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SCALEUP OF AN AIRLIFT BIOREACTOR FOR CULTIVATION OF DIATOM, *Chaetoceros calcitrans*

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ถังปฏิกรณ์ชีวภาพแบบอากาศยกขนาด 17 ลิตร ชนิดมีการให้แสงซึ่งมีความเหมาะสมต่อการเจริญเติบโตของ คีโตเซอรอส คาลซิแทรนส์ ได้แก่ถังปฏิกรณ์ที่มีค่าอัตราส่วนระหว่างพื้นที่หน้าตัดการไหลขึ้นต่อพื้นที่หน้าตัดการไหลลงของของไหล (A_d:A_r) เป็น 2.62 อัตราการเจริญเติบโตจำเพาะสูงสุดและความหนาแน่นสูงสุดของเซลล์มีค่าเป็น 0.0741 ต่อชั่วโมงและ 8.88 ล้านเซลล์ต่อมิลลิลิตร ตามลำดับสำหรับการเพาะเลี้ยงที่อัตราการให้อากาศเป็น 3 เซนติเมตรต่อวินาที จากการศึกษาพบว่าอัตราการถ่ายเทมวลระหว่างวัฏภาคก๊าซและของเหลวไม่มีผลต่อการเจริญเติบโตของไดอะตอมภายในระบบ แต่ปัจจัยสำคัญซึ่งเป็นตัวควบคุมการเจริญเติบโตของไดอะตอมในระบบได้แก่ความเข้มแสงและการลดลงของสารอาหาร จากการศึกษาพบว่าการเพาะเลี้ยงไดอะตอมในระบบแบบกะ (batch) ที่มีความเข้มข้นของสารอาหารเป็น 2 เท่าจากปกติจะให้ค่าความหนาแน่นสูงสุดของเซลล์เพิ่มขึ้นเมื่อเปรียบเทียบกับการเพาะเลี้ยงในระบบปกติ นอกจากนี้การทดลองเพาะเลี้ยงไดอะตอมในน้ำทะเลที่ผ่านการเพาะเลี้ยงเซลล์มาแล้วโดยทำการแยกเซลล์ออกพร้อมกับเติมสารอาหารใหม่จะได้ค่าความหนาแน่นสูงสุดของเซลล์ไม่ต่างจากการเพาะเลี้ยงไดอะตอมในน้ำทะเลใหม่ การบดบังแสงอันเนื่องมาจากความหนาแน่นของฟองอากาศภายในระบบก็เป็นอีกปัจจัยหนึ่งที่มีผลต่อการเจริญเติบโตของไดอะตอมเช่นเดียวกัน การเพาะเลี้ยงไดอะตอมในถังปฏิกรณ์ชีวภาพแบบอากาศยกที่มีการขยายขนาดของยอดหอสามารถลดปัญหาการการบดบังแสงได้ การแยกตัวของฟองอากาศจากระบบบริเวณส่วนยอดหอทำให้ความสามารถในการส่องผ่านของแสงเข้าสู่ระบบมีค่าสูงขึ้น สำหรับกรณีการเพาะเลี้ยงไดอะตอมในถังปฏิกรณ์ชีวภาพที่มีการขยายขนาดของยอดหอโดยมีความเร็วก๊าซเป็น 5 เซนติเมตรต่อวินาทีจะให้ค่าความหนาแน่นสูงสุดของเซลล์ลดลงเล็กน้อยเมื่อเทียบกับการเพาะเลี้ยงในระบบดังกล่าวด้วยความเร็วก๊าซอื่นๆอันเป็นผลมาจากการลดลงของความเข้มแสงจากการบดบังของฟองอากาศ นอกจากนี้การบดบังแสงโดยตัวไดอะตอมเองก็มีผลต่อการเจริญเติบโตของไดอะตอมเช่นเดียวกันซึ่งสามารถแก้ไขได้โดยการเพาะเลี้ยงไดอะตอมในระบบถังปฏิกรณ์ชีวภาพที่ต่อแบบอนุกรมซึ่งจะช่วยปรับปรุงประสิทธิภาพการส่องผ่านของแสงในระบบ การเจริญเติบโตของไดอะตอมในระบบดังกล่าวดีขึ้นกว่าระบบแบบกะ 24.8 เปอร์เซ็นต์ นอกจากนี้แล้วการเพาะเลี้ยงไดอะตอมในระบบแบบกึ่งต่อเนื่องและระบบแบบต่อเนื่องยังสามารถเพิ่มอัตราการผลิตไดอะตอมชนิดนี้ได้อีกด้วย จากการวิเคราะห์พบว่าการเพาะเลี้ยงไดอะตอมในระบบแบบต่อเนื่องที่มีอัตราการป้อนของสารอาหารเป็น 3 มิลลิลิตรต่อนาทีจะเป็นระบบที่มีความเหมาะสมทางเศรษฐศาสตร์มากที่สุด นอกจากนี้การเพิ่มกำลังการผลิตไดอะตอมยังสามารถทำได้ในระบบขนาดใหญ่ได้แก่ ถังปฏิกรณ์ให้อากาศแบบธรรมดา (Bubble column), ถังชีวปฏิกรณ์แบบอากาศยก (airlift bioreactor) และ ถังชีวปฏิกรณ์แบบอากาศยกที่มีท่อในหลายท่อ (Multiple draft tubes) ซึ่งไม่มีผลต่อการเจริญเติบโตของไดอะตอมมากเท่าใดนัก

ภาควิชา วิศวกรรมเคมี ลายมือชื่อนิสิต.....

สาขาวิชา วิศวกรรมเคมี ลายมือชื่ออาจารย์ที่ปรึกษา.....

ปีการศึกษา 2548 ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

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KEY WORD: AIRLIFT PHOTOBIOREACTOR / *CHAETOCEROS CALCITRANS* / SCALE UP

SONTAYA KRICHNAVARUK: SCALEUP OF AN AIRLIFT BIOREACTOR FOR CULTIVATION OF DIATOM, *Chaetoceros calcitrans*. THESIS ADVISOR: ASSOCIATE PROFESSOR PRASERT PAVASANT, Ph.D., THESIS CO-ADVISOR: SORAWIT POWTONGSOOK, Ph.D., 119 PP. ISBN 974-53-2894-4

The optimal system configuration for the growth of *Chaetoceros calcitrans* in the 17 L airlift photobioreactor (ALPBR) was the system with the ratio between downcomer to riser cross sectional areas (A_d:A_r) of 2.62. The maximum specific growth rate and the maximum cell concentration were 7.41×10^{-2} h^{-1} and 8.88×10^{6} cells mL^{-1} respectively for the cultivation with gas velocity (u_{sg}) of 3 cm s^{-1}. The growth of the diatom in the system did not depend on the level of gas-liquid mass transfer, but rather, it was controlled primarily by the light intensity. A few factors were found to have great influence on the growth characteristics. Firstly, the growth of the diatom was limited by the nutrient depletion. The cultivation of the diatom with two fold nutrient concentration increased the final cell concentration, and also the growth of the diatom in the refreshed spent medium resulted in almost the same growth rate and maximum cell concentration as obtained from the batch cultivation. However, the increase in the nutrient concentration could not enhance the growth rate of the diatom. Bubbles shading and self shading effects also affected the growth of the diatom by obstructing the light penetration into the system. The expanded top ALPBR was proposed to deal with the bubble shading effect as this system allowed an effective removal of small bubbles at the liquid surface. It was shown that no bubble shading effect could be seen in this expanded top ALPBR, but only a slight decrease in maximum cell concentration was observed at u_{sg} = 5 cm s^{-1} as this rather high u_{sg} could see an escape of small bubbles into the downcomer and decreasing the light intensity. Self shading effect could be dealt with using the ALPBRs-in-series setup when two or three culturing columns were connected in series. This setup allowed a better light penetration in the first column and confined the region of low light intensity to only the last column. The experiment demonstrated that the growth rate could be enhanced by 24.8% when compared with the conventional ALPBR. The diatom could be cultivated in both semi-continuous and continuous modes to ensure higher specific productivity. The analysis revealed that the continuous mode with medium feed rate of 3 mL min^{-1} was the most economical choice for the growth of this diatom. The diatom was also cultivated as a mass production in the large scale system. The maximum cell concentration and specific growth rate in the large scale bubble column, concentric ALPBR and multiple draft tubes ALPBR were not significantly different which implied that the effect of airlift inside such large system was only marginal.

Department	Chemical Engineering	Student's signature	
Field of study	Chemical Engineering	Advisor's signature	
Academic year	2005	Co–advisor's signature	

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CHAPTER 1

Introduction

1.1 General Ideas

Plankton can be classified into two major types; plankton plants are called phytoplankton and plankton animals called zooplankton. Both phytoplankton and zoo plankton play significant roles in human daily life as they are widely used as nutrition food or health food such as spirulina which contains nine necessary amino acids and rich in natural protein. Some plankton are abundant in valuable chemical substances, coloring agents and also pharmaceutical products, i.e. carotenoid, vitamins, antioxidant and essential fatty acids (Healthnotes, Inc., 2004). Phytoplankton is particularly important in ocean ecosystems as the single cell organism with chlorophyll content for photosynthesis which yields oxygen which is necessary for animal and also serves as the beginning of food chain. One of the commonly known phytoplankton is the diatom, the microorganism surrounded with box-like shell with silica content. Diatoms serve as the producer in aquatic animals' food chain (Miyamoto *et al*., 1988; Borowitzka, 1997; and Lee, 2001), and as a result, they are considered important natural food sources in commercial aquacultural industries or hatcheries, i.e. larvae of mollusk, fish, prawn, and also for larger microorganisms, such as rotifers (Belay *et al*., 1997; and Borowitzka 1997). Diatoms are also considered an alternative natural resource for Poly Unsaturated Fatty Acids (PUFAs) which is necessary for the metabolism reproduction of aqua organisms, this included immune function and intracellular transport (Chiou *et al*., 2001; University of Paisley, 2004).

The rapid decline in natural fish stocks led to an increase in aquaculture industries (Borowitzka, 1997). The growth of aquaculture industries can be strongly illustrated by shrimp industries which began in early 1970s and continued to grow until 1990s (Liang *et al*., 1997 and Smith, 1998). Among the various types of common microorganisms, the diatom *Chaetoceros calcitrans* is one of the most popular strains used in feeding shrimp larvae. *Chaetoceros calcitrans* is a diatom with

chlorophyll content and usually cultivated using similar techniques as single cell algae. The cultivation of *Chaetoceros calcitrans* in Thailand is generally performed as a two step, batch process. Firstly, the diatom is cultivated as dense cell culture in a one-liter-glass bottle for 2–3 days. This is followed by a medium size tank of a low culture density for one day. This is often done by adding up approx. 500 liters of fresh sea water (30 ppt) together with necessary nutrients for the growth of the diatom into a 500L tank. After this stage, the diatom is then moved to larger ponds, 2–3 tons in volume, and left growing for one day before it is ready to be used in feeding the shrimp larvae. However, the drawbacks of this method can be classified into two main groups. Firstly, the diatom is cultivated in an open pond, where all kinds of contamination are welcome. These contaminants may be harmful for the aquatic cultures as it causes many subsequent destructive diseases. Secondly, the old fashioned cultivation yields a low specific growth rate which provides enough time for the blooming of the unwanted species in the systems. Also the climate conditions directly influence the growth of the diatom. The design of closed system bioreactors is therefore treated as a solution to these problems.

Many investigations on the development of bioreactor had been carried out for the production of high cell density culture. Several new designs and configurations were introduced which led to a successful production of such high density culture. Examples of the novel designed photo-bioreactors include a tubular reactor (Lee and Low, 1991 and Grima *et al*., 1996) and the flat plate bioreactor (Richmond and Zou, 1999). However, these existing closed systems often suffered serious drawbacks from poor mixing and low gas-liquid mass transfer as the design only aimed at effective light utilization.

Airlift bioreactor (ALBR) is one of the alternatives for the cell cultivation (Merchuk *et al*., 1996) especially in bioprocesses (Blenke, 1979; and Orazem *et al.*, 1979). The reactor is separated into two main sections, riser and downcomer. The fluid flow pattern in both sections is induced by the density difference of the fluid. Many advantages had been reported such as good mixing, well-defined fluid flow pattern, relatively high gas-liquid mass transfer rate, and low capitals and operating costs. The mixing in the ALBR could be obtained without

causing excessive shear force in the liquid phase which, otherwise, could inhibit the growth of the microorganism. In addition, Merchuk *et al*. (1998) mentioned that the well defined circulation pattern resulted in a better light exposure which induced the growth of the diatom.

This work aims to determine the optimal design configurations for the growth of *Chaetoceros calcitrans* which yields high density cell concentration and finally proposes the continuous production system for high cell density diatom.

1.2 Objectives

The objectives of this work are to:

1. Determine the optimal growth conditions and effects of design configurations and operating conditions for the cultivation of *Chaetoceros calcitrans* in airlift bioreactor.
2. Design the semi-continuous, continuous airlift bioreactor and airlift bioreactor in series systems for the cultivation of *Chaetoceros calcitrans*.
3. Scale-up of airlift bioreactor for the cultivation of *Chaetoceros calcitrans*
4. Determine factors that limit the growth of the diatom.

1.3 Working Scopes

1.3.1 Equipment limitation

1. In 17 L airlift bioreactor, the Lux meter can be located only in the reactor with the A_d:A_r smaller than 2.62 due to the geometrical design of the reactor.
2. In 170 L airlift bioreactor, the superficial gas velocity is limited by the air compressor with the volumetric flow rate of 250 mL s^{-1}.

1.3.2 Type of bioreactors

Bioreactors employed in this work include:

- 2.8 L concentric airlift photobioreactor
- 2.8 L concentric airlift photobioreactors in series
- 17 L bubble column

- 17 L concentric airlit photobioreactor
- 23 L expanded top airlift photobioreactor
- 170 L bubble column
- 170 L concentric airlift photobioreactor
- 170 L multiple draft tubes airlift photobioreactor

CHAPTER 2

Backgrounds and Literature Review

2.1 Airlift Bioreactor

2.1.1 Classification

Airlift bioreactors can be classified into two major types; the internal loop and external loop, as shown in Figure 2.1. The internal loop airlift bioreactor is the cylindrical column with a concentric cylindrical tube that separates the column into four sections; riser, downcomer, gas separator, and bottom section (Figure 2.1a).

In external loop airlift bioreactors, 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.

2.1.2 Transport mechanism in ALCs

An airlift bioreactor can be divided into four main parts, riser, downcomer, gas separator and bottom section as shown in Figure 2.1. A concentric airlift bioreactor (Figure 2.1 a) is used to describe the function of each of the section.

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, similar to that in CSTRs. In this section, a large portion of gas disperses out at the liquid surface and creates heavier fluid.
3. Downcomer is the section that the heavier fluid from the gas separator section and some remaining gas bubbles flow downwards.
4. The final bottom section is provided to allow the liquid to re-enter the riser again 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 *Chaetoceros calcitrans*

Chaetoceros sp. is one of the well known diatoms as it serves as nutritious food for marine hatcheries. It is widely found in warm and cold waters with 17-25 ppt salinity, temperature around 20–30^{o}C and light intensity of 500–10,000 lux. Several species of *Chaetoceros* sp. can be identified, i.e. *Chaetoceros gracilis, Chaetoceros debilis* and *Chaetoceros calcitrans*. However, the most popular strain is *Chaetoceros calcitrans* which is found in small unicellular rectangular diatom, 4–10 µm in size, with two long pairs of setae at both sides. The cell is found rich in protein (27%), nucleic acid (10%) and lipid (11%) (Zhukova and Aizdaicher, 1995).

2.3 Culture Systems for Diatom and Single Cell Algae

The cultivation of microalgae can be performed in various types of ponds and bioreactors. Many factors should be considered in selecting the suitable technology, i.e. the nature of the cells, the cost of land, water supply, energy, labor, climate conditions and finally the specification of the finished products (Borowitzka, 1999). Various scale culture systems need to be compared on their basic properties *e.g.* light utilization efficiency, temperature control, hydrodynamic properties, stress placed on algal cell and the ability to be free from other contaminants (Borowitzka, 1999). Many investigations were carried out for the cultivation of microalgae in large scale systems. The large scale cultivation can be made both in opened and closed systems, however, a common problem encountered in the large scale system is the ease of contamination from other unwanted species, control difficulties, and relatively lower reliability than the lab scale.

2.3.1 Opened 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 (Figure 2.2). Often, the system consists of mechanical equipment 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.

The circular shallow ponds with a rotating scraper are widely used in Japan, Taiwan and Indonesia. The size of the pond varies from as small as 1 m^3 to as large as 50 meters in diameter (Lee, 1997). The raceway-shape ponds with agitated paddle wheel were used in Israel, the United States of America, China, Thailand and in many other countries. Example of the open ponds also include the work of Setlik *et al.* (1970) who developed open cascade system in Czech Republic with only 1 cm in depth for the cultivation of *Chlorella*, and achieved 10 g L^{-1} cell density and the productivity of 25 g m^{-2} d^{-1}. Fertilizer was used as culture medium for the cultivation of *Dunaliella* and yields 0.5 g L^{-1} cell concentration with about 25 g m^{-2} d^{-1} productivity (Richmond *et al.*, 1990). The Aquaculture Department of the Southeast Asian Fisheries Development Center cultivated *Chaetoceros calcitrans* outdoor in 1 ton fiber glass tank. The productivity obtained from the cultivation was reported to be 2.65×10^6 cells mL^{-1} after 4 days (Samonte *et al.*, 1993). Pushparaj *et al.* (1994) cultivated *Nodularia harveyana* in a 8 cm deep open pond with a paddle wheel where the maximum productivity was reported at 12.0 g dry weight $m^{-2}d^{-1}$.

Algal productivity in these culture systems are insufficient, leading to low productivities and less reliable culture. The drawbacks of the systems are the ease of contamination and difficulties in control, especially temperature control as they are highly fluctuates during day and night (Richmond *et al.*, 1993). Many species, such as *Chlorella*, *Spirulina* and *Dunaliella*, were successfully cultivated in opened systems as these species could grow in an extreme culturing-environment that allow them to grow freely from any other contaminants or protozoa (Borowitzka, 1999). *Chlorella* grows well in rich media, *Dunaliella* in high salinity system, whilst *Spirulina* requires high pH and bicarbonate concentration (Borowitzka, 1999; Borowitzka, 1988; Soong, 1980 and Belay, 1997). Furthermore, inadequate light utilization could limit the growth of the microalgae due to self shading effect. It was found that the depth of the cultivation pond directly affected the photo synthesis ability of algae. Hence, careful management is strongly required which leads to the increase in labor costs (O' Meley and Daintith, 1993; Fulks and Main, 1991; De Pauw and Persoone, 1988 and Fox,

1983). Examples of this system for the cultivation of photo-plankton are detailed in Table 2.1.

2.3.2 Closed system

Advanced methods have been introduced for a large scale culture of microalgae, not only in opened systems, but also in closed systems (Borowitzka, 1996; Chaumont, 1993 and Tredici and Materassi, 1992). The aims of these systems are to increase the volumetric yield and biomass quality under the controlled conditions by proficiency experiences. 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 (mentioned in Section 2.3.1). 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). Examples of algal species that have been cultivated in such systems are *Skeletonema*, *Chaetoceros*, *Thalassiosira*, *Tetraselmis*, and *Isochrysis* (Borowitzka, 1999).

In recent years there have been several advances in the design and operation of closed photo bioreactors for algal culture and several systems will be reliable commercial realities in the near future (Borowitzka, 1999). Merchuk *et al.* (1998) cultivated *Porphyridium* sp. in a bubble column bioreactor and reported that the maximum cell number of 22×10^6 cells L^{-1} d^{-1}(detailed in Table 2.2). Grima *et al.* (1996) cultivated *Phaeodactylum tricornutum* in a 3.0 cm diameter tubular reactor and yielded 2.7 g L^{-1} d^{-1} productivity. Pushparaj *et al.* (1994) cultivated *Nodularia harveyana* in both tubular bioreactor and paddle wheel open pond. The results revealed that the higher productivity obtained in tubular bioreactor was 14.0 g dry weight m^{-2} d^{-1} compared with paddle wheel open pond. Lee and Low (1991) cultivated *Chlorella pyrenonoidosa* in a 1.2 cm diameter tubular bioreactor and yielded 3.64 g L^{-1} d^{-1} biomass productivity. The newly introduced systems are often capable of increasing biomass productivity, however, technical experts are required as

the system is more complicated. Additional energy is often needed for cooling, lighting and air supply.

Two major types of closed bioreactor were proposed, tubular and flat plate bioreactors (Figures 2.3 and 2.4) (Campo *et al*., 2001; Lee, 2001; Torzillo, 1997; Tredici and Zitelli, 1997; Borowitzka, 1996; Hu *et al.*, 1996; Pulz, 1994; Richmond, 1993 and Tredici *et al*., 1991). One of the primary advantages of the systems is that the light input per unit volume is enhanced to minimize the shading due to the over-grown high density culture. The tubular bioreactors are designed to be small tubes to provide a large ratio of surface area to culture volume for the light absorption efficiency. However, mixing problem is commonly encountered as the main drawback of the system. This is particularly important for the growth of filamentous microorganisms, where a highly viscous suspension can lead to a poor mixing even at low biomass concentration (Torzillo *et al.*, 1993).

Several designs of closed tubular photo bioreactors have recently been proposed (details in Table 2.3) all of which were reported to yield high algal productivity. For instance, the alpha-shaped photobioreactor with cross tubes was proposed by Lee *et al.*, in 1995 for the cultivation of *Chlorella pyrenoidosa* which yielded 10 g dry weight L^{-1} of productivity (72 g dry weight m^{-2} land d^{-1}). The other type of the tubular bioreactor is the helical bioreactor (Figure 2.5). Borowitzka *et al.* (1999) introduced a biocoil system, 700 L helical tubular photobioreactor, for the cultivation of *Tetraselmis chuii* and *Isochrysis galbana*. The productivities were reported as 1.2 g L^{-1} d^{-1} and more than 1.0 g L^{-1} d^{-1} for the two strains, respectively. Hall (2003) introduced an outdoor helical reactor and found an increasing productivity with superficial gas and liquid velocities. The maximum growth rates and maximum biomass productivities were reported at 0.068 h^{-1} and 1.4 g L^{-1} d^{-1}, respectively. The applications of helical tubular bioreactor are listed in Table 2.4.

The plate bioreactor has also been one of the most employed closed systems. This system is usually inclined to induce the movement of liquid due to the gravitation. In some cases, the bioreactors were located vertically (Lee, 2001; Hu *et al.*, 1996; Pulz, 1994 and Tredici *et al.*, 1991). It was found that the high turbulence flow was achieved in the flat plate bioreactor which allowed better light exposure, and

also the mass transfer of gas and nutrient in the systems (Lee, 2001; Grobbelaar *et al.*, 1996; Hu and Richmond, 1996 and Grobbelaar, 1994). For instance, Richmond and Zou (1999) obtained the culturing of *Nannochloropsis* sp. in flat plates with narrow light path (10 cm) with the volumetric biomass productivity of 1.4 g L^{-1} d^{-1}. Hu *et al.*, (1996) introduced a flat plate bioreactor with 1.3 cm light path for the cultivation of *Spirulina platensis* and yielded 4.30 g L^{-1} d^{-1} productivity. Table 2.5 provides more information regarding the usages of these flat-plate photobioreactors.

In conclusions, there are pros and cons in using the closed photobioreactors when compared to the open pond systems. The major advantages and disadvantages in using both types of culture systems are summarized in Table 2.6.

2.4 Cultivation in Airlift Bioreactor

Few attempts have been carried out for the design of microalgae in airlift bioreactors. Merchuk *et al.* (1998) investigated the productivity of the red microalgae *Porphyridium* sp. for the cultivation in the airlift bioreactor (ALBR) when compared with the conventional bubble column. It was found that a relatively higher cell number of the red microalgae *Porphyridium* sp. in the ALBR could be obtained than in the bubble column.

Recently, airlift bioreactors have been employed for the cultivation of *Chaetoceros calcitrans* at the Biochemical Engineering Laboratory, Department of Chemical Engineering, Faculty of Enginering, Chulalongkorn University. Brief detail on this is provided here below.

Loataweesup (2002) cultivated the diatom *Chaetoceros calcitrans* in a small glass bubble column and airlift bioreactor to investigate the optimal conditions for the growth of the diatom. The optimal light intensity which yielded the maximum cell concentration was reported to be 400 μ molphoton $m^{-2}s^{-1}$. It was reported that the suitable nutrient concentration was a two fold of silica and phosphorus concentrations in a modified standard F/2 (Guillard's) medium, which resulted in a better specific growth rate of the diatom, whilst vitamin B_{12} did not significantly affect the growth of

the diatom. The maximum cell concentration for the cultivation in a 2.5 L glass bubble was reported to be 5.8×10^{6} cells mL^{-1} with a maximum specific growth rate of 3.80×10^{-2} h^{-1}.

The investigation also discovered that the cultivation of *Chaetoceros calcitrans* in the 17 L airlift bioreactor yielded higher specific growth rate and cell concentration when compared with the bubble column of the same size. The maximum cell concentration in a batch culture was obtained at the superficial gas velocity of 3 cm s^{-1}, which achieved the highest cell concentration of 8.88×10^{6} cells mL^{-1} (specific growth rate = 7.41×10^{-2} h^{-1}). It was concluded that the well defined flow pattern in airlift bioreactor provided an effective light utilization for the diatom particularly at high cell concentration as the cells were regularly circulated to the reactor outer surface where light was supplied. This circulation was not found in other bioreactors such as bubble column where a liquid circulation was poor. Examples of airlift reactor systems for the production of other types of microorganism are detailed in Table 2.7.

Table 2.1 Examples of open pond system for the cultivation of photo-plankton.

Type	Species of microorganism	Productivity	Reference
Raceway ponds	*Spirulina platensis*	14.5 g $m^{-2}d^{-1}$	Pushparaj *et al*., 1997
Open raceway ponds	*Anabaena siamensis*	0.086 g $L^{-1}d^{-1}$	Richmond *et al*., 1993
Fiber glass tanks	*Chaetoceros calcitrans*	2.65×10^{6} cells mL^{-1}	Samonte *et al*., 1993
Paddle-wheel raceway ponds	*Spirulina platensis*	20.8 g $m^{-2}d^{-1}$	Vonshak and Guy, 1992

Table 2.2 Examples of column system for the cultivation of photo-plankton.

Type	Species of microorganism	Productivity	Reference
Curved chamber	*Spirulina platensis*	1.64 g $L^{-1}d^{-1}$	Tredici and Zittelli, 1998
Bubble column	*Porphyridium sp.*	22×10^{6} cells $L^{-1}d^{-1}$	Merchuk *et al*., 1998

Table 2.3 Examples of tubular reactor system for the cultivation of photo-plankton.

Type	Species of microorganism	Productivity	Reference
Outdoor tubular	*Muriellopsis sp.*	40 g $m^{-2}d^{-1}$	Del Campo *et al*., 2001
Horizontal tubular	*Spirulina platensis*	1.26 g $L^{-1}d^{-1}$	Tredici and Zittelli, 1998
Coiled tubular	*Spirulina platensis*	0.9 g $L^{-1}d^{-1}$	Tredici and Zittelli, 1998
Tubular	*Chlorococcum sp.*	13.5 g $L^{-1}d^{-1}$	Zhang *et al*., 1997
Tubular	*Phaeodactylum tricornutum*	2.76 g $L^{-1}d^{-1}$	Grima *et al*., 1996
Tubular	*Spirulina sp.*	27.8 g $m^{-2}d^{-1}$	Torzillo *et al*., 1996
Tubular	*Spirulina platensis*	27.9 g $m^{-2}d^{-1}$	Torzillo *et al*., 1996
Tubular	*Chlorella sorokiniana*	127 g $m^{-2}d^{-1}$	Lee *et al*., 1996
α-type tubular	*Chlorella pyrenoidosa*	72.5 g $m^{-2}d^{-1}$	Lee *et al*., 1995
Tubular	*Nodularia harveyana*	14 g $m^{-2}d^{-1}$	Pushparaj *et al*., 1994
Tubular	*Isochrysis galbana*	0.32 g $L^{-1}d^{-1}$	Grima *et al*., 1994
Tubular	*Spirulina platensis*	27.0 g $m^{-2}d^{-1}$	Richmond *et al*., 1993
Tubular	*Anabaena siamensis*	0.55 g $L^{-1}d^{-1}$	Richmond *et al*., 1993
Tubular loop	*Chlorella pyrenoidosa*	130 g $m^{-2}d^{-1}$	Lee and Low, 1991

Table 2.4 Examples of helical reactor system for the cultivation of photo-plankton.

Type	Species of microorganism	Productivity	Reference
Helical reactor	*Phaeodactylum tricornutum*	1.4 g $L^{-1}d^{-1}$	Hall et al., 2003
Helical tubular	*Monodus subterraneus*	1.7 g $L^{-1}d^{-1}$	Lu *et al*., 2001
Conical helical tubular	*Chlorella sp.*	31 g $m^{-2}d^{-1}$	Morita et al., 2001
Conical helical	*Chlorella sp.*	28.3 g $m^{-2}d^{-1}$	Morita *et al*., 2000
Helical tubular	*Spirulina platensis*	0.51 g $m^{-2}d^{-1}$	Watanabe and Hall, 1996
Helical tubular	*Phaeodactylum tricornutum*	0.133 g $L^{-1}d^{-1}$	Chrismadha and Borowitzka, 1994

Table 2.5 Examples of flat plate reactor system for the cultivation of photo-plankton.

Type	Species of microorganism	Productivity	Reference
Flat plate vertical glass reactor (3 cm light path length)	*Chaetoceros muelleri*	0.10 g $L^{-1}d^{-1}$	Göksan et al., 2001
Flat plate vertical glass reactor (1 cm light path length)	*Chaetoceros muelleri*	0.22 g $L^{-1}d^{-1}$	Göksan et al., 2001

Table 2.5 Examples of flat plate reactor system for the cultivation of photo-plankton. [Cont.]

Type	Species of microorganism	Productivity	Reference
Vertical Flat plate glass	*Nannochloropsis sp.*	12.1 g $m^{-2}d^{-1}$	Richmond and Cheng-Wu, 2001
Modular flat panel	*Nannochloropsis sp.*	1.45 g $L^{-1}d^{-1}$	Zittelli *et al*., 1999
Flat plate glass	*Nannochloropsis sp.*	22.4 g $m^{-2}d^{-1}$	Richmond and Zou, 1999
Flat plate inclined	*Spirulina platensis*	9 g $L^{-1}d^{-1}$	Qiang *et al*., 1996
Flat plate inclined	*Anabaena siamensis*	1.43 g $L^{-1}d^{-1}$	Qiang *et al*., 1996 (a)
Flat plate inclined	*Spirulina platensis*	51 g $m^{-2}d^{-1}$	Qiang *et al*., 1996 (b)
Horizontal flat panel	*Spirulina platensis*	1.09 g $L^{-1}d^{-1}$	Tredici and Zittelli, 1998
Flat chamber	*Spirulina platensis*	1.93 g $L^{-1}d^{-1}$	Tredici and Zittelli, 1998
Sun-oriented panel	*Spirulina platensis*	24 g $m^{-2}d^{-1}$	Tredici and Materassi, 1992
Vertical Alveolar Panel (VAP)	*Anabaena azollae*	11 g $m^{-2}d^{-1}$	Tredici *et al*., 1991

Table 2.6 Comparison between opened and closed systems.

Opened 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 2.7 Examples of airlift system system for the cultivation of photo-plankton.

Type	Species of microorganism	Productivity	Reference
Airlift	*Synechococcus sp.*	1.4 g $L^{-1}d^{-1}$	Suh and Lee, 2001
Airlift	*Porphyridium sp.*	1.656×10^{10} cells L^{-1} (2 days)	Merchuk *et al*., 1998
Tubular airlift	*Spirulina platensis*	27.8 g $m^{-2}d^{-1}$	Torzillo *et al*., 1993

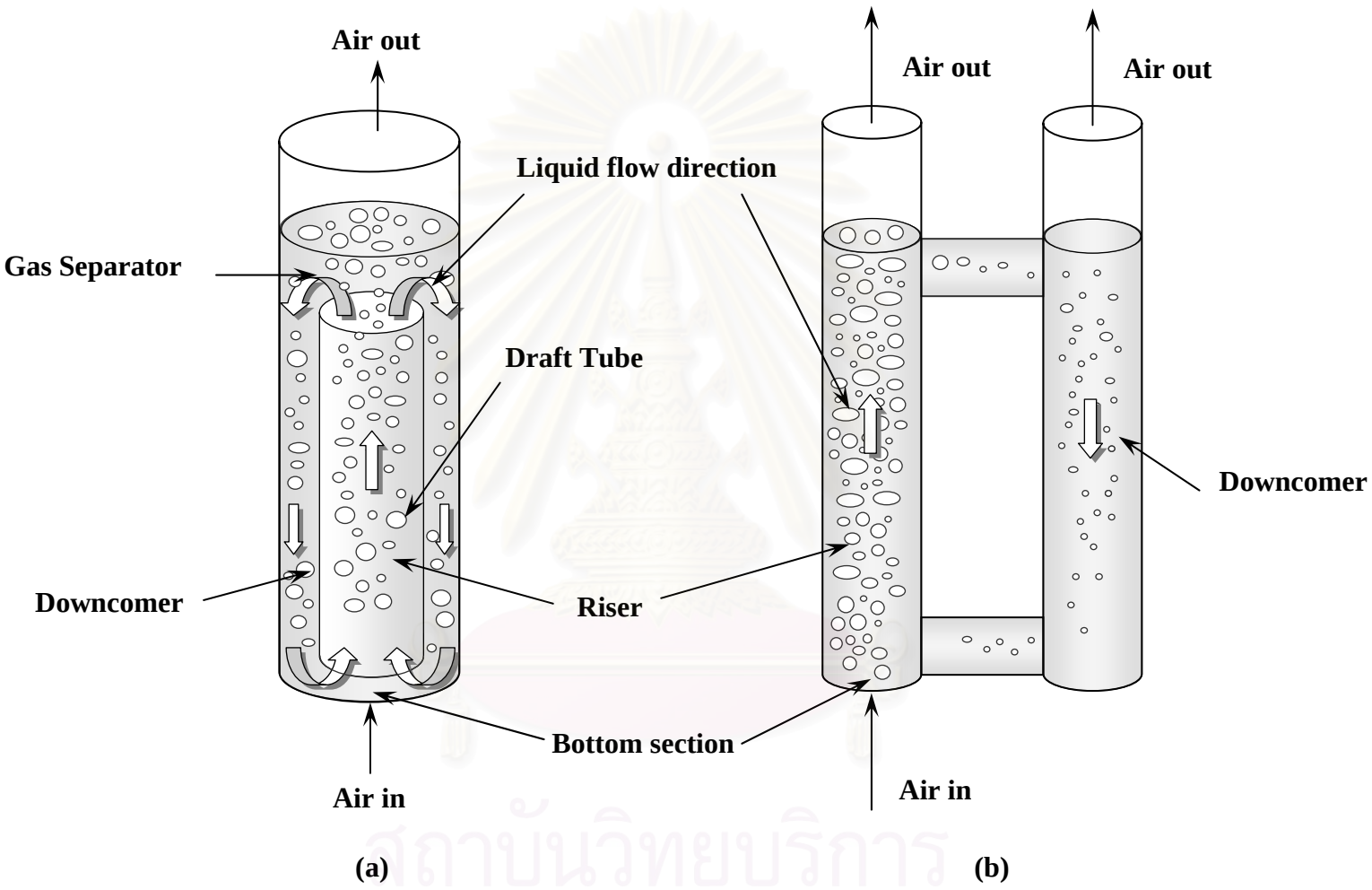


Figure 2.1 Classification of airlift bioreactor: (a) internal loop (b) external loop

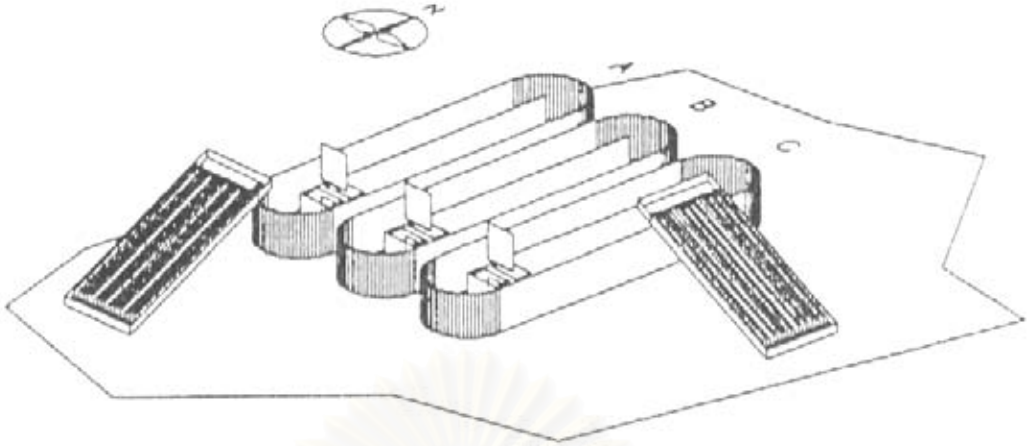


Figure 2.2 Raceway Pond (Pushparaj *et al*., 1997)

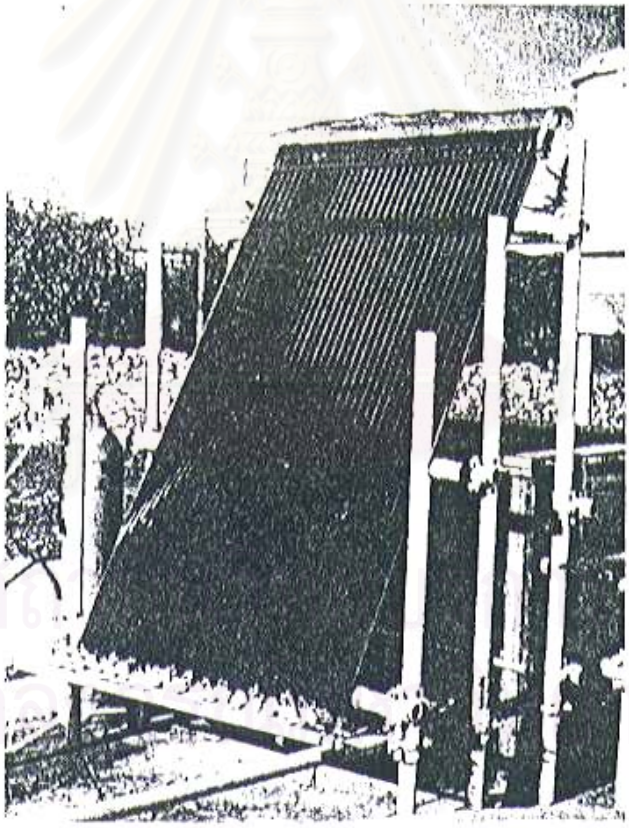


Figure 2.3 Vertical Alveolar Panel (VAP) (Tredici *et al*., 1991)

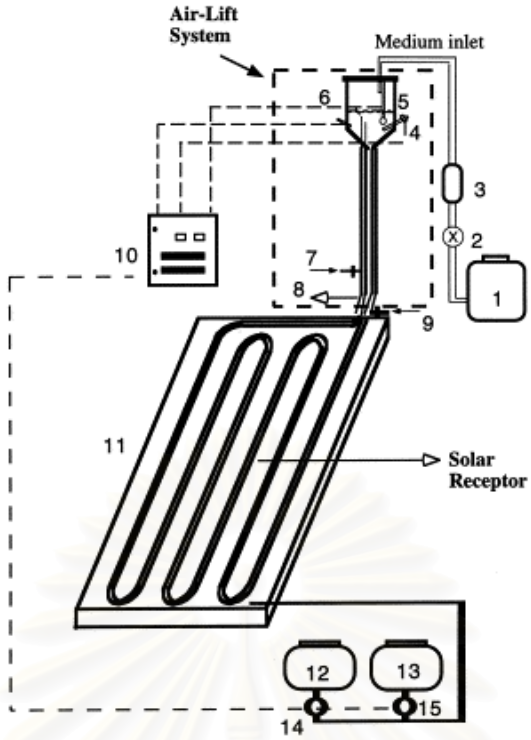


Figure 2.4 Outdoor Tubular Bioreactor (Campo *et al*., 2001)

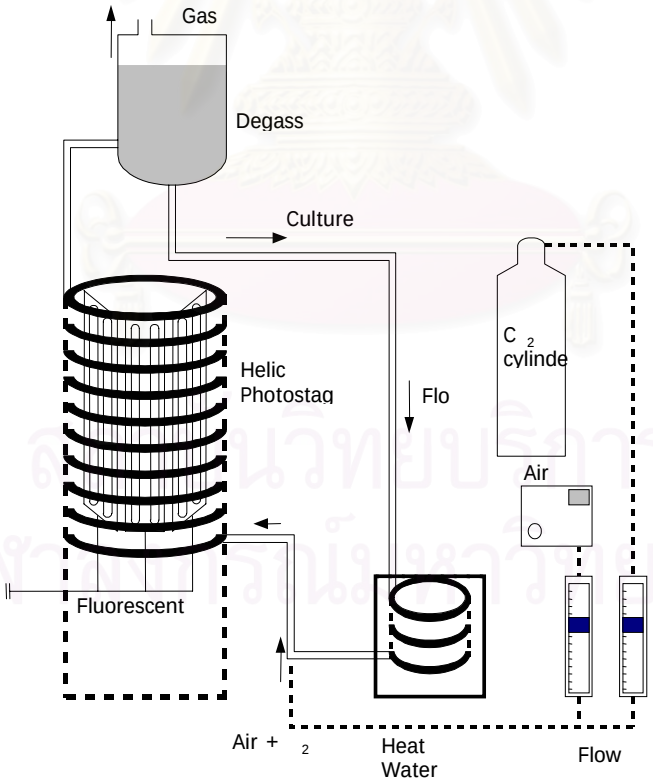


Figure 2.5 Helical Tubular Photobioreactor (Watanabe *et al*., 1995)

CHAPTER 3

Experiments

3.1 Experimental Apparatus

The bioreactors employed in this investigation are made of clear acrylic plastic to allow the visual observation of the ongoing phenomena, and also to allow the passage of light through the column.

3.1.1 Batch and semi-continuous cultures

The airlift photo bioreactor (ALPBR) employed in this investigation was the conventional ALPBR (ALPBR-1) which was separated into two main sections (riser and downcomer) by two cylindrical tubes as shown in Figure 3.1 (all dimensions are detailed in Table 3.1). The inner tube (draft tube) was varied with different tube diameters to determine the optimal configuration for the growth of the diatom. These draft tubes diameter are detailed in Table 3.2. A calibrated rotameter was used to control the gas volumetric flow rate which was supplied through a porous gas sparger at the base of the column. Fluorescent lighting bulbs (120 cm long each) were provided on both sides of the column. These light bulbs were placed 10 cm away from the column. The light intensity was measured by "Digicon LX-50 lux meter" around the column, and this was converted into μ molphoton $m^{-2}s^{-1}$ by using Equation 3.3. The investigation was carried out at room temperature which was around 27–32 ^{o}C. The schematic diagram for the experimental setup is shown in Figure 3.1. The experiments were also operated in the conventional bubble column (BC-1) with the same configurations in the ALPBR but without the insertion of draft tube.

3.1.2 Continuous and reactors-in-series cultures

The ALPBR (ALPBR-2) employed in this investigation was the 2.8 L concentric airlift bioreactor. The schematic diagram of this system is illustrated in Figure 3.2 with dimensions as also detailed in Table 3.3. Compressed air was supplied through a porous sparger located centrally at the base of the column. Fluorescent lighting bulbs were supplied on one side of the column for photosynthesis. The

temperature of the system was controlled around 28–32 ^{o}C. The sterilized F/2 medium was then pumped into the column with varying volumetric flow rate as detailed in Figure 3.2. The overflow stream was provided to control the total volume at 2.8 L. Samples were taken every 3 hours to determine the cell concentration and specific growth rate. For the cultivation in reactors in series, several ALPBR-2s were connected by the connecting tube as detailed in Figure 3.3. The investigation was done in three systems: System II with two connected columns; System III for the three connecting columns and the single column in System I as a blank. Medium feed rates were varied as detailed in the same figure.

3.1.3 Expande- top ALPBR culture

The expanded-top ALPBR (ALPBR-3) was the modification of ALPBR-1 for the investigation of bubbles shading effects on the growth of the diatom. The gas separating section of the ALPBR-1 was enlarged to disperse gas bubbles as illustrated in Figure 3.4. Compressed air was supplied through a porous sparger located centrally at the base of the column as in ALPBR-1. Fluorescent light bulbs were supplied with 20 cm space provided around the column for photosynthesis. The temperature of the system was controlled around 28–32 ^{o}C.

3.1.4 Large scale cultivation

The large scale photobioreactors employed in this work are illustrated in Figures 3.5, 3.6 and 3.7 The systems included the conventional bubble column (BC-2), the conventional concentric ALPBR (ALPBR-4) and the multiple draft tube ALPBR (ALPBR-5). Light source (fluorescent lighting bulbs) was supplied at the top and also at the outer wall around column. Compressed air was supplied through the porous spargers located at the bottom of the system to induce liquid circulation. All the dimensions of these large scale systems are detailed in Table 3.3.

3.2 Experimental Methods

3.2.1 Experimental preparation

3.2.1.1 Treatment of fresh sea water

Fresh sea water used in this investigation is obtained from salt farms around Chonburi province with the original concentration around 100-120 ppt (parts per thousand). Fresh sea water is prepared step by step as follows:

1. Dilute the sea water to 30 ppt with fresh water.
2. Disinfect the sea water with 50 ppm (parts per million) of chlorine (as sodium hypochloride).
3. Supply compressed air through all over the pond for about 3 days or until no remaining chlorine is detected.

3.2.1.2 Preparation of culture medium

The standard F/2 (Guillard's) medium is prepared according to the composition shown in Table 3.4.

3.2.1.3 Preparation of pure culturing strain

The pure culture is prepared for the investigation following the steps described below:

1. Prepare culturing medium in a 1 L Erlenmeyer flask (500 mL of 30 ppt fresh seawater with standard F/2 medium).
2. Add to the medium 4 g of agar powder, this yields 0.8%wt agar in the medium.
3. Clean the Petri dishes and dry at room temperature.
4. Autoclave the agar medium and the dried Petri dishes at 120 ^{o}C for 20 min.
5. Pour 30 mL of sterilized agar medium into sterile plates and leave it to solidify at room temperature in a laminar flow hood.
6. Streak or spread the sample on the agar surface.
7. Cover the plates and seal the dishes with parafilm.
8. Place the plates upside-down under the lighting bulbs.

9. After the growing colonies are observed, pick an isolated colony and streak it again onto the new agar medium plates prepared according to the procedure mentioned above.
10. When pure colonies are observed, transfer it to the test tube filled with 2–3 mL of sterilized culture medium and incubated at 28 $\pm 1^{o}$C for 7-10 days.
11. Scale up the inoculums to 250 mL and 500 mL sterilized culturing mediums and shake well during this period.

3.2.2 Determine the effects of designed configurations on the growth of Chaetoceros calcitrans

1. Assemble the ALPBR-1 by using the 8 cm diameter cylindrical tube as a draft tube.
2. Sterilize the column with 50 ppm chlorine (as sodium hypochloride).
3. Supply air through the porous sparger located centrally at the bottom of the column for 1 day.
4. Drain all the water and rinse the column with sterilized water to eliminate the remaining chlorine.
5. Fill in the column with sterilized sea water, culture medium together with the pure culture, adjust the total volume to 17 L. The initial cell concentration is controlled at 1×10^{5} cells mL^{-1}.
6. Cover the column with a plastic funnel to minimize airborne contamination.
7. Supply sterilized compressed air (treated with 30%wt copper sulfate solution) through a porous sparger and adjust the superficial gas velocity to 2 cm s^{-1}.
8. Supply both sides of column with five 40W fluorescent light bulbs, placed along the column height.
9. Take samples and count for the cell density, using a Haemacytometer (mention in Sec 3.3.1) and measure the light intensity in the draft tube every 3 hours until the staionary growth is observed.
10. Calculate the specific growth rate by using Equation 3.2, the productivity by using Equation 3.4 and the specific productivity by using Equation 3.6.

11. Repeat Steps 1–10 again with superficial gas velocity changed from 2 $cm \cdot s^{-1}$ to 3, 4 and 5 cm s^{-1} respectively.
12. Repeat Steps 1–10 with the draft tube diameter changed to 4, 9 and 10 cm .
13. Repeat Steps 2–10 again in the BC-1 with the optimal aeration rate (u_{sg}) obtained from the investigation in ALPBR-1.

3.2.3 Semi-continuous culture system

1. Sterilize the ALPBR-1 with 50 ppm chlorine (as sodium hypochloride).
2. Sparge air through the porous sparger at the bottom of the column for 1 day.
3. Drain all the water and rinse the column with sterilized water to remove residual chlorine.
4. Fill in the column with sterilized sea water, culture medium together with the pure culture, adjust the total volume to 17 L. The initial cell concentration is controlled at 1×10^5 cells mL^{-1}.
5. Cover the column with a plastic funnel to minimize airborne contamination.
6. Supply sterilized compressed air (treated with 30%wt copper sulfate solution) through a porous sparger and adjust the superficial gas velocity to the optimal gas velocity (determined from the previous experiment).
7. Supply both sides of column with five 40W fluorescent light bulbs along the column height.
8. Take samples and count for the cell density by using Haemacytometer every 3 hours until the stationary growth is observed.
9. Calculate the specific growth rate by using Equation 3.2, the productivity by using Equation 3.4 and the specific productivity by using Equation 3.6.
10. Subculture the cell and adjust its concentration to 1×10^5 cells mL^{-1} and the culture medium as necessary for the quantity of the additional sea water.
11. Take samples after 12 hours of the cultivation, determine the cell density and repeat Step 9 again every 12 hours or other appropriate time as will be estimated from the batch culture.

3.2.4 Continuous culture system

1. Fill the ALPBR-2 with 2.8 L of water and disinfect with 50 ppm chlorine.
2. Sparge air through the porous sparger at the bottom of the column for 1 day.
3. Drain all the water and rinse the column with sterilized water to remove residual chlorine at the bottom of the column.
4. Fill in the column with sterilized sea water, culture medium together with the pure culture, adjust the total volume to 2.8 L. The initial cell concentration is controlled at 1×10^5 cells mL^{-1}.
5. Cover the column to prevent contaminants in the column.
6. Supply sterilized compressed air (treated with 30%wt copper sulfate solution) through porous sparger and adjust the gas velocity to 3 cm s^{-1}.
7. Take samples every 3 hours and examine the cell density by using a Haemacytometer until the stationary growth is initially observed.
8. Connect the overflow line to the column to control the volume of the medium to 2.8 L.
9. Pump in the culture medium by using a peristaltic pump with the volumetric flow rate of 2 mL min^{-1}.
10. Take samples every 3 hours and count for the cell concentration using Haemacytometer.
11. Calculate the specific growth rate by using Equation 3.2, the productivity by using Equation 3.5 and the specific productivity by using Equation 3.6.
12. Repeat Steps 1–11 again with medium feed rate of 3 and 4 mL min^{-1}.

3.2.5 Reactor- in- series culture system

1. Connect two ALPBR-2s for the cultivation in series as shown in Figure 3.3 (System II).
2. Fill each column with 2.8 L of water and disinfect with 50 ppm chlorine.
3. Sparge air through the porous sparger at the bottom of the columns for 1 day.
4. Drain all the water and rinse the columns with sterilized water to remove residual chlorine at the bottom of the columns.

5. Fill in the columns with sterilized sea water, culture medium together with the pure culture, adjust the total volume to 2.8 L. The initial cell concentration is controlled at 1×10^5 cells mL^{-1}.
6. Cover the column to prevent contaminants in the column.
7. Supply sterilized compressed air (treated with 30%wt copper sulfate solution) through porous sparger and adjust the gas velocity to 3 cm s^{-1}.
8. Take samples every 3 hours and examine the cell density by using a Haemacytometer until the stationary growth is initially observed.
9. Pump in the culture medium by using a peristaltic pump with the volumetric flow rate of 2 mL min^{-1}.
10. Take samples every 3 hours and count for the cell concentration using Haemacytometer.
11. Calculate the specific growth rate by using Equation 3.2, the productivity by using Equation 3.5 and the specific productivity by using Equation 3.6.
12. Repeat Steps 2–11 again with medium feed rate of 4 mL min^{-1}.
13. Connect three ALPBR-2s for the cultivation in series as shown in Figure 3.3 (System III).
14. Repeat Steps 2–11 again with medium feed rate of 3, 4 and 6 mL min^{-1}.

3.2.6 Expanded-top ALPBR culture system

1. Assemble the ALPBR-3 (23 L capacity) by using the 8 cm diameter cylindrical tube as a draft tube.
2. Sterilize the column with 50 ppm chlorine (as sodium hypochloride).
3. Supply air through the porous sparger located centrally at the bottom of the 23 L column for 1 day.
4. Drain all the water and rinse the column with sterilized water to eliminate the remaining chlorine.
5. Fill in the column with sterilized sea water, culture medium together with the pure culture, adjust the total volume to 23 L. The initial cell concentration is controlled at 1×10^5 cells mL^{-1}.
6. Supply sterilized compressed air (treated with 30%wt copper sulfate solution) through a porous sparger and adjust the superficial gas velocity to 2 cm s^{-1}.

7. Supply the column with five 40W fluorescent light bulbs around the column, placed along the column height.
8. Take samples and count for the cell density, using a Haemacytometer (mention in Sec 3.3.1) and measure the light intensity in the draft tube every 3 hours until the stationary growth is observed.
9. Calculate the specific growth rate by using Equation 3.2
10. Repeat Steps 1–8 again with superficial gas velocity changed from 2 cm s^{-1} to 3, 4 and 5 cm s^{-1}, respectively.

3.2.7 Large scale cultivation

1. Fill 170 L water into the BC-2, and disinfect with 50 ppm chlorine.
2. Supply air through the porous sparger located at the bottom of the column for 1 day.
3. Drain all the water and rinse the column with sterilized water to eliminate the remaining chlorine.
4. Fill in the column with sterilized sea water, culture medium together with the pure strain culture, adjust the total volume to 160 L. The initial cell concentration is controlled at 1×10^5 cells mL^{-1}.
5. Supply sterilized compressed air (treated with 30%wt copper sulfate solution) through a porous sparger.
6. Supply 16 fluorescent light bulbs (18W each) around the cultivation tank and also the same 5 fluorescent bulbs on the top of the column.
7. Take samples every 3 hours until the stationary growth is observed and count for the cell density, using a Haemacytometer.
8. Calculate the specific growth rate by using Equation 3.2, the productivity by using Equation 3.4 and the specific productivity by using Equation 3.6.
9. Repeat Steps 1–8 again with the ALPBR-4 where the single riser was inserted concentrically (as shown in Figure 3.6).
10. Repeat Steps 1–8 again with the ALPBR-5 where 4 draft tubes was inserted (as shown in Figure 3.7).

3.3 Analyses

3.3.1 Determination of cell concentration

The cell concentration can be determined by using a normal blood cell counting slide, Haemacytometer. The depth of the counting grid and the area are 0.1 mm and 25 mm^2 respectively. The cell concentration can be determined as follows:

1. Dilute the sample if the sample is too dense
2. Clean the counting slide and cover glass
3. Fill the slide with sample
4. Cover the slide with cover glass, avoid of bubbles
5. Count the cell in five large squares on the grid (25 small squares per 1 large square)
6. Repeat Steps 1–5 again
7. Calculate the cells number, using Equation 3.1:

$$N = 5 \times d \times n \times 10^4 \qquad (3.1)$$

where

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

n = number of cells on five large squares (cells)

d = dilution factor (–)

3.3.2 Determination of specific growth rate

The specific growth rate can be calculated from Equation 3.2 as follows:

$$\mu = \frac{\ln(N_2) - \ln(N_1)}{t_2 - t_1} \qquad (3.2)$$

where

μ = specific growth rate (h^{-1})

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

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

t_1 = first sampling time (h)

t_2 = second sampling time (h)

3.3.3 Determination of light intensity

The light intensity can be calculated from Equation 3.3 as follows:

$$I \approx \frac{E}{50} \tag{3.3}$$

where

I = light intensity (μ molphoton $m^{-2}s^{-1}$)

E = light intensity (lux)

3.3.4 Determination of productivity

The productivity of the diatom was calculated from Equation 3.4 as follows:

For batch and semi-continuous cultivation;

$$P = \left(\frac{N_2 - N_1}{t_2 - t_1}\right) \times \frac{V \times 1000}{3600} \tag{3.4}$$

where

P = productivity (cells s^{-1})

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

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

t_1 = first sampling time (h)

t_2 = second sampling time (h)

V = harvest volume (L)

For continuous cultivation;

$$P = \frac{N \times q}{60} \tag{3.5}$$

where

P = productivity (cells s^{-1})

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

q = volumetric flow rate of fresh medium (mL min^{-1})

3.3.5 Determination of specific productivity

The specific productivity for the cultivation of the diatom in the ALPBR can be calculated from Equation 3.5:

$$SP = \frac{P}{V} \tag{3.6}$$

where

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

P = productivity (cells s^{-1})

V = harvest volume (L)

3.3.6 Determination of remaining chlorine in the solution

The remaining chlorine in the solution is determined by adding a small amount of potassium iodide into the solution. Should the color of the mixture turn to yellow, there is still chlorine remaining in the solution in the level that is harmful for the diatom.

3.3.7 Determination of salinity

The salinity of the sea water was determined by using a “Refractometer”, and adjusted the salinity of the water to 30 ppt (parts per thousand).

Table 3.1 Dimensions of conventional concentric ALPBR (ALPBR-1) employed in this work.

Parameters	Dimensions (cm)
Column diameter (D)	15
Column height (H)	120
Draft tube diameter (d)	8
Draft tube height (h)	100

Table 3.2 Design configurations of ALPBR-1 for the investigation on optimal configuration for the growth of *Chaetoceros calcitrans*.

Draft tube diameter (cm)	A_d:A_r (-)
4	16.55
8	2.62
9	1.79
10	1.22

Table 3.3 Dimensions of bubble columns and concentric airlift bioreactors employed in this investigation.

Types	Working volume	A_d:A_r	Reactor		Draft tube		Number of light source		Light intensity
			Diameter, D (cm)	Height, H (cm)	Diameter, d (cm)	Height, h (cm)	Wall	Top	(μ molphoto $m^{-2}s^{-1}$)
BC-1	17 L	-	15	120	-	-	10 (36W)	-	384.33
BC-2	170 L	-	79	66	-	-	16 (18W)	5 (18W)	72
ALPBR-1	17 L	2.62	15	120	8*	100*	10 (36W)	-	384.33
ALPBR-2	2.8 L	1.53	8	66	5	55	5 (36W)**	-	118
ALPBR-3	23 L	2.62	15	130	8	100	8 (36W)	-	130
ALPBR-4	170 L	1.47	79	66	50	50	21 (18W)	5 (18W)	72
ALPBR-5	170 L	2.82	79	66	18***	40***	21 (18W)	5 (18W)	72

* Actual configurations for ALPBR-1, otherwise specified.
** The light source was supplied perpendicular to the column on one side of the column.
*** Four draft tubes were placed in the reactor and air was supplied through the porous sparger located at the bottom of each draft tubes.

Table 3.4 Chemical composition of standard F/2 (Guillard's) stock solution*

Solution A: Nitrate and Phosphate stock solution (1 L)

Nutrient	Amount
$NaNO_3$	84.15 g
$Na_2HPO_4 \cdot H_2O$	6.0 g
$FeCl_3 \cdot 6H_2O$	2.90 g
$Na_2EDTA \cdot 2H_2O$	10.0 g

Solution B: Silicate stock solution (1 L)

Nutrient	Amount
$Na_2SiO_3 \cdot 9H_2O$	33.0 g

Solution C: Trace Metal stock solution (1 L)

Nutrient	Amount
$CuSO_4 \cdot 5H_2O$	1.96 g
$ZnSO_4 \cdot 7H_2O$	4.40 g
$Na_2MoO_4 \cdot 2H_2O$	1.26 g
$MnCl_2 \cdot 4H_2O$	36.0 g
$CoCl_2 \cdot 6H_2O$	2.0 g

Solution D: Vitamins stock solution (1 L)

Nutrients	Amount
Vitamin B1	0.4 g
Vitamin B12	0.002 mg
Biotin	0.10 mg

* To prepare the culture medium for *Chaetoceros calcitrans*, simply add 2 mL of Solutions A and B and 1 mL of Solutions C and D in 1 L of fresh sea water.

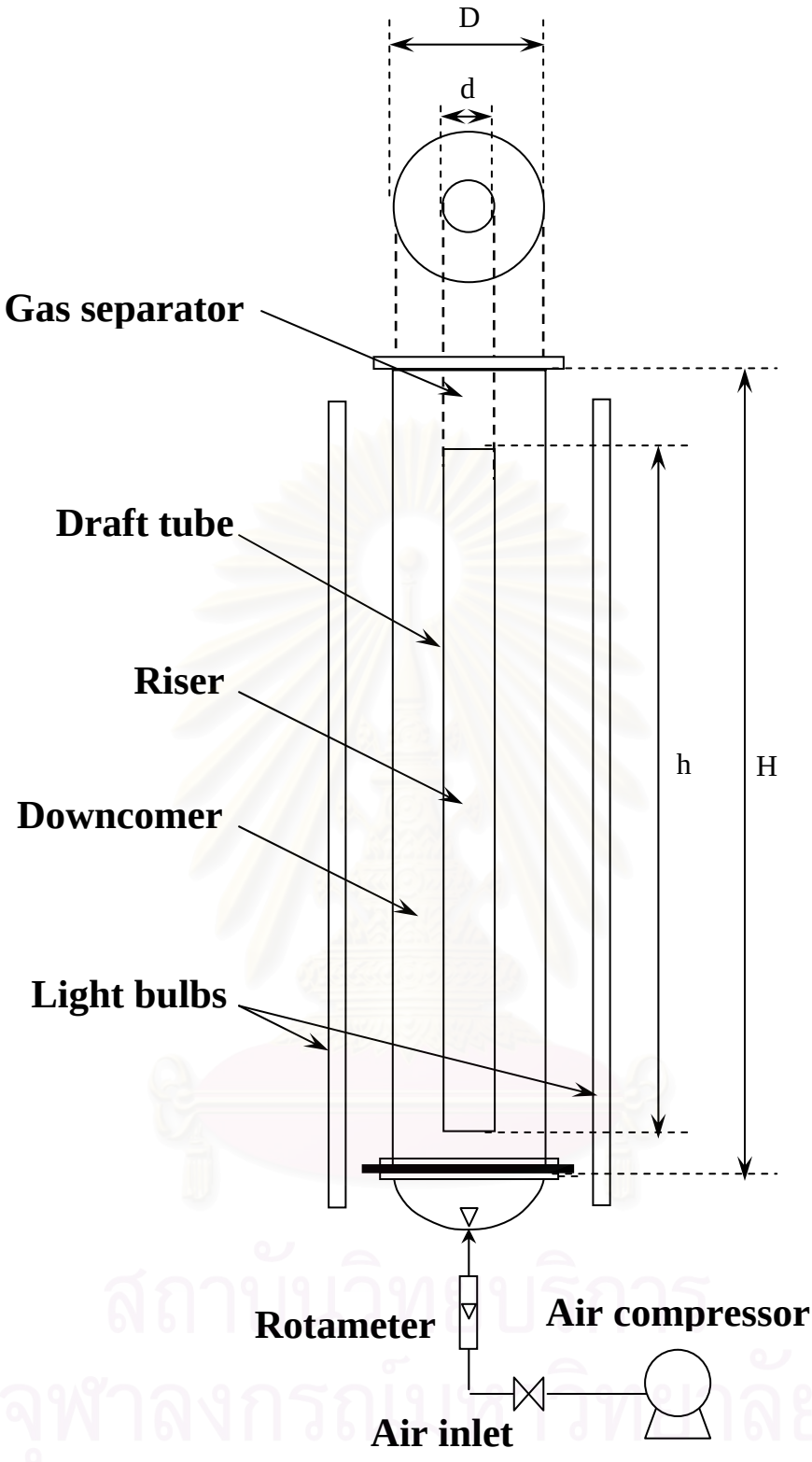
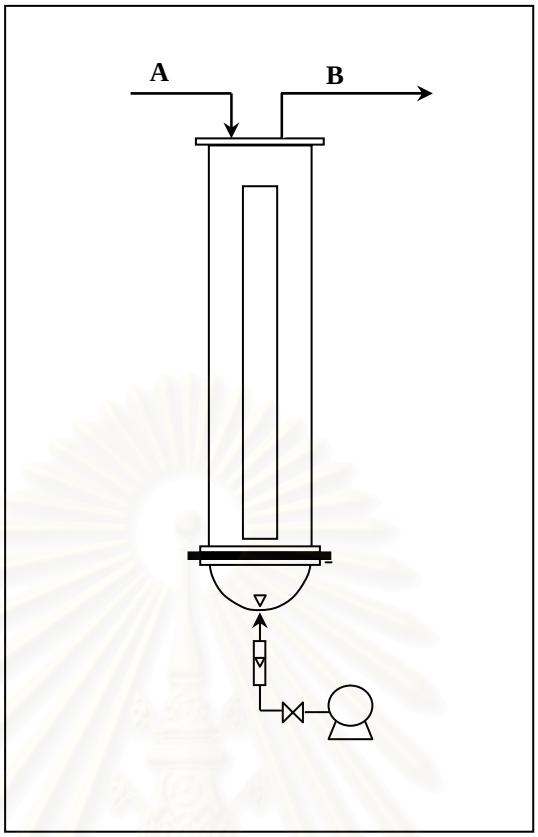


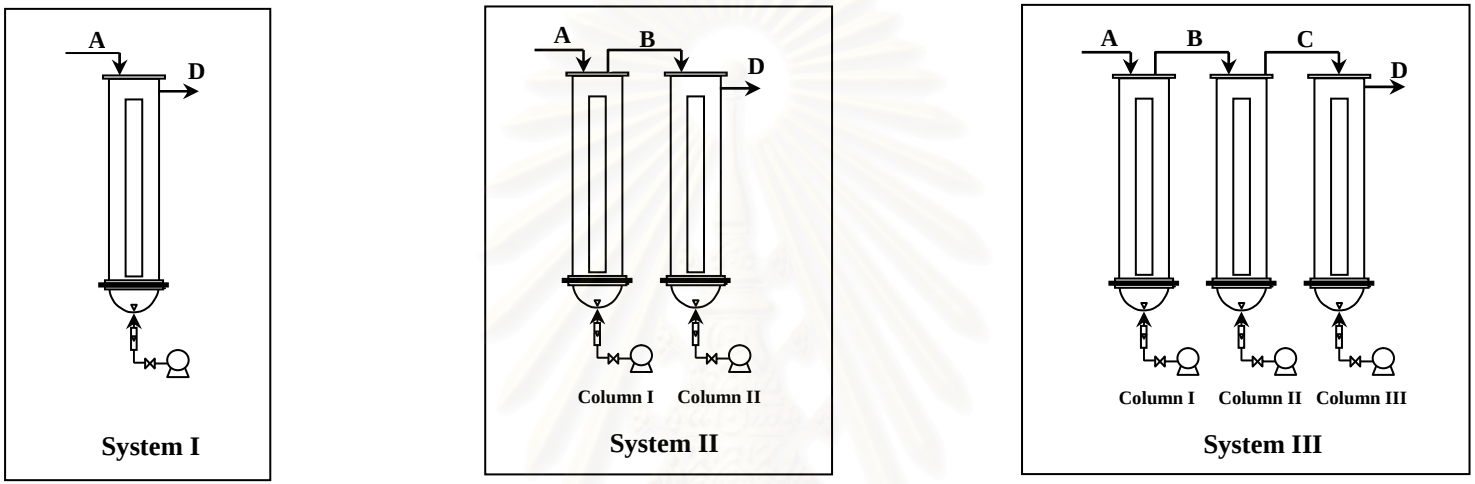
Figure 3.1 Experimental setup for the cultivation of *Chaetoceros calcitrans* in ALPBR-1 and ALPBR-2



Experiment	Fluid volumetric flow rates	
	Stream A	Stream B
I	2 mL min^{-1}	Over flow
II	3 mL min^{-1}	Over flow
III	4 mL min^{-1}	Over flow

Figure 3.2 Experimental setup for the cultivation of *Chaetoceros caltrans* in the continuous systems with various medium feed rates

Figure 3.3 Operating condition for the cultivation of *Chaetoceros calcitrans* in ALPBR in series



Experiment	System	Medium feed rates			
		Stream A	Stream B	Stream C	Stream D
Set 1	I	1 mL min^{-1}	-	-	Over flow
Set 2	I	2 mL min^{-1}	-	-	Over flow
Set 3	II	2 mL min^{-1}	2 mL min^{-1}	-	Over flow
Set 4	II	4 mL min^{-1}	4 mL min^{-1}	-	Over flow
Set 5	III	3 mL min^{-1}	3 mL min^{-1}	3 mL min^{-1}	Over flow
Set 6	III	6 mL min^{-1}	6 mL min^{-1}	6 mL min^{-1}	Over flow

Five 40 W fluorescent light bulbs were located perpendicular on one side of the columns for photosynthesis

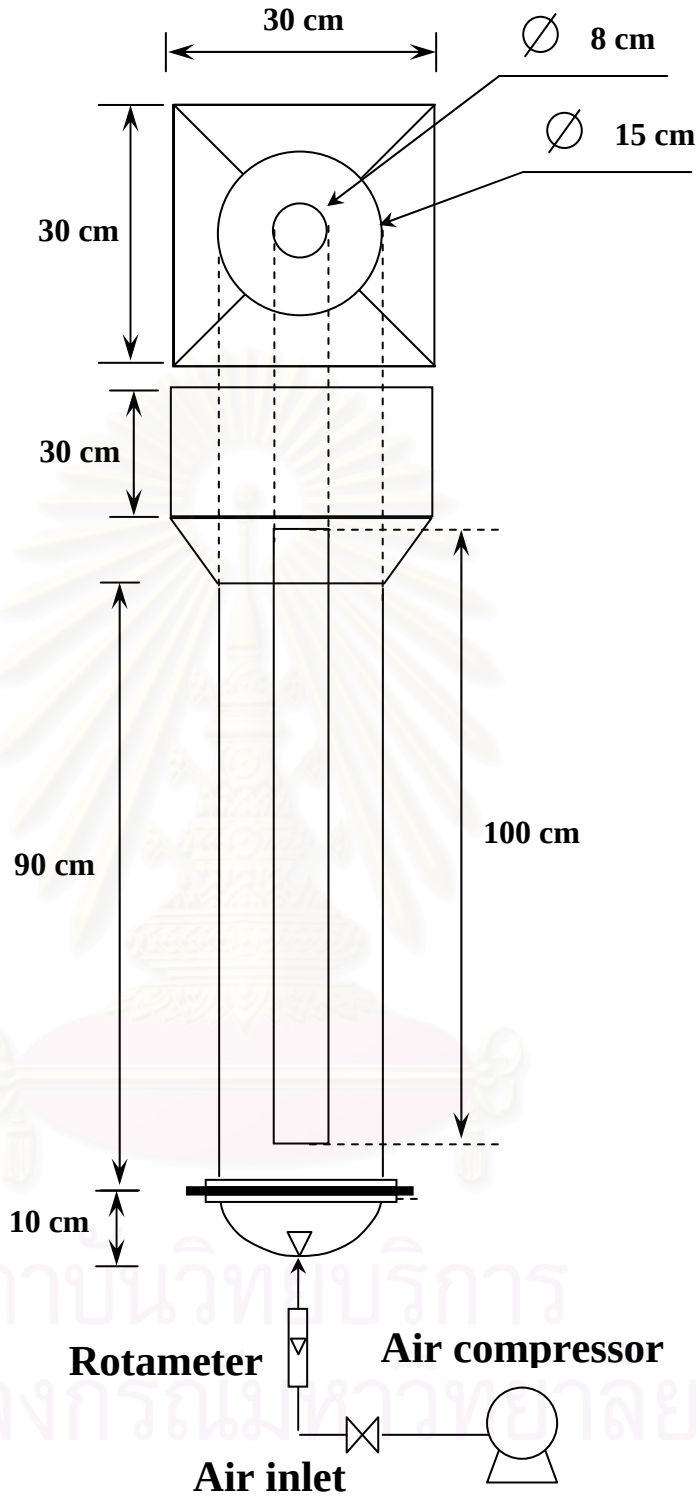


Figure 3.4 Experimental setup for the cultivation of *Chaetoceros calcitrans* in ALPBR-3

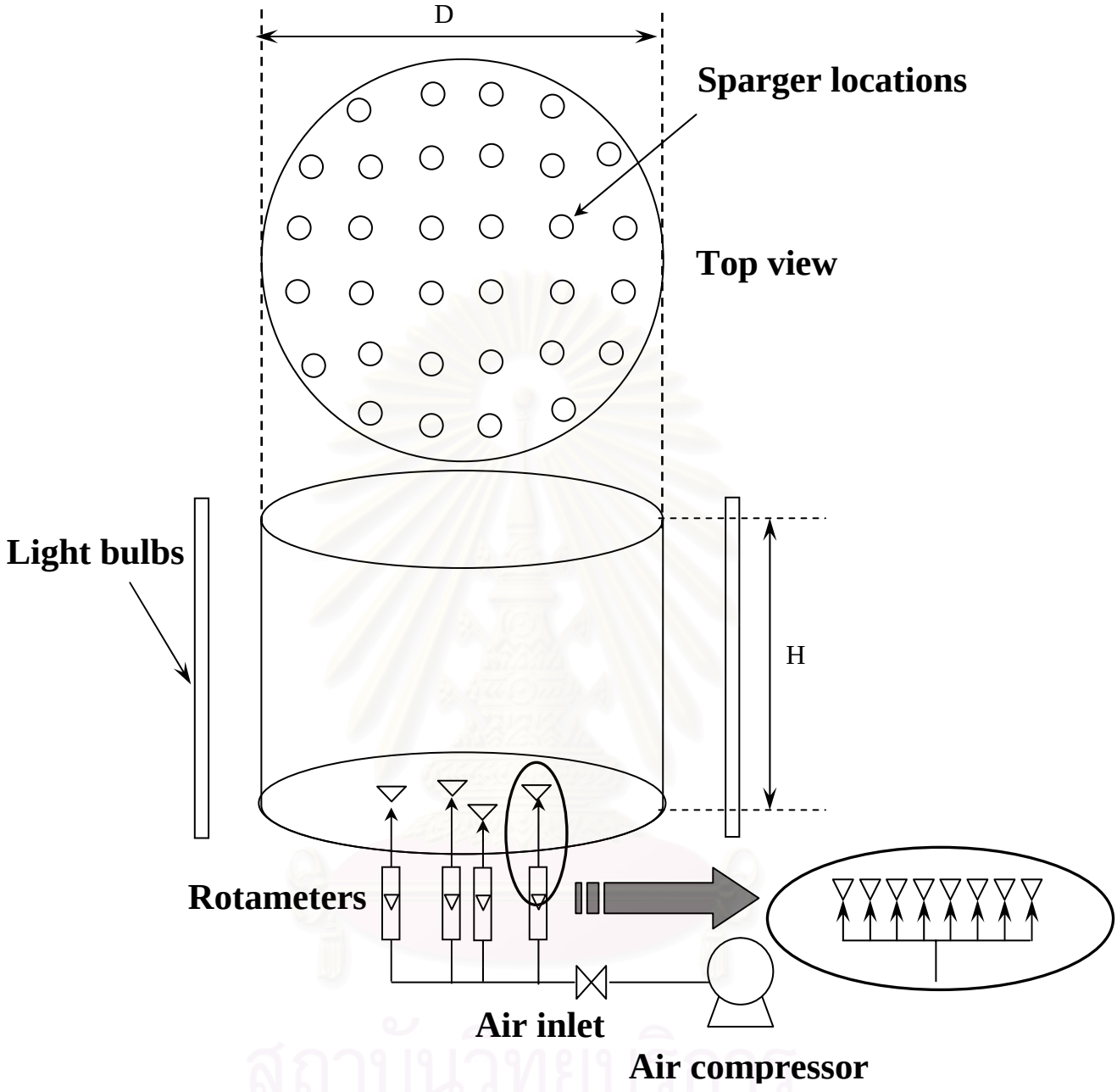


Figure 3.5 Experimental setup for the cultivation of *Chaetoceros calcitrans* in BC-2

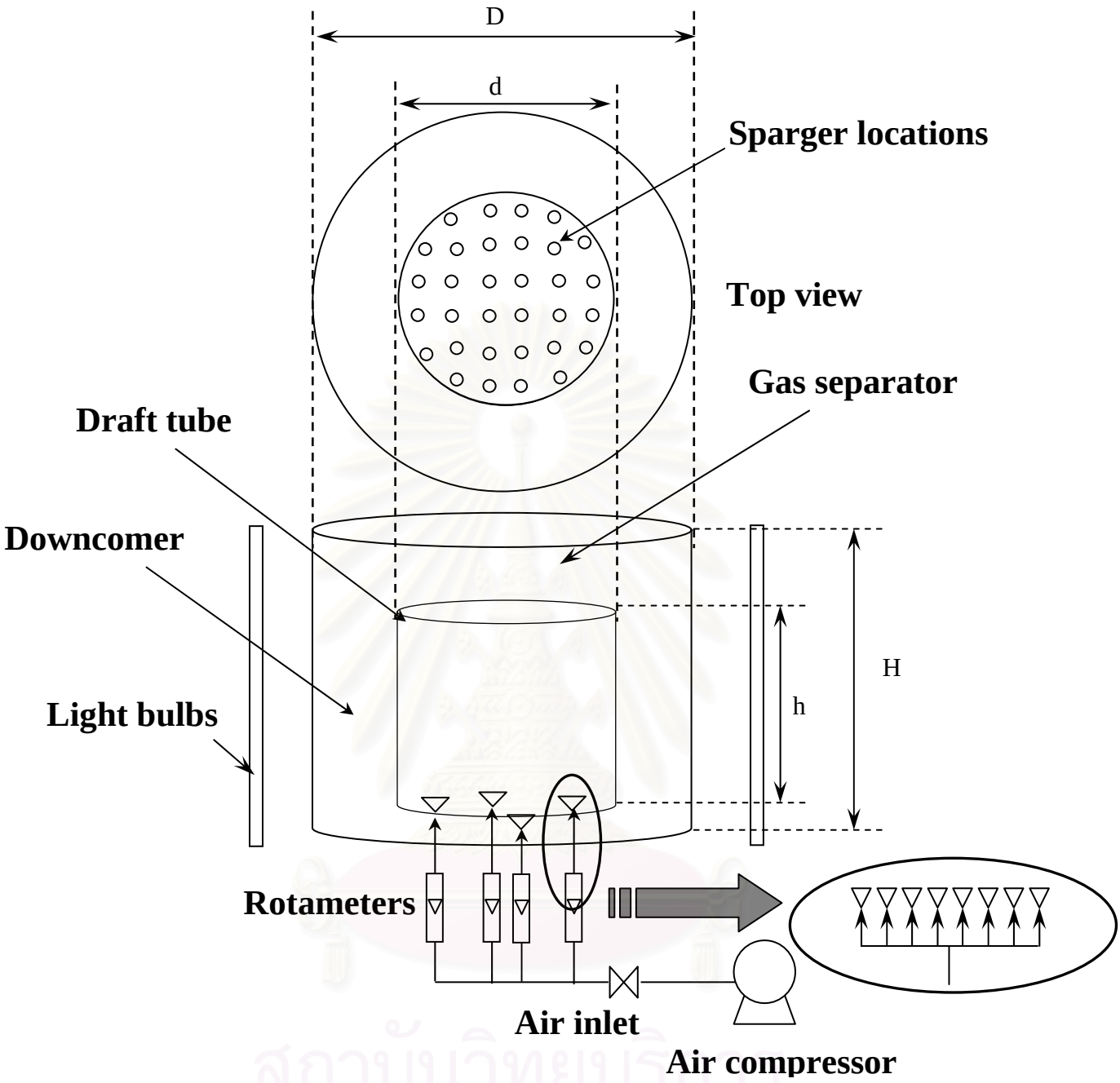


Figure 3.6 Experimental setup for the cultivation of *Chaetoceros calcitrans* in ALPBR-4

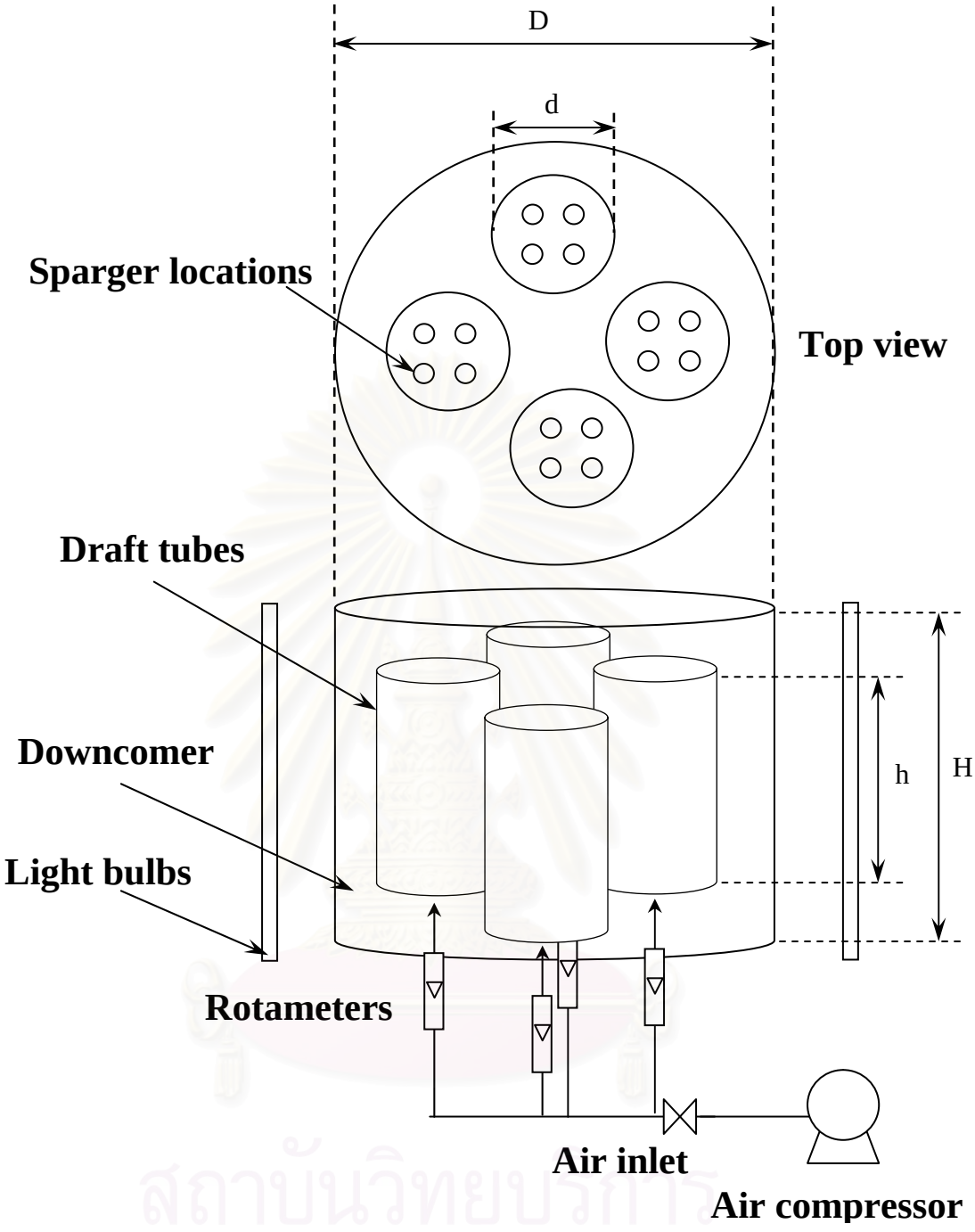


Figure 3.7 Experimental setup for the cultivation of *Chaetoceros calcitrans* in ALPBR-5

CHAPTER 4

Optimal Reactor Configuration for the Cultivation of *Chaetoceros calcitrans* in Airlift Photobioreactor

4.1 Summary

Optimal conditions for the growth of *Chaetoceros calcitrans* were investigated. A maximum cell concentration was obtained from the cultivation of *Chaetoceros calcitrans* in the 17 L airlift photobioreactor (ALPBR) with superficial gas velocity of 3 cm s^{-1} and the ratio between downcomer to riser cross sectional areas (A_d:A_r) of 2.62 where the maximum specific growth rate of 7.41×10^{-2} h^{-1} with a maximum cell concentration of 8.88×10^{6} cells mL^{-1} could be achieved in a batch culture. The operating conditions were known to have influence on the reactor behavior where an increase in the aeration rate could lead to a number of hydrodynamic variations in the system, including an increase in liquid circulation, a higher gas-liquid mass transfer, and a larger bubble size. It was illustrated that gas-liquid mass transfer rate did not have significant effect on the growth of the diatom within the range of aeration rate examined in this work. Therefore the effect of aeration rate on the growth of the diatom was regulated by the changes in the circulation velocity and bubble sizes. A higher circulation velocity caused a better circulation of diatom to the high light intensity area (in downcomer) and promoted a rapid growth. The effect of changes in bubble size was more complicated. The main message obtained from this work was not to let the bubbles flow down the downcomer as they would block the light penetration, resulting in a lower diatom growth. The most productive aeration rate (measured in terms of superficial velocity) was 3 cm s^{-1}. Similar to the influence of aeration rate on cell growth, the changes in A_d:A_r could lead to hydrodynamic changes. The situation where (i) cells were well circulated and (ii) less bubbles in downcomer were most favorite for the growth.

4.2 Backgrounds

The cultivation of the diatom in biological systems was investigated in many bioprocesses (Baquerisse *et al*., 1999). Several factors should be considered which include: types of the diatom, cost, labor, energy, climate and the type of final product (Borowitzka, 1992). One of the most classical systems for the cultivation of the diatom was the opened pond, i.e. tank, raceway pond and shallow pond with paddle wheels or aeration equipment supplied into the system (Lee, 1997).

A number of closed cultivating systems have been developed and introduced as detailed in Tables 2.2–2.7, most of which aimed to achieve the high efficiency in light utilization, increase the productivity, and also produce the pure culturing strain (Lee, 1986). For instance, tubular bioreactors were introduced due to their advantages in obtaining higher light intensity per unit volume. However, mixing problems were often encountered in the system resulting in the limitation of the system for the cultivation of the diatom.

The diatom *Chaetoceros calcitrans* is considered one of the most popular strains in Thailand used as a feed for shrimp larvae. Very few investigations on the examination of optimal condition for the growth of *Chaetoceros calcitrans* are available. The literature reported that the highest yield for this diatom was obtained at an initial silica concentration of 400 µg L^{-1} (as sodium metasilicate) (Merchuk and Stein, 1981). The Aquaculture Department of the Southeast Asian Fisheries Development Center cultivated *Chaetoceros calcitrans* in the outdoor fiber glass tank (1 ton capacity) achieved the final density of 2.65×10^{6} cells mL^{-1} within 4 days operation (Samonte *et al*., 1993.) This was considered a rather good productivity rate, however, the report did not contain adequate technical information and hence, the applicability of such report is highly questionable.

Airlift bioreactors (ALBR) have recently become an attractive alternative for cell cultivation (Merchuk *et al.*, 1996). This might be due to several main advantages such as good mixing, well-defined fluid flow pattern, relatively high

gas-liquid mass transfer rate, and low capitals and operating costs. Laotaweesup (2002) investigated the optimal condition for the growth of *Chaetoceros calcitrans* in the conventional airlift photobioreactor, ALPBR and reported that the highest growth rate could be obtained with the light intensity of around 400 μ molphoton m^{-2} s^{-1}, and with the aeration rate (u_{sg}) of 3 cm s^{-1} (see summary in Table 4.1). Also a two fold of silica and phosphorus concentrations in the standard F/2 medium introduced a better growth of this diatom whilst vitamin B_{12} (1 to 3 μg L^{-1}) was essential but its concentration did not significantly affect the growth of the diatom. This work aimed at the continuity of the work by focusing particularly on the examination of appropriate operating and design parameters that supported the growth of the diatom. Note that the modified F/2 medium as suggested by Laotaweesup (2002), although could lead to an enhancement in the diatom growth, but the effect was only marginal and should not be economically attractive. Therefore the standard F/2 medium was used throughout this investigation.

4.3 Experimental

The experiment was carried out as batch cultivation in the 17L ALPBR (ALPBR-1) and the 17 L bubble column (BC-1) as described in Section 3.1.1 with experimental preparation as reported in Section 3.2.1. The determination of optimal growth condition was conducted based on the procedure in Section 3.2.2.

4.4 Growth Dependency on The Operating Condition in Airlift System

For batch culturing systems, the only operating parameter in airlift photobioreactor (ALPBR) is the rate of aeration. In this section the cultivation of *Chaetoceros calcitrans* was carried out in the 17 L ALPBR with the ratio between downcomer to riser cross sectional areas (A_d:A_r) of 1.79 and 2.62. The aeration rate (measured as superficial velocity, u_{sg}) was varied in the range of 2 to 5 cm s^{-1}. The results as demonstrated in Figures 4.1 and 4.2 revealed the significance of the aeration on the growth of the diatom where the maximum growth rate was obtained at u_{sg} = 3 cm s^{-1} in both systems, above or below which the growth rate dropped. The

comparison between maximum cell concentration and specific growth rate are already summarized in Table 4.2.

It was observed that at a lower range of aeration (u_{sg}= 2–3 cm s^{-1}), an increase in the aeration rate greatly induced mixing, liquid circulation rate and also the mass transfer between gas and liquid in the system (Merchuk and Stein, 1981 and Gavrilescu and Tudose, 1998). The increase in aeration rate also resulted in a better mixing between the medium and diatom which led to efficient nutrients utilization. In addition, the increase in aeration rate means that more gas bubbles were supplied into the riser of the system, increasing gas holdup and potentially enhancing the overall volumetric gas-liquid mass transfer. A higher mass transfer might also facilitate the removal of metabolic gases such as oxygen, preventing its accumulation which, otherwise, might adversely affect the growth rate (Tung *et al.*, 1998).

It was usually found that gas holdup and liquid velocity in the ALC were correlated in the form of:

$$\varepsilon \propto u_{sg}^{\alpha} \tag{4.1}$$

where

ε = gas holdup (–)

u_{sg} = superficial gas velocity (cm s^{-1})

α = constant (–)

In air-water mixture, the constant in Equation 4.1, α, was found to vary in a range from 0.2–1.26 depending on the configurations of the ALC (Chakravarty *et al.*, 1973, Chisti *et al.*, 1988 and Tung *et al.*, 1997).

Previous investigation at the Department of Chemical Engineering, Faculty of Engineering at Chulalongkorn University (Limpanuphap, 2003) showed that the correlation between gas holdup (ε), riser liquid velocity (v_{Lr}), aeration rate (u_{sg}), ratio between downcomer to riser cross sectional area (A_d:A_r) and overall volumetric mass transfer coefficient (k_La) in the fresh sea water (30 ppt) could be

described as a function of area ratio and superficial gas velocity according to the following equations:

$$\varepsilon_o = 0.5166(A_d{:}A_r)^{-0.5223}(u_{sg})^{0.5994} \quad (4.2)$$

$$\varepsilon_d = 0.3099(A_d{:}A_r)^{-0.6282}(u_{sg})^{0.4238} \quad (4.3)$$

$$\varepsilon_r = 0.1662(A_d{:}A_r)^{0.5198}(u_{sg})^{0.4048} \quad (4.4)$$

$$v_{Lr} = 1.7931(A_d{:}A_r)^{0.1244}(u_{sg})^{0.5029} \quad (4.5)$$

$$k_La = 0.0611(A_d{:}A_r)^{-0.3571}(u_{sg})^{0.1314} \quad (4.6)$$

where

v_{Lr} = riser liquid velocity (m s^{-1})

v_{Ld} = downcomer liquid velocity (m s^{-1})

u_{sg} = superficial gas velocity (m s^{-1})

ε_o = overall gas holdup (–)

ε_r = riser gas holdup (–)

ε_d = downcomer gas holdup (–)

k_La = overall volumetric mass transfer coefficient (s^{-1})

$A_d{:}A_r$ = ratio between downcomer to riser cross sectional area (–)

The overall volumetric mass transfer coefficient, k_La, at various gas velocities were calculated from Equation 4.6 and are illustrated in Figure 4.3. To demonstrate the influence of the gas-liquid mass transfer on the growth of the diatom, these changes in k_La was plotted along with the maximum achievable cell concentration at the same operating condition. The results illustrate that, while an increase in k_La was obtained due to a better mixing at higher aeration rate, the cell concentration diminished as u_{sg} increased above 3 cm s^{-1}. This leads to a potential conclusion that the growth of the diatom in the ALPBR did not depend on the mass transfer in the system.

At low aeration rate (u_{sg} < 3 cm s^{-1}), it was observed that there existed a large pool of small bubbles in the downcomer. This was because there was a low level of bubble coalescence at this condition and small bubbles could be easily

dragged down the downcomer due to the liquid circulation in the system. The presence of bubble swarm in the downcomer inhibited the light penetration in the column when compared with the system running at u_{sg} = 3 cm s^{-1} (see Figure 4.4 for the illustration of this phenomenon). This reduced the intensity of light to which the diatom was exposed, and therefore a low growth rate was observed.

At higher aeration rate (u_{sg} = 4–5 cm s^{-1}), the growth rate of *Chaetoceros calcitrans* was also found to decline. The high aeration rate converted the flow regime in the ALPBR to a more turbulent condition where small bubbles coalesced and formed very large bubbles (Laotaweesup, 2002 and Limpanuphap, 2003). Large bubbles moved up at higher speed and this caused the liquid to circulate at higher speed due to the momentum transfer from the gas bubbles. Higher circulation rate meant that the liquid could bring a number of larger bubbles down to the downcomer section and these bubbles, again, hindered the light penetration through the column (see Figure 4.4). Figure 4.5 indicates that as much as 40% of light intensity could be suppressed in the riser (or in the draft tube) with the presence of gas bubbles in the downcomer. As a result, the diatom was subject to a lower light intensity and a lower growth rate was observed.

4.5 Influence of Design Parameter on Cell Growth

The previous section described the effect of aeration rate on the growth of the diatom in the ALPBR for the cultivation of the diatom *Chaetoceros calcitrans* in the concentric ALPBR with A_d:A_r = 1.79 and 2.62. The maximum cell concentration and the maximum growth rate in both systems were obtained at u_{sg} = 3 cm s^{-1} and this level of aeration was remained in this discussion. In this section, the effect of an important design parameter for airlift system, A_r, or more specifically, the ratio between downcomer and riser cross sectional area, A_d:A_r, on the growth of the diatom was discussed. The diatom was cultivated in the ALPBR where the draft tube could be changed to vary the cross sectional area ratio between downcomer and riser, A_d:A_r.

Figure 4.6 illustrates the relationship between A_d:A_r and the cell growth for the cultivation of the diatom. It was found that the maximum cell concentration could be obtained for the cultivation of the diatom in the ALPBR with A_d:A_r = 2.62 with the maximum cell concentration of approx 8.88×10^6 cells mL^{-1} and the specific growth rate of 7.41×10^{-2} h^{-1}. The operation in the system with A_d:A_r = 1.79 yielded a lower maximum cell concentration and specific growth rate, i.e. at 7.37×10^6 cells mL^{-1} and 5.78×10^{-2} h^{-1}, respectively. Although the maximum cell concentration was only just 17% difference, the operation in the system with A_d:A_r = 2.62 was considered more economical as it needed lower air compressor power input to achieve the aeration rate. (129.09 mL s^{-1} for A_d:A_r = 2.62 and 166.2 mL s^{-1} for A_d:A_r = 1.79).

According to the mass conservation law, the relationship between riser liquid velocity and downcomer liquid velocity can be correlated as shown in the following equation:

$$v_{Lr}A_r(1-\varepsilon_r) = v_{Ld}A_d(1-\varepsilon_d) \qquad (4.7)$$

where

v_{Lr} = riser liquid velocity (cm s^{-1})

v_{Ld} = downcomer liquid velocity (cm s^{-1})

ε_r = riser gas holdup (–)

ε_d = dowmcomer gas holdup (–)

A_r = riser cross sectional area (cm^2)

A_d = downcomer cross sectional area (cm^2)

Equation 4.7 suggests that riser liquid velocity in the system with large A_d:A_r (small A_r) was higher than that obtained from the system with lower A_d:A_r (large A_r). A small riser space resulted in bubbles coalescence in which a large fraction of gas bubbles coalesced to form larger bubbles. These bubbles posed high buoyant force and also moved at high speed and easily left the liquid at the top surface. In addition, the mass conservation law, Equation 4.7, suggested that, for the case of large downcomer cross sectional area, the local liquid velocity in downcomer would be low, and not adequate to drag the bubbles down into the downcomer. Hence, less gas

bubbles in the downcomer were observed and resulting in higher light penetration all through the system. This high light intensity enhanced the growth of the diatom due to the effective photosynthesis.

For the system with smaller A_d:A_r (large A_r), high liquid velocity in downcomer was observed. The smaller A_d enhanced the entrainment of gas bubbles to the downcomer part, and hence, resulting in a higher downcomer gas holdup. Light intensity was consequently blocked by these swarm bubbles which reduced the light penetration into the system. The gas fraction for the system with smaller A_d:A_r was rather high when compared to the system with larger A_d:A_r. This behavior suppressed and/or inhibited an effective growth of the diatom.

Figure 4.7 schematically illustrates the effect of A_d:A_r on the formation of gas bubbles which affected the light penetration of the system. In addition to the light penetration mechanism due to the presence of bubbles, the diatom in the system with larger A_r spent a longer time in the riser due to low riser liquid velocity, and consequently lowered the residence time of the diatom along the downcomer along which high light intensity was supplied. This might also diminish the growth of the diatom in the system.

Limpanuphap, 2003 and Tanthikul, 2004 reported that a greater k_La was observed in the system with smaller A_d:A_r. However, the experimental results indicated that the cultivation of the diatom in the system with greater A_d:A_r yielded the higher maximum cell concentration. It is interesting to note that although the ALPBR with small A_d:A_r have a greater k_La than the system with a large A_d:A_r, the behavior of such system approached to that of the bubble column where a low liquid velocity and a large internal recirculation inside the draft tube took place. Furthermore, as discussed in the previous section, k_La did not play a significant role in dictating the growth of the diatom in the airlift system. The schematic diagram for the phenomena in the ALPBR with different A_d:A_r is illustrated in Figure 4.7 with the comparison of hydrodynamic properties as summarized in Table 4.3.

4.6 Concluding Remarks

The optimal conditions for the cultivation of the diatom *Chaetoceros calcitrans* in the concentric ALPBR were investigated. The maximum cell concentration of 8.88×10^{6} cells mL^{-1} could be achieved with the specific growth rate of 7.41×10^{-2} h^{-1}. The growth of the diatom depended strongly on the level of aeration in the airlift system. Increasing the aeration rate resulted in a better circulation of cells within the system and this sufficed the need of the diatom in obtaining high light intensity for their photosynthesis. The optimal circulation rate was observed at u_{sg} = 3 cm s^{-1} which resulted in the maximum cell concentration and specific growth rate. On the other hand, a further increase in gas velocity to 4 and 5 cm s^{-1} resulted in a decrease in maximum cell concentration and also specific growth rate. This was because of the poor light penetration ability caused by bubble shading effect. This point was being analyzed in detail in the following chapter.

The optimal ratio between downcomer and riser cross sectional area (A_d:A_r) was found to be 2.62. In fact, this might not be an exactly "optimal" ratio as the investigation only covered the area ratio of 1.79 and 2.62. Lowering or increasing the area ratio from this range was found to be not quite practical as the space between the inner and outer columns would be very low and difficult to operate. The results did reveal that a smaller area ratio (of 1.79, or which meant a larger riser) led to a higher downcomer liquid velocity and a subsequent increase in downcomer gas holdup. The high velocity of liquid dragged some gas bubbles down into the annulus which inhibited the light penetration necessary for the photosynthesis.

This chapter demonstrates that the cultivation of the diatom *Chaetoceros calcitrans* in the ALPBR could be successfully achieved with reasonably good outcomes, i.e. a high cell concentration and high growth rate could be obtained. In the following chapters, the bottlenecks of the ALPBR in the cultivation of such diatom were analyzed along with the suggestions on the design of ALPBR to obtain higher cell productivity.

Table 4.1 Optimal growth factors for the cultivation of *Cheatoceros calcitrans* in airlift photobioreactor (Laotaweesup, 2002).

Growth factor	Optimal value
Light intensity (μ molphoton $m^{-2}s^{-1}$)	400
Aeration rate (cm s^{-1})	3
Silica concentration (mg Na_2SiO_3 L^{-1})	3.2
Phosphorus concentration (mg Na_2HPO_4 L^{-1})	2.4
Nitrogen concentration (mg $NaNO_3$ L^{-1})	14
Vitamin B_{12} concentration (μg L^{-1})	1
Specific growth rate (h^{-1})	7.4×10^{-2}
Maximum cell concentration (cells mL^{-1})	8.64×10^{6}

Table 4.2 Comparison between maximum cell concentration and specific growth rate for the cultivation of *Chaetoceros calcitrans* in ALPBR-1 with A_d:A_r = 1.79 and 2.62 at various aeration rates.

Superficial velocity (cm s^{-1})	Maximum cell concentration (cells mL^{-1})		Specific growth rate, μ (h^{-1})	
	A_d:A_r = 1.79	A_d:A_r = 2.62	A_d:A_r = 1.79	A_d:A_r = 2.62
2	5.36×10^{6}	4.84×10^{6}	4.84×10^{-2}	5.69×10^{-2}
3	7.37×10^{6}	8.88×10^{6}	5.78×10^{-2}	7.41×10^{-2}
4	6.24×10^{6}	6.12×10^{6}	5.60×10^{-2}	5.78×10^{-2}
5	1.88×10^{6}	2.82×10^{6}	3.81×10^{-2}	5.01×10^{-2}

Table 4.3 Comparison of hydrodynamic properties of the conventional concentric ALPBR with difference A_d:A_r.

Hydrodynamic properties	Small A_d:A_r	Large A_d:A_r
Riser cross sectional area	High	Low
Gas velocity (same aeration rate)	Low	High
Riser liquid velocity (same aeration rate)	Low	High
Downcomer liquid velocity (same aeration rate)	High	Low
Riser gas holdup	Low	High
Downcomer gas holdup	High	Low
Mass transfer area	High	Low
Mass transfer coefficient	High	Low
Light penetration ability	Low	High

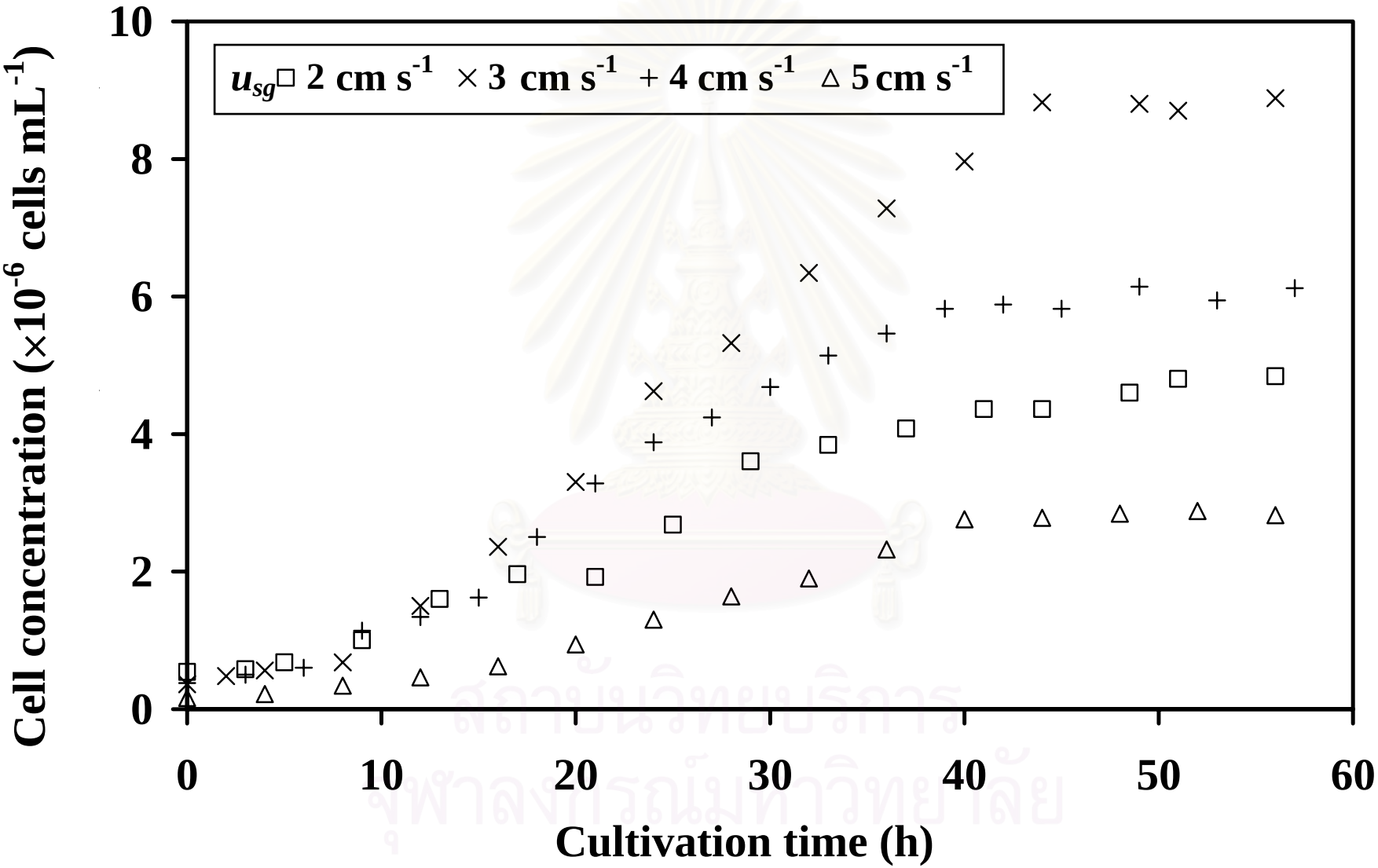


Figure 4.1 Effect of superficial gas velocity on the growth of *Chaetoceros calcitrans* (*Ad:Ar* = 2.62).

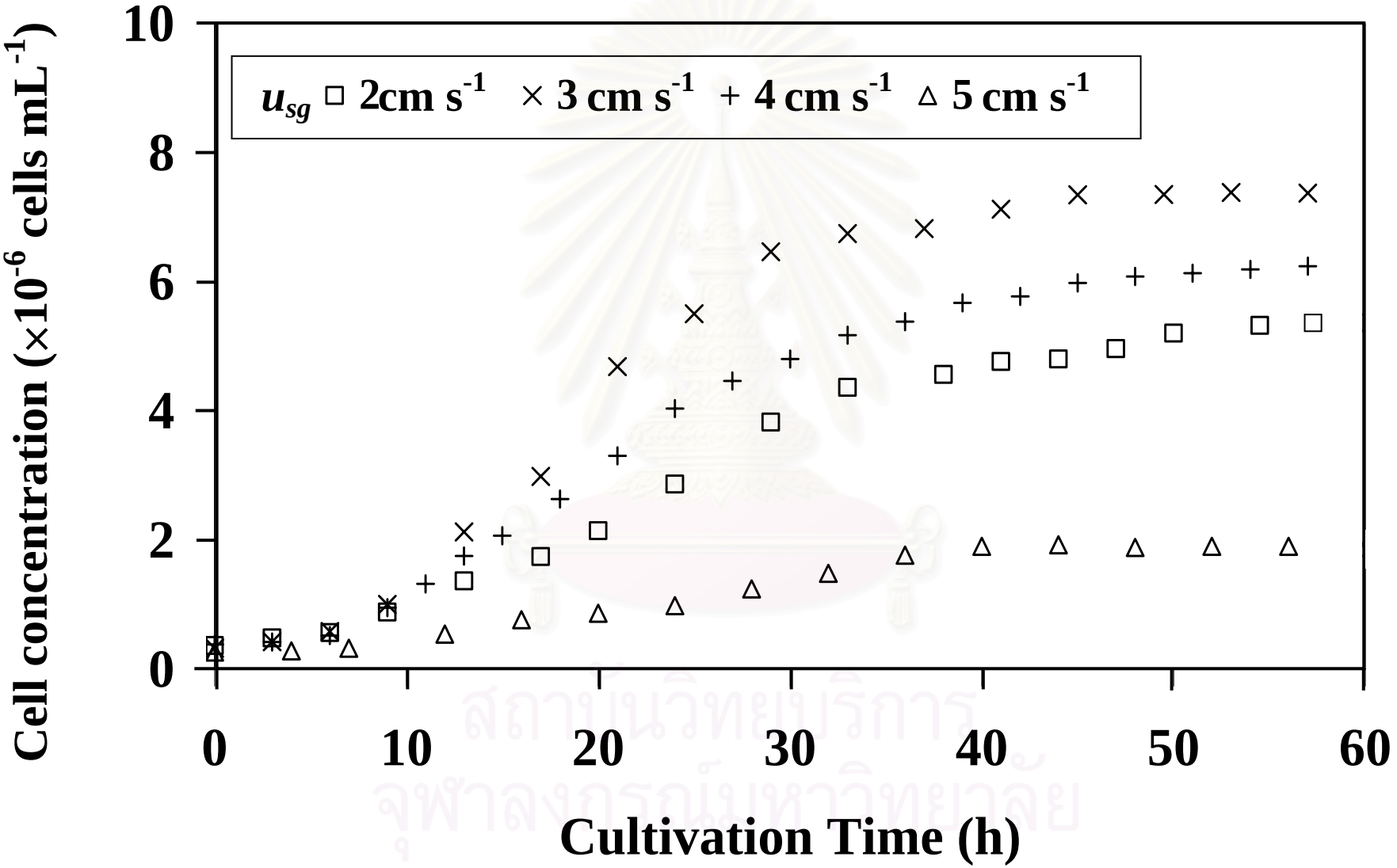


Figure 4.2 Effect of superficial gas velocity on the growth of *Chaetoceros calcitrans* (*Ad:Ar* = 1.79).

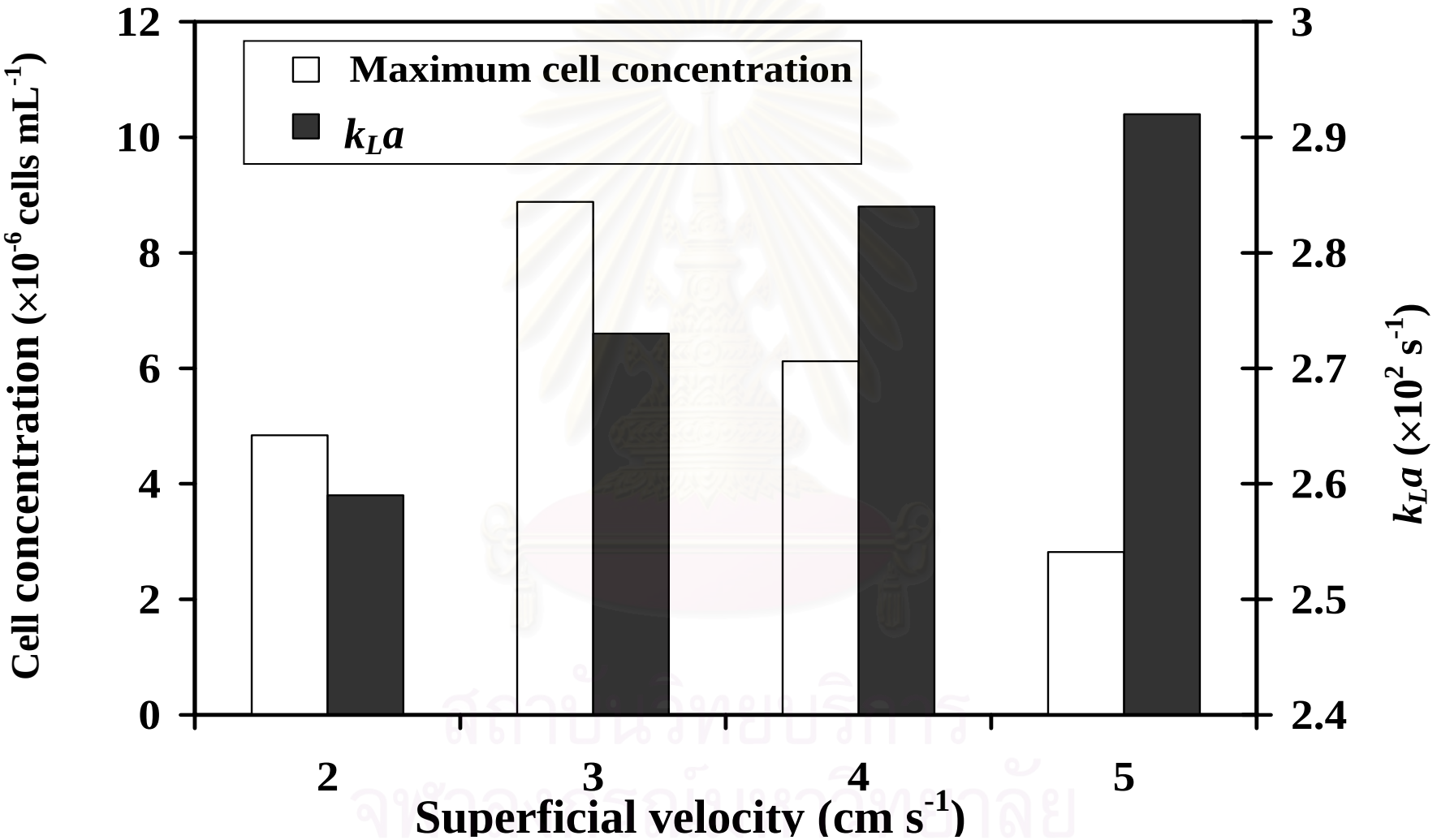


Figure 4.3 Relation ship between maximum cell concentration and $k_l a$.

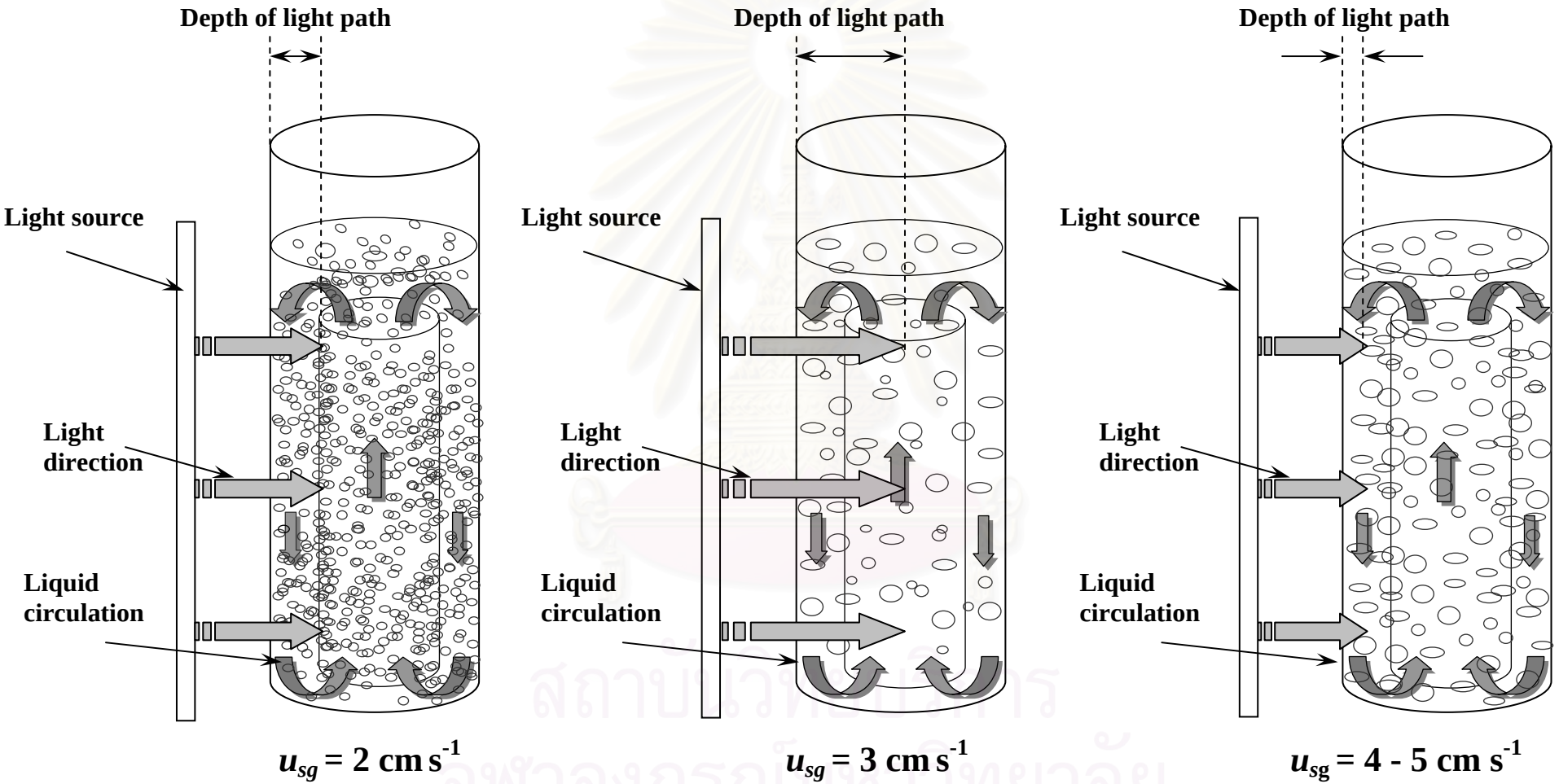


Figure 4.4 Effect of superficial gas velocity on bubble formation and light penetration in ALPBR

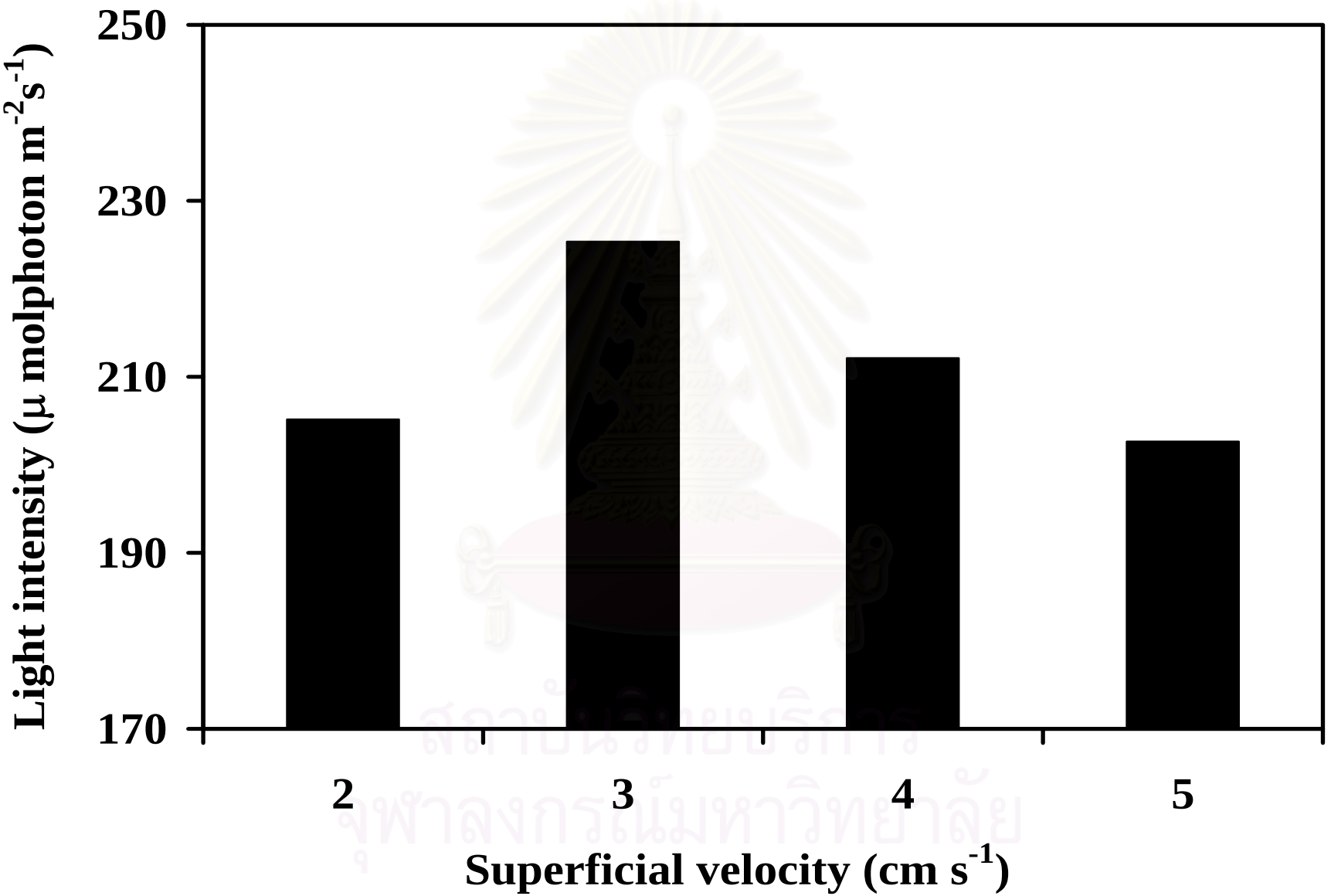


Figure 4.5 Effect of superficial gas velocity on light intensity in ALPBR-1

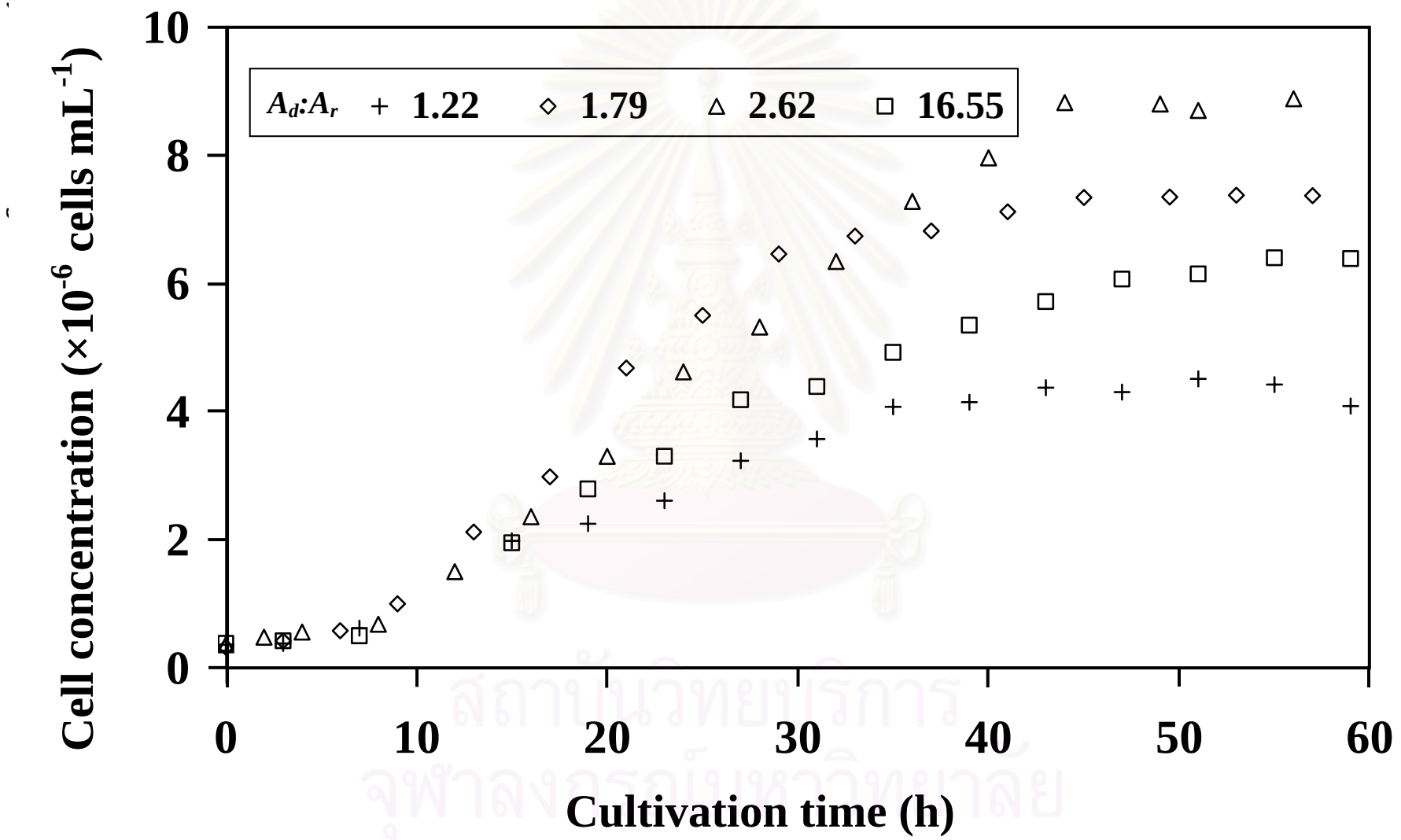
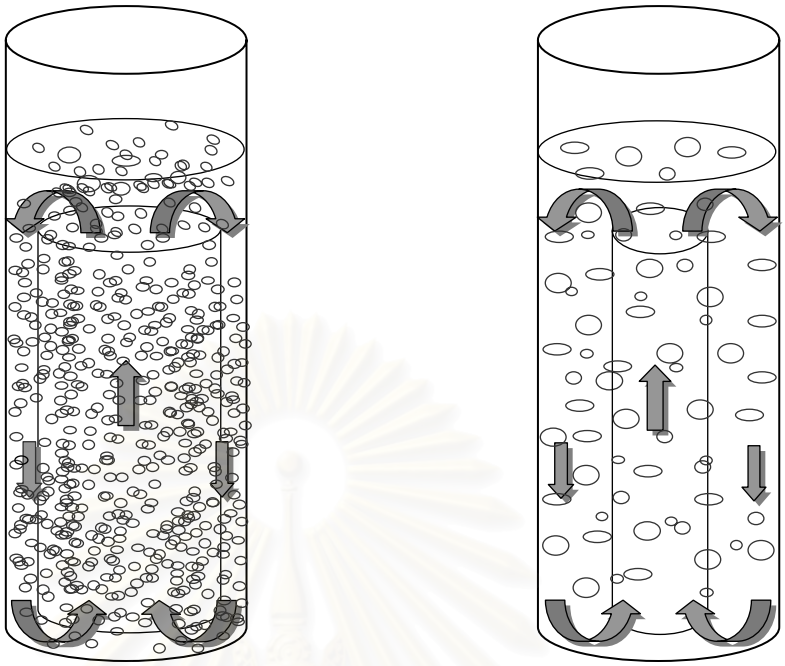


Figure 4.6 Effect of A_d:A_r on the growth of *Chaetoceros calcitrans* in ALPBR-1 with u_{sg} = 3 cm s^{-1}



Small A_d:A_r **Large A_d:A_r**

Figure 4.7 Effect of A_d:A_r on bubble formation in ALPBR.

CHAPTER 5

Enhanced Productivity of *Chaetoceros calcitrans* in Airlift Photobioreactors

5.1 Summary

The cultivation of *Chaetoceros calcitrans* in airlift photobioreactor (ALPBR) was examined. The culture was cultivated in the batch, semi-continuous, continuous and large scale systems. The cultivations in both semi-continuous and continuous systems resulted in high cell productivity although the steady state cell concentrations in both systems were lower than that obtained from the batch system. The behavior of the large scale airlift system was not significantly different from the conventional bubble column where a large amount of the diatom could be produced at low cell density. The main limiting factor for the large scale airlift culture was the availability of light. In all systems, the continuous cultivation in the 2.8L ALPBR with a medium feed rate of 3 mL min^{-1} was most economical where the operation cost could be maintained at a minimum of approx. 7.95×10^{-4} THB $L^{-1}h^{-1}$. However, this continuous small scale system suffered from relatively low cell productivity (8.10×10^{4} cells s^{-1}).

5.2 Backgrounds

Conventionally, the cultivation of the diatom, *Chaetoceros calcitrans*, is cultivated in the opened pond system. However, the system faces severy drawbacks due to the contamination and low specific growth rate which led to low productivity. Airlift photobioreactor (ALPBR) was proposed as an alternative for the cultivation system for *Chaetoceros calcitrans* where a high maximum cell concentration of 8.88×10^{6} cells mL^{-1} could be achieved from the batch cultivation with a maximum specific growth rate of 7.41×10^{-2} h^{-1} at u_{sg} = 3 cm s^{-1} (see Chapter 4). Nevertheless, batch operation is often encountered drawbacks regarding the production scheduling, reactor maintenance, etc. Hence, the cultivation of the diatom in semi-continuous and

continuous system was introduced as an alternative to achieve greater productivity. Furthermore, our previous study pointed out that the growth of the diatom in ALPBR strongly depended on system performances i.e. flow behavior and light utilization. As a result, the modification of airlift system together with the mode of operation could therefore lead to the achievement of higher productivity. Thus far, the investigation on the growth of *Chaetoceros calcitrans* in various designs of ALPBR was not available in literature. The aim of this chapter was therefore to investigate the effect of modes of operation for the cultivation of *Chaetoceros calcitrans* in various types of ALPBR, i.e. batch, semi-continuous, continuous and the large scale cultivations.

5.3 Experimental

The experiment was carried out as batch cultivation and semi-continuous cultivation in the 17L ALPBR (ALPBR-1) as described in Section 3.1.1. The continuous cultivation was also investigated in the 2.8 L ALPBR (ALPBR-2) as described in Section 3.1.2 together with the large scale cultivation in 170 L reactors in BC-2, ALPBR-4 and ALPBR-5 as described in Section 3.1.4. The experimental preparation was held as reported in Section 3.2.1 and the growth behavior in each system was determined based on the procedure in Sections 3.2.3, 3.2.4 and 3.2.7.

5.4 Cultivation in Batch System

Figure 5.1 illustrates the comparison between the performance of BC-1 and ALPBR-1 for the cultivation of *Chaetoceros calcitrans*. The growth behavior in the airlift system clearly outperformed that in the bubble column as the cultivation in the airlift system provided almost a two-fold productivity of the diatom than that in the bubble column. The reason for this could be due to the differences in liquid flow behavior in both systems. In ALPBR, the diatom was carried along the column height due to the energy transfer from gas bubbles. As the bubbles were separated from the liquid at the top surface, the heavier liquid and the diatom moved down the column in the downcomer section and re-entered the riser through the space provided at the bottom of the system. This circulation allowed the diatom to constantly move to the “high light intensity” area, i.e. in downcomer section. Apparently, this was proven to

be beneficial for photosynthesis of the diatom. In the case of BC, the movement of liquid was random as the liquid and bubbles were completely mixed in one chamber. The low liquid velocity condition did not support a proper circulation of the diatom, and the sedimentation of the diatom could, at times, be observed at the bottom of the column. Therefore the diatom could not be exposed to high light intensity, and hence, ineffective photosynthesis was resulted and this led to a low productivity.

Figure 5.2 illustrates the growth curve of the diatom in the airlift system where the culture was started with an initial cell concentration of approx. 1×10^5 cells mL^{-1} (u_{sg} = 3 cm s^{-1}). After a 9 hour lag phase, the diatom entered its exponential growth period where the cell concentration rapidly increased before reaching the stationary phase where the maximum cell density was approx. 8.88×10^6 cells mL^{-1}. This growth process reached the maximum cell concentration in 44 hours which rendered a specific growth rate of about 7.41×10^{-2} h^{-1}. This was equivalent to a productivity of 9.42×10^5 cells s^{-1} or a specific productivity of 5.61×10^4 cells L^{-1} s^{-1}.

5.5 Cultivation in Semi-continuous System

The batch cultivation, although could provide a relatively high final cell concentration, often suffered from an initially slow growth regime, not to mention the loss of time during the final system cleansing. The cultivation of *Chaetoceros calcitrans* in the semi-continuous system was therefore investigated to examine the probability of enhancing the productivity of the diatom. Figure 5.3 illustrates the result for the cultivation of *Chaetoceros calcitrans* in the 17 L semi-continuous system where the diatom was cultivated with an initial cell concentration of approx. 1×10^5 cells mL^{-1} and u_{sg} = 3 cm s^{-1}. The diatom was initially cultured as a batch system for 30 hours to ensure an active cell condition before harvesting. Approximately 12 L of culture was harvested where a fresh F/2 solution was then added to ALPBR-1 to replace the harvested volume. This diluted the culture down from approx. 3.86×10^6 to 1.27×10^6 cells mL^{-1}. The culture was then continued for 12 hours before the next harvest cycle to allow the diatom to reach the mid point of the exponential phase where the diatom was most active. An average maximum cell concentration achieved from each cycle was around 3.86×10^6 cells mL^{-1} with a

specific growth rate of about 9.25×10^{-2} h^{-1}. The summary of the performance in each cycle of this semi-continuous system is provided in Table 5.1. The cultivation of the diatom in the semi-continuous system achieved an average productivity of approx. 7.19×10^{5} cells s^{-1} or equivalent to a specific productivity of 5.56×10^{4} cells $L^{-1}s^{-1}$ which was only marginally lower than those obtained from the batch system.

5.6 Prediction of Airlift System Performance

The prediction of system performance for batch and semi-continuous cultures in ALPBR-1 could be achieved through the following derivations. For the batch system, the mass balance around the reactor results in:

$$V\frac{dx(t)}{dt} = \mu x(t)V \qquad (5.1)$$

The Logistic law (Bailey and Ollis, 1986) was assumed to explain the growth rate of the diatom where

$$\mu = \mu_m(1-\frac{x(t)}{x_m}) \qquad (5.2)$$

Substitute Eq. (5.2) into Eq. (5.1) yields:

$$V\frac{dx(t)}{dt} = \mu_m(1-\frac{x(t)}{x_m})x(t)V \qquad (5.3)$$

This could be integrated to:

$$x(t) = \frac{x_o x_m e^{\mu_m t}}{x_m - x_o + x_o e^{\mu_m t}} \qquad (5.4)$$

where $x(t)$ = cell concentration (cells mL^{-1})

x_o = cell concentration (cells mL^{-1})

x_m = maximum cell concentration (cells mL^{-1})

t = culturing time (h)

μ = specific growth rate (h^{-1})

μ_m = maximum specific growth rate (h^{-1})

V = cultivating volume (L)

The maximum cell concentration (x_m) and the maximum specific growth rate (μ_m) are system dependent and were therefore obtained from the experiment in the batch cultivation, and in this case, these two parameters were found to be 6.64×10^6 cells mL^{-1} and 1.4×10^{-1} h^{-1}, respectively. Eq. (5.4) was then used to simulate the growth curve of the diatom in both batch and semi-continuous modes. Figure 5.3 illustrates that the model predictions agreed reasonably well with experimental results. For the simulation of the semi-continuous culture, the initial cell concentration for each cycle was controlled at approx. 1.2×10^6 cells mL^{-1} and the harvesting period was maintained at 12 hours. The model was then used to estimate the cell concentration in each harvesting cycle. The predicted productivity of the semi-continuous system using this model was found to be 6.32×10^5 cells s^{-1}, which was slightly lower than the actual level of 7.19×10^5 cells s^{-1}. This was equivalent to an error of approximately 12%.

The model was further implemented to predict the harvesting period for which the maximum productivity could be achieved in both batch and semi-continuous systems. The maximum productivity for the batch operation was predicted by using the following correlations:

$$\text{Productivity} = \left(\frac{x(t)-x_o}{t-t_o}\right)\times V \qquad (5.5)$$

To obtain the maximum productivity, the time differentiation of Eq. (5.5) must be equal to zero:

$$V\frac{d}{dt}\left(\frac{x(t)-x_o}{t}\right)=0 \qquad (5.6)$$

Substitute Eq. (5.4) into Eq. (5.6) yields:

$$V\frac{d}{dt}\left(\frac{\dfrac{x_o x_m e^{\mu_m t}}{x_m - x_o + x_o e^{\mu_m t}} - x_o}{t}\right) = 0 \qquad (5.7)$$

Eq. (5.7) suggests that the maximum productivity would be obtained if the system was allowed to operate for 41 hours, and with x_o = 1.03×10^5 cells mL^{-1}. The relationship between the cell productivity in the batch system and the culturing time was shown in Figure 5.4.

For the semi–continuous cultivation, the maximum productivity can be calculated from:

$$\frac{d}{dt}\left(\frac{x(t)-x_o}{t-t_o}\right)\left(V_o - V(t)\right) = 0 \qquad (5.8)$$

where V_o = initial cultivating volume (L)

$V(t)$ = remaining volume after the harvest (L)

From the balance of component *x*;

$$V_o x_o = V(t)x(t) \qquad (5.9)$$

$$V(t) = \frac{V_o x_o}{x(t)} \qquad (5.10)$$

Eqs. (5.4) and (5.10) can be substituted into Eq. (5.8) which allowed the determination of the cycle time required to yield the maximum productivity (with V_o = 17L and x_o = 1.26×10^6 cells mL^{-1}). In this case, it was predicted that the maximum productivity of 7.22×10^5 cells s^{-1} could be achieved after 19 hours of cultivating period as illustrated in Figure 5.5. However, this predicted maximum productivity was quite close to the actual attainable productivity with 12 hours cultivation.

5.7 Continuous Cultivation System

This section focused on the continuous cultivation of the diatom *Chaetoceros calcitrans* in ALPBR-2. The culture was remained in the batch mode in the first 30 hours to ensure that the cells already passed through their lag phase. As the diatom reached its exponential phase (with cell concentration of approx. 2.2–2.5×10^6 cells mL^{-1}), the mixture between fresh sea water and F/2 medium was then fed to the system at the flow rate in the range of 2-4 mL min^{-1}. The overflow at the top section of the column allowed an effective level control of this system.

As the system was switched to the continuous mode, the cell concentration started to drop due to the dilution effect (see Figure 5.6). After another 10 hours or so, the steady state could be observed. The cultivation of the diatom with the nutrient feed rates of 2, 3 and 4 mL min^{-1} resulted in the dilution rates of 4.29×10^{-2}, 6.43×10^{-2} and 8.57×10^{-2} h^{-1}, respectively. The maximum final cell concentration of approx. 1.95×10^6 cells mL^{-1} was obtained at the feed rate of 2 mL min^{-1}. At this condition, the attainable specific productivity was 2.32×10^4 cells $L^{-1}s^{-1}$ (or equivalent to a productivity of 6.5×10^4 cells s^{-1}). Due to a higher dilution effect, the maximum steady state cell concentration for the cultivation at the medium feed rate of 3 mL s^{-1} was only 1.62×10^6 cells mL^{-1}. Although this value was lower than that obtained from the case with the medium feed rate of 2 mL^{-1}, the productivity was higher, at approx. 8.10×10^4 cells s^{-1} or equivalent to a specific productivity of 2.89×10^4 cells L^{-1} s^{-1}. For the case where the medium feed rate was maintained at 4 mL min^{-1}, the final cell concentration was unable to reach the steady state concentration as the diatom seemed to be continually diluted by the feed of nutrient. In conclusion, for the range of operating condition employed in this work, the continuous ALPBR system should be conducted with the nutrient feed rate of 3 mL min^{-1} to ensure the highest level of cell productivity.

5.8 Large Scale Cultivation

Conventionally, the diatom *Chaetoceros calcitrans* was cultivated in a large scale pond. The main reason for this was perhaps the economic of scale as a large scale system often requires a lower investment when compared to small systems with the same volume. This section examined the performance of such system compared with that of smaller systems. This is to investigate whether the large scale cultivation was, in fact, suitable to the growth of the diatom.

The results from the cultivation of *Chaetoceros calcitrans* in the large scale system was illustrated in Figure 5.7. It was found that the maximum cell concentration of the diatom in ALPBR-4 (2.96×10^{6} cells mL^{-1}) and in ALPBR-5 (3.04×10^{6} cells mL^{-1}) were slightly higher than that from BC-2 (2.51×10^{6} cells mL^{-1}). However, the specific growth rate of the diatom in the large scale cultivation for the cultivation in bubble column and in both ALPBRs was not significantly different, i.e. $\mu = 2.46\times10^{-2}$ h^{-1} for BC-2, 2.58×10^{-2} h^{-1} for ALPBR-4 and 2.52×10^{-2} h^{-1} for ALPBR-5. Wongsuchoto *et al*. (2003) reported that there existed non-ideal flow particularly in airlift systems with large riser where the internal circulation took place within the riser itself. This local circulation caused the system to behave like a bubble column. Therefore, in ALPBR-5, the riser was divided into 4 sub-sections, each with a smaller diameter. This was to minimize the local circulation in the riser and a better liquid circulation within the system was anticipated. However, the large system was limited by the capacity of the air pump and the maximum aeration rate of 250 mL s^{-1} could only be achieved. Therefore, under the operating conditions employed in this work, the difference in the behaviors of the three systems might not be enough to have significant effect on the growth of the diatom, as obvious from the resulted mentioned above.

The cultivation of the diatom in these large scale systems was demonstrated to increase the productivity of the diatom. This was mainly due to the increase in the harvested volume when compared to the small systems.

5.9 Limiting Factors for The Operation in Large Scale Systems

One of the main drawbacks for the cultivation of the diatom in large scale systems was the poor light utilization. The light source could only be supplied around the wall and at the top of the tank, and with this setup, the light intensities measured in the system were around 48–50 μ mol photon $m^{-2}s^{-1}$ near the inner wall, 72 μ mol photon $m^{-2}s^{-1}$ at the outer wall, 52 μ mol photon $m^{-2}s^{-1}$ near the surface of liquid level, and approx. 30 μ mol photon $m^{-2}s^{-1}$ at the center of the tank. In particular, when the cells grew high in density, poor light penetration became a serious problem that retarded the effective growth of the diatom.

To further investigate the effect of light intensity on the growth of the diatom, the cultivation of the diatom was carried out in a smaller scale 2.8L ALPBR (ALPBR-2) at similar light intensity levels to the large tank. Figure 5.8 demonstrates the comparison between the cultivation in these two different scale ALPBRs. It was obvious that the growth rate in ALPBR-4 at the light intensity (at the wall) of 75 μ molphoton $m^{-2}s^{-1}$ was even lower than that in ALPBR-2 with the light intensity of 64 μ molphoton $m^{-2}s^{-1}$. However, it should be mentioned that the ability of the passage of light through the large scale system was much lower than that in the small scale system. Therefore the higher light intensity at the wall in the large scale system did not necessarily mean that the light intensity would remain high within the culture. Unlike the large system, the light intensity in the small scale system should remain almost constant throughout the column. Nevertheless, as the performance of ALPBR-2 with low light intensity became closer to that of ALPBR-4 at the same range of light intensity, this suggested that the findings in the small scale could, to some extent, be used to explain the phenomena within the large scale system.

Figure 5.8 also reveals that the light intensity could significantly affect the growth of the diatom, and in this case, the greater final cell density of around 7.08×10^{6} cells mL^{-1} with 4.24×10^{-2} h^{-1} specific growth rate was obtained at the light intensity of 110 μ molphoton $m^{-2}s^{-1}$ whereas the lower light intensity (64 μ molphoton $m^{-2}s^{-1}$) yielded a much lower cell concentration of 3.84×10^{6} cells mL^{-1} (3.42×10^{-2} h^{-1} specific growth rate). This finding implied that insufficient light intensity could

negatively affect the performance, and this was the case of the large scale system, and as long as the light intensity could not be maintained at higher level (without causing excessive heat), the cultivation in the large scale system could only be subject to low cell density.

5.10 Economics of Cultivation Systems for *Chaetoceros calcitrans*

The economical analysis for the cultivation of the diatom *Chaetoceros calcitrans* in various scales of ALPBRs was carried out where the results are shown in Table 5.3. The systems involved in this analysis were the batch cultivation (ALPBR-1), semi-continuous cultivation (ALPBR-1), continuous cultivation (ALPBR-2) and the large scale cultivation (ALPBR-4). This analysis was based on the production of 1×10^{12} cells. It was observed that the most attractive system for the cultivation of the diatom in marine hatcheries was the continuous cultivation of the diatom in the 2.8L ALPBR (ALPBR-2) with the medium feed rate of 3 mL min^{-1}. The lowest operating cost, approx. 7.95×10^{-4} THB $L^{-1}h^{-1}$, was obtained which was the lowest when compared to any other systems. Surprisingly, the productivity of this system was found to be the lowest among the four systems. The benefits of this system were derived from a number of advantages when compared to the larger systems, i.e. low overhead charge due to the labor cost, minimal lost of time during the start up and shut down period or for system maintenance. As the cells remained active at all time in the continuous system, the culture medium could be switched to the new reactor as soon as the maintenance was needed without disturbing the growth of the diatom. The maintenance and equipment costs in this system, i.e. reactor, air compressor and light source, were also lower than the set up for larger systems.

5.11 Concluding Remarks

This investigation described the achievement in the cultivation of *Chaetoceros calcitrans* in various reactor modes. It was found that, the semi-continuous and the continuous systems could be successfully implemented to minimize the maintenance of the batch system. The large scale system was also practiced, but could only reach a small final cell concentration. Due to the size of the

harvested volume, the large scale was found to produce cell at the highest productivity. The main drawback for this large system was the limitation on the availability of light which restricted the growth of the diatom. The economical analysis pointed out that small culture system running in continuous mode seemed to be the most attractive choice for the cultivation of *Chaetoceros calcitrans*.

Table 5.1 Initial and harvest cell concentrations, productivity and specific growth rate of *Chaetoceros calcitrans* with semi-continuous culturing system in ALPBR-1 (12 L harvested at every 12 h).

Cycle	Initial cell concentration (cells mL^{-1})	Harvested cell concentration (cells mL^{-1})	Specific growth rate (h^{-1})	Productivity (cells s^{-1})
1	1.26×10^{6}	3.86×10^{6}	9.33×10^{-2}	7.22×10^{5}
2	1.28×10^{6}	4.08×10^{6}	9.66×10^{-2}	7.78×10^{5}
3	1.27×10^{6}	3.66×10^{6}	8.82×10^{-2}	6.64×10^{5}
4	1.28×10^{6}	3.92×10^{6}	9.33×10^{-2}	7.33×10^{5}
5	1.27×10^{6}	3.78×10^{6}	9.09×10^{-2}	6.97×10^{5}
Average	**1.27×10^{6}**	**3.86×10^{6}**	**9.25×10^{-2}**	**7.19×10^{5}**

Table 5.2 Comparison between maximum cell concentration, productivity, specific productivity and specific growth rate for the cultivation of *Chaetoceros calcitrans* in BCs and ALPBRs in various modes of operation.

Systems	Harvest time (h)	Maximum cell concentration (cells mL^{-1})	Productivity (cells s^{-1})	Specific productivity (cells $L^{-1}s^{-1}$)	Specific growth rate (h^{-1})
Batch BC-1	60 h	4.96×10^{6}	3.83×10^{5}	2.25×10^{4}	2.88×10^{-2}
Batch ALPBR-1	44 h	8.88×10^{6}	9.42×10^{5}	5.54×10^{4}	7.41×10^{-2}
Semi-continuous ALPBR-1	> 12 h	3.86×10^{6}	7.19×10^{5}	6.01×10^{4}	9.25×10^{-2}
Continuous* ALPBR-2	> 30 h	1.62×10^{6}	8.10×10^{4}	2.89×10^{4}	6.42×10^{-2}
Large scale BC-2	80 h	2.51×10^{6}	1.21×10^{6}	7.12×10^{3}	2.46×10^{-2}
Large scale ALPBR-4	80 h	2.96×10^{6}	1.48×10^{6}	8.68×10^{3}	2.58×10^{-2}

*continuous culturing system with a medium feed rate of 3 mL min^{-1}

Table 5.3 Economical analysis for the cultivation of 1×10^{12} cells of *Chaetoceros calcitrans* in ALPBRs.

		Working volume			
		Batch (ALPBR-1)	**Semi-continuous (ALPBR-1)**	**Continuous* (ALPBR-2)**	**Large scale (ALPBR-4)**
Effective volume (L)	**[A]**	17	12	–	170
Cycle time (h)	**[B]**	44	12	–	80
Productivity (cells s^{-1})**	**[C]**	9.42×10^{5}	7.19×10^{5}	8.10×10^{4}	1.48×10^{6}
Specific productivity (cells $L^{-1}s^{-1}$)**	**[D]**	5.54×10^{4}	5.99×10^{4}	2.89×10^{4}	8.68×10^{3}
Cultivation time (h)	**[E=(1×10^{12}×3,600)÷C]**	295	386	3,429	188
Number of cycle (–)	**[F=E÷B]**	6.70	32.17	–	2.35
Total volume of sea water used (L)	**[G=A×F]**	114	386	617	400
Cost of water, 0.06 THB L^{-1} (THB)	**[H=0.06×G]**	6.84	23.16	37.03	23.97
Cost of nutrient, 1 THB L^{-1} (THB)	**[I=G×1]**	114	386	617	400
Power of air compressor (W)	**[J]**	300	300	60	1,600
Power of light source (W)	**[K]**	400	400	40	378
Total electrical unit (units)	**L=(J+K)×E÷1,000]**	207	270	343	372

Electrical cost, 3 THB per unit (THB)	**[M=3×L]**	620	811	1,029	1,116
Total investment cost (THB)	**[N=H+I+M]**	740	1,220	1,683	1,539
Investment cost per hour (THB h^{-1})	**[O=N÷E]**	2.51	3.16	0.49	8.19
Specific investment cost (THB $L^{-1}h^{-1}$)	**[P=O÷G]**	2.20×10^{-2}	8.19×10^{-3}	7.95×10^{-4}	2.05×10^{-2}

*continuous cultivation with a medium flow rate of 3 mL min^{-1}
** from Table 5.2

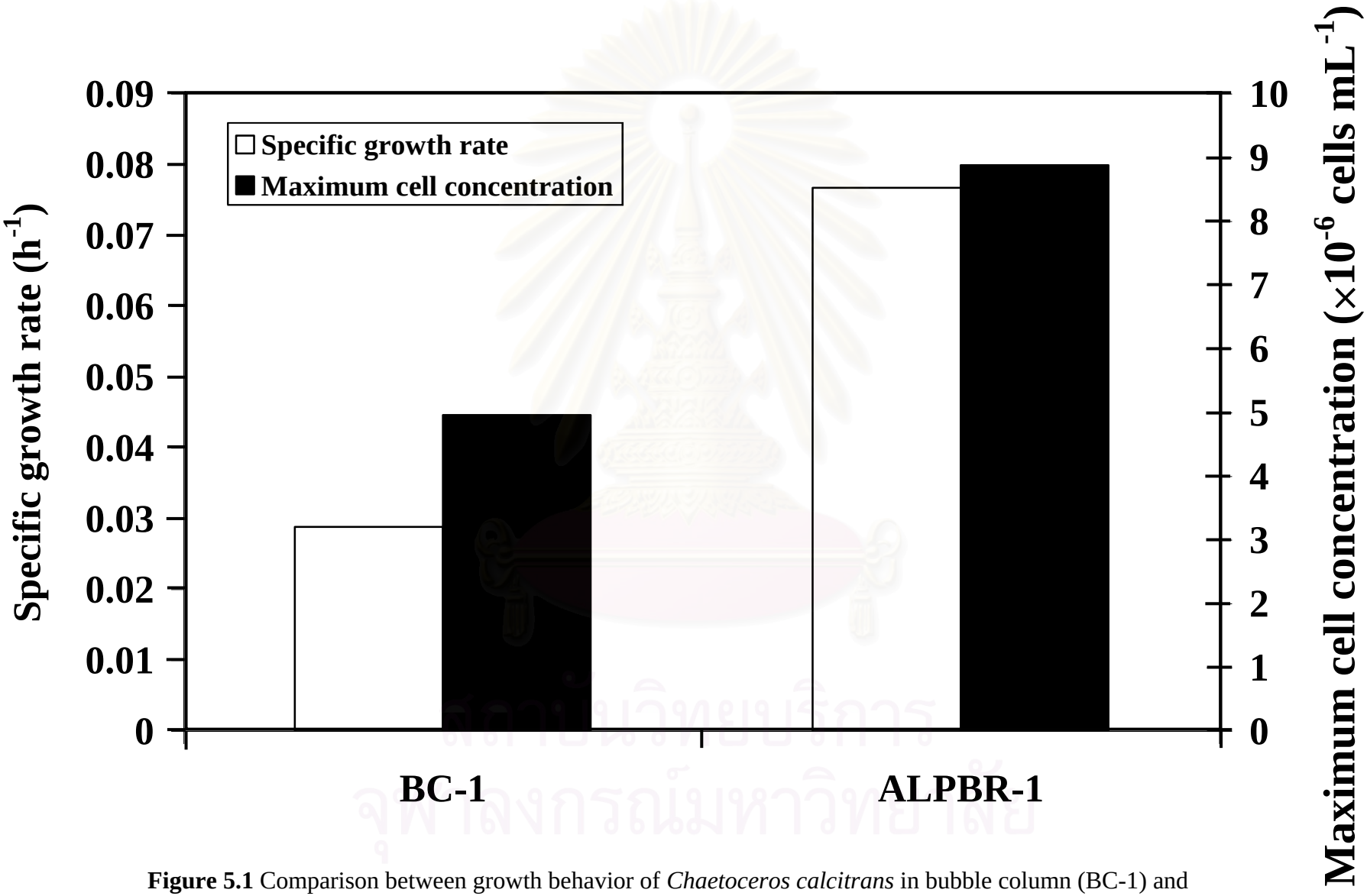


Figure 5.1 Comparison between growth behavior of *Chaetoceros calcitrans* in bubble column (BC-1) and airlift photobioreactor (ALPBR-1) .

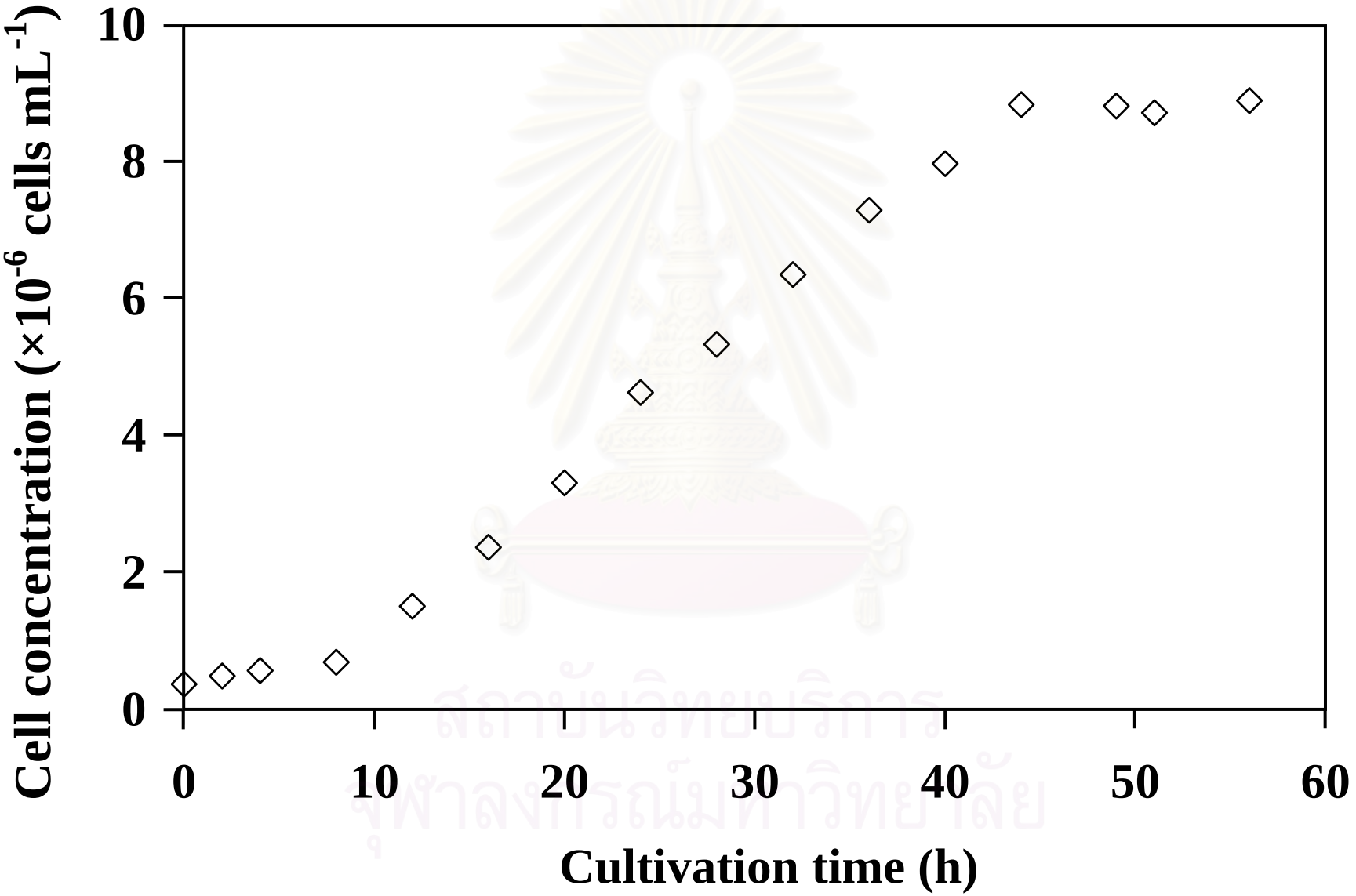


Figure 5.2 Growth curve of *Chaetoceros calcitrans* in batch culturing system (ALPBR-1 with u_{sg} = 3 cm s^{-1}).

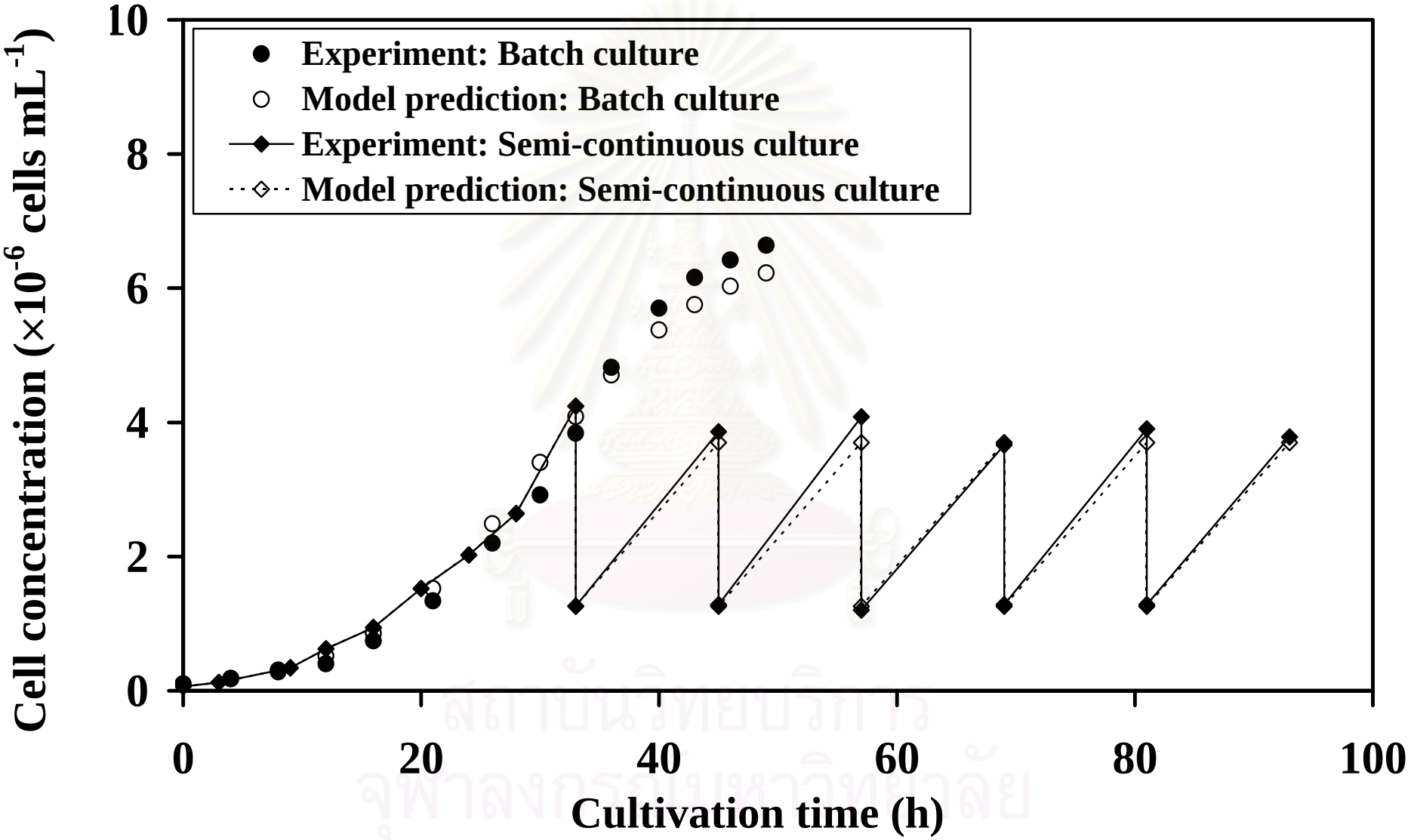


Figure 5.3 Comparison between growth behavior of *Chaetoceros calcitrans* for the cultivation in batch and semi-continuous culturing system (ALPBR-1 with u_{sg} = 3 cm s^{-1}) and growth behavior from model prediction.

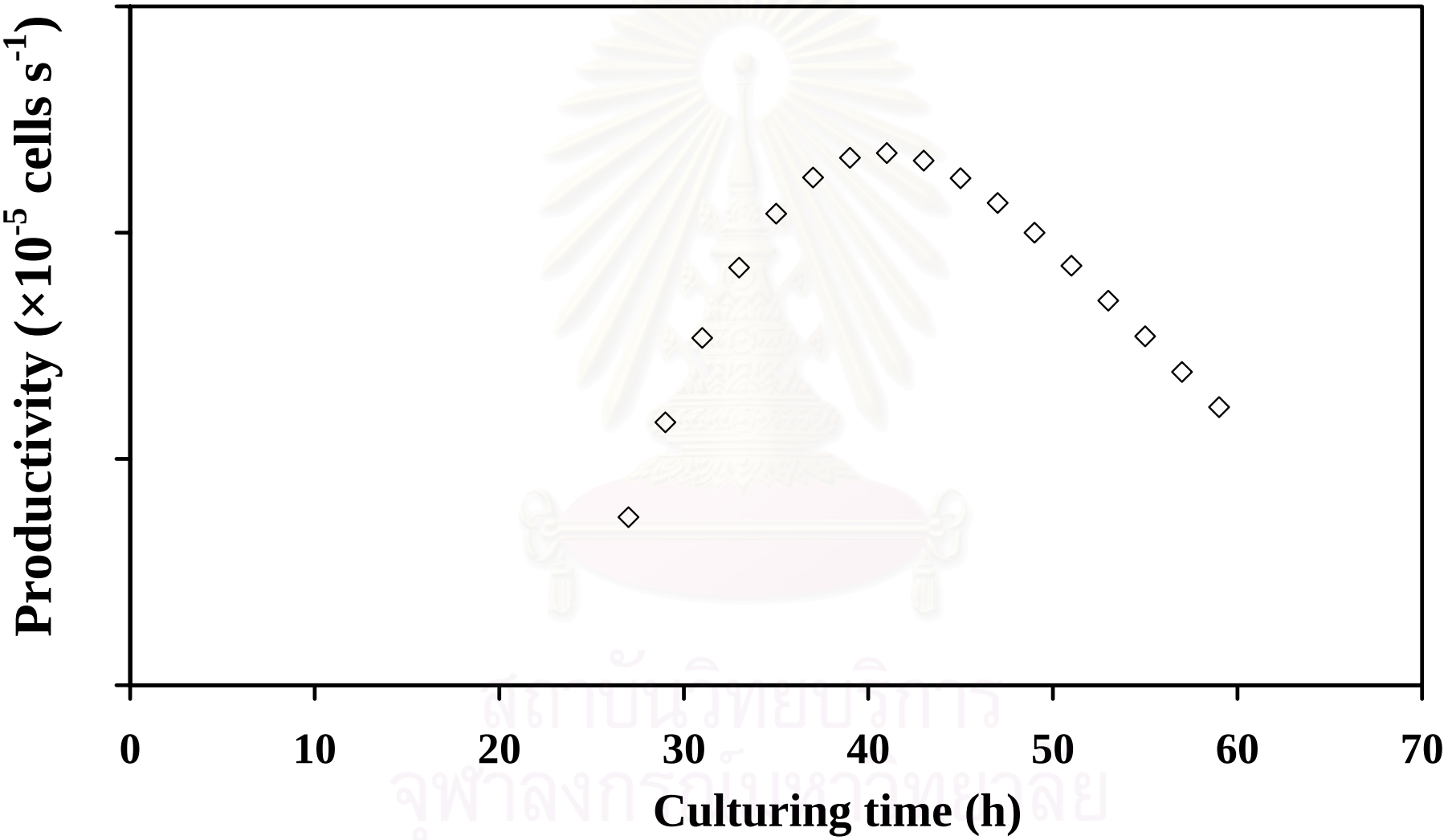


Figure 5.4 Prediction of productivity at various culturing time for the batch cultivation of *Chaetoceros calcitrans* in ALPBR-1 (u_{sg} = 3 cm s^{-1}).

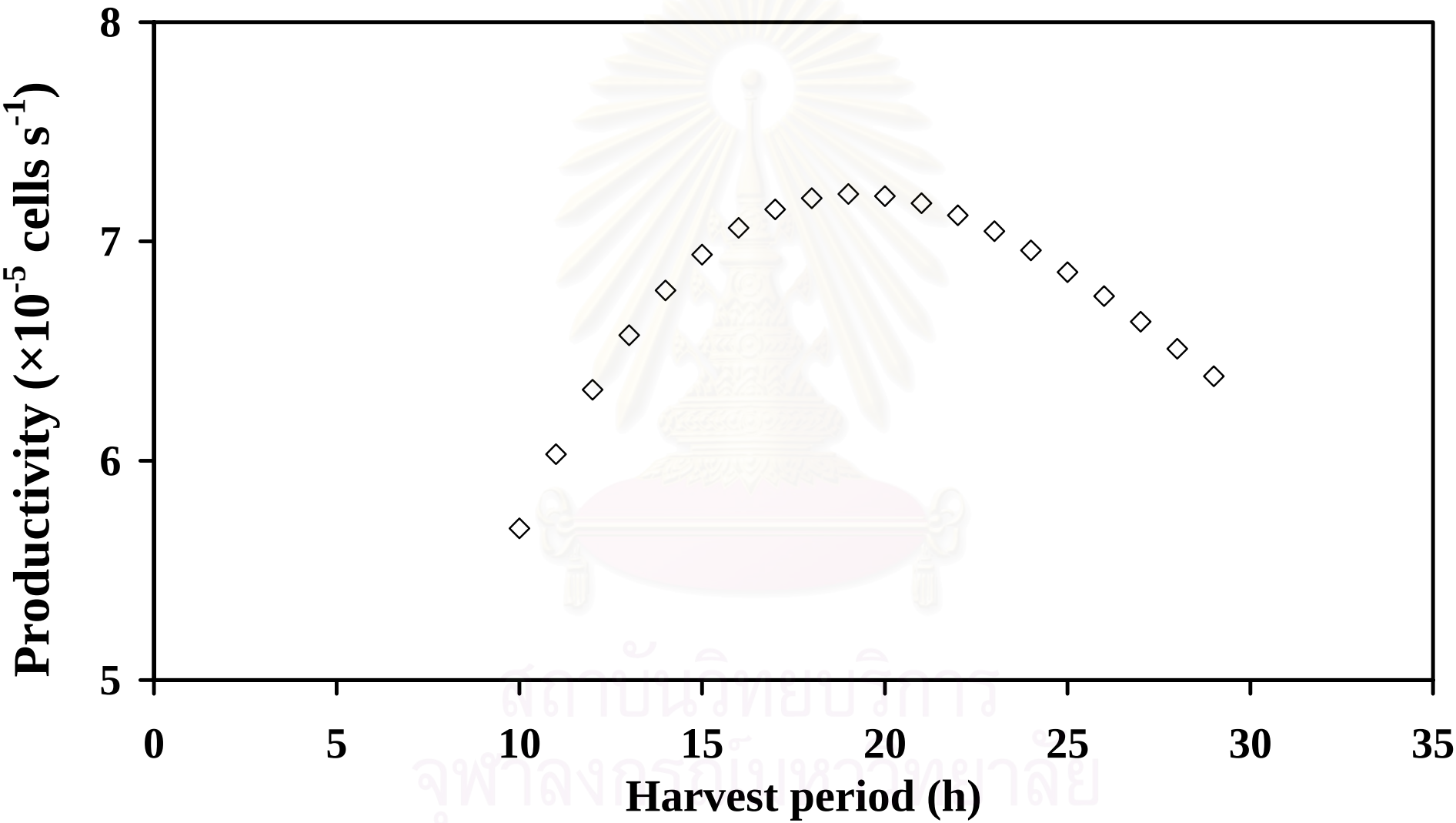


Figure 5.5 Prediction of productivity at various culturing time for the semi-continuous cultivation of *Chaetoceros calcitrans* in ALPBR-1 (u_{sg} = 3 cm s^{-1}).

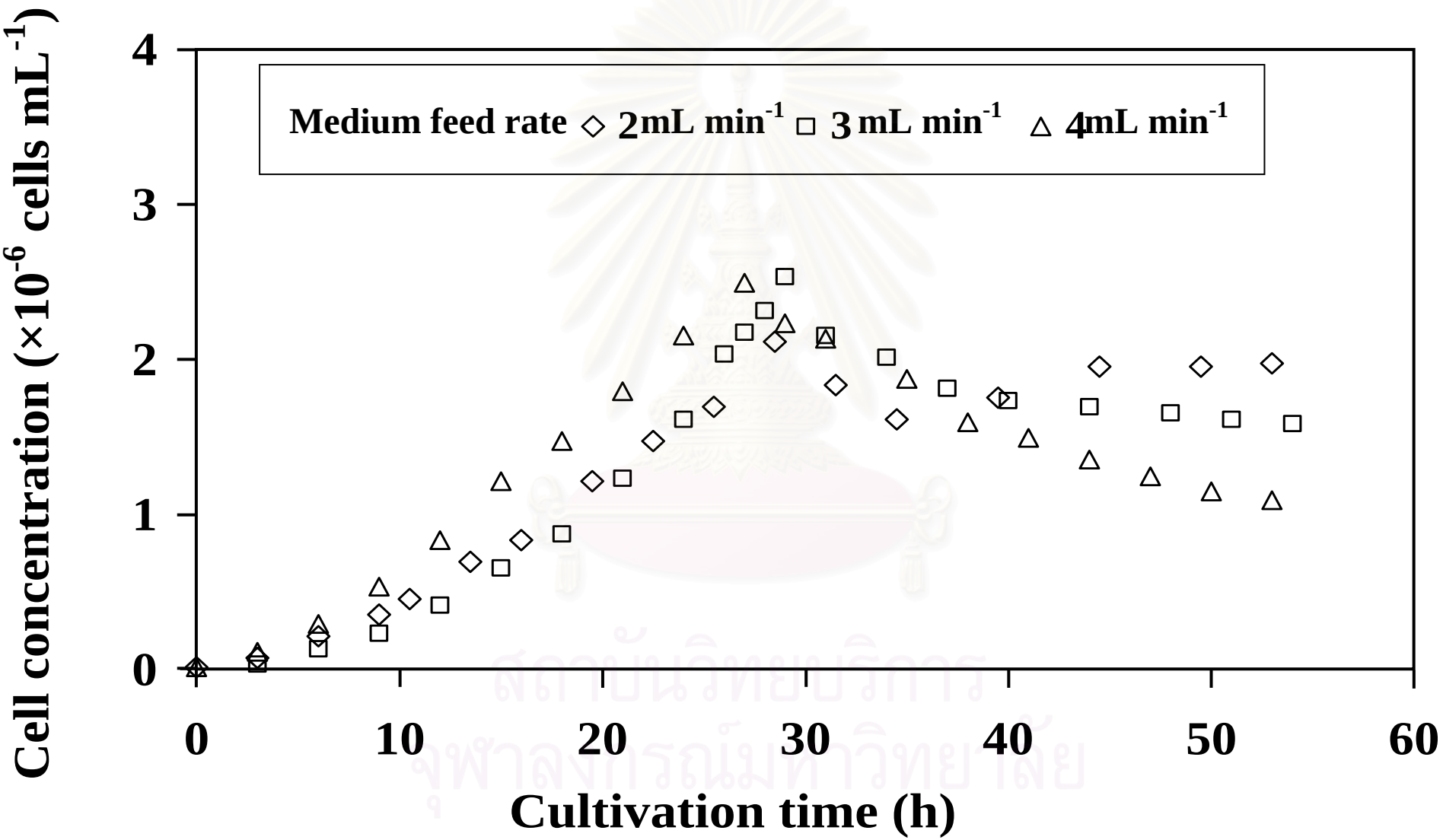


Figure 5.6 Growth behaviors of *Chaetoceros calcitrans* in continuous culturing system with various medium feed rates (ALPBR-2 with u_{sg} = 3 cm s^{-1}).

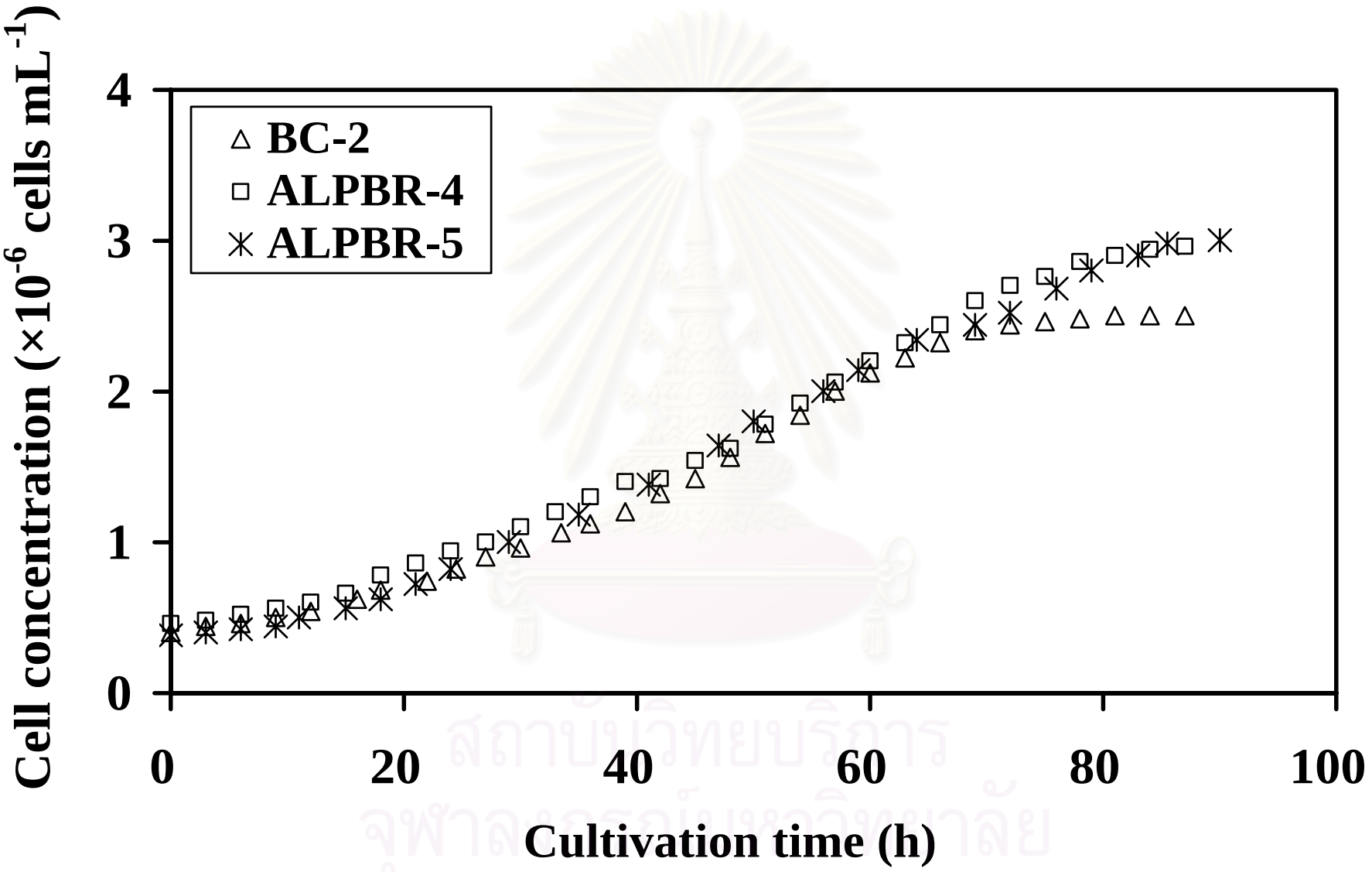


Figure 5.7 Growth curves of *Chaetoceros calcitrans* in large scale systems (BC-2, ALPBR-4 and ALPBR-5 with u_{sg} = 1 cm s^{-1}).

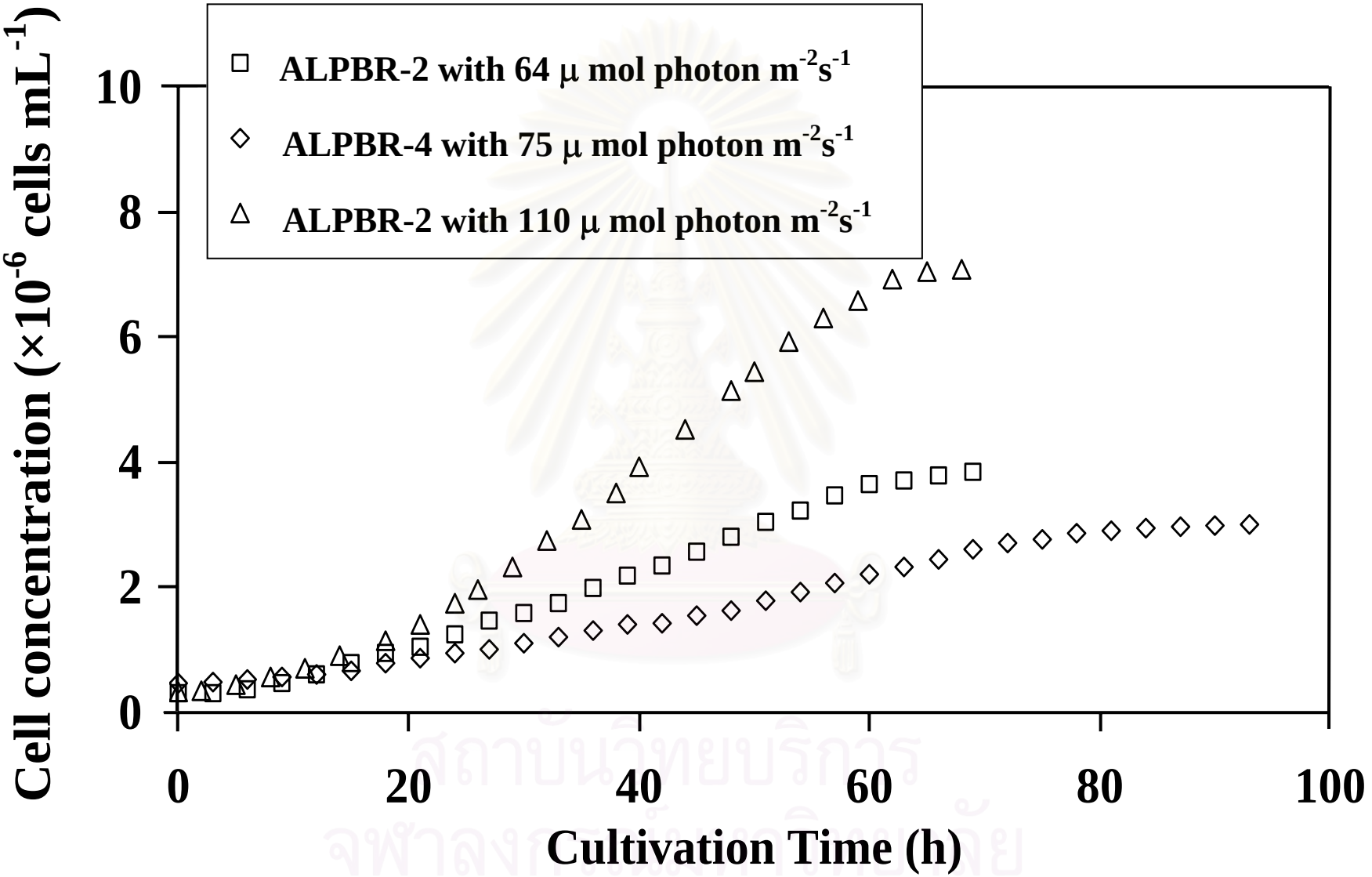


Figure 5.8 Comparison between the growth of *Chaetoceros calcitrans* in ALPBR-2 (initial cell concentration of approx. 3×10^5 cells mL^{-1}, u_{sg} = 3 cm s^{-1}) with the variation in light intensity and the cultivation in ALPBR-4.

CHAPTER 6

Design of Airlift Photobioreactor for The Cultivation of High Density Cell Concentration of *Chaetoceros calcitrans*

6.1 Summary

Three growth limiting factors were investigated for the cultivation of the diatom, *Chaetoceros calcitrans*, i.e. nutrient limitation, bubble shading, and self shading effects. In adequate nutrient in the standard F/2 medium was found to limit the maximum cell concentration during the batch experiment. An addition of nutrient during the stationary phase or a two-fold increase in the nutrient concentration at the beginning of the cultivation could lead to an increase in the maximum attainable cell concentration from 6.03×10^5 to 8.76×10^6 cells mL^{-1}. However, an increase in the nutrient concentration could not enhance the growth rate of this diatom. Light obstruction due to the presence of excessive bubble in the airlift system was also found to limit the growth of the diatom, and this was regarded as "bubble shading effect". The airlift system operated at high aeration rate could result in a large number of bubbles being dragged into the downcomer which obstructed the light path and decreased cell growth. The cultivation of the diatom in the expanded-top airlift photobioreactor facilitated the disengagement of the bubbles at the top section of the system and reduced the bubble shading effect. However, although the shading effect at high aeration rate was eliminated, the growth was not significantly enhanced as the diatom seemed to reach its maximum growth rate at u_{sg} = 3 cm s^{-1}. At high cell concentration, the growth was limited by the self shading effect where the light penetration was obstructed by the dense cell culture. The use of reactors-in-series could lessen this problem as cells were allowed to grow in separate compartments connected in series. In this configuration, the first compartments contained culture at low concentration and therefore was exposed to high light intensity, and only the last compartment would contain high cell density. The effect of self shading therefore could be limited to the last column in the series. The maximum specific productivity

(6.08×10^5 cells L^{-1} s^{-1}) for the steady cell concentration could be obtained when two columns were connected in series with the nutrient feed rate of 2 mL min^{-1}.

6.2 Backgrounds

Thus far, we have shown that airlift photobioreactor could be effectively employed as a closed cultivation system for *Chaetoceros calcitrans*. In the operation of airlift systems, several operational conditions were defined by the aeration rate and the configuration of the reactor, as stated in Krichnavaruk, 2000, Suksoir, 2000, Wongsuchoto, 2002, Limpanuphap, 2003 and Tanthikul, 2004. In previous chapters, we had not attempted to investigate the rate limiting step for the growth of such diatom. The determination of rate limiting steps is important in the design perspective as we could then further improve the performance of the system that initially considered as "optimal". In other words, it was difficult to conclude on the optimal condition of the reactor, unless the rate limiting steps were identified. Therefore this chapter was set out to examine such limiting steps for the cultivation of *Chaetoceros calcitrans*. These included nutrient depletion and shading effects. The modified ALPBRs were proposed to tackle each individual problem in order to achieve a better growth rate.

6.3 Experimental

The experiment was carried out as batch cultivation in the 17L ALPBR (ALPBR-1) as described in Section 3.1.1. whose result was compared with the cultivation in the expanded-top ALPBR (ALPBR-3) as described in Section 3.1.3. The the application of the continuous cultivation in the 2.8 L ALPBR (ALPBR-2) was introduced as the ALPBRs-in-series and was involved in the investigation as described in Section 3.1.2. The cultivation of the diatom in the 23 L expanded-top ALPBR (ALPBR-3) was established to investigate the effect of bubble shading effect on the growth of the diatom as detailed in Section 3.1.3. The experimental preparation was held as reported in Section 3.2.1. and the growth behavior was conducted based on the procedure in Sections 3.2.5 and 3.2.6.

6.4 Growth Limiting Factors

The growth curve of *Chaetoceros calcitrans* in the 17L airlift system was illustrated in Figure 6.1. This shows that the lag phase of this specific microorganism lasted for about 9 hours, followed by a 35 hour exponential phase, and the stationary region where the cell concentration was not further increased. The maximum cell concentration was reported at approx. 8.88×10^{6} cells mL^{-1} with a specific growth rate of $7.41x10^{-2}$ h^{-1}. Krichnavaruk *et al.* (2005) reported that an increase in each major component in the standard F/2 medium could enhance the growth of the diatom but not to a significant level. Despite so, it was suspected that insufficient nutrient might play significant role in controlling the cell growth in the large scale culture. This aspect would be examined further in this article.

Figure 6.2 demonstrates that the effect of aeration rate could be categorized into two regions. The first region was when the aeration was low, and the growth rate depended on the rate of aeration. However, at high aeration rate, the growth seemed to be deteriorated as the aeration became stronger. It was generally known that aeration rate had a strong impact on the level of gas-liquid mass transfer within the airlift system (Wongsuchoto *et al*., 2003; and Krichnavaruk and Pavasant, 2002), but this increase in mass transfer rate did not seem to have influence on the cell growth. Figure 6.2 demonstrates that, although the gas-liquid mass transfer rate (represented by the overall volumetric mass transfer coefficient, K_La) increased with aeration rate, the maximum cell achievable in the system did not increase, particularly at high aeration rate. It was therefore concluded that the growth of the diatom could not be promoted just by improving the gas-liquid mass transfer rate.

On the other hand, the aeration caused a better liquid circulation in the system and liquid circulation allowed an effective circulation of cell within the system. This, in turn, facilitated the circulation of the diatom to the high light intensity area, i.e. downcomer, where cells could effectively utilize the light for their photosynthetic activities. In the low aeration zone ($u_{sg} \leq 3$ cm s^{-1}), the growth was enhanced with aeration, but at the high aeration zone, opposite result was observed. Figure 6.3 demonstrates that the effect of aeration on the light intensity in the airlift system

followed the same trend as that of the growth rate, i.e. higher light intensity was obtained with higher aeration when $u_{sg} \leq 3$ cm s^{-1} and lower light intensity at $u_{sg} > 3$ cm s^{-1}. It was therefore concluded that the growth of the diatom was, in fact, controlled by the level of light intensity in the system which was a function of aeration rate.

6.5 Nutrient Limitation for The Cultivation of *Chaetoceros calcitrans*

To determine the nutrient depletion effect, two experiments were carried out. The first system was cultivated with the spent F/2 medium where cells were already removed. The second column was cultivated with the spent medium refreshed with F/2 stock solution. The spent medium was prepared by cultivating the diatom in the fresh standard F/2 medium with the initial cell concentration of approx. 1×10^5 cells mL^{-1} (u_{sg} = 3 cm s^{-1}) for 44 hours to make sure that the diatom reached its stationary phase where the maximum cell concentration of around 7×10^6 cells mL^{-1} was obtained. The diatom was then separated from the culture medium by centrifuge at 3000 rpm. The resulting clear solution was used as spent medium. In the second case, the refreshed nutrient had the same concentration level as that of the standard F/2 medium.

Figure 6.4 illustrates that the cultivation of the diatom in the refreshed spent medium resulted in almost the same specific growth rate as those obtained from the new medium (approx. 5.08×10^{-2} h^{-1}) although the steady state cell concentration was slightly lower (about 15.5% reduction in maximum cell concentration was observed). On the other hand, the cultivation of the diatom in the non-refreshed spent medium was clearly inferior to that with the refreshed medium where both specific growth rate and the maximum cell concentration were found to be relatively low, at 2.32×10^{-2} h^{-1} and 1.88×10^6 cells mL^{-1}, respectively. This suggested that the refreshed used medium could support the growth of the diatom with no significant difference in maximum cell concentration and specific growth rate when compared with the cultivation with a newly prepared F/2 standard medium.

The next experiment was set up to verify the result of the nutrient depletion effect. In this case, the amount of nutrient remaining in the system once it reached the stationary phase was thought not adequate for further growth of the diatom. At this period, the fresh F/2 stock solution was added into the batch culturing system to replace the depletion of the nutrient and to refresh the medium back to the standard F/2 concentration level again. The experimental results as shown in Figure 6.5 reveal that the diatom was able to grow further and to reach a new maximum cell concentration of approx. 8.96×10^{6} cells mL^{-1} within 33 h. This increase in cell concentration was equivalent to 61% original maximum cell concentration. The time required for the diatom to reach the second stationary phase was less than those in the first section as the initial cell concentration for the second period was much greater than that of the first, and hence, the nutrient consumed by the diatom in the second section was also faster. At this point, the fresh nutrient stock solution was again added into the system, but the cell concentration could not be further enhanced. This was because cells were already quite dense in the solution and this prevented an effective passage of light through the medium. This aspect was examined in the next section.

The diatom was also cultivated in ALPBR-1 using the nutrient with a two-fold concentration of the standard F/2 solution. This medium was employed directly at the beginning of the experiment to examine the effect of nutrient concentration on the growth of the diatom. Figure 6.5 demonstrates that the maximum cell concentration for this case was not significantly different from those obtained from the previous experiment, at around 8.76×10^{6} cells mL^{-1}.

These verification tests supported a conclusion that cells did not produce inhibitory metabolic substance during the growth and therefore the spent nutrient could be refreshed and reused. However, if cells were not separated from the spent nutrient, the dense cells remained in the system would block the light penetration and ceased the growth.

6.6 Bubble Shading Effect (Cultivation in The Expanded-top ALPBR)

As described earlier, the maximum light intensity for the cultivation of diatom in ALPBR-1 was obtained at superficial velocity (u_{sg}) = 3 cm s^{-1}. This condition permitted the light to pass through the column at the highest intensity. It was noticed that, at gas velocity lower or higher than 3 cm s^{-1}, a large number of gas bubbles were dragged down into the downcomer and obstructed the light penetration. In this work, this phenomenon was named "bubble shading effect" and is schematically illustrated in Figure 6.6. In this figure, it was illustrated that there existed a large amount of small bubbles in the downcomer at low u_{sg}. This was because this condition led to a system with a relatively low liquid circulation rate and the small bubbles with lower terminal velocity than this circulation velocity would be dragged down into the downcomer. As the aeration rate increased, bubble coalescence became significant which resulted in bubbles with larger size. At this condition, only a small fraction of bubbles could move into the downcomer as the terminal velocity exceeded that of circulating velocity. However, when u_{sg} reached about 4-5 cm s^{-1}, the liquid circulation rate became stronger and a large number of large bubbles could be brought down the downcomer and, again, caused an obstruction to the light penetration.

The expanded-top ALPBR (ALPBR-3) was used for the investigation of bubble shading effect on the growth of *Chaetoceros calcitrans*. The expanded-top ALPBR was the conventional airlift column where the gas separating section was enlarged to support the disengagement of gas bubbles from liquid surface (detailed in Figure 3.4). The waterproof probe was inserted into the riser to determine the light intensity in the riser at various gas velocities. Figure 6.7 demonstrates that, with the enlargement of gas separator, an increase in gas superficial velocity (u_{sg}) from 2 to 4 cm s^{-1} did not have notable effect on the level of light intensity in the column, where a slight decrease in light intensity could be seen when u_{sg} was increased to 5 cm s^{-1} (approximately 10% lower than the intensity at u_{sg} = 4 cm s^{-1}). This was because at this high u_{sg}, there were a rather large number of small bubbles that could still escape into the downcomer. This finding suggested that the light penetration ability in the

system could be improved by allowing bubbles to leave the system, and for this case, this was achieved by enlarging the gas separator section of ALPBR. It should be noted here that the light intensity supplied for ALPBR-3 could not be controlled at the same range as in ALPBR-1 as the setup of the system did not support a installation of the light bulbs with the same pattern as that of ALPBR-1.

Although there was no further blockage of light pathway, the growth in ALPBR-3 at high aeration rate was still found to be similar to that at lower aeration rate. However, a higher aeration rate supported a faster circulation of the fluid in the airlift system. Hence, this finding stated that the growth diatom could no longer be enhanced by the liquid circulation, and the optimal gas superficial velocity for the cell growth was still found to be at 3 cm s^{-1} (detailed in Figure 6.7).

6.7 Self Shading Effect (Cultivation in ALPBRs-in-series)

Previous experiments demonstrated that the maximum cell concentration obtained from the cultivation of *Chaetoceros calcitrans* in the 2.8L ALPBR with two fold nutrient concentration was greater than that obtained in the batch cultivation. Similarly, an increase in cell concentration could be obtained when fresh nutrient was added into the nutrient-depleted culture at the time where the diatom reached its stationary cell concentration. These observations indicated that there was a time where the nutrient was depleted and diminished further growth of the diatom. However, after the second stationary phase was reached, a supplement of fresh nutrient could not further support the growth. This was because the culture was too dense for the light to penetrate and therefore the cell suffered from the inadequate light intensity and no further growth was observed. This condition was generally called “self shading effect”.

The cultivation of the diatom *Chaetoceros calcitrans* in ALPBRs-in-series was carried out to investigate the self shading effect due to the dense cell culture which could occur during the later stage of the cultivation. In this system, the diatom was firstly cultivated in the 2.8L ALPBR(s) in a similar fashion to the batch cultivation. Compressed air was supplied with u_{sg} = 3 cm s^{-1} and light intensity was

controlled at approx. 110 μmolphoton m^{-2} s^{-1} (measured at the outer wall). When the growth of the diatom reached the mid exponential region, the columns were connected in series and fresh medium was fed into the first column (Column I). The schematic diagram of this setup is illustrated in Figure 3.3. The volumetric feed rates (q) were manipulated using peristaltic pumps. The reactor-in-series setup separates the growth of the diatom into several stages, each with different level of cell concentration. In the first column, the cell concentration was remained at low level to ensure effective light penetration and a high level of photosynthesis. On the other hand, the last column would contain culture at high cell density, as required for the harvest. Although this high density would block the light penetration, the low light intensity zone would be limited only to the last column instead of having only one column which would cause the whole culture to be subject to low light condition.

Figure 6.8 illustrates the result from the cultivation of the diatom in System I with q = 1 mL min^{-1} and System II with q = 2 mL min^{-1} (both with the same residence time of 46.7 h). It was observed that, the ALPBR-in-series provided a higher harvest cell concentration than the original one reactor continuous system. The productivities were approx. 0.83×10^5 and 2.07×10^5 cells s^{-1} for Systems I and II, respectively. This growth behavior was also observed in the ALPBRs-in-series with q = 4 mL min^{-1} in comparison with the continuous cultivation in System I with q = 2 mL min^{-1} (both at the same residence time of 23.3 h) as illustrated in Figure 6.9. The productivities obtained from these two systems were 3.45×10^5 and 1.73×10^5 cells s^{-1}, respectively. An increase in the harvesting productivity was supported by an increase in light penetration into the systems. The cell concentration in Column I was lower than that in Column II because of the dilution effect in the first columns which increased the chance of the diatom to be exposed to higher light intensity.

The ALPBRs-in-series (System II) was further modified by adding the third column following the second column (System III and the setup shown in Figure 3.3). The cultivation of the diatom was carried out with medium feed rates (q) adjusted to 3 and 6 mL min^{-1}. The results of these experiments are shown in Figures 6.10 and 6.11, respectively. At this condition, a clearer culturing medium was, again, observed in the first column as the diatom was diluted with the fresh medium which allowed the

diatom in this column to be exposed to high light intensity and enhanced the photosynthesis. The harvest productivities from the cultivation of the diatom in the ALPBRs-in-series (3 columns) with nutrient feed rates q = 3 and 6 mL min^{-1} were 4.25×10^5 and 4.54×10^5 cells s^{-1}, respectively.

However, the results as illustrated in Figures 6.10 and 6.11 revealed that the use of the third column, in fact, did not have significant effect on cell growth as the cell was already quite dense from the second column, particularly at q = 3 mL min^{-1} (see Figure 6.10). At this condition, no further growth in the third column was observed as the maximum cell concentration was in the same range with Column II. The maximum cell concentration obtained from Column II was approx 8.24×10^6 cells mL^{-1} which was only marginally lower than 8.50×10^6 cells mL^{-1} obtained from Column III. And in this case, the productivity obtained at the exit of Column II was around 4.12×10^5 cells s^{-1}, a mere 3% lower than the productivity obtained from the operation with three columns. In addition, this level of productivity was found to be the highest among all the cases evaluated in this work. It was therefore concluded that the operation at the feed rate of 3 mL min^{-1} was the most optimal for the system employed in this work.

6.8 Concluding Remarks

The growth of the diatom in the ALPBRs was limited by a number of factors including nutrient concentration and shading effects by gas bubbles together with the dense culture of the diatom. Increasing the standard F/2 nutrient concentration could help enhance the final cell concentration but not the growth rate. This was caused by the self shading effect. For the cultivation of photosynthetic microorganisms, the culture could practically be limited by the self shading effect caused by the dense cell culture. However, in the airlift system, the bubble shading effect caused by the presence of bubble swarm in the downcomer was also observed and this resulted in a blockage of the light penetration. The two shading effects could be dealt with by modifying the airlift structure. The bubble shading problem was shown to be overcome using an expanded-top ALPBR which allowed most of the bubbles to disengage from the system at the liquid surface. The self shading effect could be

lessened using the ALPBRs-in-series system, where a part of cell culture could remain in the high light intensity section.

Table 6.1 Comparison between productivity and specific productivity for the cultivation of *Chaetoceros calcitrans* in ALPBRs-in-series systems.

Experiment	System	Medium feed rate in Stream A (mL min^{-1})	Residence time (h)	Productivity in Stream D (cells s^{-1})
Set 1	I	1	46.66	0.83×10^5
Set 2	I	2	23.33	1.73×10^5
Set 3	II	2	46.66	2.07×10^5
Set 4	II	4	23.33	3.45×10^5
Set 5	III	3	46.66	4.25×10^5
Set 6	III	6	23.33	4.54×10^5

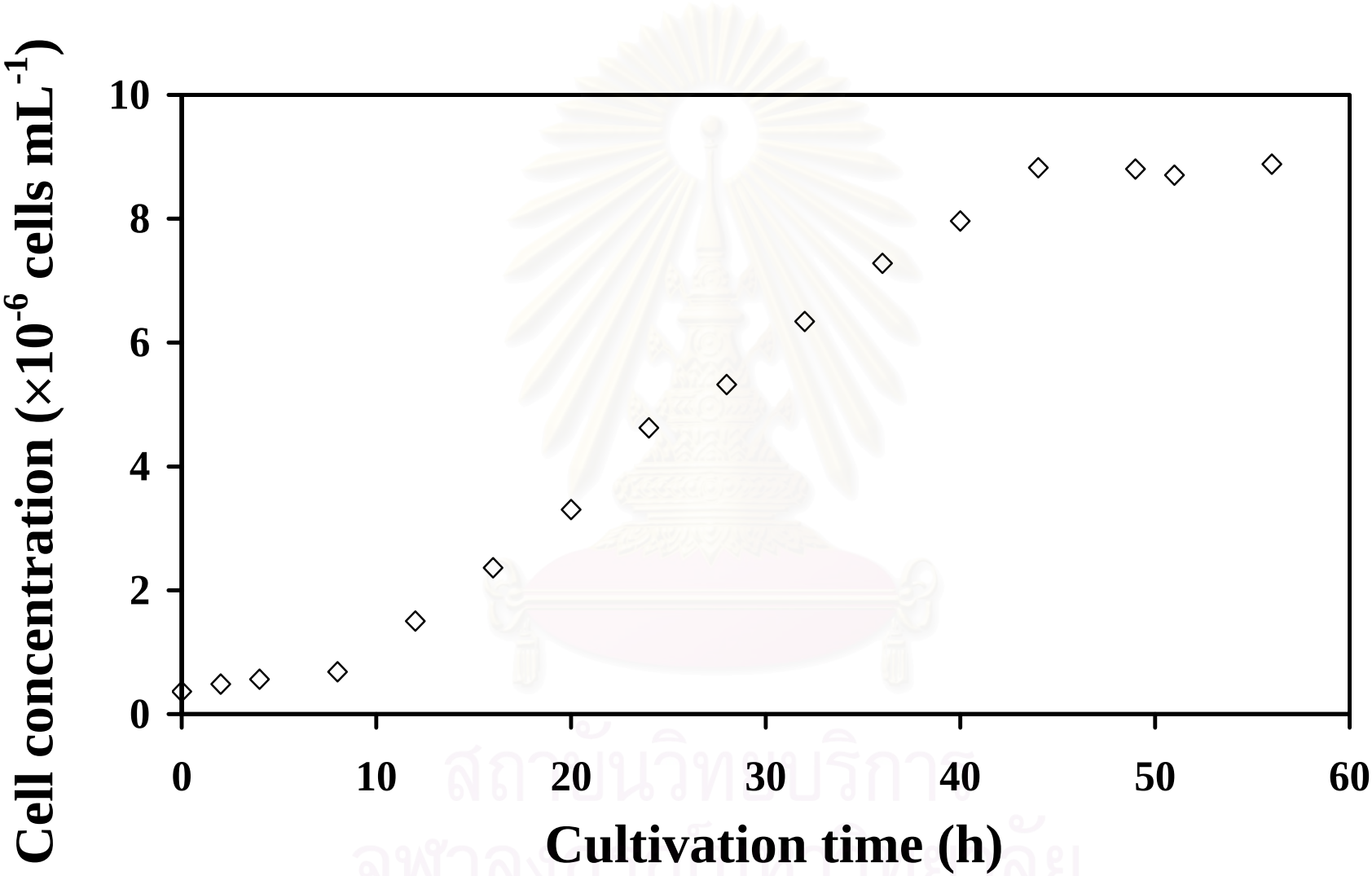


Figure 6.1 Growth behavior for the batch cultivation of *Chaetoceros calcitrans* in ALPBR-1 (u_{sg} = 3 cm s^{-1}).

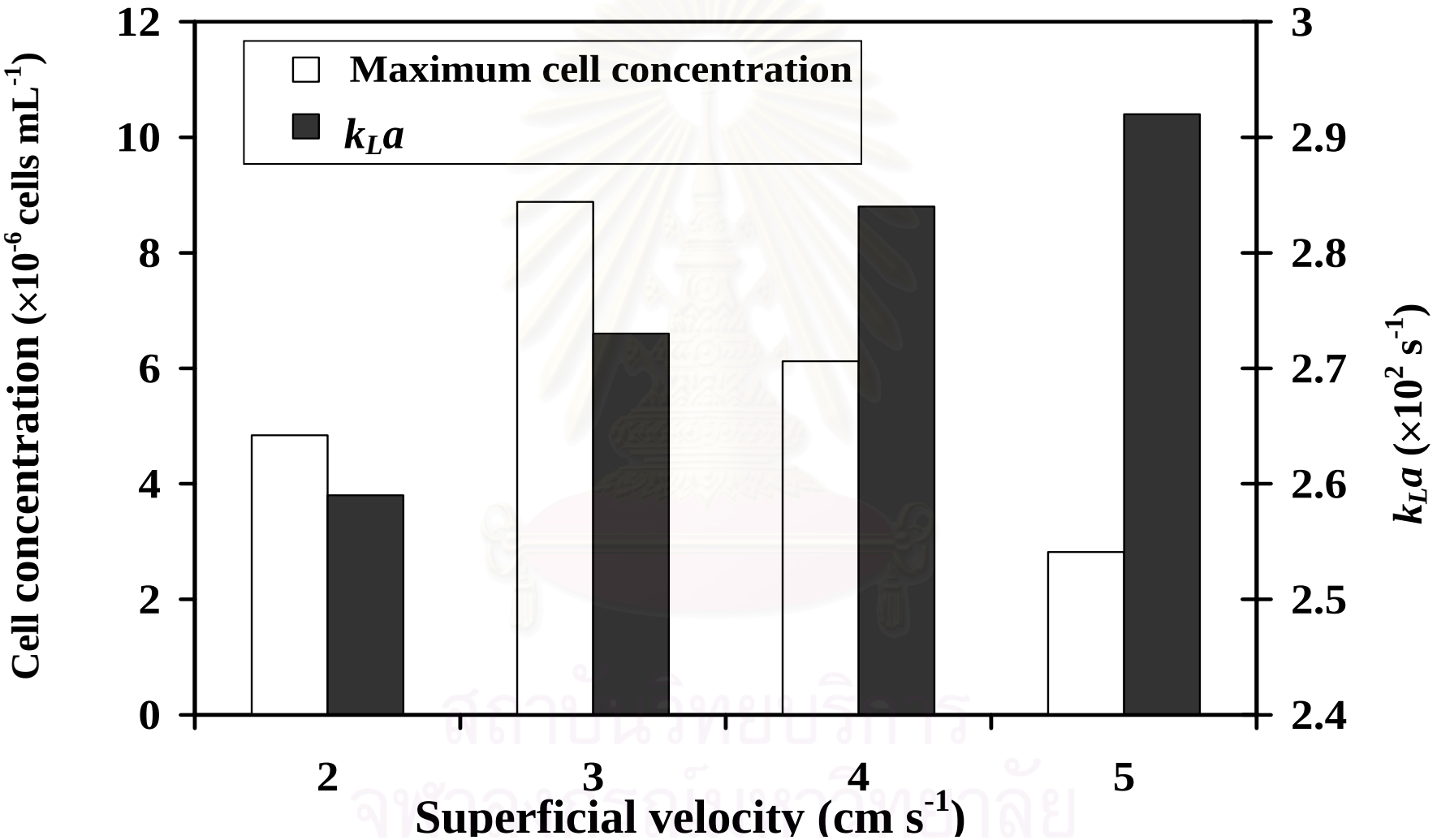


Figure 6.2 Comparison between specific mass transfer coefficient (K_La) and maximum cell concentration for the cultivation of *Chaetoceros calcitrans* in ALPBR-1 at various gas velocities.

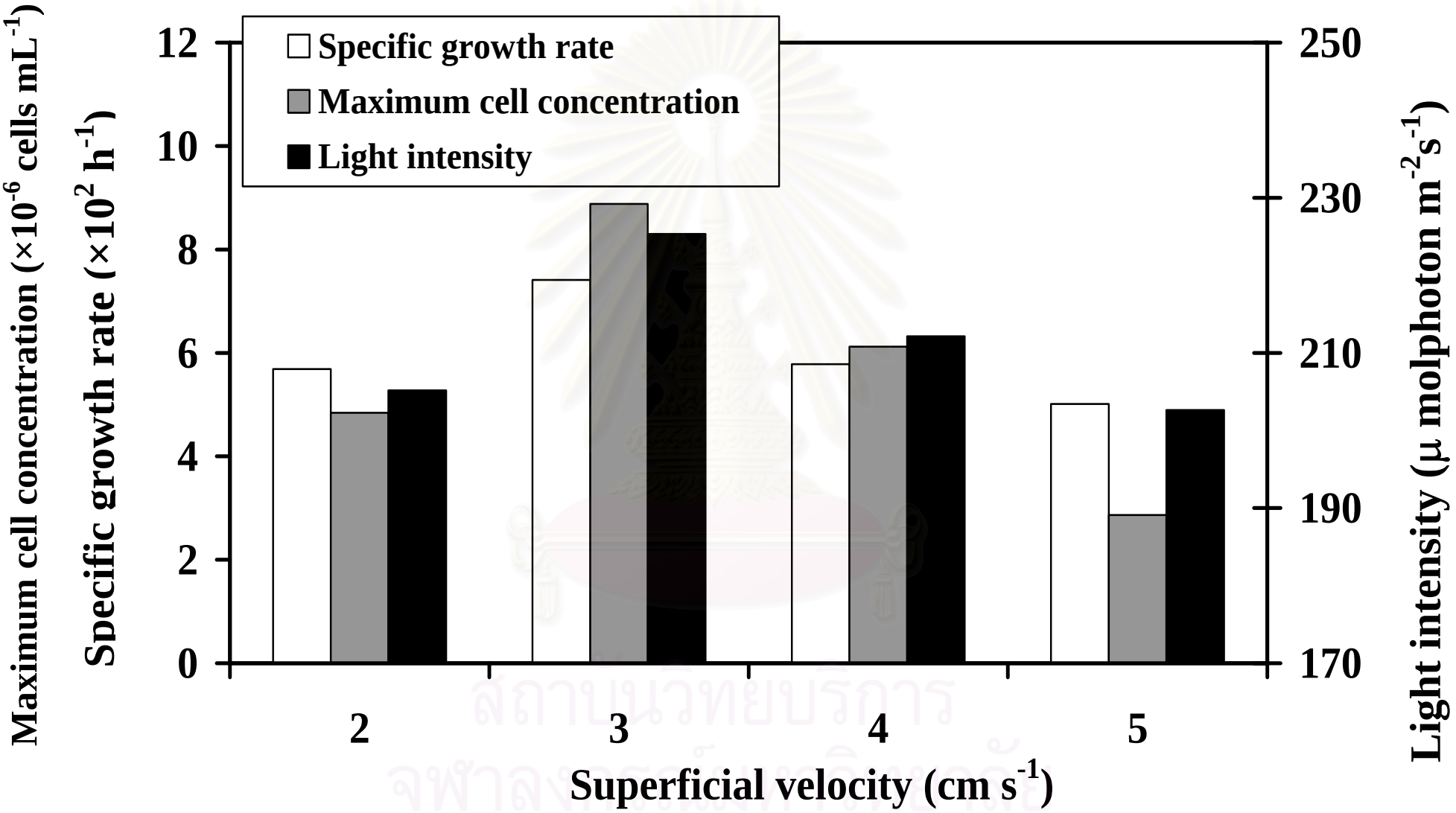


Figure 6.3 Relationship between specific growth rate, maximum cell concentration and light intensity in conventional ALPBR-1 at various gas velocities.

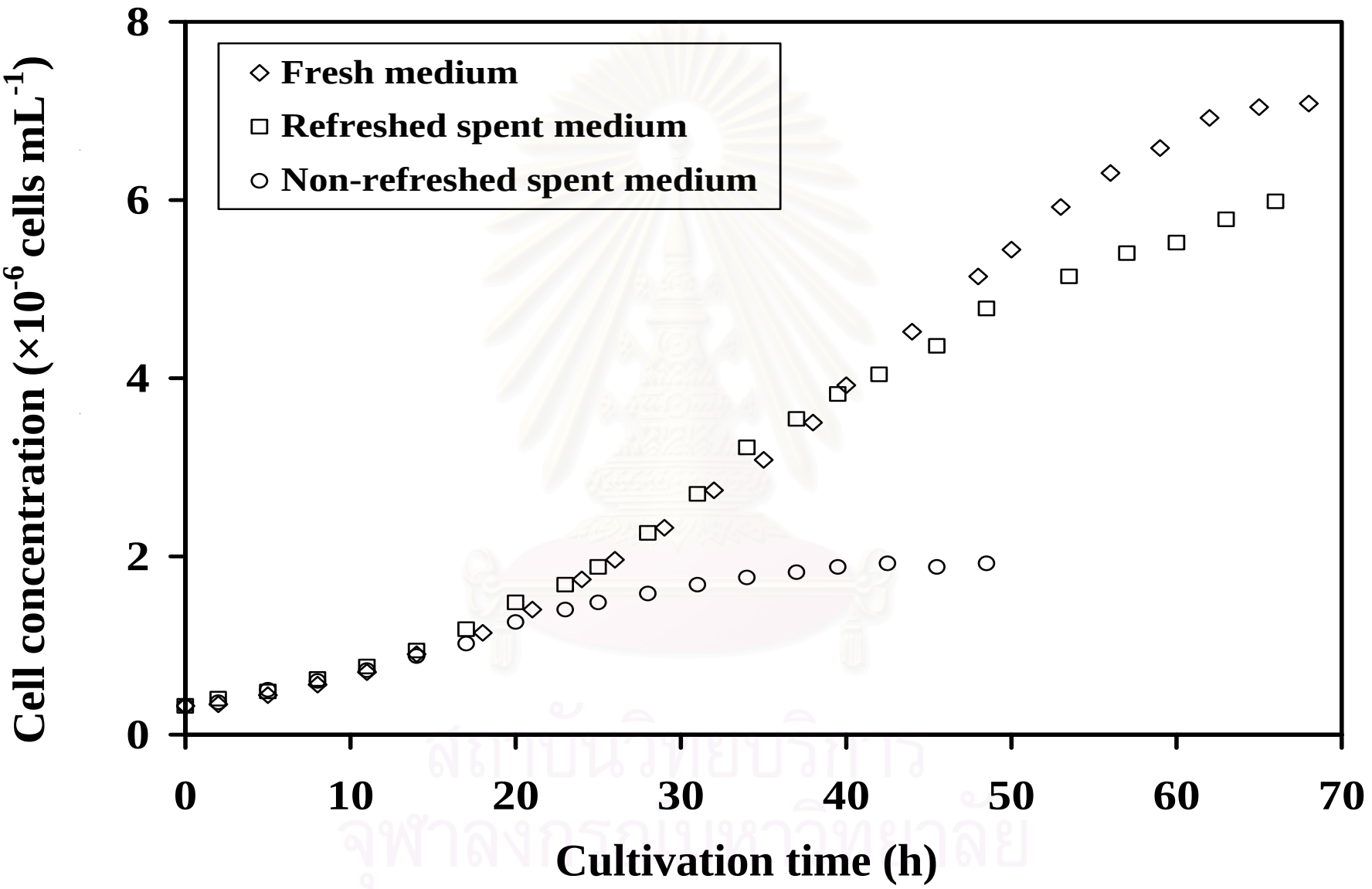


Figure 6.4 Growth behaviors for the cultivation of *Chaetoceros calcitrans* in fresh medium, refreshed spent medium and non-refreshed spent medium.

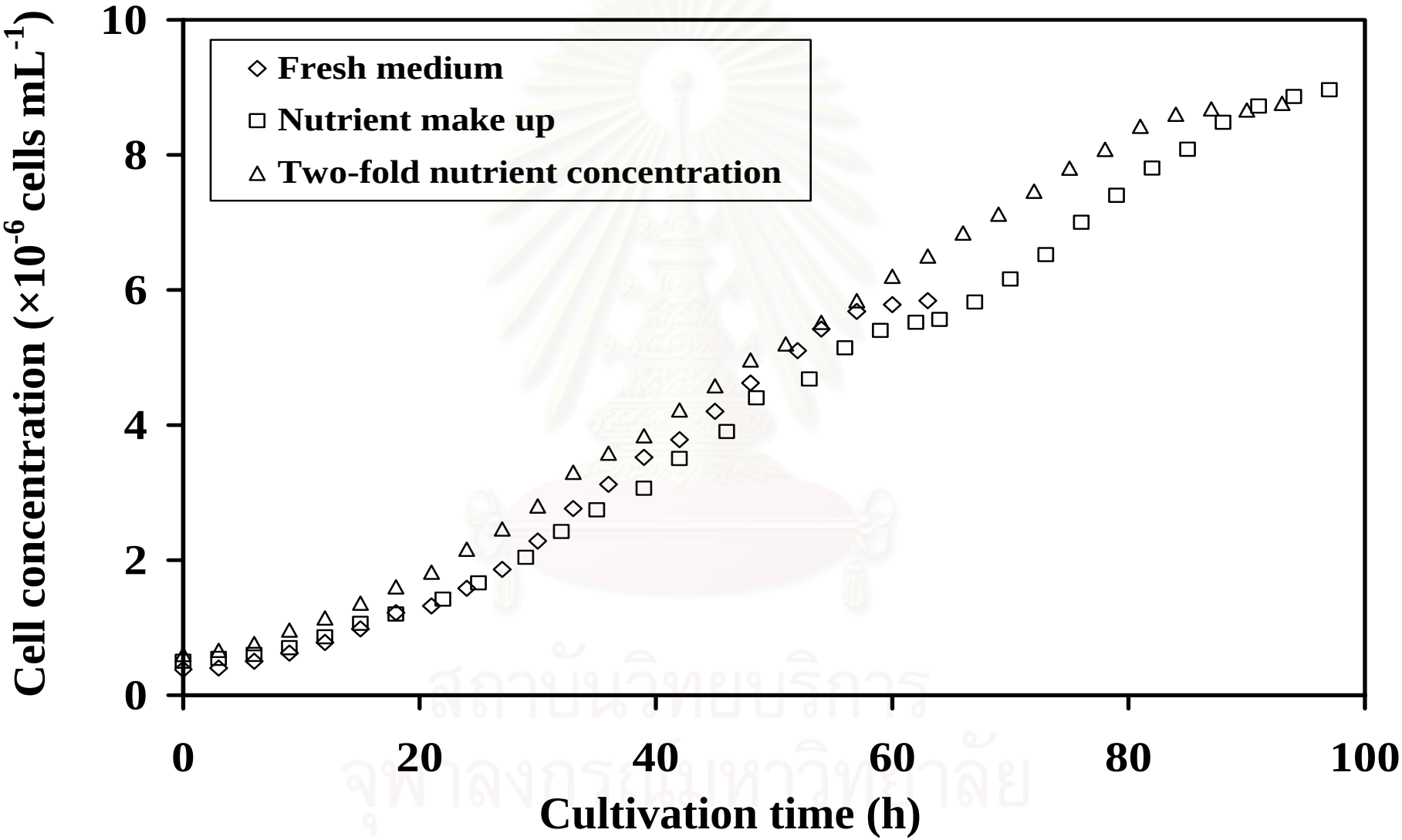


Figure 6.5 Effect of nutrient depletion on the growth of *Chaetoceros calcitrans*.

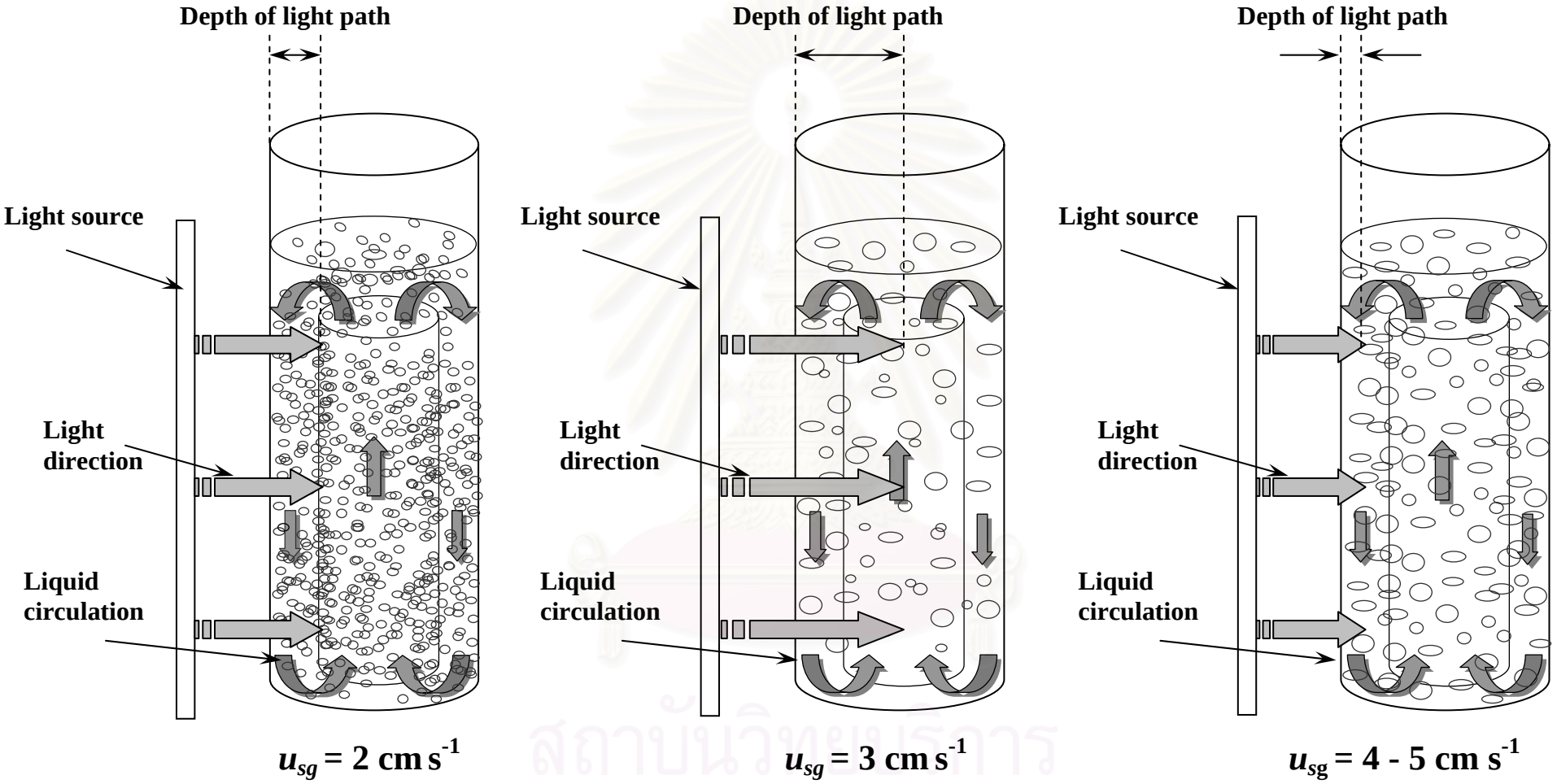


Figure 6.6 Bubbles flow characteristic and light penetration ability in conventional ALPBR with various aeration rates.

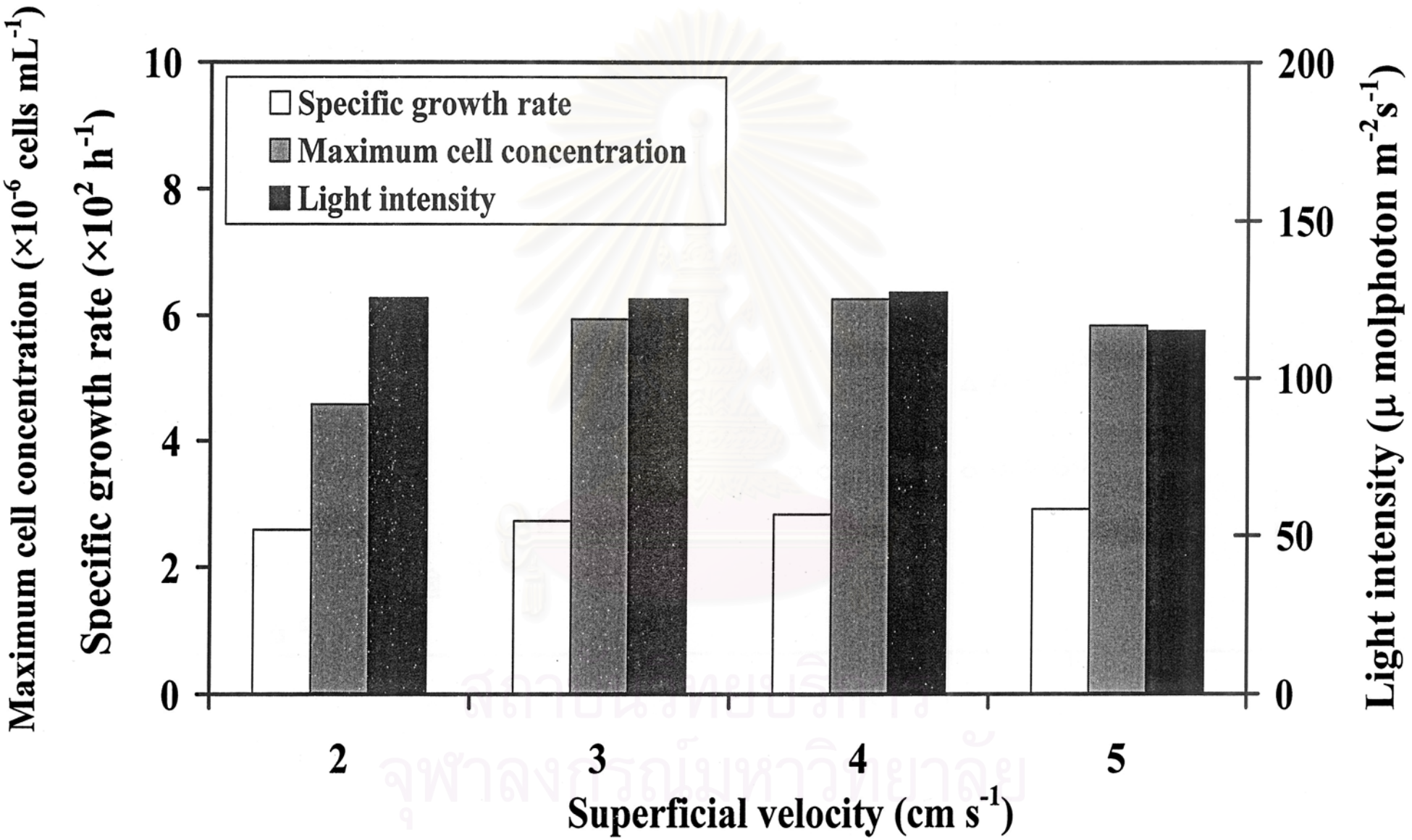


Figure 6.7 Relationship between specific growth rate, maximum cell concentration and light intensity in conventional ALPBR-2 at various gas velocities.

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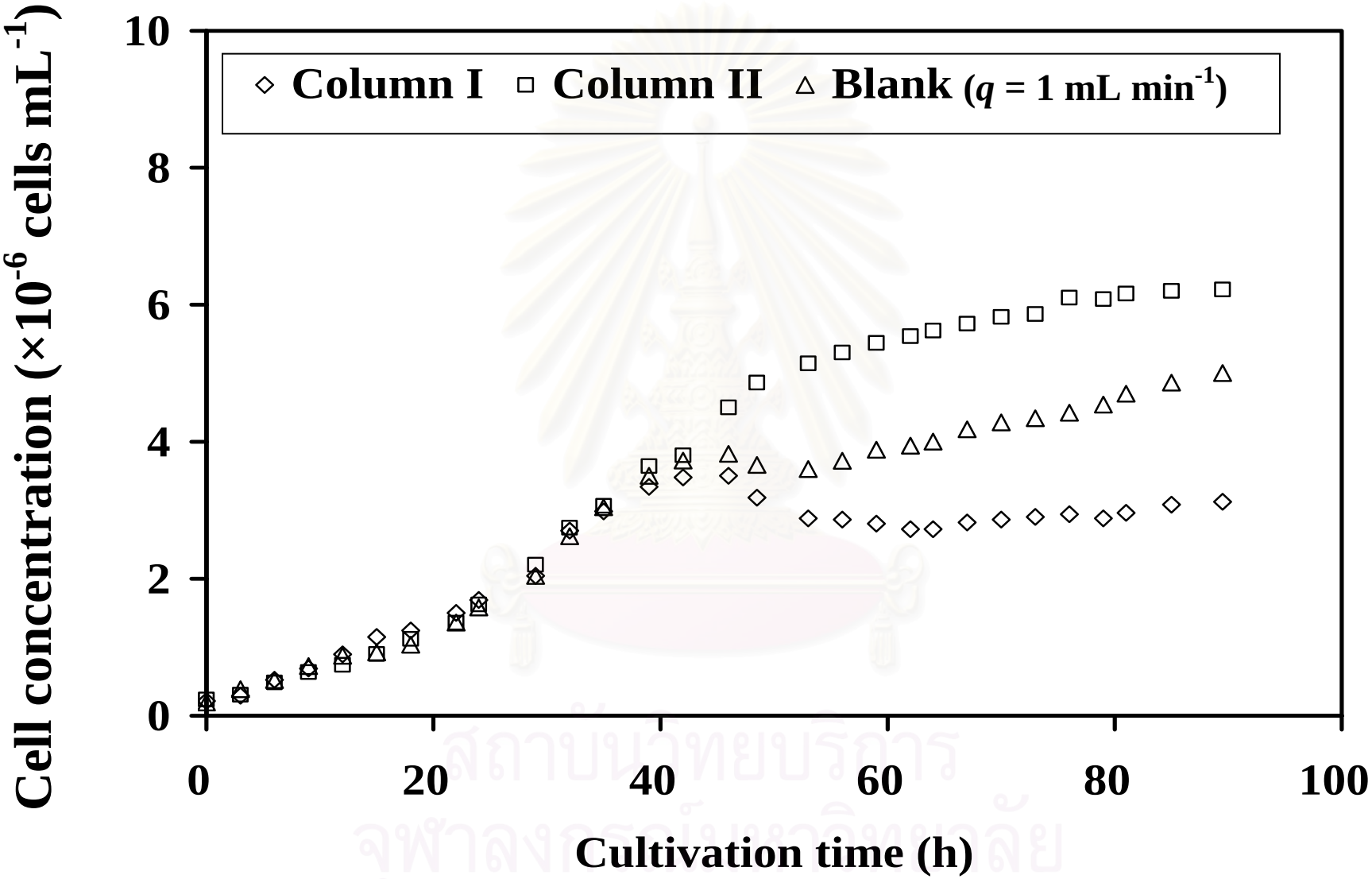

Figure 6.8 Growth behavior for the cultivation of *Chaetoceros calcitrans* in a single 2.8 L ALPBR (blank) with medium feed rate, q = 1 mL min^{-1}, and ALPBRs-in-series (System II) with medium flow rate, q = 2 mL min^{-1}.

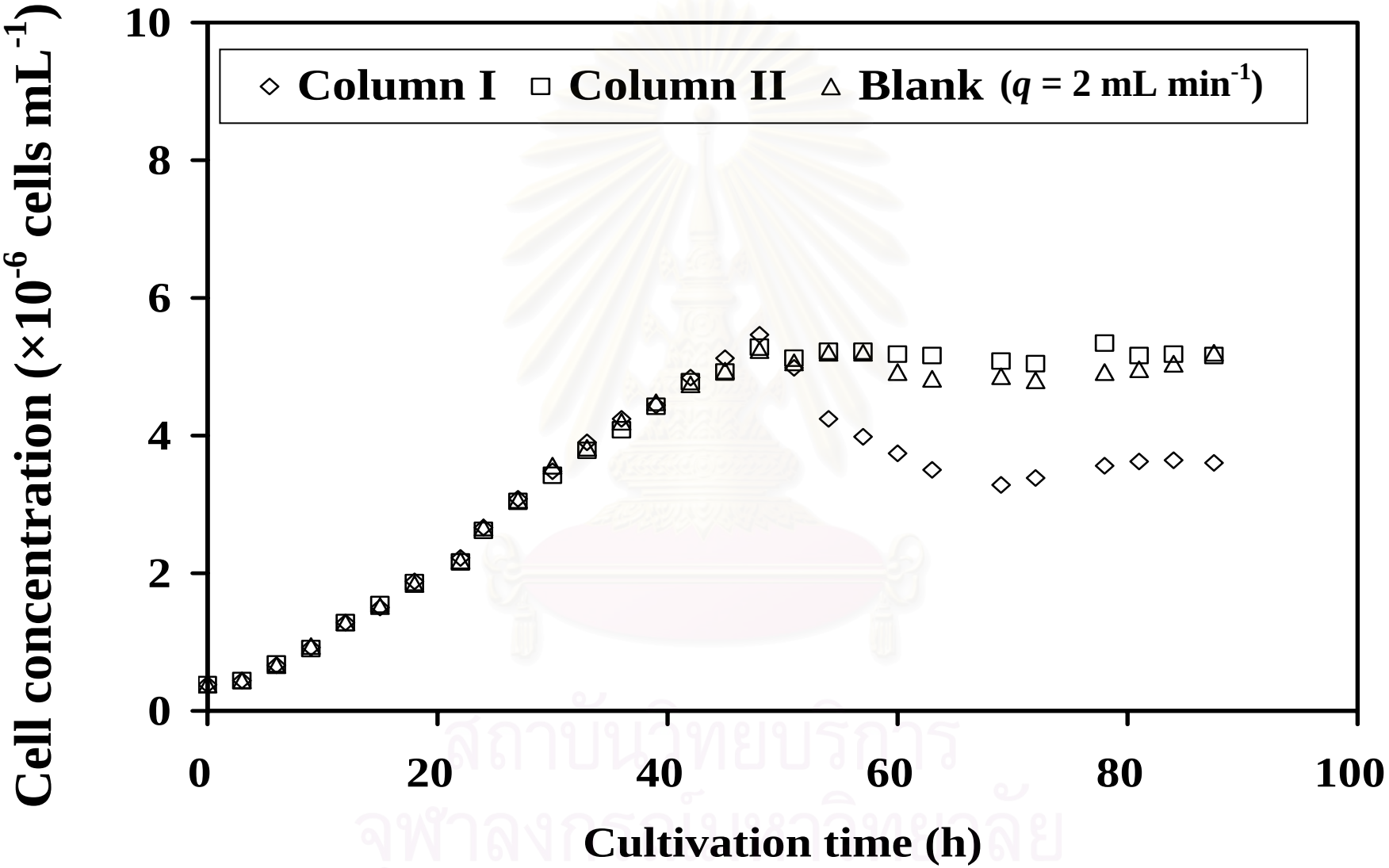

Figure 6.9 Growth behavior for the cultivation of *Chaetoceros calcitrans* in a single 2.8 L ALPBR (blank) with medium feed rate, q = 2 mL min^{-1}, and ALPBRs-in-series (System II) with medium flow rate, q = 4 mL min^{-1}.

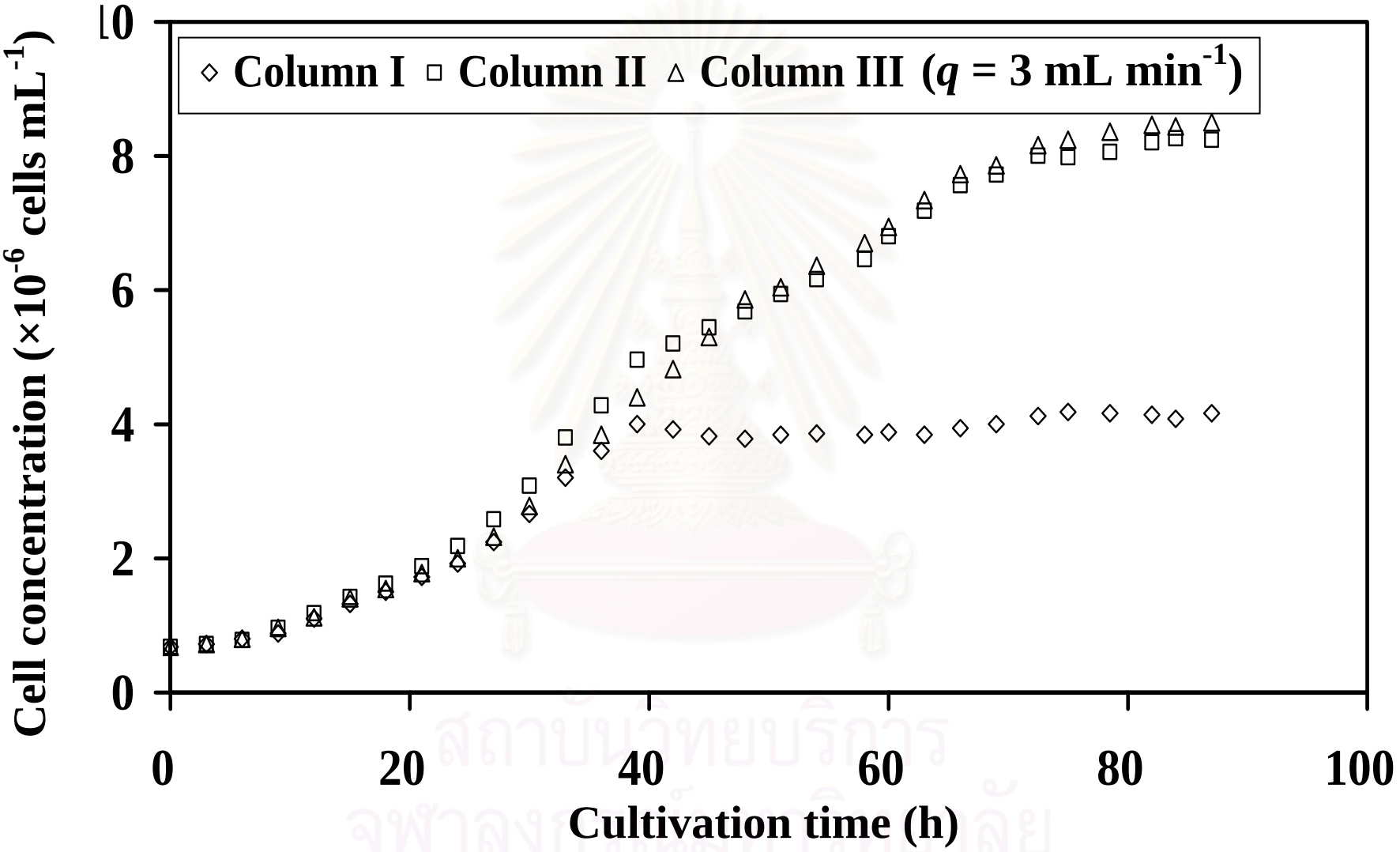

Figure 6.10 Growth behavior for the cultivation of *Chaetoceros calcitrans* in ALPBRs-in-series (System III) with medium feed rate, q = 3 mL min^{-1}.

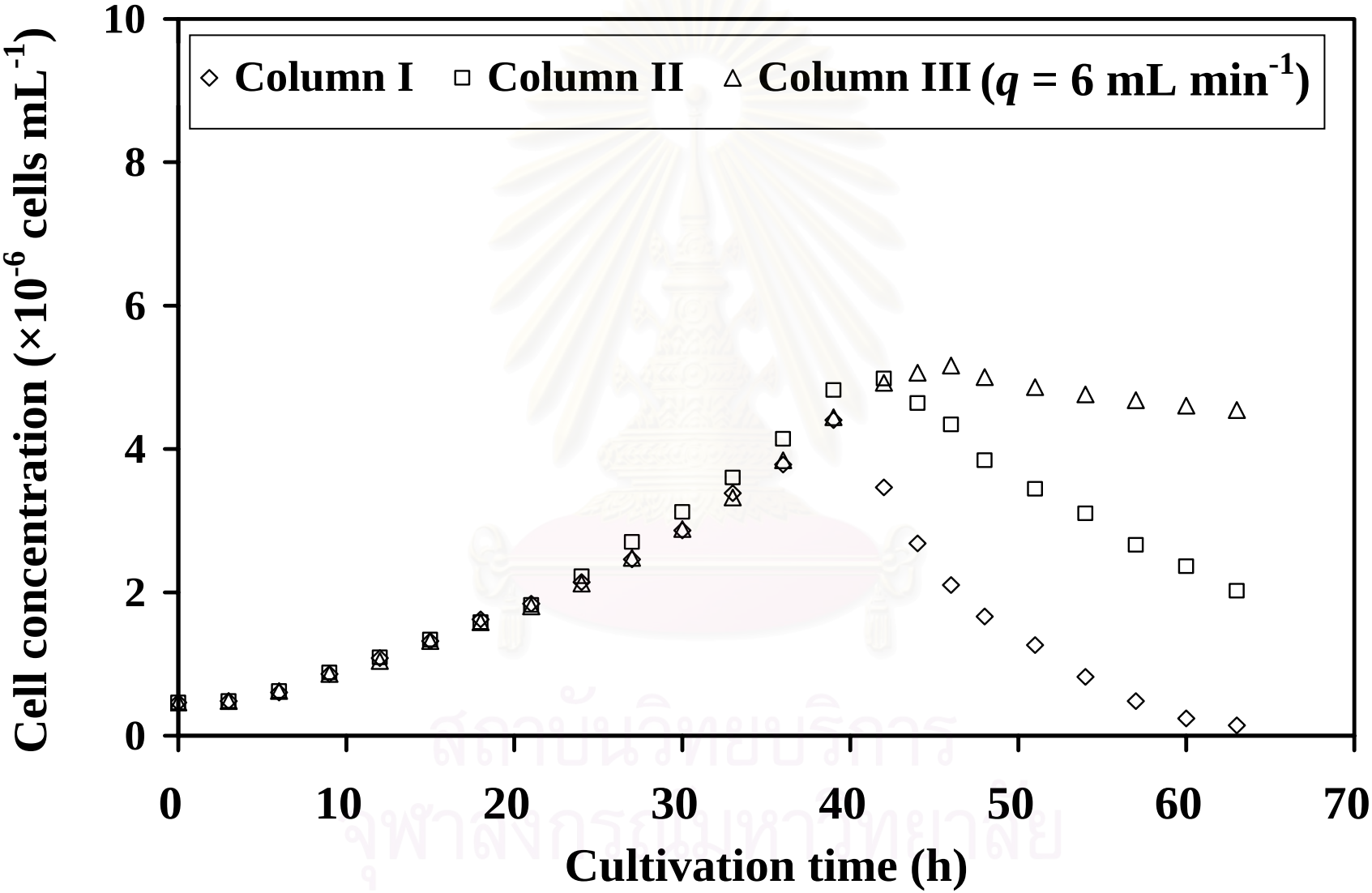

Figure 6.11 Growth behavior for the cultivation of *Chaetoceros calcitrans* in ALPBRs-in-series (System III) with medium feed rate, q = 6 mL min^{-1}.

CHAPTER 7

Conclusions and Recommendations

7.1 Main Achievements

This work demonstrates the success of the cultivation of the diatom *Chaetoceros calcitrans* in the airlift photobioreactor (ALPBR) which explicitly out performed the bubble column in achieving both higher specific growth rate and higher maximum cell concentration. This was due to a better circulation which enhanced the light exposure frequency of the diatom where all cells were constantly exposed to high light intensity zone near the wall of the column. The optimal reactor configurations for the growth of the diatom in the 17L ALPBR was the system with A_d:A_r = 2.62 and u_{sg} = 3 cm s^{-1}.

7.2 Research Breakthrough

The investigation revealed the facts regarding the growth limiting factors for *Chaetoceros calcitrans* in conventional cultivation systems. Three rate limiting aspects have been illustrated to have significant effect on growth and they are summarized below.

7.2.1 Nutrient depletion

The standard F/2 medium (Guillard, 1975) was found to be reasonably good for the growth of *Chaetoceros calcitrans*, although a slight modification of some components in the medium was shown to have beneficial influence as reported by Laotaweesup (2002). However, it was proven that the final cell concentration was partially limited by the depletion in nutrient. Increasing the nutrient concentration did not enhance the growth rate but it could raise the final cell concentration up to the point where the system reached “self shading” condition where a further increase in the nutrient could no longer support growth.

7.2.2 Bubble shading effect

The conditions that allowed bubbles to move down the downcomer caused light obstruction and reduced the growth of the diatom. There were two cases where this occurred: (i) obstruction by small bubbles at low gas velocity condition, and (ii) obstruction by large bubbles at high gas velocity. A large number of small bubbles were present in the system at low gas velocity condition. These bubbles could be easily dragged down the downcomer and became obstacle for the light path. Bubble coalescence was intense at high gas velocity condition. The resulting high liquid velocity was capable of bringing the large bubbles down into the downcomer and obstructed the light penetration. For both cases, the diatom was exposed to lower light intensity, and consequently, lower cell growth could be observed. An expanded top ALPBR was proposed to deal with this problem. The expanded top ALPBR allowed liquid to stay for a longer period in the gas separator section and lowered down the liquid velocity. Therefore a large fraction of bubbles left the system instead of traveling down the downcomer. However, the significance of this system was still a question as, although no light obstruction was observed in the expanded top ALPBR, the growth of the diatom was hardly noticed at a higher superficial gas velocity than 3 cm s^{-1}. It was possible that the increase in the circulation rate for the diatom above the level at u_{sg} = 3 cm s^{-1} did not have substantial influence on the cell growth.

7.2.3 Self shading effect

The growth of the diatom could be self-inhibiting as the dense cell culture obstructed the light path. It was proven in the investigation that the growth of the cell was initially limited by the lack of nutrient, but later on, this was limited by the low light intensity caused by the dense cell culture which did not allow an effective passage of light. This problem could be solved by having a continuous culture in reactors-in-series systems. The diatom in the first reactor would be constantly exposed to high light intensity and accelerate the growth. Although those cells in the final reactor would be limited to the low light intensity condition, this configuration was demonstrated to have positive effect on the overall growth.

7.3 Best Solutions for the Cultivation of *Chaetoceros calcitrans*

Chaetoceros calcitrans can be cultivated in many modes apart from batch cultivation. The semi-continuous cultivation could provide a reasonably good balance between productivity and system maintenance. The continuous system was also proven to have good stability and attractive economical outcome. Furthermore, a large cultivating system was demonstrated to be possible and had a future in further development, although there were some difficulties in achieving a practically good circulation within the system.

Based on economical break down, the most suitable system for the cultivation of *Chaetoceros calcitrans* for the application in marine industries was the 2.8 L continuous system with a medium feed rate of 3 mL min^{-1}. The advantages of this system were the ease of operation and the low investment and operating cost. The lost of operating time due to the startup and shut down periods was minimal. The growth of the diatom in this system could be maintained as the diatom was, most of the time, active. This means whenever the system maintenance was needed, the diatom could be immediately moved to other columns without significantly disturbing its growth. Moreover, the compactness of the system also made the system easy for personal operated which saves the operating area and labor employment.

7.4 Experimental Limitations and Recommendations

Some other aspects of work should still be held for further development of the cultivation of *Chaetoceros calcitrans* in the ALPBR systems, and they are summarized as follows:

1. Effect of salinity on the growth of the diatom

   The cultivation of *Chaetoceros calcitrans* was actually done in the 30 ppt salinity seawater, although it was reported that water salinity could be varied between 20-30 ppt. The actual feeding water for prawn larvae was also subject to a variation in salinity. It is therefore interesting to understand the influence of salinity on the growth of this diatom.

2. Effect of carbon dioxide concentration on the growth of the diatom.

Apart from light for photosynthesis, carbon dioxide is also another factor for the growth of the diatom. Dissolved carbon dioxide may affect the growth behavior as carbon dioxide is the main carbon source for the growth of the diatom. However, extra care should be practiced as the addition of carbon dioxide is often subject to a much higher operating cost. Furthermore, dissolved carbon dioxide will decrease the pH of the culturing medium which may inhibit the growth of the diatom.

3. The probability for the development of the downstream process *i.e.* the separation of the diatom out from the culture medium.

Currently, the downstream process for the production of *Chaetoceros calcitrans* was not considered. The diatom together with the culturing medium was directly used as a feed for the larvae of aquatic animals. This, not only, presents a risk for the contamination from the culturing medium, but also requires an additional resource for the transportation of the diatom culture. The development of the downstream process, such as concentration unit, extraction processes, may result in an easy handling and transportation of the diatom and eventually prevents the risk of contamination.

REFERENCES

Bailey, J. E. and Ollis, D. F. Biochemical Engineering Fundamentals. New York: McGraw-Hill, 1986.

Baquerisse, D., Nouals, S., Isambert, A., Santos, P. F., Durand, G. Modeling of a Continuous Pilot Photobioreactor for Microalgae Production J. Biotechnol. 70 (1999): 335-342.

Belay, A. Mass Cultivation of *Spirulina* Outdoors-The Earthrise Farms Experience. (1997), Sited in: Vonshakm A. (Ed.), *Spirulina platensis* (*Arthrospira*): Physiology, Cell Biology and Biotechnology. Taylor and Francis, London, pp. 131-158.

Blenke H. Loop reactors Advanc. Biochem. Eng. 13 (1979): 121-124.

Borowitzka M. A. Algal Biotechnology Products and Processes: Matching Science and Economics. J. Appl. Phycol. 4 (1992): 267-279. Cited in: Borowitzka, M. A. Microalgae for Aquaculture: Opportunities and Constraints. J. Appl.Phycol. 9 (1997): 393-401.

Borowitzka M. A. Microalgae for Aquaculture: Opportunities and Constraints J. Appl.Phycol. 9 (1997): 393-401.

Borowitzka, M. A. Commercial Production of Microalgae: Ponds, Tanks, Tubes and Fermenter. J. Biotechnol. 70 (1999): 313-321.

Borowitzka, M.A. Closed Algal Photobioreactor: Design Considerations for Large-scale Systems. J. Mar. Biotechnol. 4 (1996): 185-191.

Borowitzka, M.A., and Borowitzka, L.J. "*Dunaliella*." Sited in: Borowitzka, M.A., and Borowitzka, L.J. (Eds.), Micro Algal Biotechnology. Cambridge: Cambridge University Press, 1988.

Chakravarty, M., H. D. Singth, J. N. Baruah and M. S. Iyengar Gas Holdup Distribution in A Gas-Lift Column. Biotechnol. Bioeng. Symp. 4 (1973): 363-378.

Chaumont D. Biotechnology of Algal Biomass Production-A Review of Systems for Outdoor Mass Culture. J. appl. Phycol. 5 (1993): 593-604.

Chiou, S. Y., Su, W. W., Su, Y. C. Optimizing Production of Polyunsaturated Fatty Acids in *Machantia polymorpha* Cell Suspension Culture. J. Biotechnol.. 85 (2001): 247-257.

Chisti, M. Y., and M. Moo-Young Gas Holdup in Pneumatic Reactors Chem. Eng J. 38 (1988) 149-152.

Chrismadha, T., Borowitzka, M. A. Effect of Cell Density and Irradiance on Growth, Proximate Composition and Eicosapentaenoic Acid Production of *Phaeodactylum* Grown in A Tubular Photobioreactor. J. Appl. Phycol. 6 (1994): 67-74.

de Pauw, N. and Persoone, G. Microalgae for Aquaculture. In Borowitzka, M. A., Borowitzka Lj (eds), Bio-algal Biotechnology. Cambridge: Cambridge University Press, 1988.

del Campo, J. A., Rodriguez, H., Moreno, J., Vergas, A., Rivas, J., Guerrero, M. G. Lutein Production by *Muriellopsis* sp. in an Outdoor Tubular Photobioreactor. J. Biotechnol. 85 (2001): 289-295.

Fox J. M. Intensive Algal Culture Techniques. In McVey JP (ed.), CRC Handbook of Mariculture. Crustacean Aquaculture. CRC. Press, Inc., Bocca Raton, Florida, 1983. Cited in: Borowitzka, M. A. Microalgae for aquaculture: Opportunities and Constraints J. appl.Phycol. 9 (1997): 393-401.

Fulks W. and Main K. L. The Design and Operation of Commercial Scale Feeds Production Systems. In Fulks, W., Main, K. L. (eds), Rotifer and microalgae systems. Honolulu: The Oceanic Institute, 1991.

Gavrilescu, M., Tudose, R. Z. Modelling of Liquid Circulation Velocity in Concentric-tube Airlift Reactors. J. Chem. Eng. 69 (198): 85-91.

Göksan T., Durmaz Y. and Gökpinar S. Effects of Light Path Lengths and Initial Culture Density on The Cultivation of *Chaetoceros muelleri*. Aquaculture 217 (2001): 431-436.

Grima, E. M., Sanchez Perez A. S., Camacho F. G., Garcia Sanchez, J. L., Acien Fernandez F. G., Alonso, D. L. Productivity Analysis of Outdoor Chemostat Culture in Tubular Air-lift Photobioreactors. J. Appl. Phycol. 8 (1996): 369-380.

Grima, E. M., Sanchez Perez A. S., Camacho F. G., Garcia Sanchez, J. L., Acien Fernandez F. G., Alonso, D. L. Outdoor Culture of *Isochrysis galbana* ALII-4 in a Closed Tubular Photobioreactor. J. Biotechnol. 37 (1994): 159-166.

Grobbelaar J. U. Turbulence in Mass Algal Cultures and The Role of Light/dark Fluctuations. J. appl. Phycol. 6 (1994): 331-335.

Grobblelaar J. U., Nedbal L. and Tichy V. Influence of High Frequency Light/dark Fluctuations on Photosynthetic Characteristics of Microalgae Photoacclimated to Different Light Intensities and Implications for Mass Algal Cultivation. J. appl. Phycol. 8 (1996): 335-343.

Guillard, R. L. Culture of Phytoplankton for Feeding Marine Invertebrates. 1975, In W. L. Smith, M. H. Chanley (eds.), Culture of Marine Invertebrates Animal. New York: Plenum Press, (For general algae culture techniques).

Hall D. O., Acin Fernandez F. G., Guerrero E. C., Rao K. K., Grima E. M. Outdoor Helical Tubular Photobioreactors for Microalgal Production: Modeling of Fluid-dynamics and Mass Transfer and Assessment of Biomass Productivity. Biotechnol Bioeng. 82 (2003): 62-73.

Healthnotes, Inc., “Blue-Green Algae”, Available from: _ HYPERLINK "http://www.vitacost.com/science/hn/Supp/Blue_Green_Algae.htm" __http://www.vitacost.com/science/hn/Supp/Blue_Green_Algae.htm_., (August 18, 2004).

Hu Q. and Richmond A. A Flat Inclined Modular Photobioreactor (FIMP) for Outdoor Mass Cultivation of Photoautotrophs. Biotechnol. Bioeng. 51 (1996): 51-60.

Krichnavaruk, S. and Pavasant, P. Analysis of Gas-liquid Mass Transfer in An Airlift Contactor with Perforated Plates. Chem. Eng. J. 89 (2002): 203-211.

Lee Y. K. and Richmond A. Bioreactor Technology for Mass Cultivation of Photoautotropic Microalgae. (1998). Cited in Fingerman M., Nagabhushanam R. and Thompson M. Recent Advances in Marine Technology. Env. Marine. Biotechnol., Vol 2, Oxford & IBH, New Delphi, 271-288.

Lee Y. K. Commercial Production of Microalgae in The Asia-Pacific Rim. J. Appl. Phycol. 9 (1997): 403-411.

Lee Y. K., Ding S. Y., Chang Y. C., Forday W. L., Chew P. C. and Low C. S. Design and Performance of An (α-type Tubular Photo-bioreactor for Mass Cultivation of Microalgae. J. Appl. Phycol. 7 (1995): 47-51.

Lee Y. K., Ding S. Y., Hoe C. H. and Low C. S. Mixotrophic Growth of *Chlorella sorokiniana* in Outdoor Enclosed Photobioreactor. J. Appl. Phycol. 8 (1996): 163-169.

Lee, Y. K. and Low, C. S. Effect of Photobioreactor Inclination on The Biomass Productivity of An Outdoor Algal Culture. Biotechnol. Bioeng. 38 (1991): 995-1000.

Lee, Y. K. Commercial Production of Microalgae in The Asia-Pacific Rim J. Appl. Phycol. 9 (1997): 403-411.

Lee, Y. K. Enclosed Bioreactors for The Mass Cultivation of Photosynthetic Microorganism: The Future Trend TIBTECH. 4 (1986): 186-189. Cited in Rebolloso Fuentes, M. M., Garcia Sanchez, J. L., Fernandez Sevilla, J. M., Acien Fernandez, F. G., Sanchez Perez, J. A., Grima, E. M. Outdoor Continuous Culture Of *Porphyridium Cruentum* in A Tubular Photobioreactor: Quantitative Analysis of The Dialy Cyclic Variation of Culture Parameters. J. Biotechnol. 70 (1999): 271-288.

Lee, Y. K. Micro Algal Mass Culture System and Methods: Their Limitation and Potential. J. Appl. Phycol. 13 (2001): 307-315.

Lee, Y. K., Low, C. S. Effect of Photobioreactor Inclination on The Biomass Productivity of An Outdoor Algal Culture. Biotechnol. Bioeng. 38 (1991): 995-1000.

Liang B. H., Leung P. S. and Shang Y. C. Overview of World Shrimp Industry Aquaculture Asia 2-3 (1997): 28-31.

Limpanuphap, A. A Hydrodynamics and Gas-liquid Mass Transfer in Internal Loop Airlift Contactor with Sea Water. Master thesis Chemical Engineering Chulalongkorn University, 2003.

Loataweesup W. Cultivation of A Diatom Chaetoceros Calcitrans in Airlift Bioreactor. Master thesis Chemical Engineering Chulalongkorn University, 2002.

Lu, C., Rao, K., Hall, D., Vonshak, A. Production of Eicosapentaenoic Acid (EAP) in *Monodus Subterraneus* Grown in A Helical Tubular Photobioreactor As Affected by Cell Density and Light Intensity. J. Appl. Phycol. 13 (2001): 517-522.

Merchuk, J. C., Ladwa, N. Cameron, A., Bulmer, M., Berzin, I., Pickett, A. M. Liquid Flow Mixing in Concentric Tube Air-Lift Reactors. J. Chem. Tech. Biotechnol. 66 (1996): 174-182.

Merchuk, J. C., Ronen, M., Geris, S., Arad, S. Light/dark Cycles in the Growth of the Red Microalga *Porphylidium sp.* Biotechnol. Bioeng. 59 (1998): 705-713.

Merchuk, J. C., Stein, Y. Local Hold-Up and Liquid Velocity in Air-lift Reactors. AIChE J. 27(3) (1981): 377-388.

Miyamoto, K., Wable, O., and Benemann, J. R. Vertical Tubular Reactor for Microalgae Cultivation. Biotech. Letters. 10 (1988): 703-708.

Morita, M., Watanabe, Y., Saiki, H. Investigation of Photobioreactor Design for Enhancing The Photosynthetic Productivity of Microalgae. Biotechnol. Bioeng. 69 (2000): 693-698.

O' Meley, C. and Daintith, M. Algal Culture for Matrine Hatcheries. Aquaculture Source books, Launceston: 1993.

Orazem, M.E. and Erickson, L.E. Oxygen-Transfer Rates and Efficiencies in One- and Two-Stage Airlift Towers Biotechnol. Bioeng. 21 (1979): 69-88.

Pulz O. Open-Air and Semi-Closed Cultivation Systems for The Mass Cultivation of Microalgae. Kuala Lumpur: University of Malaya, 1994.

Pushparaj, B., Pelosi, E., Torzillo, G., Carlozzi, P., Sacchi, A., Materassi, R. Growth Physiology of A Marine Nitrogen-Fixation Cyanobacterium (*Nodularia Harveyana*) in Outdoor Culture. J. Appl. Phycol. 6 (1994): 533-537.

Pushparaj, B., Pelosi, E., Tredici, M. R., Pinzani, E., Materassi, R.An Integrated Culture System for Outdoor Production of Microalgae and Cyanobacteria. J. Appl. Phycol. 9(2) (1997): 113-119.

Qiang, H., Guterman, H., Richmond, A. A Flat Inclined Modular Photobioreactor for Outdoor Mass Cultivation of Photoautotrophs. Biotech. Bioeng. 51 (1996): 51-60.

Qiang, H., Guterman, H., Richmond, A. Physiological Charateristics of *Spirulina Platensis* (Cyanobacteria) Cultured at Ultrahigh Cell Density. J. Phycol. 32 (1996): 1066-1073.

Qiang, H., Richmond, A. Productivity and Photosynthetic Efficiency of *Spirulina Platensis* as Affected by Light Intensity, Algal Density and Rate of Mixing in A Flat Plate Photobioreactor. J. Appl. Phycol. 8 (1996): 139-145.

Richmond A. Efficient Utilization of High Irradiance for Production of Photoautotropic Cell Mass. A survey. J. appl. Phycol. 8 (1996) 381-387.

Richmond A., Boussiba S., Vonshak A. and Kopel R. A New Tubular Reactor for Mass Production of Microalgae Outdoors. J. Appl. Phycol. 5 (1993) 327-332.

Richmond A., Lichtenberg E., Stahl B. and Vonshak A. Quantitative Assessment of The Major Limitations on Productivity of *Spirulina Platensis* in Open Raceways. J. Appl. Phycol. 2 (1990) 195-206.

Richmond, A., Cheng-Wu, Z. Optimization of a Flat Plate Glass Reactor for Mass Production of *Nannochloropsis* sp. Outdoors. J. Biotechnol.. 85 (2001) 259-269.

Richmond, A., Zou, N. Efficient Utilization of High Photon Irradiance for Mass Production of Photoautotrophic Micro-organisms. J. Appl. Phycol. 11 (1999) 123-127.

Samonte G. PB., Espergadera C. C. and Caturao R. D. Economics of Microalgae (*Chaetoceros Calcitrans*) Production Using The Multi-Step Method in The Philippines. Aquaculture 112(1) (1993): 39-45.

Setlik I., Veladimir S. and Malek I. Dual Purpose Open Circulation Units for Large Scale Culture of Algae in Temperature Zones. I., Basic Design Considerations and Scheme of Pilot Plant. Algol. Stud. (Trebon) 1 (1970): 11.

Setlik, I., Veladimir, S., Malek, I. Dual Purpose Open Circulation Units for Large Scale Culture of Algae in Temperature Zones. I., Basic Design Considerations and Scheme of Pilot Plant Algol. Stud. (Trebon) 1 (1970): 11. Cited in Lee, Y. K. Microalgal Mass Culture Systems and Method: Their Limitation and Potential. J. Appl. Phycol. 13 (2001) 307-315.

Smith D.A., “The Honduran Shrimp Farming Industry: Social and Environmental Impacts”, New York University School of Law, (1998), Available from: _ HYPERLINK "http://www.earthsummitwatch.org/shrimp/national_reports/crhond1.html"

__http://www.earthsummitwatch.org/shrimp/national_reports/crhond1.html_, (July 5, 2004).

Soong, P. Production and Development of *Chlorella* and *Spirulina* in Taiwan. (1880). Sited in: Shelef, G., Soeder, C.J. (Eds.), Algae biomass. Amsterdam: Elsevier/North Holland Biomedical Press, 97-113.

Suh, I. S., Lee, S. B. Cultivation of A Cyanobacterium in An Internally Radiating Air-Lift Photobioreactor. J. Appl. Phycol. 13 (2001): 381-388.

Suksoir, V. Transient Models for Oxygen Mass Transfer in Airlift Contactor. Master Thesis Chemical Engineering Chulalongkorn University, 2000.

Tanthikul N. Hydrodynamics and Mass Transfer Behavior in Large Scale Multiple Draft Tube Airlift Contactors**.** Master thesis Chemical Engineering Chulalongkorn University, 2004.

Torzillo, G. Tubular Bioreactor. Sited in: Vonshak, A. (Ed.), (*Spirulins platensis* (*Arthrospira*): Physiology, cell biology and biotechnology. Taylor and Francis London: 1977.

Torzillo, G., Carlozzi, P., Pushparaj, B., Montaini, E. A Two-Plane Tubular Photoioreactor for Outdoor Culture of *Spirulina* Biotechnol. Bioeng. 42 (1993): 891-898.

Tredici M. R. and Materassi R. From Opened Ponds to Vertical Alveolar Panels-The Italian Experience in The Development of Reactors for The Mass Cultivation of Phototropic Microorganisms. J. appl. Phycol. 4 (1992): 221-231.

Tredici M. R., Carlozzi P., Zittelli C. G. andd Materassi R. A Vertical Alveolar Panel (VAP) for Outdoor Mass Cultivation of Microalgae and Cyanobacteria. Biores. Technol. 38 (1991): 153-159.

Tredici, M. R., Zittelli, G. C.Efficiency of Sunlight Utilization: Tubular versus Flat Photobioreactors. Biotechnol. Bioeng. 57(2) (1998): 187-197.

Tredici, M.R., Zitelli, G.C. Cultivation of *Spirulina* (*Arthrospira*) *Platensis* in Flat Plate Reactors. (1997). Cited in: Vonshak, A. (Ed.), *Spirulina platensis* (*Arthrospira*): Physiology, cell biology and biotechnology. Taylor and Francis, London: 117-130.

Tung, H. L., Tu, C. C., Chang, Y. Y., Wu, W. T. Bubble Characteristics and Mass Transfer in An Airlift Reactor with Multiple Net Draft Tubes Bioproc. Eng. 18 (1998): 323-328.

Tung, H.L., Chiou, S.Y., Tu, C.C. and Wu, W.T. An Airlift Reactor with Double Net Draft Tubes and Its Application in Fermentation. Bioproc. Eng. 17 (1997) 1-5.

University of Paisley, Department of Biological Sciences, “PUFA Biological Functions”, Available from: _ HYPERLINK "http://www-biol.paisley.ac.uk/Courses/Enzymes/glossary/PUFA.html"__http://www.biol.paisley.ac.uk/Courses/Enzymes/glossary/PUFA.html_, (August 15, 2004).

Vonshak A. Outdoor Mass Production of *Spirulina*: The basic concept. Cited in Vonshak A. (ed), *Spirulina platensis* (*Arthrospira*): Physiology, Cell Biology and Biotechnology. Taylor and Francis, London: 1997.

Vonshak, A., Guy, R. Photoresponse, Photoinhibition and Productivity in The Blue-Green Alga, *Spirulina Platensis*, Grown Outdoors. Plant Cell Environ. 15 (1992): 613-616. Cited in Rebolloso Fuentes, M. M., Garcia Sanchez, J. L., Fernandez Sevilla, J. M., Acien Fernandez, F. G., Sanchez Perez, J. A., Grima, E. M. Productivity and Photosynthetic Efficiency of *Spirulina Platensis* as Affected by Light Intensity, Algal Density And Rate Of Mixing In A Flat Plate Photobioreactor. J. Biotechnol. 70 (1999): 271-288.

Watanabe, Y., Noue, J., Hall, D. O. Photosynthetic Performances of A Helical Tubular Photobioreactor Incorporating The Cyanobacterium *Spirulina Platensis*. Biotech. Bioeng. 47 (1995): 261-269.

Wongsuchoto P. Bubble Characteristics and Liquid Circulation in Internal Loop Airlift Contactors Doctor thesis Chemical Engineering Chulalongkorn University, 2002.

Wongsuchoto, P., Charinpanitkul, Y., and Pavasant, P. Bubble Size Distribution and Gas-Liquid Mass Transfer in Airlift Contactors. Chem. Eng. J. 92 (2003): 81-90.

Zhang, D. H., Lee, Y. K., Ng, M. L., Phang, S. M. Composition and Accumulation of Secondary Carotenoids in *Chlorococcum* Sp. J. Appl. Phycol. 9 (1997): 147-155.

Zhukova, N. V., Aizdaicher, N. A. Fatty Acid Composition of 15 Species of Marine Microalgae. Phytochem. 39(2) (1995): 351-356.

Zittelli, G. C., Lavista, F., Bastianini, A., Rodolfi, L., Vincenzini, M. Production of Eicopentaenoic Acid by *Nannochloropsis* Sp. Cultures in Outdoor Tubular Photobioreactors. J. Biotechnol. 70 (1999): 299-312.

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