were varied from 0.1-0.3 by changing the vertical plate position. A schematic diagram of 2 dimension airlift photobioreactor was demonstrated in Figure 3.1.

The liquid culture in the airlift photobioreactor was agitated by supplying air bubbles at the bottom of the airlift column. The small 17 L reactor consists of 3 gas sparger at the bottom of the reactor that help distribute gas bubbles within the contactor. Air from an air compressor was metered through a flowmeter, sterilized with a 0.22 μm Gelman filter, and passed into the culture at the bottom of the reactor. The CO_2 enriched air was sterilized with a 0.22 μm Gelman filter, mixed with air, before entering the system at the bottom side of the reactor. The concentration of CO_2 was lower than 1% v/v, which was found not to affect the pH significantly as CO_2 was continuously taken up by the algae during the course of cultivation. The schematic diagram of experimental setup in airlift photobioreactor is shown in Figure 3.2.

Light for the airlift photobioreactor was supplied from the vertical 18 W fluorescent lamps. Two lamps were provided for adequate intensity, one on the right hand side and the other on the left hand side of the column. Each lamp was distanced around 1-5 cm from outer surface column depending on the required light intensity at the reactor surface. The illumination intensity incident to airlift bioreactor outer surface was measured with a digital LX-5 Lux meter, where Photon flux density and irradiance

was readily interconverted as (Thimijan et al., 1982):

$$1\ \mu mol\ (m^{-2}s^{-1}) = 1\ \mu E\ m^{-2}s^{-1} = \frac{119.7}{\lambda}\ W\ m^{-2} = 74\ Lux \qquad (3.1)$$

where λ = the wave length of the light in nanometer

3.1.2 Preparation of the bioreactor

1) setup the bioreactor as described in Section 3.1.1
2) fill the tap water into the bioreactor
3) sparge 200 L min^{-1} of ozone through the 0.22 µm Gelman filter and a flow meter into the water at the base of the reactor for 1h in order to clean the whole system
4) sparge the air through the 0.22 µm Gelman filter and a flow meter into the water at the base of the reactor for 3-4h to remove residual ozone in the water

3.2 Experimental procedure

3.2.1 Preparation of inoculum

1) obtain a green alga, *H. pluvialis* strain NIES-144 from Center of Excellence for Marine Biotechnology, Faculty of science, Chulalongkorn University, Thailand. The original strain was from the National Institute of Environmental Studies, Japan.
2) prepare F1 medium with chemical composition as indicated in Table 3.2
3) sterilize the medium in autoclave at 121 ^{0}C for 20 min
4) inoculate 10% by volume of cell into 500 mL sterilized fresh F1 medium in 1,000 mL Erlenmeyer flask
5) incubate the flask at 27 ^{0}C and supply continuous light intensity of 20 µmol photon.m^{-2}.s^{-1} to the surface of the culture vessel
6) manually shake the flask daily
7) harvest the active green motile cells under exponential phase and used as inoculum for other experiments

3.2.2 Growth experiment

1) Set the airlift system according to the description in Section 3.1. A_d/A_r was firstly fixed at 0.1. This parameter was manipulated within the range of 0.1-3.0 by changing the location of the plate.
2) fill the bioreactor with 3000 mL of culture with approximately 250 mL of starter innoculum. The exact quantity of the starter medium depended on the required initial concentration in the airlift photobioreactor. An initial density of *H. pluvialis* for all experiments was fixed at 2×10^4 cell mL^{-1}.
3) sparge a mixture of air and CO_2 (from the preset concentration) through the reactor bottom at superficial gas velocity of 0.4 $cm.s^{-1}$. This superficial gas velocity was altered within a short range (e.g. from 0-2 $cm.s^{-1}$)
4) illuminate the photobioreactor with the fluorescent lamp (18W) at continuous light intensity of 20 μmol $photon.m^{-2}.s^{-1}$ to the surface of the bioreactor. This light intensity was also varied by moving the lamp closer (higher intensity) or away (lower intensity) from the reactor surface. The range of light intensity examined in this work is from 10-100 20 μmol $photon.m^{-2}.s^{-1}$. The temperature was controlled in the range of 27 ± 1 ^0C
5) measure cell properties as described in Section 3.3.

Experiments were carried out to determine the effect of different factors on the cell growth, the superficial gas velocity, the ratio between the downcomer and riser cross sectional area, and illumination intensity. All experiments were carried out in duplicate. In the batch operation, the culture was grown in the bioreactor until the stationary phase was reached, whereas in the semi-continuous mode, the cultivation was started off as a batch culture, and once the cell concentration reaches a required value, 50% volume of culture broth was harvested. This harvested portion was replaced with a fresh medium.

3.3 Determination of growth

Alga cell growth was determined by cell density and specific growth rate. The productivity of the cell in each reactor condition was also investigated.

3.3.1 Determination of cell density

Cell density was measured by microscope and the counting of cells were performed using an improved Neubauer haemacytometer (Figure 3.3).

1) take two 25 µL drop of culture and place them on a clean haemacytometer
2) place clean cover slip on the drop so that the drop was evenly dispersed under the cover slip
3) count cells under a microscope (objective 10x) (see Figure 3.4)
4) calculate the number of cells as follows :

$$N = \frac{n_1 + n_2}{8} \times 10^4 \quad (3.2)$$

where N = Cell number (cell mL^{-1})
n_1 and n_2 = number of cells count in upper and lower grid (cells)

3.3.2 Determination of specific growth rate

The specific growth rate is calculated from:

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

where μ = specific growth rate ($\mu;d^{-1}$)
N_1 and N_2 = cells density at t_1 and t_2 (cell mL^{-1})
t = time (day)

3.3.3 Determination of productivity

The productivity is calculated by the following equation:

$$\text{Productivity} = \frac{C_2 - C_1}{t_2 - t_1} \quad (3.5)$$

where C_1 and C_2 = cells density at t_1 and t_2 (cell mL^{-1})
t = time (day)

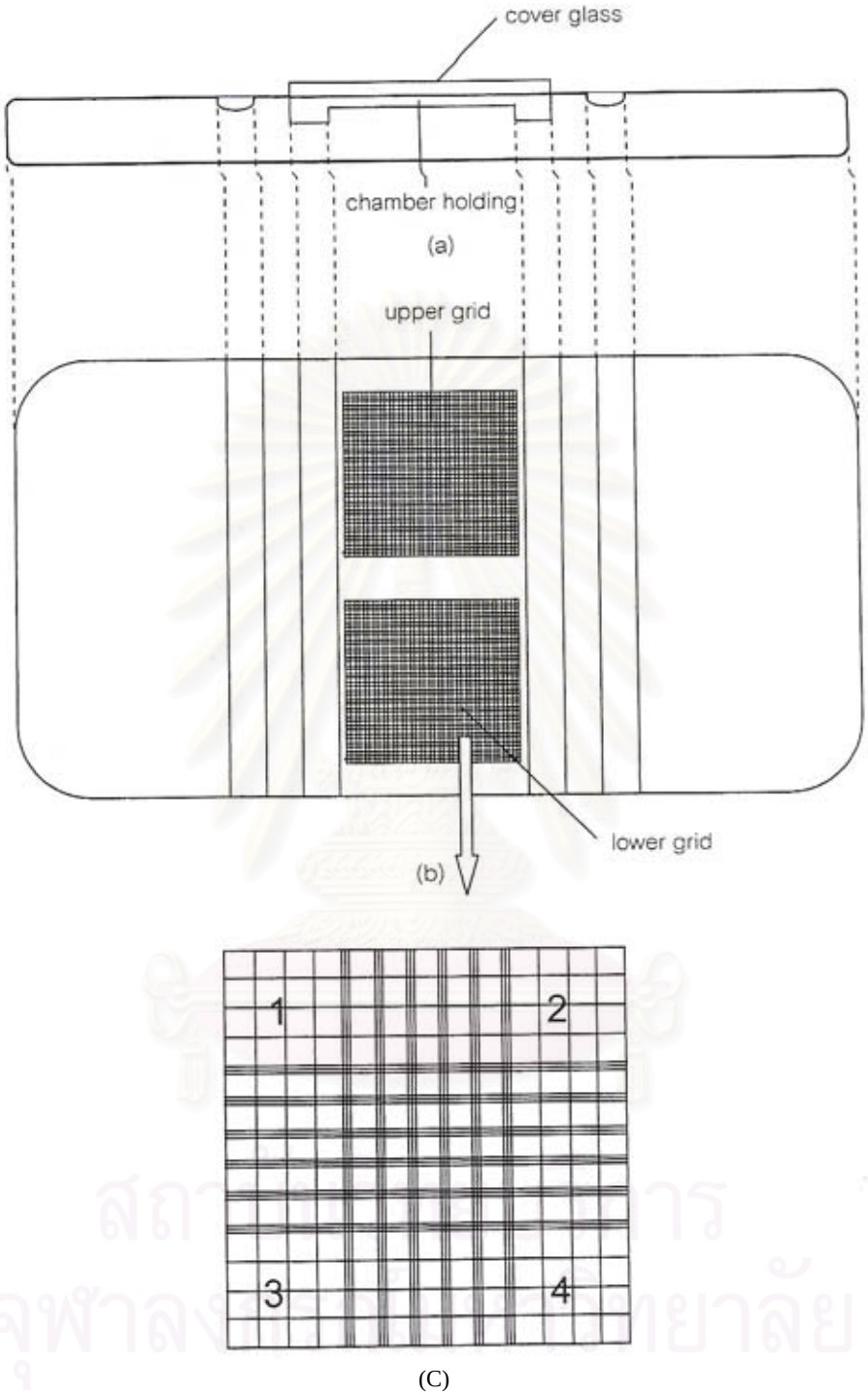
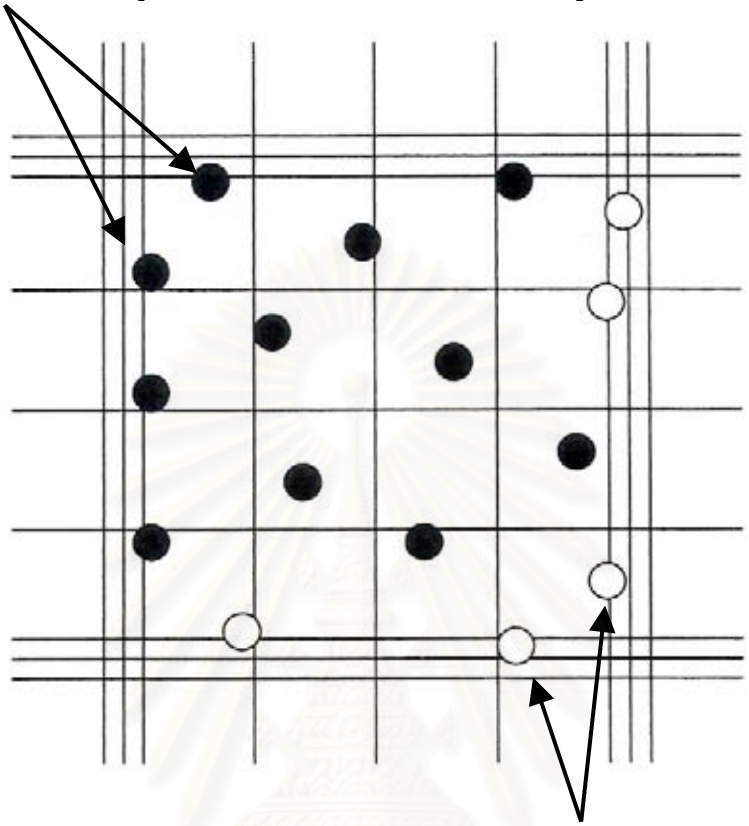


Figure 3.3 (a) Side view of the cell counting chamber showing the cover glass and the space beneath it that holds a microalgae suspension. (b) Top view of the chamber. The chamber has two grids located in the center of the slide. (c) An enlarged view of the grid. The microalgae in the Squares 1,2,3 and 4 are used for cell count.

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



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

Figure 3.4 Counting cell density

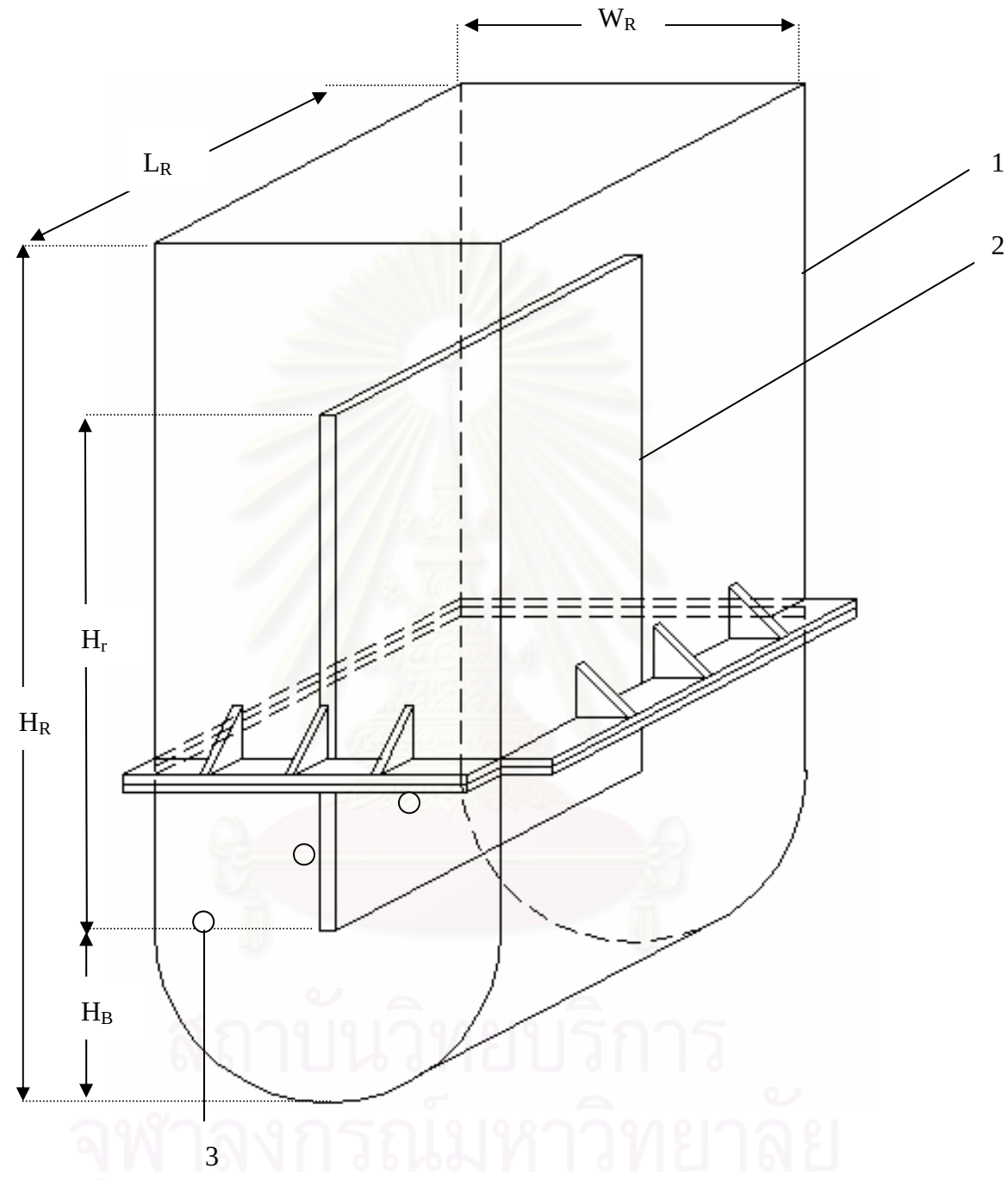


Figure 3.1 Schematic diagram of flat panel airlift photobioreactor
Symbol, 1 = reactor column; 2 = vertical plate; 3= spargers;
H_R = column height; L_R = column length; W_R = column width; H_r = plate height; H_B = bottom clearance

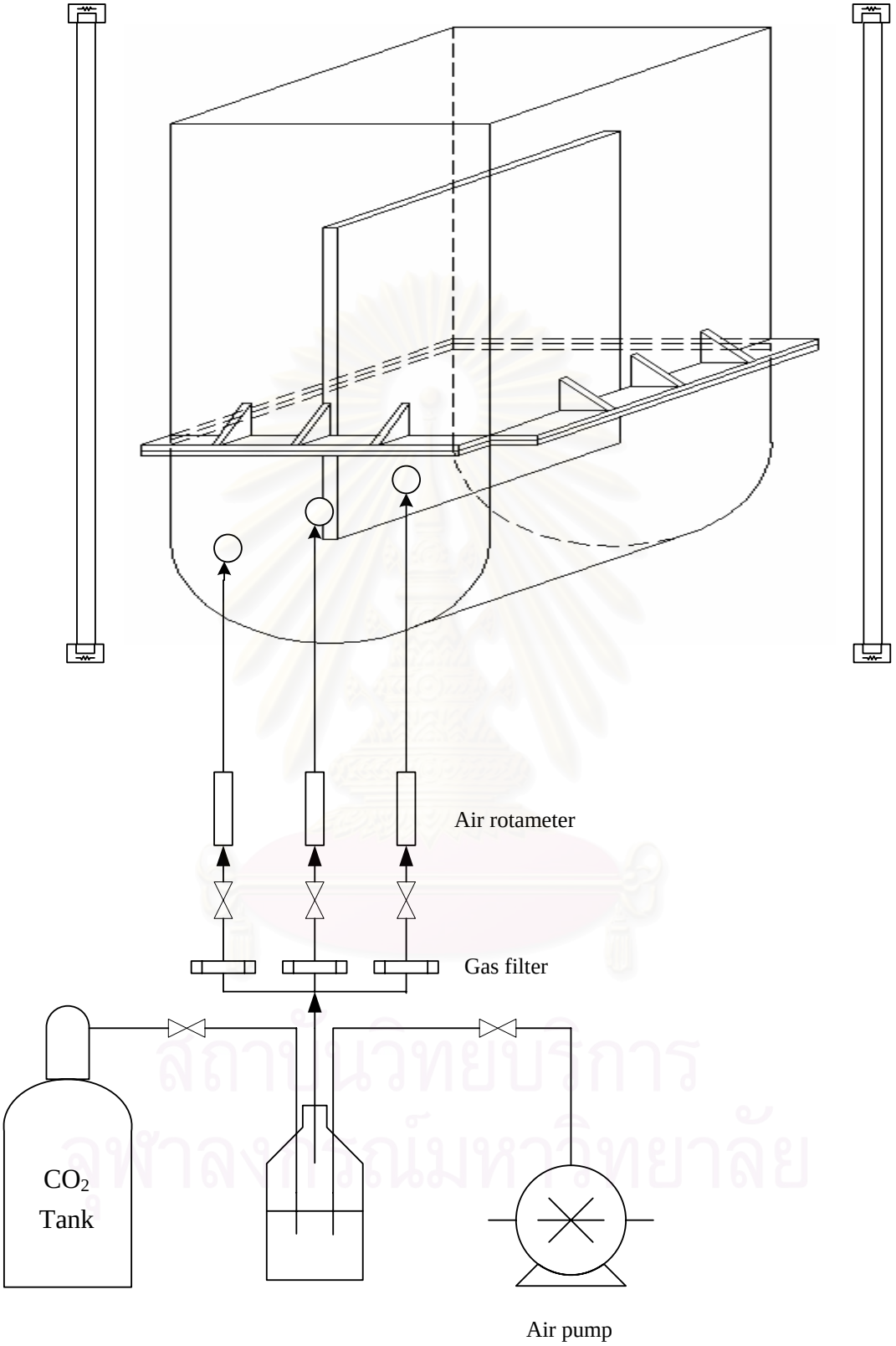


Figure 3.2 Experimental setup for flat panel airlift photobioreactor

Table 3.1 Geometric details of flat panel airlift photobioreactor

		Symbol	17 L Flat panel airlift photobioreactor	90 L Flat panel airlift photobioreactor
Total volume	(L)		19	95
Working volume	(L)		17	90
Column height	(cm)	H_R	50	50
Column length	(cm)	L_R	20	100
Column width	(cm)	W_R	20	20
Draft tube height	(cm)	H_r	30	30
Bottom clearance	(cm)	H_B	10	10

Table 3.2 Composition of F1 medium (Fabregas et al., 1998)

Substances	Amount (per liter)	
$CaCl_2.2H_2O$	9.87	mg
KNO_3	0.41	g
Na_2HPO_4	0.03	g
$C_6H_5FeO_7.5H_2O$	2.22	mg
$MgSO_4.7H_2O$	16.41	mg
$CuSO_4.5H_2O$	0.008	mg
$Na_2MoO_4.2H_2O$	0.08	mg
MoO_3	0.66	mg
Cr_2O_3	0.05	mg
SeO_2	0.036	mg
$CoCl_2.6H_2O$	0.0078	mg
$NH_4Fe(C_6H_5O_7)$	6	mg

CHAPTER IV

RESULTS AND DISCUSSION

The cultivation of *Haematococcus pluvialis* was investigated in internal loop airlift systems by Kaewpintong et al. (2006) but that work only focused on the small scale 3L cylindrical geometry system where the specific growth rate and the maximum cell attainable were 0.45 d^{-1} and $77x10^4$ cell mL^{-1}. This was already considered one of the highest growth cultivation systems when compared with the literature of the same era. After that work, Panitchakarn (2007) tried to upscale this system to 17L but contamination seemed to be the main problems and the resulting growth was quite unattractive with the specific growth rate and the maximum cell density of 0.4 d^{-1} and $21x10^4$ cell mL^{-1}. Up to the time of this work, there were very few attempts for the scale up of such system, there was only one statement in 1996 where the cultivation in the 30L airlift was conducted where the maximum cell density was $25x10^4$ cell mL^{-1} and the specific growth rate of 0.13 d^{-1} (Harker et al., 1996a). This work therefore focused on the establishment of growth and environmental conditions that could sustain high production of green vegetative cells of *H.pluvialis*. In the following discussion, each of the conditions is discussed in detail.

4.1 Cultivation of *H. pluvialis* in 3L and 17L cylindrical airlift photobioreactor (ALPBR)

The cultivation of *H.pluvialis* in this work was conducted in an evaporative room where the temperature was well controlled under 30°C. This was different than the work of Kaewpintong et al. (2006) and Panichakarn (2007) in which the temperature was controlled with the air conditioned room. The use of evaporative room was selected to save the energy during the cultivation and to ensure that the cultivation was performed in the condition close to the actual one in industrial scale. The cultivations of *H. pluvialis* in 3L and 17L airlift photobioreactor were started off using optimal conditions reported in the two previous experiments as stated above, i.e. superficial gas velocity = 0.4 cm s^{-1} with an addition of 1% CO_2, pH=7, light intensity = 20 μmol photon m^{-2} s^{-1}. The geometric detail of the cylindrical airlift photobioreactors

is demonstrated in Table 4.1.

Fig. 4.1(a) shows the comparison between the performance of these two systems which illustrated that the cell density of *H. pluvialis* in 3L ALPBR was nearly $90x10^4$ cell mL^{-1} with specific growth rate of 0.45 d^{-1}, and for the 17L system, these two quantities were $28x10^4$ cell mL^{-1} and 0.38 d^{-1}, respectively. The cultivation of *H.pluvialis* in the both systems was shown in Fig. 4.1(b). When compared with the previous attempt which was conducted in the air conditioned room, the cultivation in the evaporative room was found to yield slightly better results except for the specific growth rate in the 17L system where Panitchakarn (2007) obtained a slightly better level (but with lower cell concentration). This demonstrates the potential for the cultivation in the evaporative room which was found to be quite effective and the contamination seemed to be better controllable.

The 17L was clearly out-performed by the 3L, and this could be due to several reasons. Firstly, the cultivation in large scale systems is always subject to a greater requirement in terms of both materials and energy supplied to the bioreactors. In this case, it was possible that the cultivation in 17L required a stronger supply of light intensity at the reactor surface. With the same level of light intensity, the penetration depth of light into the airlift columns was the same for both 3L and 17L. However, this penetration depth was relatively large for the 3L when compared with the actual diameter of the 17L system, as illustrated in Fig. 4.2. The next reason could be due to the nonideal behavior in the large scale airlift. Fig. 4.3 illustrates that, as the column becomes quite large, there would exist local internal liquid circulation (Wongsuchoto and Pavasant, 2004). In addition, the inherited larger riser often destroys the flow pattern in riser itself as it allowed local backflow of the liquid which reduced the liquid circulation rate and also the gas-liquid mass transfer rate. This could then result in the precipitation of the over-grown cells which then reduced the growth rate of the alga. Apart from these two reasons, the hydrostatic pressure acted on the cell could be quite high if the system is physically tall. This effect of hydrostatic pressure, although not readily proven to exist, could therefore limit the growth in the 17L airlift system as it was approx. 60 cm taller than the 3L.

4.2 Cultivation of *H. pluvialis* in 17L flat panel airlift photobioreactor (FP-ALPBR)

One of the difficulties in designing the close cultivation system is the scale up and the results obtained from the previous section demonstrated that, even with the simple cylindrical airlift system, the scale up could be quite troublesome. It was therefore proposed that the upscale was achieved just by increasing the length of the airlift without changing the width and the height. This type of airlift should be designed as a flat panel so that the increase in the length was easy and simple. In this section, the behavior of such system was investigated but only with the volume of 17L. This was to compare the results with the cylindrical configuration with the same volume.

4.2.1 Effect of downcomer and riser cross sectional area (A_d/A_r)

In this section, the effect of design configuration in the flat panel airlift photobioreactor (FP-ALPBR) was investigated. A basic design parameter in the airlift system was the ratio between cross sectional area of downcomer and riser (A_d/A_r). For this experiment, the parameter could be simply altered by changing the plate position (see Fig. 3.1) and this area was fixed at 3.0, 1.5, 0.8, and 0.4 respectively. For comparison, the cultivation in the bubble column was also carried out and this was performed in the same system without the installation of the separation plate, and this is labeled as “control” in Fig. 4.4. Fig. 4.4(a) shows the growth curves of *H. pluvialis* in 17L FP-ALPBRs with the modified F1 medium. The superficial velocity was selected at 0.4 cm s^{-1}, based on the previous report by Kaewpintong et al. (2006). Other controlled conditions are: 1% CO_2 in the air supply, pH=7, light intensities using fluorescence lamps at approx. 20 μmol photon m^{-2} s^{-1}.

The results as shown in Fig. 4 suggested that the maximum cell density and specific growth rate were obtained from the FP-ALPBR with the smallest A_d/A_r of 0.4 (cell density = $41x10^4$ cell mL^{-1} and specific growth rate = 0.52 d^{-1}) and the second best was from A_d/A_r of 0.8 ($39x10^4$ cell mL^{-1} with specific growth rate of 0.49 d^{-1}) followed by A_d/A_r of 1.5 ($29x10^4$ cell mL^{-1} with specific growth rate of 0.42 d^{-1}) and A_d/A_r of 3.0 ($24x10^4$ cell mL^{-1} with specific growth rate of 0.37 d^{-1}), respectively. As expected

bubble column seems to provide the poorest performance ($19x10^4$ cell mL^{-1} with specific growth rate of 0.40 d^{-1}) (Fig. 4.4(b)). Note that small A_d/A_r reflects the airlift with large riser and small downcomer cross sectional area. The exact reason why this cell grew best at this condition was still not evaluated properly, however, it was expected that the system with large riser could exhibit lower liquid circulation velocity than that obtained in the system with small riser. However, the liquid flowrate in the downcomer was high as the cross sectional area of the downcomer was rather small. This high liquid circulation velocity in downcomer section might significantly support the uplift of the cells which formed aggregation at the bottom of the column and also small portions of the cells attached on the wall of the column. This aggregation of the cells at the bottom of the column could be observed after the first few days of cultivation and this sediment increased continually to the final stage (15 day). Therefore cell cultivation was simply promoted by controlling the ratio between downcomer and riser cross sectional area at minimum, and in this case, this was equal to 0.4.

It should be noted, however, that airlift with large riser cross sectional area could support gas flow without inducing strong liquid flow in the system. Therefore this might result in a greater difference between bubbles and liquid velocities, and as a result, a larger shear force acting on the cells. Subsequently this might exert some negative impact on the growth. However, Wongsuchoto et al. (2003) and Ruen-ngam (2008) both agreed that the alteration of A_d/A_r did not seem to have significant influences on the mass transfer coefficient (k_L), particularly in pure water system. Hence, as k_L depends largely on the difference between liquid velocity (V_L) and gas velocity (V_g), this level of difference in velocity was not strong enough to yield noticeable results in the shear rate acting on the cells.

Fig. 4.5 demonstrates the morphological changes of the cell structure, i.e. green vegetative cell, immature cell and cyst cell. This finding illustrated that the morphology changes in *H. pluvialis* was not obvious particularly during the growth stage, and only some immature cells were observed at the end of each batch (around Days 13-15). This was investigated with the whole range of A_d/A_r and the results were quite similar to each other, i.e. the effect of A_d/A_r on the morphological changes of *H.pluvialis*. Cyst was not at all observed during this experiment. Hence, only the green vegetative cells would be measured and reported in the following discussion.

4.2.2 Effect of aeration

During the vegetative growth stage of *H. pluvialis*, cells have two flagella and can move around by themselves. Hence, even though there was no circulation provided, the cells should be able to swim around by themselves to the location suitable for their growth (perhaps in the region of high light and nutrient concentration). This movement of such cell was slightly slow and could be affected significantly by the liquid flow induced by aeration. This was why it was important to initially check whether the circulation was beneficial for the growth of such microorganism.

This experiment was performed in the 17L FP-ALPBR with the A_d/A_r of 0.4 (form the above section), illuminated at 20 µmol photon m^{-2} s^{-1}, and with pH =7. Fig. 4.6(a) illustrates the growth of *H. pluvialis* in the 17L FP-ALPBR running at different levels of aeration rate (which was measured in terms of superficial gas velocity) whereas Fig. 4.6(b) summarizes the cell density and specific growth rate of *H.pluvialis* cultivated under various aeration conditions. It was observed clearly that the optimum aeration rate occurred at 0.4 cm s^{-1} where a high cell density of $41x10^4$ cell mL^{-1} and specific growth rate of 0.52 d^{-1} were resulted. This level of growth was relatively high when compared with those reported in literature. This finding agreed well with that of Kaewpintong et al., (2006) who reported that the suitable aeration rate for the growth of such cells was 0.4 cm s^{-1}. Reducing the aeration rate to 0.2 cm s^{-1} saw a decrease in cell density and specific growth rate ($38x10^4$ cell mL^{-1} and 0.44 d^{-1}, respectively). This could be due to inadequate level of circulation in the system. Increasing the aeration above 0.4 cm s^{-1}, the growth seemed to slow down quite significantly, and at 0.8 cm s^{-1}, the system could only reach the maximum cell density of $24x10^4$ cell mL^{-1} and specific growth rate of 0.41 d^{-1}. Therefore, a much too high aeration rate did not show benefits for the growth. Although an increase in aeration rate gave rise to the mass transfer which facilitated the removal of gases such as oxygen, preventing the accumulation of such gas, growth seemed to be drastically slowed down by excessive aeration. This might be due to the excessive shear force which was induced at high aeration. This indicated that the cell of *H. pluvialis* was highly shear sensitive and even the shear caused by aeration could deteriorate the growth. This explanation was supported by several past reports. For instance, Gudin and Chaumont (1991) stated that the key problem in the cultivation of microalgae in photobioreactors was cell damage due to

shear stress. Hata et al. (2001) illustrated that the culture of green vegetative cell in exponential phase of growth required a low liquid velocity due to its fragility.

4.3 Semi-continuous cultivation of *H. pluvialis* in airlift systems

Semi-continuous cultivation was conducted in order to examine the potential of having a large-scale culture system that could operate economically. The three types of airlift mentioned above, i.e. 3L, 17L cylindrical ALPBR, and 17L FP-ALPBR were employed for this comparative purpose. The cultivation of *H.pluvialis* in 3L (Fig. 4.7(a)) and 17L cylindrical ALPBR (Fig. 4.7(b)) were carried out under the most suitable conditions obtained from the aforementioned experiments, i.e. A_d/A_r = 3.0, u_{sg} = 0.4 cm s^{-1}, light intensity = 20 μmol photon m^{-2} s^{-1}, pH = 7. Fig. 4.7(c) shows the semi-continuous cultivation in 17L FP-ALPBR which was also operated with the most optimal conditions as examined in the previous section: A_d/A_r = 0.4, u_{sg} = 0.4 cm s^{-1}, light intensity = 20 μmol photon m^{-2} s^{-1}, pH = 7.

At the beginning of the cultivation, the culture was allowed to grow until the maximum cell density for each system was obtained, i.e. $90x10^4$, $32x10^4$, and $35x10^4$ cell mL^{-1} in 3L, 17L cylindrical ALPBR and 17L FP-ALPBR, respectively. For the 3L system, the highest cell density occurred at about days 9-11 whereas the two 17L ALPBRs saw their maximum cell at about days 6-8 of cultivation. After that, the culture was harvested. The harvested volume must be calculated to yield the initial cell concentration for the next cycle at $10x10^4$ cell mL^{-1}. The harvested volume was replaced with the newly prepared medium. The culture was then left to grow in the each system until it reached the exponential growth phase or near the stationary phase (to ensure that cells were most active) where the next harvest was carried out. The harvested cell density (from Cycle 2 onwards) obtained from 3L, 17L cylindrical and 17L FP- ALPBRs were approximately $70x10^4$ cell mL^{-1}, $30x10^4$ cell mL^{-1} and $32x10^4$ cell mL^{-1}, respectively. It was proven that with this harvesting cycle, the cell could maintain its vegetative form and, for the 3L system, cell density increased up to the level obtained in the previous cycle within 7 days period, and for the two 17L systems, each cycle required only about 3-4 days. The productivity of semi-continuous culture in 3L, 17L cylindrical ALPBR and 17L FP-ALPBR were $9x10^7$ cell L^{-1} d^{-1}, $7x10^7$ cell L^{-1} d^{-1} and $7.7x10^7$ cell L^{-1} d^{-1}, respectively. This level of productivity was comparable, within the same order of magnitude with that reported by Hata et al. (2001) who

achieved the productivity of 6.8 cells mL^{-1} d^{-1} in the small scale (0.5L Erlenmeyer flask) semi-continuous culture.

4.4 Performance of large scale FP-ALPBR on the cultivation of *H. pluvialis*

This section described the cultivation of *H. pluvialis* in the large scale flat plate airlift photobioreactor (FP-ALPBR). The 90L airlift system was fabricated with dimension as stated in Table 3.1, and up to the present time, this was perhaps the largest ever reported closed system for *H.pluvialis*. The cultivation was conducted with the most suitable operating conditions obtained from Sections 4.2.1 and 4.2.2, i.e. A_d/A_r = 0.4, u_{sg} = 0.4 cm s^{-1}, light intensity = 20 μmol photon m^{-2} s^{-1}, pH = 7. With a proper control of contamination, the system could be operated successfully and Fig 4.8(c) illustrates that this system could achieve the maximum cell density of $40x10^4$ cells mL^{-1} and the specific growth rate of 0.39 d^{-1} whereas Fig. 4.9 summarizes the cell density and specific growth rate of *H.pluvialis* cultivated under various type of ALPBR.

The economical analysis of the various airlift systems for the cultivation of *H.pluvialis* was demonstrated in Table 4.2. This includes not only the airlift systems employed in this work, but also those bioreactors proposed in literature. With the local cost of utilities in Thailand, the results from the analysis indicated that the operation of the 90L FP-ALPBR was most attractive as it was the cheapest choice for the cultivation of *H.pluvialis*. It should be noted, however, that this analysis did not include the installation cost for the equipments.

Note that, the cultivation of *H.pluvialis* in 17L and 90L FP-ALPBR: growth stage at suitable condition: u_{sg} = 0.4 cm s^{-1}, pH = 7, light intensity = 20 μmol photon m^2 s^{-1} are shown in Fig. 4.10(a) and Fig. 4.10(b), respectively.

Table 4.1 Geometric details of cylindrical airlift photobioreactor

		3L cylindrical airlift photobioreactor	17L cylindrical airlift photobioreactor
Total volume	(L)	3.6	19
Working volume	(L)	3	17
Column height	(cm)	60	120
Diameter of column	(cm)	9	15
Diameter of draft tube	(cm)	4.5	7.5
Bottom clearance	(cm)	5	10
A_d/A_r		3.0	3.0

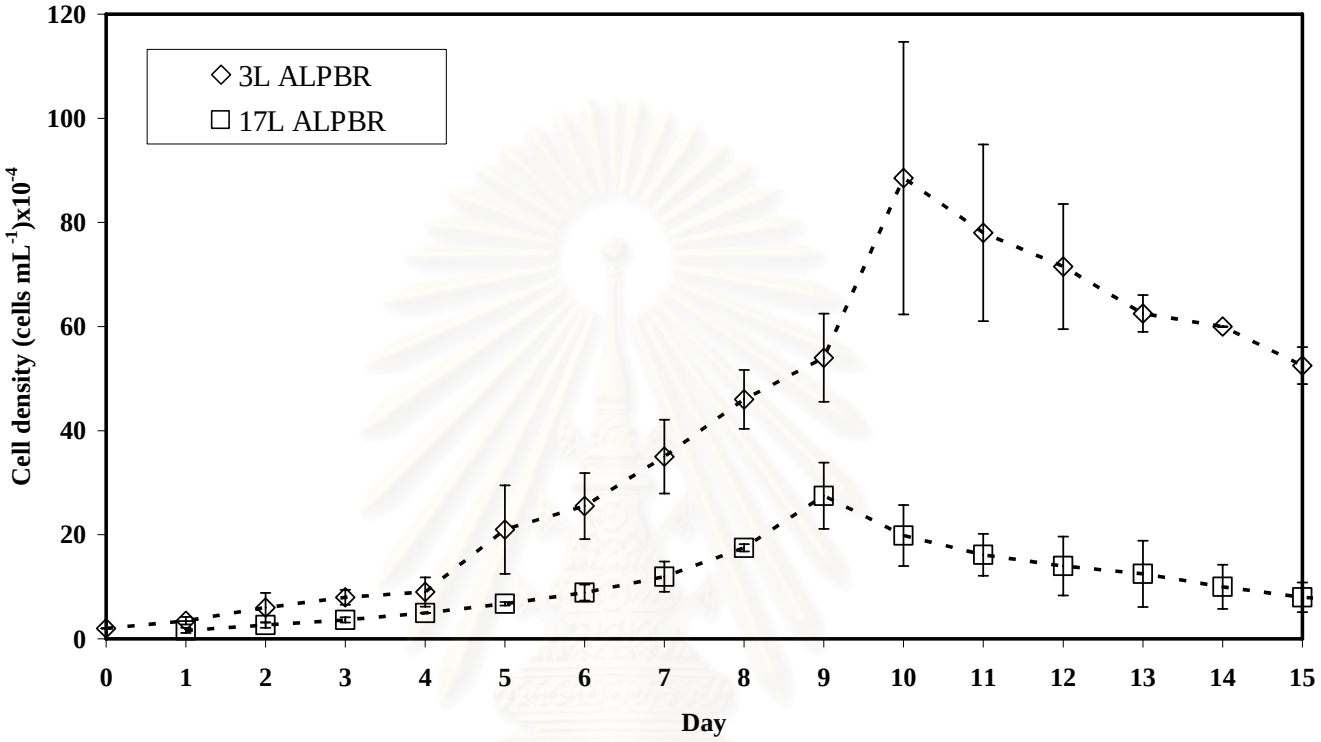


Figure 4.1(a) Cultivation of *H.pluvialis* in 3L and 17L of airlift photobioreactors at u_{sg} = 0.4 cm s^{-1}, pH = 7, light intensity = 20 μmol photon m^{-2} s^{-1}

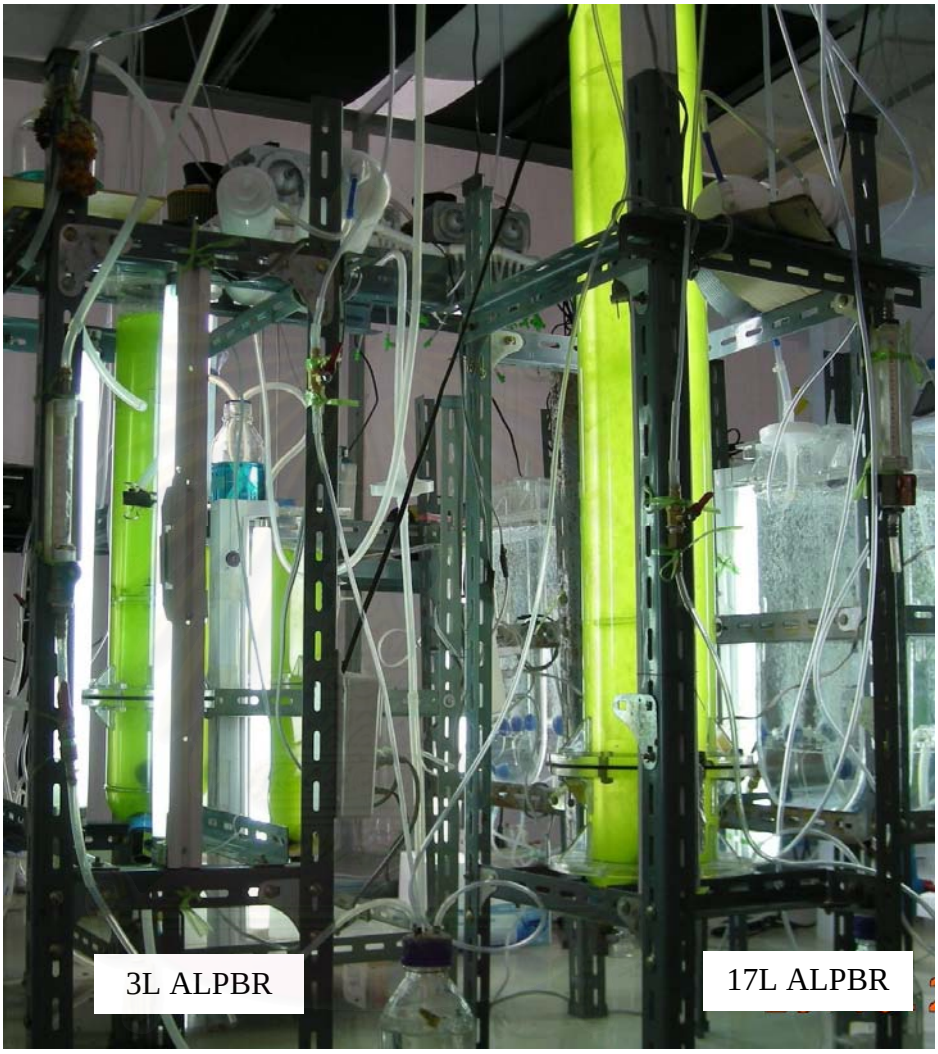


Figure 4.1(b) Cultivation of *H.pluvialis* in 3L and 17L of airlift photobioreactors at u_{sg} = 0.4 cm s^{-1}, pH = 7, light intensity = 20 μmol photon m^{-2} s^{-1}

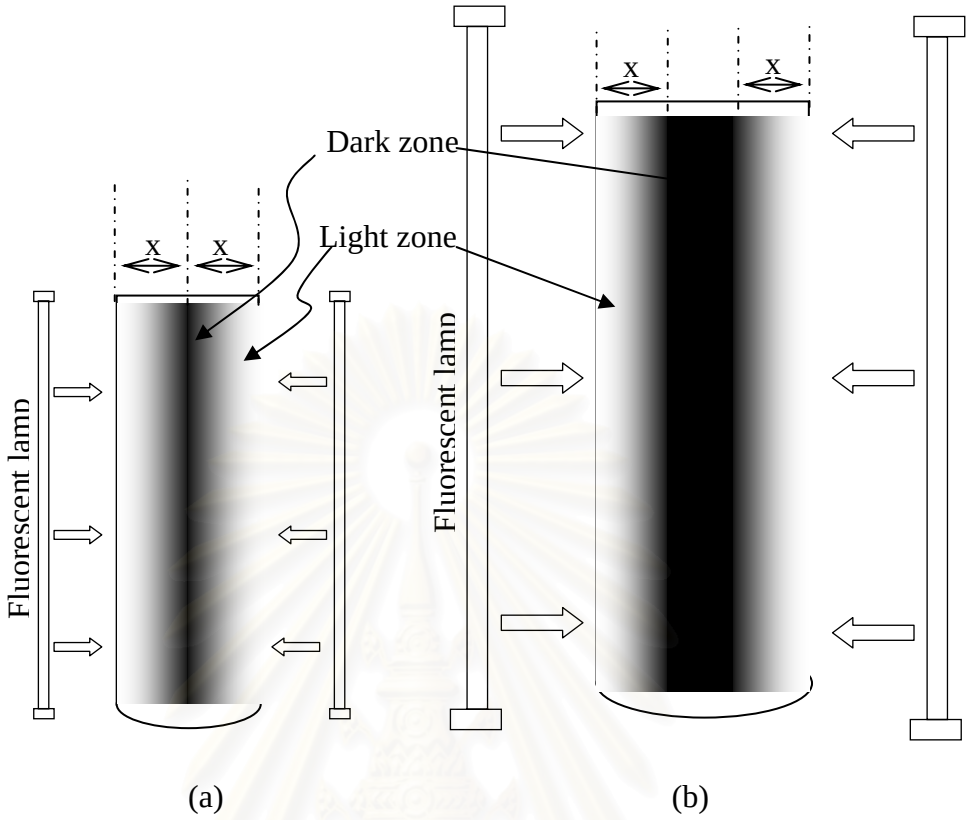


Figure 4.2 Light penetration in airlift systems: (a) 3L ALPBR and (b) 17L ALPBR

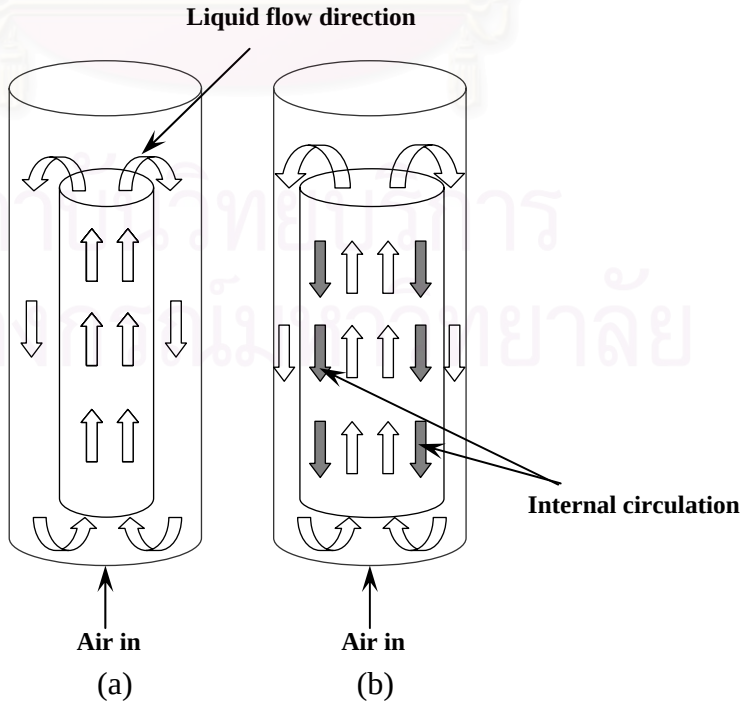
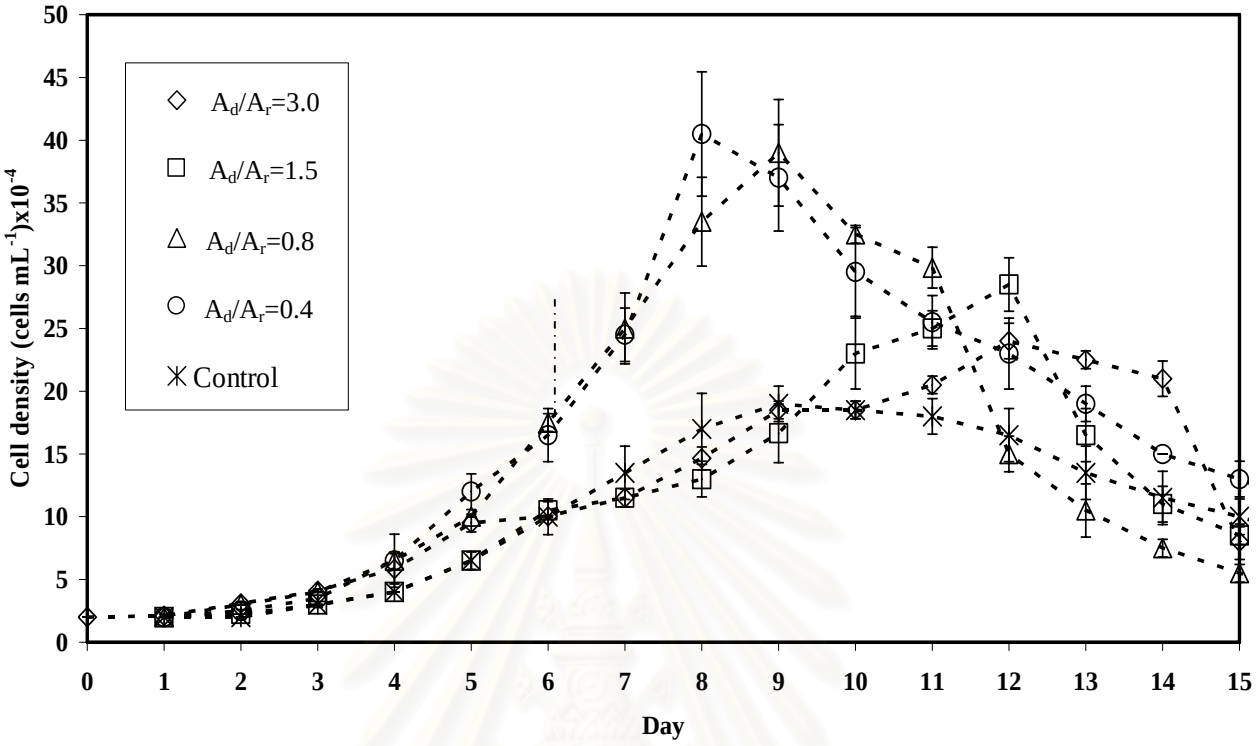
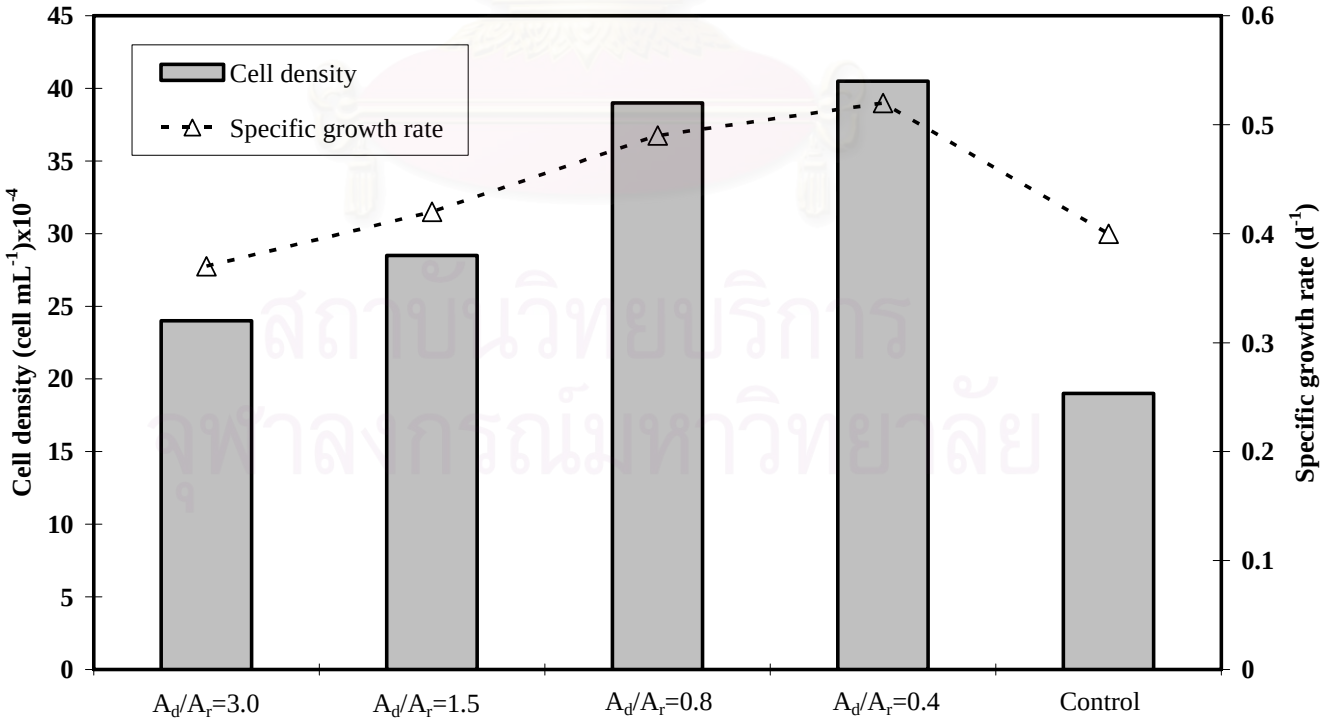


Figure 4.3 Internal circulation in airlift systems: (a) small riser (b) large riser



(a) growth curve



(b) maximum cell density and specific growth rate

Figure 4.4 Effect of downcomer and riser cross sectional area (A_d/A_r) on cell density of *H. pluvialis* in 17 L flat panel airlift photobioreactor

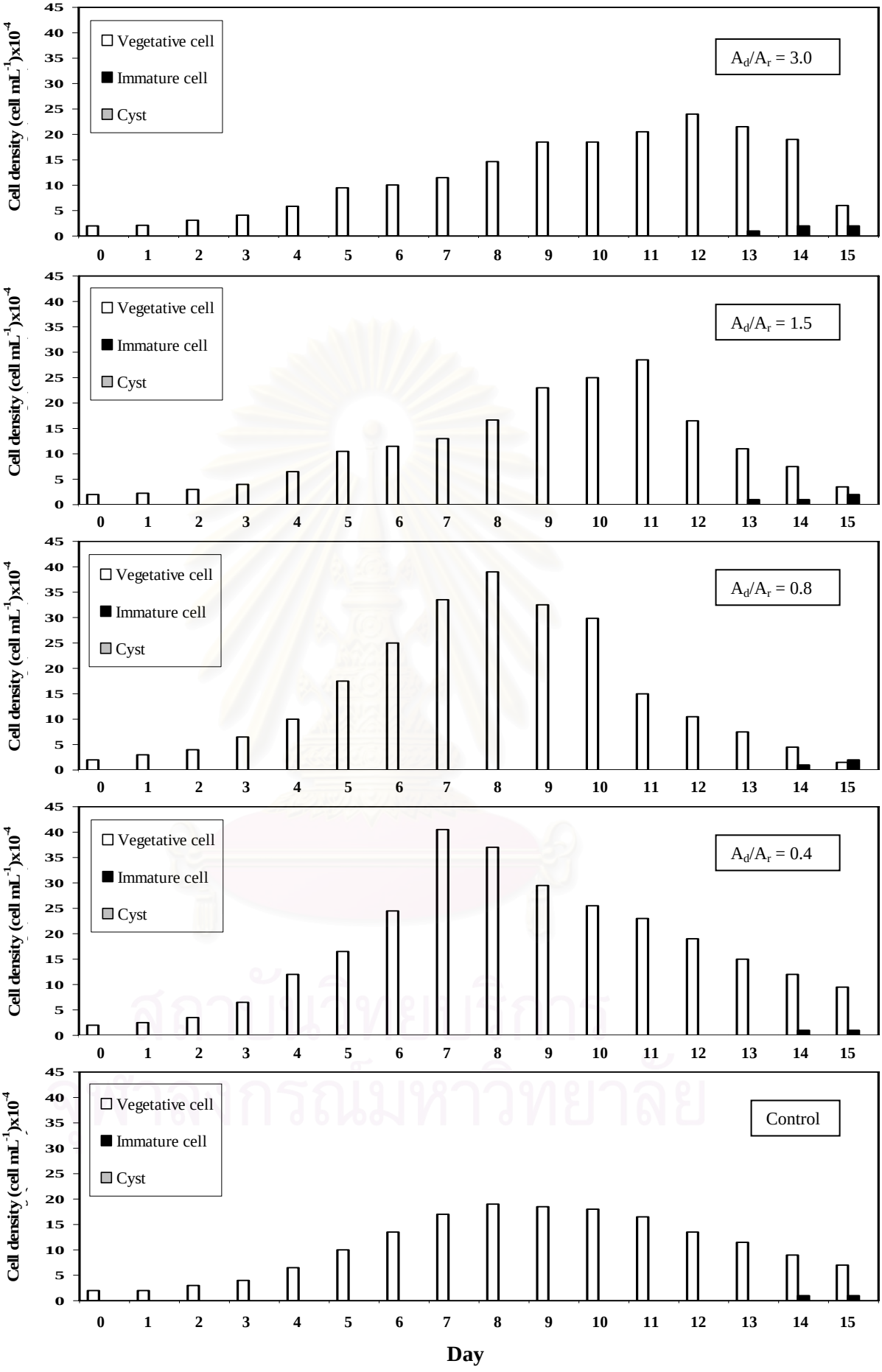
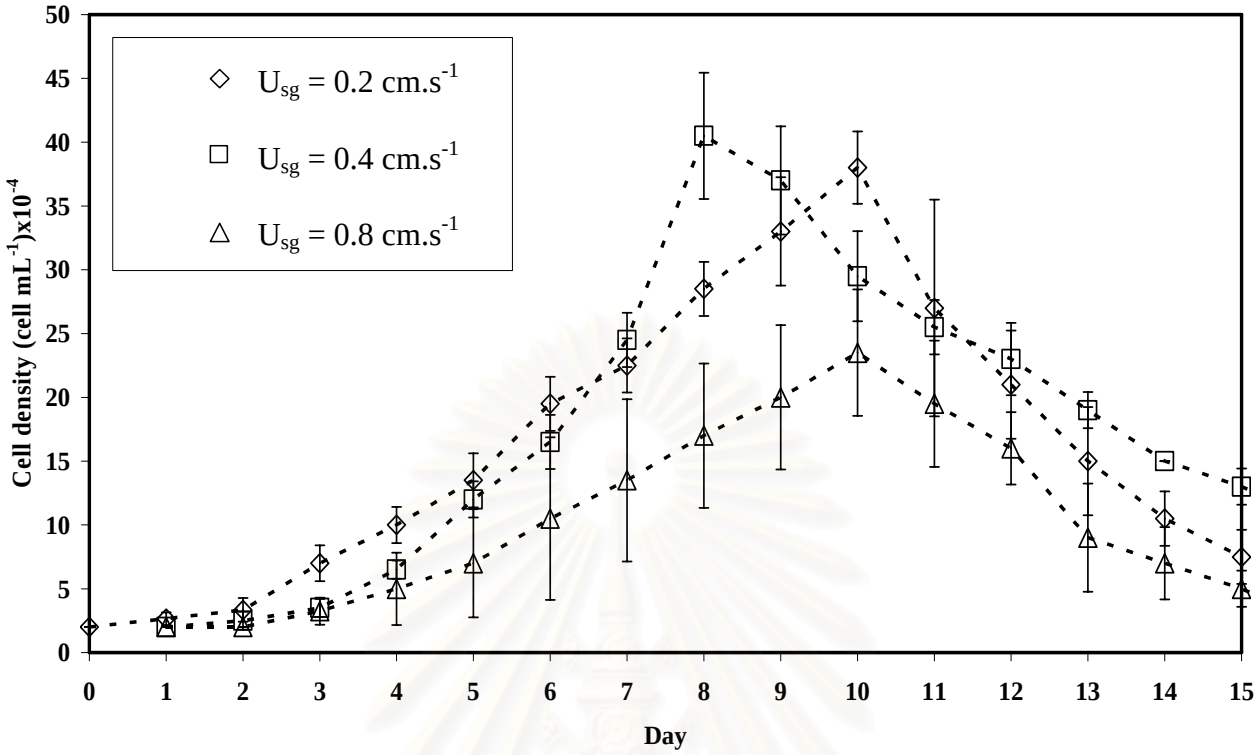
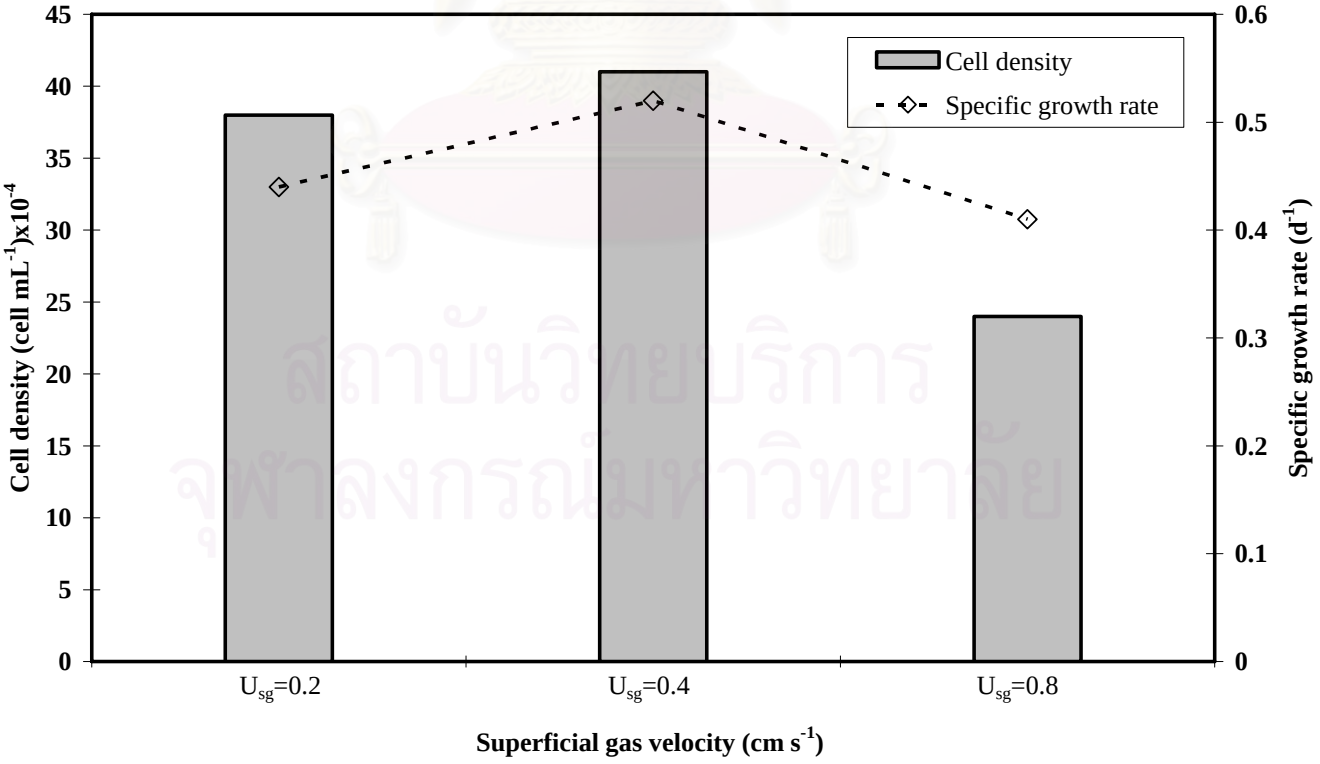


Figure 4.5 Effect of downcomer and riser cross sectional area (A_d/A_r) on morphology of *H. pluvialis* in 17 L flat panel airlift photobioreactor



(a) growth curve



(b) maximum cell density and specific growth rate

Figure 4.6 Effect of aeration on cell density of *H. pluvialis* in 17L flat panel airlift photobioreactor

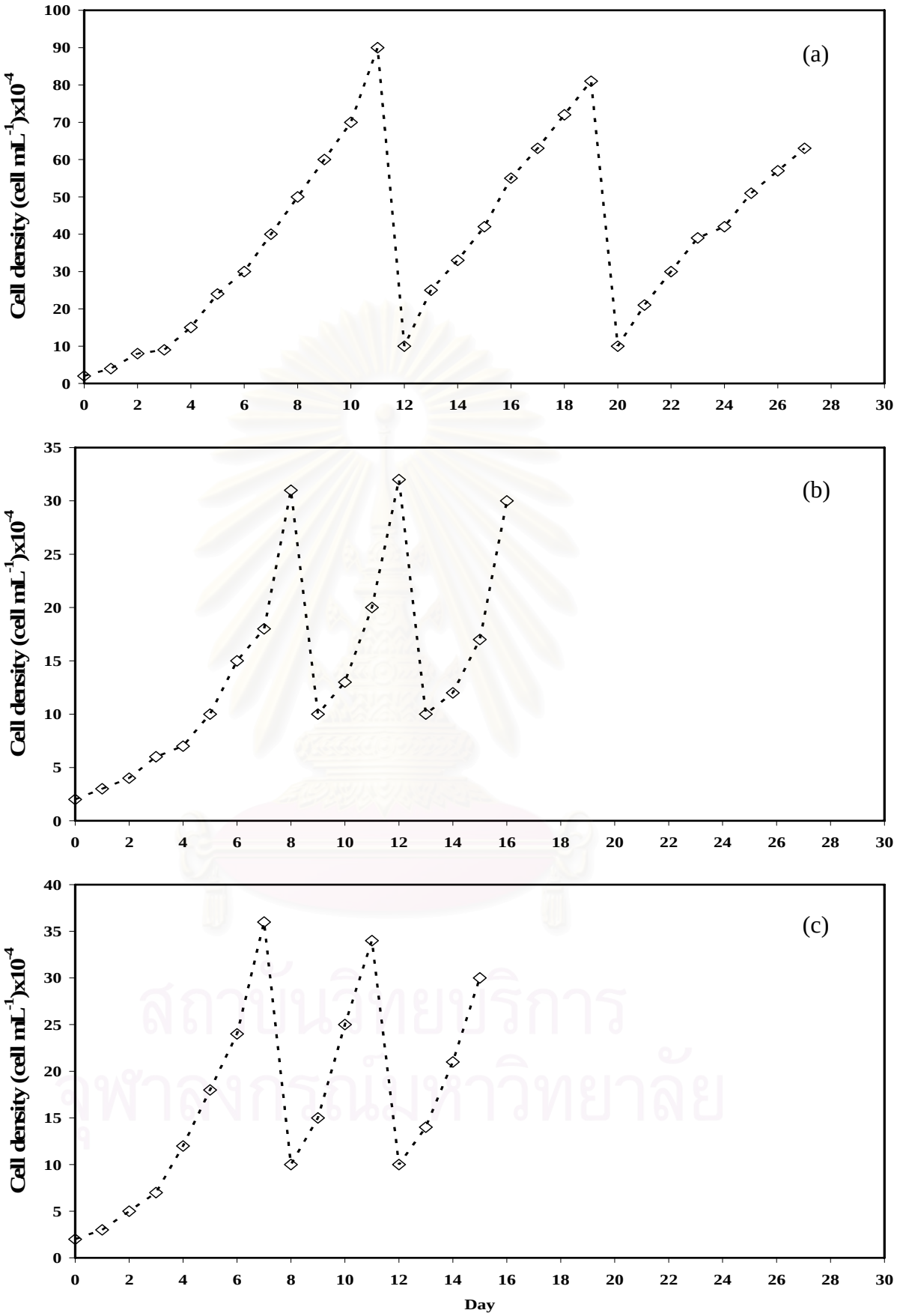


Figure 4.7 Semi continuous cultivation of *H.pluvialis* in (a) 3L ALPBR (b) 17L ALPBR and (c) 17L FP-ALPBR at u_{sg} = 0.4 cm s^{-1}, pH = 7, light intensity = 20 μmol photon m^{-2} s^{-1}

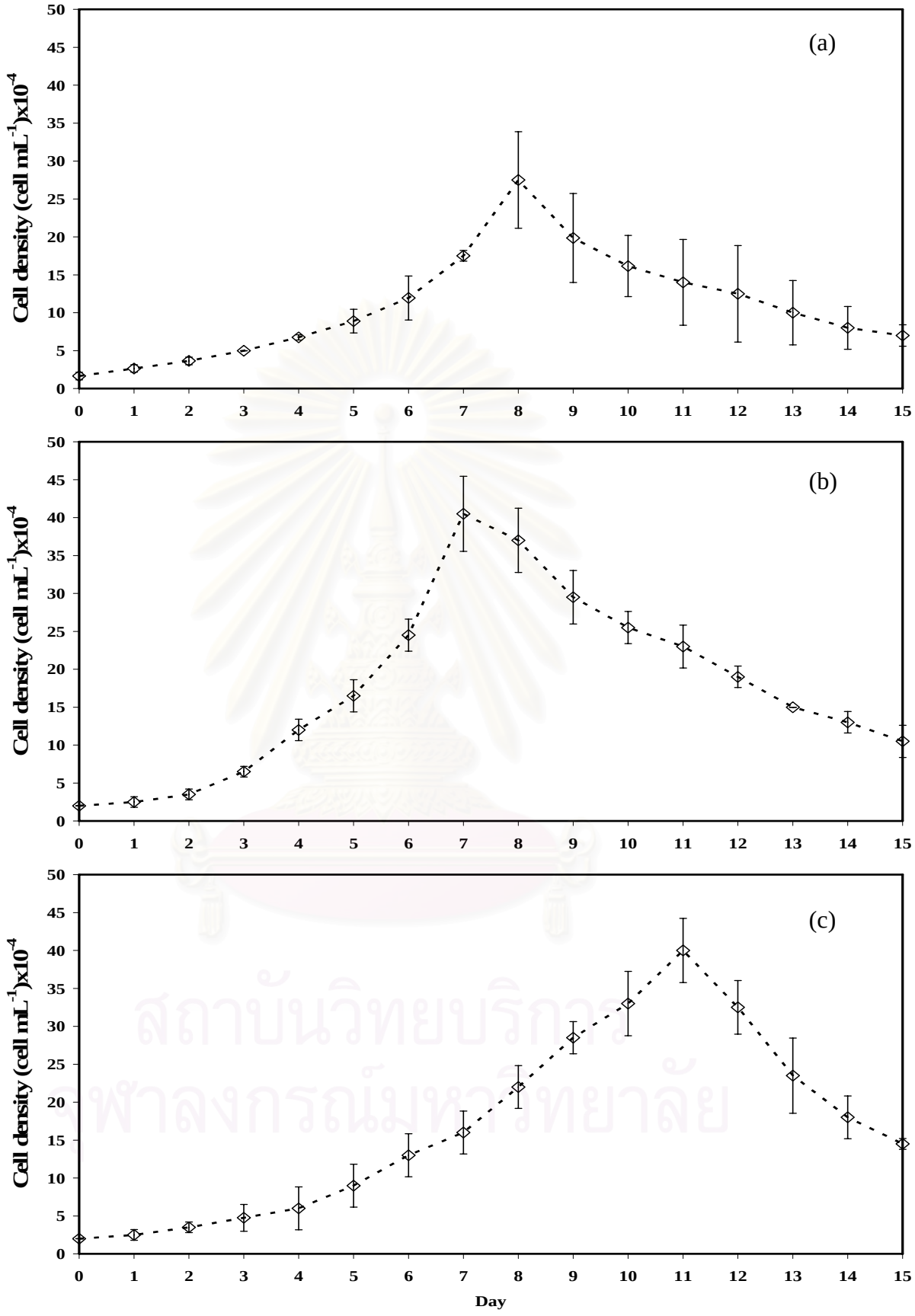


Figure 4.8 Cultivation of *H.pluvialis* in (a) 17L ALPBR, (b) 17L FP-ALPBR and (c) 90L FP-ALPBR at u_{sg} = 0.4 cm s^{-1}, pH = 7, light intensity = 20 μmol photon m^{-2} s^{-1}

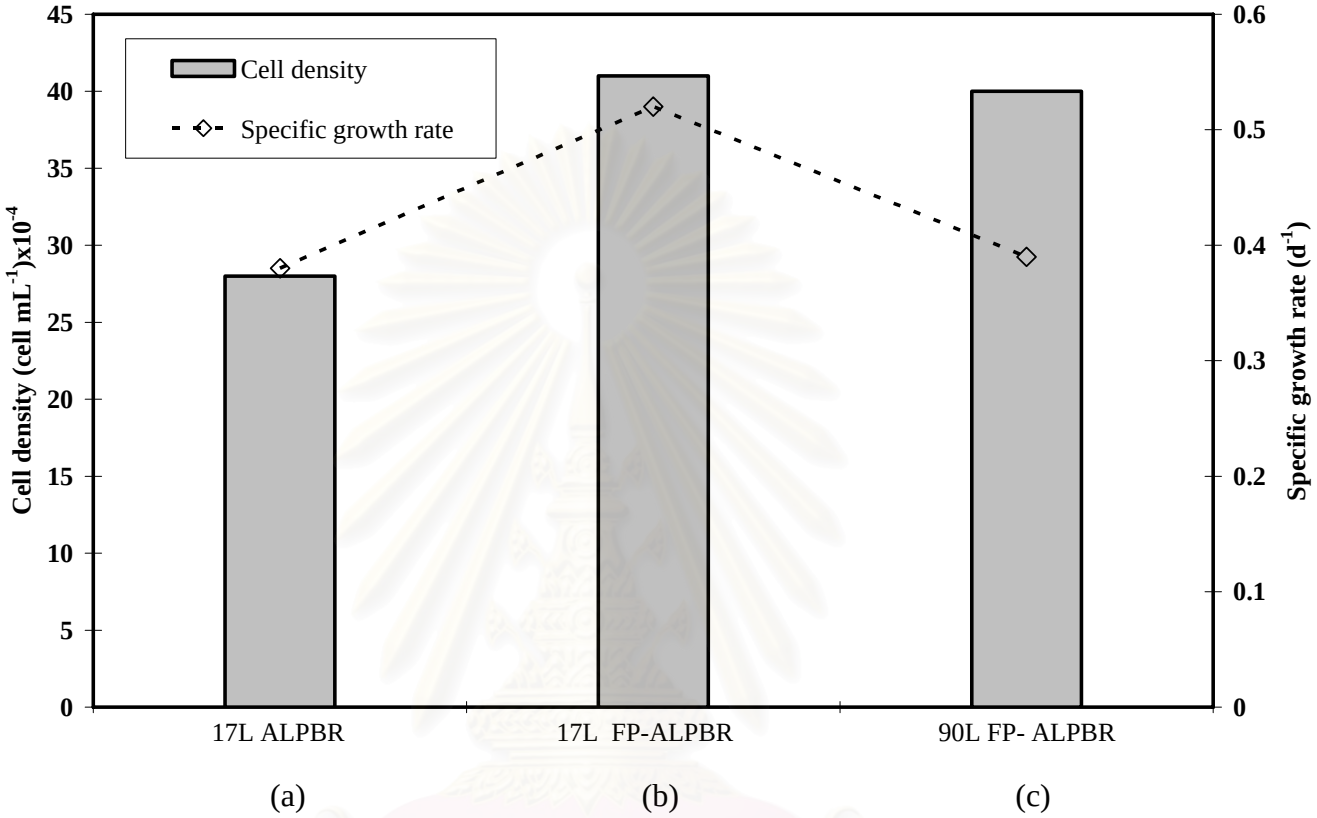


Figure 4.9 Maximum cell density and specific growth rate of *H.pluvialis* in (a) 17L ALPBR, (b) 90L FP-ALPBR and (c) 17L FP-ALPBR: growth stage at suitable condition: u_{sg} = 0.4 cm s^{-1}, pH = 7, light intensity = 20 μmol photon m^{-2} s^{-1}

Figure 4.10(a) Cultivation of *H.pluvialis* in 17L FP-ALPBR: growth stage at suitable condition: u_{sg} = 0.4 cm s^{-1}, pH = 7, light intensity = 20 μmol photon m^{-2} s^{-1}

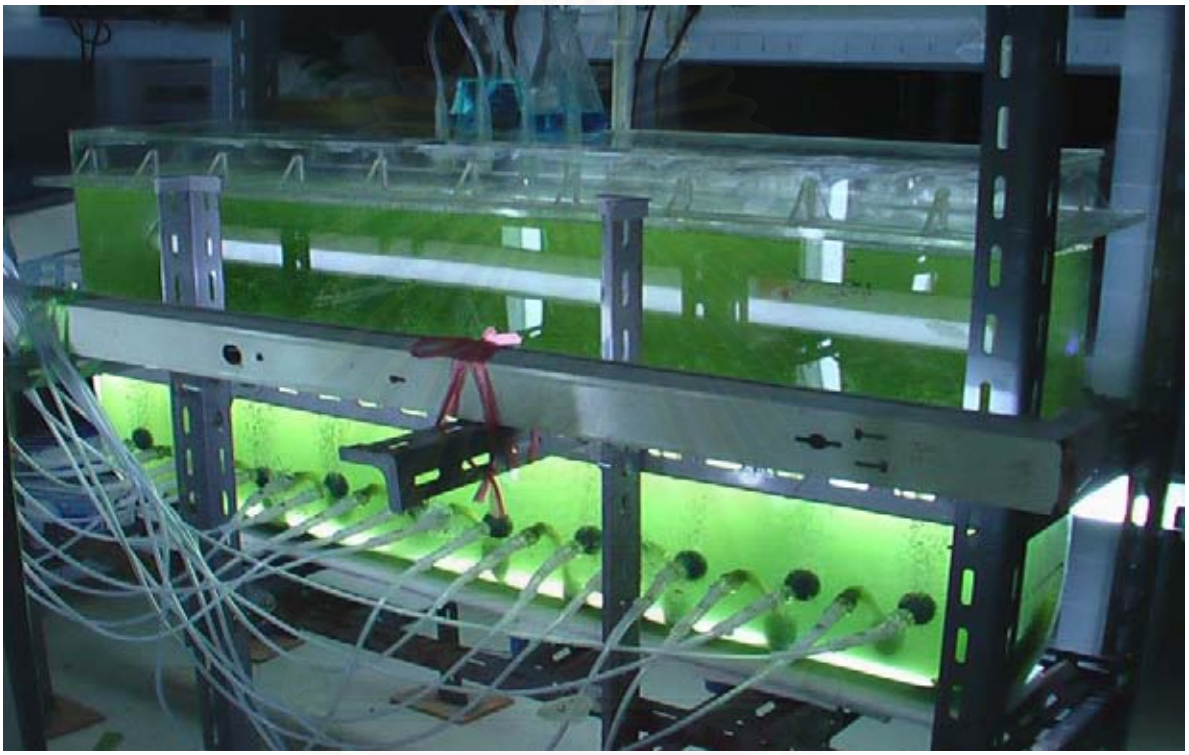


Figure 4.10(b) Cultivation of *H.pluvialis* in 90L FP-ALPBR: growth stage at suitable condition: u_{sg} = 0.4 cm s^{-1}, pH = 7, light intensity = 20 μmol photon m^{-2} s^{-1}

Table 4.2 Economicical analysis for the cultivation of $3.6x10^{10}$ cell of *Haematococcus pluvialis* in ALPBRs

		3L ALPBR	17L ALPBR	17L FP-ALPBR	90L FP-ALPBR	[A]	[B]	[C]
Harvested cell concentration (cell mL^{-1})		9.00E+05	2.80E+05	4.00E+05	4.00E+05	2.50E+05	5.50E+05	5.00E+06
Effective volume (L)	[A]	3	17	17	90	30	11	1.6
Cycle time (day)	[B]	10	9	8	11	20	12	13
Productivity (cells day^{-1})	[C]	2.70E+08	5.29E+08	8.50E+08	3.27E+09	3.75E+08	5.04E+08	6.15E+08
Specific productivity (cell L^{-1} day^{-1})	[D]	9.00E+04	3.11E+04	5.00E+04	3.64E+04	1.25E+04	4.58E+04	3.85E+05
Cultivation time (day)	[E= $(3.6x10^{10})$÷C]	1.33E+02	6.81E+01	4.24E+01	1.10E+01	9.60E+01	7.14E+01	5.85E+01
Number of cycle (-)	[F= E÷B]	1.33E+01	7.56E+00	5.29E+00	1.00E+00	4.80E+00	5.95E+00	4.50E+00
Total volume of eater used (L)	[G= AxF]	4.00E+01	1.29E+02	9.00E+01	9.00E+01	1.44E+02	6.55E+01	7.20E+00
Cost of water, 0.06 THB L^{-1} (THB)	[H= 0.06xG]	2.40E+00	7.71E+00	5.40E+00	5.40E+00	8.64E+00	3.93E+00	4.32E-01
Cost of nutrient, 1 THB L^{-1} (THB)	[I= Gx1]	4.00E+01	1.29E+02	9.00E+01	9.00E+01	1.44E+02	6.55E+01	7.20E+00
Power of air compressor (W)	[J]	60	200	200	400	200	200	30
Power of light source (W)	[K]	72	72	72	144	150	150	110
Total electrical unit (units)	[L= (J+K)x(Ex24)÷1000]	4.22E+02	4.44E+02	2.76E+02	1.44E+02	8.06E+02	6.00E+02	1.97E+02
Electrical cost, 3 THB per unit (THB)	[M= 3xL]	1.27E+03	1.33E+03	8.29E+02	4.31E+02	2.42E+03	1.80E+03	5.90E+02
Total operation cost (THB)	[N=H+I+M]	1.31E+03	1.47E+03	9.25E+02	5.26E+02	2.57E+03	1.87E+03	5.97E+02

Remark

[A] = 30L ALPBR (Harker et al., 1996a)

[B] = 11L Double layered photobioreactor (Suh et al., 2006)

[C] = 1.6L Bubble column photobioreactor (Ranjbar et al., 2007)

CHAPTER V

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Major findings from this work can be summarized as follows:

1. The highest cell density of *H.pluvialis* was accomplished by culturing cells in 3L cylindrical airlift photobioreactor. The cell density of up to $90x10^4$ cell mL^{-1} was obtained with the specific growth rate of 0.45 d^{-1}.

2. *H. pluvialis* cultured in large scale 17L flat plant airlift photobioreactor (FP-ALPBR) was achieved with the maximum cell density of 41 $x10^4$ cell mL^{-1} and the specific growth rate of 0.52 d^{-1}.

3. The suitable riser sectional area and downcomer cross sectional area (A_d/A_r) for the cultivation of *H. pluvialis* in 17L FP-ALPBR was observed at 0.4. The aeration in the 17L FP-ALPBR was crucial for a proper growth of the alga. This had to be high enough to induce adequate circulation in the reactor but not too high as the associate shear might cause negative effects on the cell growth.

4. The semi-continuous culture was successfully implemented where the harvest could be periodically performed. For the 3L system, cell density increased up to the level obtained in the previous cycle within 7 days period, and for these two 17L systems, each cycle required only about 3-4 days. The productivity of semi-continuous culture in 3L, 17L cylindrical ALPBR and 17L FP-ALPBR were $9x10^7$, $7x10^7$ and $7.7x10^7$ cell L^{-1} d^{-1}, respectively.

5. *H. pluvialis* cultured in the largest scale ever previous reported (90L) FP-ALPBR could be achieved the maximum cell density of $40x10^4$ cell mL^{-1} and the specific growth rate of 0.39 d^{-1}.

6. The operating cost, approx. $5.26x10^2$ THB, was obtained from the 90L FP-ALPBR.

This was the most attractive figure as it was the lowest when compared to any photobioreactor systems for the cultivation of *H. pluvialis*.

5.2 Contributions

This work was a continuation from the previous work of Kaewpintong (2006) and Panitchakarn (2007) who were among the first that deeply investigated the cultivation of *Haematococcus pluvialis* in the airlift photobioreactors. In these works, the success of using the 3L ALPBR was reported which opened up the research question as to whether this system could be cultivated in a larger scale system. Up to the time of this work, there were very few attempts for the scale up of such system, only one statement in 1996 where the cultivation in the 30L ALPBR was reported (Harker et al., 1996a). Even though the growth in the large scale was rather slow, it was demonstrated that the design and scale up such large scale airlift system for the production of *H. pluvialis* was possible. One of the main findings from this work was that the cultivation of such microalga was performed in an evaporative room where the temperature was well controlled under 30 ^{o}C. This system was different from the work of Kaewpintong et al. (2006) and Panichakarn (2007) in which the temperature was controlled with the air conditioned room. The use of evaporative room was selected to save the energy during the cultivation, and to ensure that the cultivation was performed in the condition close to the actual one in industrial scale. It was evidenced in this work that, with the operating constraints employed in this work, the small airlift photobioreactors were more effective than the large airlift photobioreactors for the production of biomass in terms of specific growth rate. This is common for the design of reactors as small ones usually had better performance in terms of the liquid circulation and mass transfer than the larger scale systems. However, the large scale system could be still attractive as it was easier to operate particularly if ones wanted to mass-produce the cell culture product. Furthermore, the economical analysis indicated that the operation of the large scale 90L FP-ALPBR was most attractive as it was the cheapest choice for the cultivation of *H.pluvialis*. The following table summarizes the comparison between the maximum cell density and the specific growth rate obtained from the cultivation in the various types of photobioreactors.

Table 5.1 Summary the cultivation of *H.pluvialis* in photobioreactors

Reference	Type of photobioreactor	Maximum		
		cell density (cell mL^{-1})	Specific growth rate (d^{-1})	Productivity (cell mL^{-1} d^{-1})
This study	3L ALPBR	$90x10^4$	0.45	$0.87x10^5$
	17L ALPBR	$28x10^4$	0.38	$0.62x10^5$
	17L FP-ALPBR	$41x10^4$	0.52	$0.43x10^5$
	90L FP-ALPBR	$40x10^4$	0.39	$0.37x10^5$
Kaewpintong et al. (2006)	3L ALPBR	$77x10^4$	0.45	$0.51x10^5$
Panichakarn et al. (2007)	3L ALPBR	$41 x10^4$	0.47	$0.85x10^5$
	17L ALPBR	$21 x10^4$	0.40	$0.40x10^5$
Harker et al. (1996a)	30L ALPBR	$25 x10^4$	0.12	$0.12x10^5$
Suh et al. (2006)	11L Double layered- PBR	$55 x10^4$	0.30	$0.62x10^5$
Ranjbar et al. (2007)	1.6L Bubble column- PBR	$60 x10^5$	0.22	$4.80x10^5$

5.3 Recommendation

Due to a serious time constraint of the experiment work, several parameters could not be tested for their optimality in the cultivation of *H.pluvialis*. Therefore the following future works regarding the cultivation of the alga are recommended:

1. The light intensity should be investigated in more detail. For instance, this is to make sure that it was sufficient cultivation in the large scale.
2. The CO_2 consumption rate.
3. The nutrient limitation especially nitrogen and phosphorous should be investigated.

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APPENDIX

14th REGIONAL SYMPOSIUM CHEMICAL ENGINEERING 2007
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Yogyakarta, August 29, 2007

Dear Sir/Madam,
We are pleased to acknowledge the acceptance of the abstract

Title : A Novel Two Dimensional Airlift Reactor For Cultivation Of Vegetative Cells Of Microalga *Haematococcus pluvialis*.

Author : Kerati Issarapayup

to be presented orally at the upcoming 14th Regional Symposium on Chemical Engineering (RSCE2007) to be held at Melia Purosani Hotel, Yogyakarta from 4-5 December 2007.

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A NOVEL TWO DIMENSIONAL AIRLIFT REACTOR FOR CULTIVATION OF VEGETATIVE CELLS OF MICROALGA *HAEMATOCOCCUS PLUVIALIS*

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ABSTRACT

A comparison between the cultivation of *Haematococcus pluvialis* in airlift systems with different configuration was carried out. The 3L airlift clearly outperformed the other two. However, the upscale of 3L to 17L airlift with similar design resulted in a drastically drop in the growth performance where the specific growth rate and the productivity decreased from 0.179 d^{-1} and 5.25 cell mL^{-1} d^{-1} in the 3L to 0.268 d^{-1} and 4.75 cell mL^{-1} d^{-1} in the 17L. The 2-D design airlift was proposed as alternative cultivation system where the upscale was simplified just by increasing the length of the reactor. Without a thorough investigation on the optimal design and operating conditions, the airlift was capable of giving reasonable growth character. The performance of the system was, however, still quite not satisfactorily where the attainable specific growth rate was as low as 0.137 d^{-1}.Options were proposed to deal with this problem particularly on the selection of design parameters such as the ratio between downcomer and riser cross sectional areas and the operating conditions such as the aeration rate. In addition, the 2-D design was likely to have greater length than width and therefore the system might likely be subject to internal circulation, particularly in the riser section, and hence, a higher level of aeration than in the cylinder geometry might be required.

Keywords: photobioreactor, bioreactor, micro alga, bubble column

I. INTRODUCTION

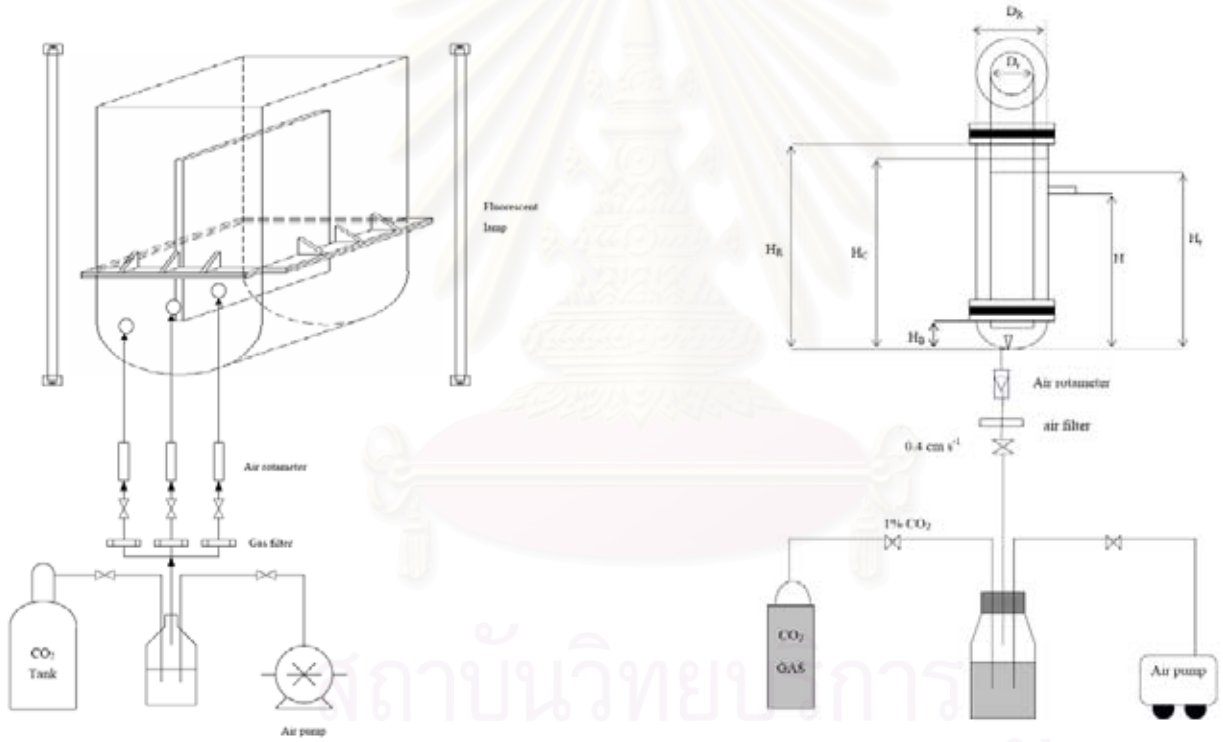
Haematococcus pluvialis has recently received increasing interest as one of the best producers of astaxanthin [1]. Astaxanthin is a red ketocarotenoid which attracts a great commercial interest due primarily to its versatile application and high production costs. Since astaxanthin holds superior antioxidant activity property than other carotenoids, it is used as potential stimulator for cancer prevention, immune response enhancement [1, 2]. The growth of *H.pluvialis* is rather slow and contamination problems are always difficult to eliminate [3]. Therefore most of the previous reports achieved successful cultivation in laboratory scale bioreactors where environmental conditions were well controlled. In addition, the upscale of the bioreactor often ended up with a much slower growth of the cell. The highest maximum cell density was reported by Hata et al., [4] who achieved the highest maximum cell density of 100 x 10^4 cells mL^{-1} in a 500 mL flask. Furthermore, the highest productivity (0.88 x 10^5 cells mL^{-1} d^{-1}) and the highest specific growth rate (0.62 d^{-1}) were obtained from Kobayashi et al., [5] who operated the cultivation in 200 mL under mixotrophic condition. In practice, the large-scale production must be carried out in a bioreactor under autotrophic condition. Recently, airlift bioreactor has been employed for the cultivation of *H. pluvialis*. Kaewpintong et al., [6] successfully achieved the cultivation of vegetative cells of *H. pluvialis*, but only in the small scale autotrophic 3 L airlift bioreactor with a maximum cell density of 77 x 10^4 cells mL^{-1} and with the maximum specific growth rate of 0.45 day^{-1}. The scaleup of such photo-bioreactor often encounters problems regarding the flow pattern and the light penetration ability and this usually lowered the growth rate of the cell. This work aims to deal with these problems by developing a 2-D airlift photo-bioreactor in which the depth of column is fixed. The upscale of such system can be achieved through the increase in the width of the reactor.

II. APPARATUS AND CALCULATION

All bioreactors employed in this wok were made of a clear acrylic plastic. Such reactors were composed of the draft tube or vertical plate installed centrally inside of column. The liquid culture in the airlift bioreactor was agitated by supplying air bubbles at the bottom of the airlift column. Air from an air compressor was metered through a flowmeter, sterilized with a 0.45 µm Gelman autoclave filter, and passed into the culture at the bottom of the reactor. The CO_2 enriched air was sterilized with a 0.45 µm Gelman autoclave filter, mixed with air, before entering the system at the bottom side of the reactor. The concentration of CO_2 used in this study was lower than 2% v/v, which was found not to affect the pH significantly as CO_2 was continuously taken up by the algae during the course of cultivation. The schematic diagram of experimental setup in airlift bioreactor is shown in Fig. 1. Light for the airlift bioreactor was supplied from the vertical 18 W fluorescent lamps. Two lamps were provided for adequate intensity, one on the right hand side and the other on the left hand side of the column. Each lamp was distanced around 1-5 cm from outer surface column depending on the required light intensity at the reactor surface. The illumination intensity incident to airlift bioreactor outer surface was measured with a digital LX-5 Lux meter, where Photon flux density and irradiance may be readily converted by:

$$1\ \mu mol\ (m^{-2}s^{-1}) = 1\ \mu E\ m^{-2}s^{-1} = \frac{119.73}{\lambda}\ W\ m^{-2} = 50\ Lux \qquad (1)$$

where λ = the wave length of the light in nanometer.



(a) 2-D airlift photobioreactor (b) Cylinder airlift photobioreactor

		Symbol	17 L Airlift bioreactor
Total volume	(L)		19
Working volume	(L)		17
Column height	(cm)	H_R	50
Column length	(cm)	L_R	20
Column depth	(cm)	D_R	20
Draft tube height	(cm)	H_r	30
Bottom clearance	(cm)	H_B	10

Geometric details	3 L Airlift photobioreactor	17 L Airlift photobioreactor
Total volume, V_T (L)	3.6	18.85
Working volume, V (L)	3	17
Reactor diameter, D_R (cm)	10	14
Column height, H_R (cm)	60	115
Liquid height, H_C (cm)	46	110.4
Draft tube height, H_D (cm)	50	96.5
Diameter of draft tube, D_t (cm)	4.4	7.2
Bottom clearance, H_B (cm)	4	10

Fig.1 Experimental setup for airlift photobioreactor and the geometric details of each reactor

Determination of cell density

Cell density was measured by microscope and the counting of cells were performed using an improved Neubauer haemacytometer and:

$$N = \frac{n_1 + n_2}{8} \times 10^3 \quad (2)$$

where N =Cell number (cell mL^{-1})
n_1 and n_2 = number of cells count in upper and lower grid (cells)

Determination of specific growth rate

The specific growth rate is calculated from:

$$\mu = \frac{\ln(N_2) - \ln(N_1)}{t_2 - t_1} \quad (3)$$

where μ = specific growth rate (μ;d^{-1})
N_1 and N_2 = cells density at t_1 and t_2 (cell mL^{-1})
t = time (day)

Determination of productivity

The productivity is calculated by the following equation:

$$\text{Productivity} = \frac{C_2 - C_1}{t_2 - t_1} \quad (4)$$

where C_1 and C_2 = cells density at t_1 and t_2 (cell mL^{-1})
t = time (day)

III. RESULTS AND DISCUSSION

Scale up is always the issue that challenges the applicability of the reactor design enterprise. Our experience in airlift design shows that the performance of the large scale airlift system does not necessarily become worse than the smaller ones provided that optimal design and operating conditions were provided. In this experiment, the cultivations of *H. pluvialis* in 3 and 17 L of airlift bioreactors were compared to illustrate the effect of scale up for such system. This was then compared with the performance of the 17L 2-D airlift photobioreactor and the discussion follows.

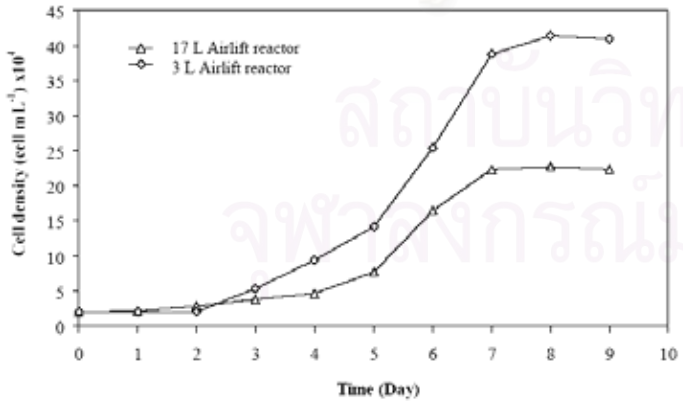


Fig.2 Cultivation of *H.pluvialis* in 3L at suitable conditions: u_{sg} = 0.4 cm s^{-1}, light intensity = 20 μmol photon m^{-2} s^{-1}, pH = 7: and 17L of airlift photobioreactor at suitable conditions: u_{sg} = 1 cm s^{-1}, light intensity = 30 μmol photon m^{-2} s^{-1}, pH = 7

The 3L airlift system was found to be best operated at superficial gas velocity of 0.4 cm s^{-1}, light intensity of 20 μmol photon m^{-2} s^{-1}, whereas the 17L was at superficial gas velocity = 1 cm s^{-1}, light intensity = 30 μmol photon m^{-2} s^{-1}. This was because the 17L had a greater thickness than the 3L and therefore the light intensity needed to penetrate through the reactor was stronger, and since the height was also greater, the higher aeration rate was necessary to generate liquid circulation. Note that both systems were found to run best at pH = 7. Fig. 2 shows the comparison between the performance of these two systems which illustrated that the cell density of *H. pluvialis* in 3 L airlift bioreactor ($41x10^4$ cell mL^{-1}) was higher than that in 17 L airlift bioreactor ($21x10^4$ cell mL^{-1}). There was a drastic drop in the airlift performance at these two conditions where the specific growth rate and the productivity decreased from 0.179 d^{-1} and 5.25 cell mL^{-1} d^{-1} in the 3L to 0.268 d^{-1} and 4.75 cell mL^{-1} d^{-1} in the 17L. Exact reasons

for this are not quite clear but this microorganism is particularly fragile to the pressure dragging upon the cell and therefore the 17L with a greater height could establish the cultivation conditions which less suited the algal requirement.

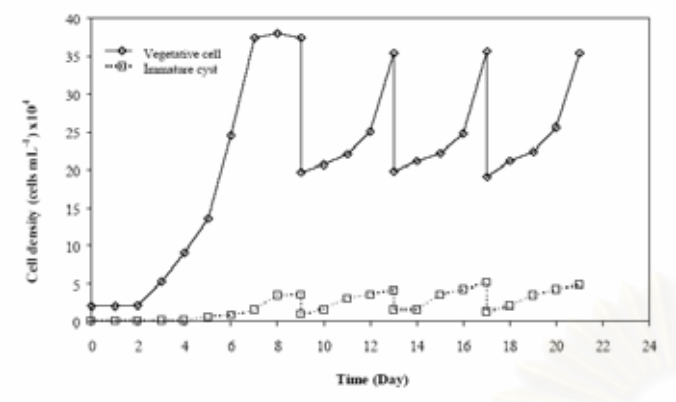


Fig.3 Cultivation of *H.pluvialis* in 3L airlift photobioreactor under semi-continuous culture

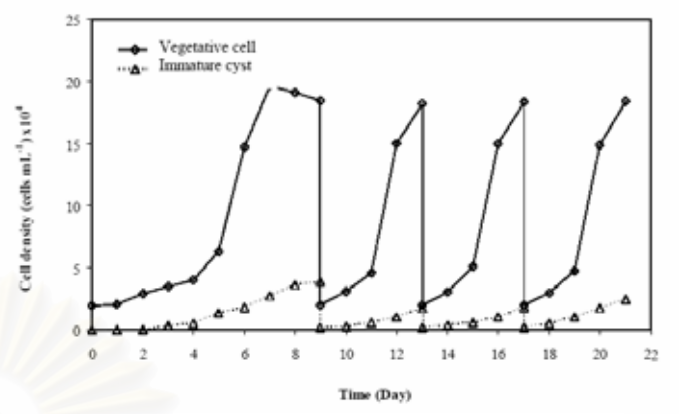


Fig.4 Cultivation of *H.pluvialis* in 17L airlift photobioreactor under semi-continuous culture

Semi-continuous cultivation was conducted in order to examine the potential of having a large-scale culture system that could operate economically. This cultivation of *H.pluvialis* in 3 and 17 L airlift photobioreactor were carried out under the most suitable conditions obtained from the aforementioned experiments, i.e. ratio between downcomer and riser cross sectional area (A_d/A_r) = 3.2, u_{sg} = 0.4 cm s^{-1}, light intensity = 20 μmol photon m^{-2} s^{-1}, pH = 7 (Fig. 3) and A_d/A_r = 2.78, u_{sg} = 1 cm s^{-1}, light intensity = 30 μmol photon m^{-2} s^{-1}, pH = 7 (Fig. 4), respectively. For the semi-continuous culture, the cultivation of 3 and 17 L airlift photobioreactor were started as a batch culture with the initial cell density of $2x10^4$ cells mL^{-1}. The cell was grown in the system until it reached the exponential growth phase after which the volume of culture broth was replaced with a fresh culture medium. Normally, cells were cultivated at every four days. The quantity taken out of the system was calculated to result in an approximately constant remaining cell density. For the 3L, this remaining cell density was controlled at around $20x10^4$ cell mL^{-1} (Fig. 3) whilst the 17L saw this remaining density at $2x10^4$ cell mL^{-1} (Fig. 4). Figs. 3 and 4 show clearly that the condition in the smaller system was more suitable for the growth of the alga, and the alga could reach a maximum cell density of $38x10^4$ cell mL^{-1} which was much greater than the larger system that could only reach around $18x10^4$ cell mL^{-1}. Both systems also contain cells in immature cyst form which surprisingly were at the same level of around $3x10^4$ cell mL^{-1}. No cyst with the containment of astaxanthin was found in both systems. The specific growth rate and productivity in these semi-continuous cultures were 0.179 d^{-1} and $5.25x10^4$ cell $mL^{-1}d^{-1}$ and 0.268 d^{-1} and $4.75x10^4$ cell $mL^{-1}d^{-1}$ in the 3 L and 17 L airlift photobioreactors, respectively.

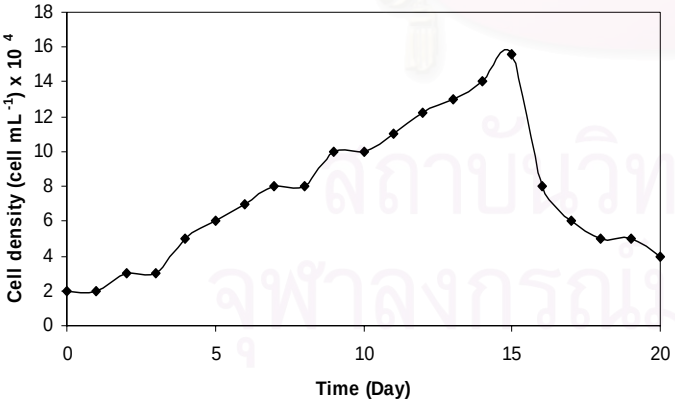


Fig.5 Cultivation of *H.pluvialis* in a 2-D (17L) airlift photobioreactor at suitable conditions: u_{sg} = 0.4 cm s^{-1}, light intensity = 20 μmol photon m^{-2} s^{-1}, pH = 7

The cultivations of *H. pluvialis* in a novel 2 dimensional (17 L) airlift bioreactor was then carried out using the conditions of the cylindrical geometry, i.e. superficial gas velocity = 0.4 cm s^{-1}, light intensity = 20 μmol photon m^{-2} s^{-1} ,A_d/A_r = 3.0 (results in Fig. 5). From this experiment, the maximum cell density obtained in this novel design was 15.5 $x10^4$ cell mL^{-1}. This was, although less than those obtained from both 3L and 17L airlift with cylindrical geometry, positive as this was only the preliminary result where investigation on the optimal conditions for such system was yet to be accomplished. Virtual examination of such system suggested that one of the problems in the 2-D system was on the distribution of the aeration in the riser which was designed to extend in one direction. Therefore the location of the gas sparger could also play a significant role in controlling the system performance otherwise the flow of liquid could be subject to internal circulation, particularly in the riser section [7] which would then suppressed cell circulation.

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BIOGRAPHY

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