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EXTERNAL LOOP AIRLIFT PHOTOBIOREACTOR FOR CULTIVATION OF *Chaetoceros calcitrans*

Ms. Piyanate Nakseedee

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By	Ms. Piyanate Nakseedee
Field of Study	Chemical Engineering
Advisor	Associate Professor Prasert Pavasant, Ph.D.
Co-advisor	Sorawit Powtongsook, Ph.D.

Accepted by the Faculty of Engineering, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

.....Dean of the Faculty of Engineering
(Associate Professor Boonsom Lerdhirunwong, Dr.Ing.)

THESIS COMMITTEE

..... Chairman
(Associate Professor Muenduen Phisalaphong, Ph.D.)

..... Advisor
(Associate Professor Prasert Pavasant, Ph.D.)

..... Co-Advisor
(Sorawit Powtongsook, Ph.D.)

..... Examiner
(Assistant Professor Artiwan Shotipruk, Ph.D.)

.....External Examiner
(Aparat Mahakhant, Ph.D.)

ปิยะเนตร นาคสีดี : ถังปฏิกรณ์ชีวภาพเชิงแสงอากาศยกแบบไหลวนภายนอกสำหรับการเลี้ยงคีโตเซอรอส คาลซิแทรนส์ (EXTERNAL LOOP AIRLIFT PHOTOBIOREACTOR FOR CULTIVATION OF *Chaetoceros calcitrans*) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: รศ.ดร. ประเสริฐ ภวสันต์, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม: ดร. สรวิศ เผ่าทองศุข, 76 หน้า.

การศึกษาสภาวะที่เหมาะสมของการเติบโตของไดอะตอมคีโตเซอรอส คาลซิแทรนส์ในถังปฏิกรณ์ชีวภาพเชิงแสงอากาศยกชนิดไหลวนภายนอกแบบอากาศยกขนาด 17 ลิตร ซึ่งมีค่าอัตราส่วนระหว่างพื้นที่หน้าตัดการไหลขึ้นต่อพื้นที่หน้าตัดการไหลลงของของไหล (A_r:A_d) เป็น 3.717 แสดงให้เห็นว่าการลดระดับความสูงของส่วนที่ให้อากาศและไม่ให้อากาศจาก 120 เซนติเมตร เป็น 100 เซนติเมตร ช่วยพัฒนาประสิทธิภาพการเติบโตของเซลล์ เนื่องจากลักษณะของถังปฏิกรณ์รูปแบบนี้มีฟองอากาศจำนวนมากในส่วนที่มีการให้อากาศ ซึ่งมีผลทำให้การถ่ายเทมวลสารระหว่างก๊าซและของเหลวได้มากขึ้น เพราะฉะนั้นก๊าซคาร์บอนไดออกไซด์สามารถถ่ายเทสู่ของเหลวเพื่อให้เซลล์ใช้เป็นแหล่งคาร์บอนได้ง่ายขึ้น ส่วนรูปแบบเครื่องแบบท่อเชื่อมระหว่างส่วนที่ให้อากาศและไม่ให้อากาศแบบเอียงนั้นช่วยให้ฟองอากาศออกจากระบบได้มากขึ้น แต่เนื่องจากความเร็วที่เพิ่มมากขึ้น ทำให้การถ่ายเทมวลสารระหว่างก๊าซและของเหลวน้อย ซึ่งไม่ส่งผลต่อการพัฒนาการเติบโตของเซลล์เมื่อเปรียบเทียบกับแบบท่อเชื่อมระดับสูง สำหรับการเลี้ยงที่อัตราการให้อากาศเป็น 3 เซนติเมตรต่อวินาที โดยเลี้ยงแบบกึ่งกะได้ความหนาแน่นสูงสุดของเซลล์มีค่าเป็น 11.9×10^6 เซลล์ต่อมิลลิลิตรและอัตราการเติบโตจำเพาะสูงสุดมีค่าเป็น 0.0846 ต่อชั่วโมง เนื่องจากที่อัตราการให้อากาศค่าน้อย (< 3 เซนติเมตรต่อวินาที) ระบบมีการถ่ายเทมวลสารระหว่างก๊าซและของเหลวน้อย ไม่ทำให้อัตราการเติบโตเพิ่มขึ้น ในขณะที่อัตราการให้อากาศสูง (>3 เซนติเมตรต่อวินาที) เป็นเหตุให้เซลล์ถูกทำลาย และที่อัตราการให้อากาศ 4 เซนติเมตรต่อวินาที เป็นอัตราการให้อากาศที่มีค่าสูงทำให้มีปริมาณฟองขนาดเล็กในส่วนที่ไม่ให้อากาศ ซึ่งทำให้ความเข้มแสงภายในถังปฏิกรณ์ลดลง และยังพบว่าประสิทธิภาพของการเติบโตของเซลล์ในถังปฏิกรณ์แบบอากาศยกชนิดไหลวนภายนอกดีกว่าชนิดไหลวนภายใน เนื่องจากปัญหาของการบังแสงจากฟองลดน้อยลงเนื่องมาจากการเลี้ยงเซลล์ในถังปฏิกรณ์แบบอากาศยกชนิดไหลวนภายนอกรูปแบบนี้ช่วยลดปริมาณฟองในส่วนที่ไม่ให้อากาศ ลดปัญหาการบังแสงจากฟองอากาศ ซึ่งเป็นปัจจัยที่จำกัดการเติบโตของเซลล์ในถังปฏิกรณ์แบบอากาศยกชนิดไหลวนภายใน และช่วยให้แสงส่องผ่านเข้าถึงเซลล์ได้ดีกว่า สุดท้ายนี้การประมาณค่าเบื้องต้นที่ของผลของความเข้มข้นของของก๊าซคาร์บอนไดออกไซด์ที่ใช้ในการเลี้ยงเซลล์คีโตเซอรอส คาลซิแทรนส์ในถังปฏิกรณ์แบบอากาศยกชนิดไหลวนภายนอกรูปแบบดังกล่าว แสดงให้เห็นว่าถึงแม้ว่าเซลล์จะสามารถเติบโตที่ความเข้มข้นคาร์บอนไดออกไซด์สูงถึง 5 เปอร์เซ็นต์ แต่การเติบโตของเซลล์ก็ไม่ได้ดีไปกว่าการเลี้ยงโดยให้อากาศเพียงอย่างเดียว ซึ่งน่าจะเป็นเหตุมาจากค่าความเป็นกรดที่มากขึ้น

ภาควิชา.....วิศวกรรมเคมี..... ลายมือชื่อนิสิต.....

สาขาวิชา.....วิศวกรรมเคมี..... ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

ปีการศึกษา.....2551..... ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม.....

5070354021 : MAJOR CHEMICAL ENGINEERING
KEYWORDS: AIRLIFT PHOTOBIOREACTOR / *CHAETOCEROS CALCITRANS* / EXTERNAL LOOP

PIYANATE NAKSEEDEE: EXTERNAL LOOP AIRLIFT PHOTOBIOREACTOR FOR CULTIVATION OF *Chaetoceros calcitrans*. ADVISOR: ASSOC. PROF. PRASERT PAVASANT, Ph.D., CO-ADVISOR: SORAWIT POWTONGSOOK, Ph.D., 80 pp

The study of the cultivation of *Chaetoceros calcitrans* was performed using the 17 L external loop airlift photobioreactor (ELAP) with the ratio between downcomer and riser cross sectional area (A_d:A_r) of 3.717. It was demonstrated that lowering the location of the connection tube from 120 cm (in UTCT-ELAP) to 100 cm (in LTCT-ELAP) helped improve the growth performance. This could be due to the improved gas-liquid mass transfer rate which allowed more CO_2 to dissolve into the medium as a carbon source. Inclining the connection tube (in ITCT-ELAP) could facilitate the release of bubbles from the system, however, the inherited higher liquid velocity in such system lowered the gas-liquid mass transfer, and no improvement in the growth was observed when compared with the performance of UTCT-ELAP. Optimal aeration superficial velocity (u_{sg}) was found to agree with the literature at 3 cm s^{-1} where the maximum cell density and specific growth rate from the semi-batch operation of LTCT-ELAP were approx. 11.9×10^6 cell mL^{-1} and 0.0846 h^{-1}, respectively. This was because at low u_{sg} (< 3 cm s^{-1}), the system was operated at low gas-liquid mass transfer which inherently could not allow high growth rate. On the other hand, high u_{sg} (> 3 cm s^{-1}) caused too much cell damage and lowered the growth performance. only a slight decrease in maximum cell concentration was observed at u_{sg} of 4 cm s^{-1} as this rather high u_{sg} could see small bubbles into the downcomer and decreasing the light intensity. When compared with the internal loop airlift photobioreactor (ILAP), ELAP clearly provided a better growth performance due to a lower light shading effect. Owing to the fact that the cultivation in ELAP helped reduce the number of bubbles in the downcomer, this lessened the bubble shading effect which was the growth limiting factor in ILAP, and promoting a better light penetration to cells. Lastly, the pre-evaluation of the effect of CO_2 for the cultivation of *C. calcitrans* in the LTCT-ELAP demonstrated that, although cells of *C. calcitrans* could grow in the system with as much as 5% CO_2, the growth was not as good as when the cells were cultivated in normal air (without CO_2). This could be attributed to the high acidity level imposed by CO_2.

Department:.....Chemical Engineering..... Student's Signature: .....
Field of Study:Chemical Engineering..... Advisor's Signature: .....
Academic Year:.....2008..... Co-advisor's Signature: .....

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CHAPTER I

INTRODUCTION

1.1 Motivations

Shrimp is regarded as one of economic animals for Thailand as the country is among the top shrimp producers in the world. One of the most significant steps in cultivating shrimp is to have an effective larviculture technique. Good feeding strategy is one the main concerns in shrimp larviculture. Shrimp larvae needs alive feed and single cell algae are often used for this purpose. Among the various types of algae available as a shrimp larvae feed, the diatom *Chaetoceos calcitrans* is one of the most popular as it provides essential nutritions for the healthy growth of the shrimp zoea stages. Therefore the cultivation of *Chaetoceros calcitrans* is one of the major upstream requirements for strong and healthy shrimp culture.

Generally, *Chaetoceros calcitrans* is cultivated in a two-step process. Firstly, the diatom is cultivated as dense cell culture in a one-liter-glass bottle for 2–3 days. The culture is upscaled into a medium size tank of a low culture density for one day 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. The diatom is finally moved to larger ponds (about 2-3 tons) and left growing for one day before it is ready to use in feeding shrimp larvae. However, this type of cultivation can only reach low cell densities resulting in a large area requirement and the diatom is easily contaminated from other microorganisms. The design of closed system bioreactors is therefore treated as a solution to these problems.

Recently, the cultivation of diatom *Chaetoceros calcitrans* in internal loop airlift photobioreactors (ILAP) was investigated at the Department of Chemical Engineering, Chulalongkorn University (Loataweesup, 2002; Krichnavaruk et al., 2005, 2007; Sriouam, 2007). There are clear evidences that this diatom could be well cultivated in ILAP. A high maximum cell concentration of 8.88 x 10^6 cells mL^{-1} could be achieved from the 17 L batch cultivation in ILAP with a maximum specific growth

rate of 7.41 x 10^{-2} h^{-1} at superficial gas velocity (u_{sg}) of 3 cm s^{-1} (Krichnavaruk et al., 2005, 2007), or with a maximum cell concentration of 7.30 x 10^{6} cells mL^{-1} with maximum specific growth rate = 7.0 x 10^{-2} h^{-1} at u_{sg} = 3 cm s^{-1} (Sriouam, 2007). This fast growth was achieved due to several main benefits from the use of airlift systems such as good mixing, well-defined fluid flow pattern, and low operating cost.

External loop airlift photobioreactor (ELAP) has two separate columns operating as riser and downcomer with connection tubes connecting the two columns together near the top and the bottom. This physical appearance allows easy alternation of the reactor configurations to suit particular needs for each application. Examples of the various design configurations of ELAP are shown in Figure 1.1. Moreover, as the supply of light to ELAP can be done both at riser and downcomer, a better light penetration to the diatom compared with ILAP is possible. ILAP and ELAP are schematically shown in Figure 1.2. It is therefore anticipated that ELAP would provide a better cultivating performance for *Chaetoceros calcitrans* when compared with ILAP of similar geometry. This work aims to determine the optimal design configurations for the growth of *Chaetoceros calcitrans* in ELAP which yields high cell concentration and proposed alternative options in terms of configuration to improve the reactor performance regarding the growth of *Chaetoceros calcitrans*.

1.2 Objectives

The objectives of this work are to:

1.2.1 Determine the optimal growth conditions and the effects of design configurations and operating conditions on the cultivation of *Chaetoceros calcitrans* in ELAP, and

1.2.2 Compare the cultivation of *Chaetoceros calcitrans* in ELAP with that in ILAP, and

1.2.3 Pre-evaluate the effect of carbon dioxide on the cultivation of *Chaetoceros calcitrans* in ELAP.

1.3 Working Scopes

1.3.1 Modified standard F/2 (Guillard's) medium and light intensity at approximate 10,000 Lux was used for the cultivation of *Chaetoceros calcitrans* as proposed by Loataweesup (2002).

1.3.2 Bioreactor employed in this work are:

- 17 L external loop airlift photobioreactor with A_d/A_r 3.717
- 17 L internal loop airlift photobioreactor with A_d/A_r 2.620

1.3.3 The range of superficial gas velocity (u_{sg}) employed in this work was between 1–4 cm s^{-1}

1.3.4 The various configurations of external loop airlift photobioreactor are shown in Figure 3.2.

1.3.5 The range of carbon dioxide in the air inlet was from 1 to 5%.

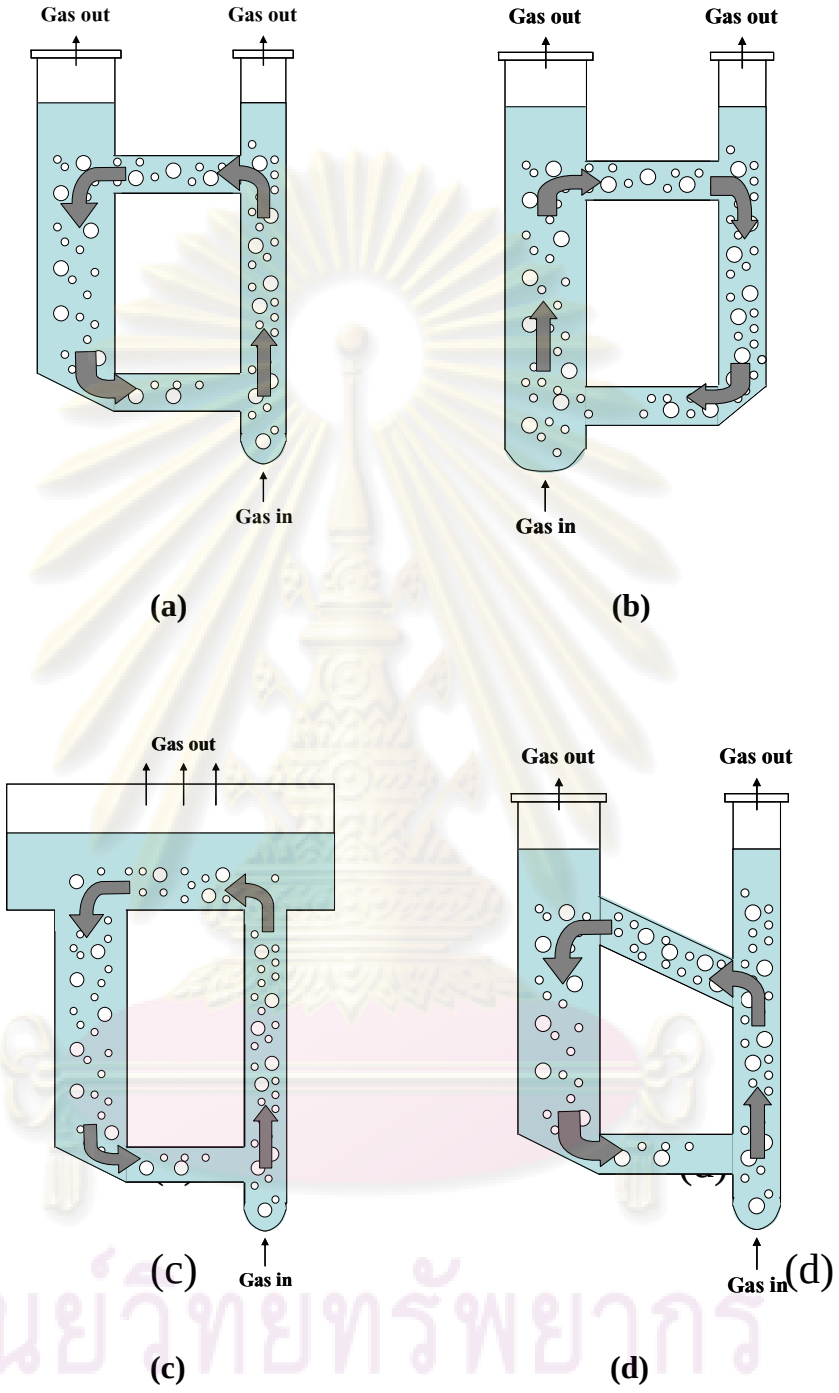


Figure 1.1 Various types of external loop airlift photobioreactor: (a) riser smaller than downcomer (b) riser bigger than downcomer (c) inclined connection tubes (d) liquid pool gas liquid separator

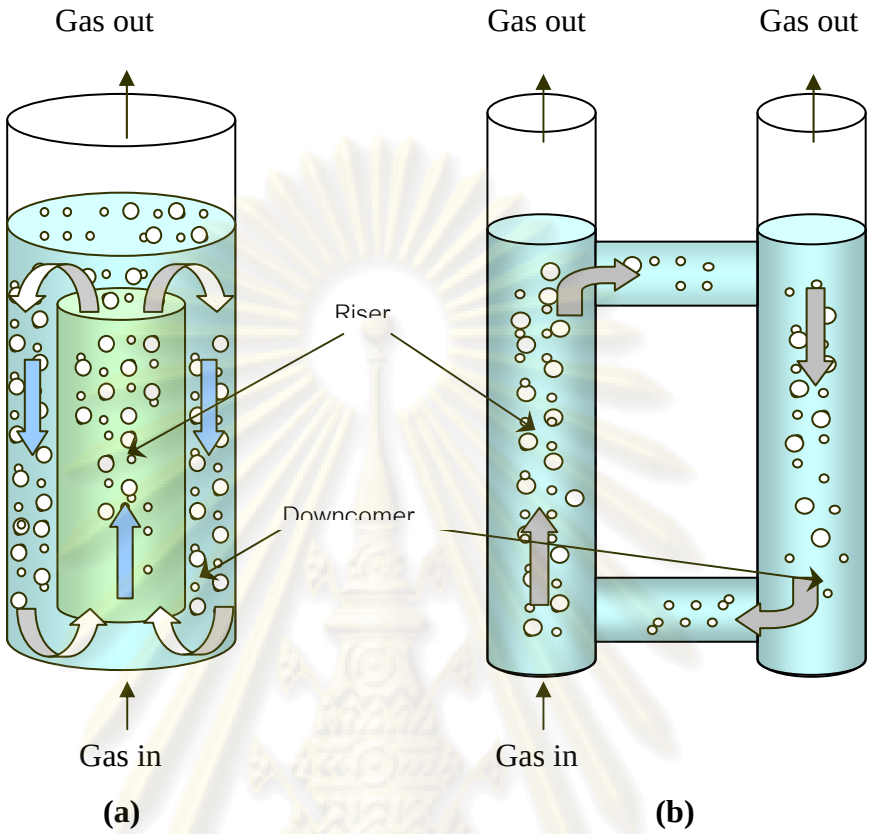


Figure 1.2 Types of airlift photobioreactor: (a) internal loop (b) external loop

CHAPTER II

BACKGROUNDS AND LITURATURE REVIEW

2.1 Introduction to algae

2.1.1 Definition

Algae are a large and diverse group of simple, typically autotrophic organisms, ranging from unicellular to multicellular forms. The largest and most complex marine forms are called seaweeds. They are photosynthetic, like plants, and "simple" because they lack the many distinct organs found in land plants (Nabors, 2004; Houghton, 2000).

The terms algae refers to both macroalgae and a highly diversified group of microorganisms known as microalgae. The profound diversity of size ranging from picoplankton only 0.2-2.0 μm in diameter to giant kelps with fronds up to 60 m in length, ecology and colonized habitats, cellular structure, levels of organization and morphology, pigments for photosynthesis, reserve and structural polysaccharides, and type of life history reflect the varied evolutionary origins of this heterogeneous assemblage of organisms. The number of algae species has been estimated to be one to ten million, and most of them are microalgae (Barsanti and Gualtieri, 2006).

2.1.2 Classification

No easily definable classification system acceptable to all exists for algae because taxonomy is under constant and rapid revision at all levels. A tentative scheme of classification is adopted mainly based on the work of Van Den Hoek et al. (1995) and compared with the classifications of Bold et al (1987). Prokaryotic members of this assemblage are grouped into two divisions: Cyanophyta and Prochlorophyta, whereas eukaryotic members are grouped into nine divisions: Glaucophyta, Rhodophryta, Heterokontophata, Haptophyta, Cryptophyta, Dinophyta,

Euglenophyta, Chlorarachniophyta, and Chlorophyta (Barsanti and Gualtieri, 2006) (Table 2.1).

2.1.3 Diatoms

Diatoms are a major group of eukaryotic algae in a class of Bacillariophyceae (Diaomaphyceae) and are one of the most common types of phytoplankton. Most diatoms are unicellular or colonial algae found floating in all the waters of the earth. The diatoms are single-celled, eukaryotic organisms, having genetic information sequestered into sub-cellular compartments called nuclei. This characteristic distinguishes the group from other single-celled photosynthetic aquatic organisms, like the blue-green algae that do not possess nuclei and are more closely related to bacteria. Diatoms also are distinct because they secrete complex outer cell walls, sometimes called skeletons. The skeleton of a diatom is properly referred to as a frustule (The silicon containing, complex, ornate cell wall structures of diatomous algae) (Round, 1990).

Diatom cells within frustules contain chloroplasts which are the organelles in photosynthetic activity. Chloroplasts contain chlorophyll which function to capture solar energy and convert it into usable chemical energy in the form of simple sugars. Due to this, diatoms are essential components of aquatic food chains. They are a major food source for many microorganisms, aquatic animal larvae, and grazing animals like mollusks (snails) (Stoermer, 1999).

2.1.4 *Chaetoceros calcitrans*

Chaetoceros is probably the largest genus of marine planktonic diatoms with approximately 400 species described. It is unicellular diatom found in both fresh and marine aquatic environments, and most remain at the upper levels of the water column where they can absorb the most light**.** One of the most popular strains for feeding shrimp larvae is *Chaetoceros calcitrans.*

Chaetoceros calcitrans is originated from the Philippines. This cell is united by their long setae to form filaments. The size of cell is about 8-12 μm in length and about 4-10 μm in width. It has a slit along the valves (the raphae) and inside the valves the cytoplasm forms a relatively thin lining surrounding a large vacuole filled with cell sap; the nucleus is central in position with cytoplasmic strands extending across the vacuole. The *Chaetoceros* is comprised of highly-unsaturated fatty acids with a good balance of vitamins. The cell is found rich in protein (27%), nucleic acid (10%) and lipid (11%) (Zhukova and Aizdaicher, 1995). The percentages of biochemical composition and total fatty acids of *Chaetoceros calcitrans* are shown in Tables 2.2 and 2.3, respectively.

2.2 Physical and chemical conditions for cultivation algae

The biochemical composition of algae is generally affected by cultivation conditions and many other factors such as nutrient composition, light intensities, pH, aeration, salinity, carbon dioxide and temperature, etc.

2.2.1 Culture medium/Nutrients

Concentrations of cells in phytoplankton cultures are generally higher than those found in nature. Algal cultures must therefore be enriched with nutrients to make up for the deficiencies in the seawater. Macronutrients include nitrate, phosphate, and silicate. Micronutrients consist of various trace metals and the vitamins, thiamin (B_1), cyanocobalamin (B_{12}) and sometimes biotin. Two enrichment media that have been used extensively and are suitable for the growth of most algae are the Walne medium (Table 2.4.) and the standard Guillard's F/2 medium (Table 2.5). Various specific recipes for algal culture media are described by Vonshak (1986). Commercially available nutrient solutions may reduce preparation labor. The complexity and cost of the above culture media often excludes their use for large-scale culture operations. Alternative enrichment media that are suitable for mass production of micro-algae in large-scale extensive systems contain only the most

essential nutrients and are composed of agriculture-grade rather than laboratory-grade fertilizers (Lavens, and Sorgeloos, 1996).

2.2.2 Light intensity

As with all plants, micro-algae photosynthesize, *e.g.* they assimilate inorganic carbon for conversion into organic matter. Light is the source of energy which drives this reaction, and in this regard, intensity, spectral quality and photoperiod need to be considered. Light intensity plays an important role, but the requirements vary greatly with the culture depth and the density of the algal culture: at higher depths and cell concentrations the light intensity must be increased to penetrate through the culture (*e.g.* 1,000 lux is suitable for Erlenmeyer flasks, 5,000-10,000 lux is required for larger volumes). Light may be natural or supplied by fluorescent tubes. Too high light intensity (*e.g.* direct sun light, small container close to artificial light) may result in photo-inhibition. Also, overheating due to both natural and artificial illumination should be avoided. Fluorescent tubes emitting either in the blue or the red light spectrum should be preferred as these are the most active portions of the light spectrum for photosynthesis (Jeffrey et al., 1977, Brown, 1980 and Glover et al., 1987). However, most algae require a photoperiod of alternating light and dark (Lunning, 1981 and Rebolloso-Fuentes et al., 1999). The duration of artificial illumination should be minimum 18 h of light per day, although cultivated phytoplankton develops normally under constant illumination.

2.2.3 pH

The pH range for most cultured algal species is between 7 and 9, with the optimum range being 8.2-8.7. Complete culture collapse due to the disruption of many cellular processes can result from a failure to maintain an acceptable pH. (Lavens, and Sorgeloos, 1996)

2.2.4 Aeration/mixing

Mixing is necessary to prevent sedimentation of the algae, to ensure that all cells of the population are equally exposed to the light and nutrients, to avoid thermal stratification (e.g. in outdoor cultures) and to improve gas exchange between the culture medium and the air. The latter is of primary importance as the air contains the carbon source for photosynthesis in the form of carbon dioxide. For very dense cultures, the CO_2 originating from the air (containing approximate 0.03% CO_2) bubbled through the culture is limiting the algal growth and pure carbon dioxide may be supplemented to the air supply (e.g. at a rate of 1% of the volume of air). Depending on the scale of the culture system, mixing is achieved by stirring daily by hand (test tubes, erlenmeyers), aerating (bags, tanks), porous gas sparger (airlift), or using paddle wheels and jet pumps (ponds). However, it should be noted that not all algal species can tolerate vigorous mixing. (Lavens, and Sorgeloos, 1996)

2.2.5 Salinity

Marine phytoplanktons are extremely tolerant to changes in salinity. Most species grow best at a salinity that is slightly lower than that of their native habitat, which is obtained by diluting sea water with tab water. Salinity of 20-24 $g.L^{-1}$ has been found to be optimal.

2.2.6 Carbon dioxide (CO_2)

Carbon dioxide (CO_2) is a principal carbon source for the algae. The supplying rate of CO_2 therefore controls the growth rate of the algae. In dense cultures, the rate of diffusion of carbon dioxide into the culture from the air becomes limiting more rapidly than the system with low population density. Improvement of the rate of aeration by shaking, stirring, or bubbling air through the culture will prolong exponential growth.

2.2.7 Temperature

The optimal temperature for phytoplankton cultures is generally between 20 and 24 °C, although this may vary with the composition of the culture medium, the species and strain cultured. Most commonly cultured species of microalgae tolerate temperature between 16-27 °C If necessary, algal cultures can be cooled by a flow of cold water over the surface of the culture vessel or by controlling the air temperature with refrigerated air-conditioning units.

2.3 Algae culture techniques

Algae can be produced using a wide variety of methods. The technical terms used to describe the type of algal culture include:

2.3.1 Open pond systems

Cultivation of algae in open ponds has been extensively studied in the past few years (Boussiba et al., 1988, Tredici et al., 1991; Hase et al., 2000) Open ponds can be categorized into natural waters (lakes, lagoons, pons) and artificial ponds or containers. The most commonly used systems include shallow big ponds, tanks, circular ponds and raceway ponds (Ugwu et al., 2008). The nutrient medium for open ponds cultures is based on that used indoors. The major advantage of open pond systems is that they are easier to construct and operate than closed systems. However, the problems of this type of cultivation include low cell densities resulting in a large area requirement, poor light utilization by the cells, case of contamination, poor batch consistency and unpredictable culture crashes caused by changes in weather, sunlight or water quality.

2.3.2 Cultivation in bioreactors

Because of the problem in open ponds systems, much attention is now focused on the development of closed systems such as flat-plate, tubular, vertical column and airlift photobioreactor

2.3.2.1 Flat-plate photobioreactors

Flat-plate photobioreactors have received much attention for cultivation of photosynthetic microorganisms due to their large illumination surface area. The preparation of flat culture vessels for cultivation of algae was presented by Milner (1953). Subsequently, the development of outdoor flat panel reactor was examined by Ramos de Ortega and Roux (1986) by using thick transparent PVC materials. Afterwards, extensive works on various designs of vertical alveolar panels and flat plate reactors for mass cultivation of different algae were reported (Tredici et al., 1991; Hu et al., 1996, Hoekema et al., 2002, Zhang et al., 2002).

Generally, flat-plate photobioreactors are made of transparent materials for maximum utilization of solar light energy. Accumulation of dissolved oxygen concentrations in flat-plate photobioreactors is relatively low compared to horizontal tubular photobioreactors.

2.3.2.2 Tubular photobioreactors

Tubular photobioreactors are designed to be small parallel tubes in order to provide a larger ratio of surface area to culture volume for effective adsorption of light. It is the one of the most suitable types for outdoor mass cultures. They can be in form of horizontal/serpentine (Chaumont et al., 1988; Molina et al., 2001), vertical (Pirt et al., 1983), near horizontal (Chini Zittelli et al., 1999), conical (Watanabe and Saiki, 1997), inclined (Lee and Low, 1991; Ugwu et al., 2005) photobioreactors. In any case, efficient light distribution to the cells can be achieved by improving the mixing system (Ugwu et al.,2005). However, it is difficult to control culture temperature in most of tubular photobioreactors types. Although they can be equipped with thermostat to maintain the desired culture temperature, this could be very expensive and difficult to implement. It should also be noted that adherence of the cells at the walls of the tubes is common in tubular photobioreactors.

2.3.2.3 Vertical-column photobioreactors

This type of bioreactors is usually a high column which may be cylinder with effective mixing and oxygen removal achieved by air bubbling. Vertical-column photobioreactors are compact, low-cost, and easy to operate monoseptically (Sanchez Mirón et al., 2002).

2.3.2.4 Airlift photobioreactors

Airlift photobioreactor is similar to bubble column reactors, but differ by the fact that they contain a draft tube which improves circulation and oxygen transfer and equalizes shear forces in the reactor. It consists of a liquid pool divided into two distinct zones only one is usually sparged by gas. Airlift photobioreactors can be classified into two major types as the internal loop, which is the cylindrical column with a vertical draft tube which a loop channel for the fluid is formed in the airlift and external loop which has two separated columns operating as riser and downcomer with connection tubes connecting the two columns together near the top and the bottom (Sriouam, 2007).

A summary of the prospects and limitation of these algae culture techniques as shown in Table 2.6

2.4 Cultivation of algae in airlift photobioreactor

With the advantages of airlift system as indicated earlier, a number of research have investigated the growth of algae in the airlift photobioreactor compared with bubble columns such as the study of Merchuk et al. (1998) which showed that a relatively high cell number of the red microalgae *Porphyridium sp.* could be obtained in the airlift bioreactor and the results indicated that the cultivation in airlift achieved higher maximum cell density than that in bubble columns. Xu et al. (2002) cultivated the brown alga *Undaria pinnatifida* in airlift reactor and this yielded higher growth rate than bubble columns. Examples for the work with airlift systems are given in Table 2.7.

For *Chaetoceros calcitrans*, airlift photobioreactors were reported to be an effective cultivated method, particularly the work carried out at the Department of Chemical Engineering, Faculty of Engineering, Chulalongkorn University (Krichnavaruk et al., 2005; 2007; Sriouam, 2007) where suitable nutrient composition, design configuration, and operating constrains have been identified. The modified F/2 medium was proposed with two fold of silica and phosphorus concentrations, optimal light intensity was found to be quite high, at 25,900-29,600 lux but this could be be applied to the bioreactor due to heating problems. With

10,000 lux, the airlift system could provide extraordinary well growth characteristics with the maximum specific growth rate of 7.41×10^{-2} h^{-1} and maximum cell density of 8.88×10^{6} cells mL^{-1}. However, all were done in internal loop airlift systems where the riser is placed inside the column over which there was no control of several important parameters such as light intensity.

Table 2.1 Classification scheme of the different algae groups (Bold, 1987)

Kingdom	Division	Class
Prokaryota eubacteria	Cyanophyta	Cyanophyceae
Eukaryota	Prochlorophyta	Prochlorophyceae
	Glaucophyta	Glaucophyceae
	Rhodophryta	Bangiophyceae
		Florideophyceae
	Heterokontophata	Chrysophyceae
		Parmophyceae
		Sarcinochysidophyce
		Xanthophyceae
		Eustigmatophyceae
		Bacillariophyceae
		Raphidophyceae
		Dictyochophyceae
		Phaeophyceae
		Oomycetes
	Haptophyta	Haptophyceae
	Cryptophyta	Cryptophyceae
	Dinophyta	Dinophyceae
	Euglenophyta	Euglenophyceae
	Chlorarachniophyta	Chlorarachniophyceae
	Chlorophyta	Prasinophyceae
		Chlorophyceae
		Ulvophyceae
		Cladophorophyceae
		Brypsidophyceae
		Zygnematophyceae
		Trentepohliophyceae
		Klebsormidiophyceae
		Clarophyceae

Table 2.2 Biochemical composition of *Chaetoceros calcitrans* expressed as a percentage of dry weight (Zhukova and Aizdaicher, 1995)

Biochemical Composition	**% Dry weight**
Protein	27.21
Nuecleic acid	10.00
Total lipids	11.80
Polyunsaturated fatty acids (PUFAs)	0.90

Table 2.3 Fatty acids composition of *Chaetoceros calcitrans* expressed as a percentage of the total fatty acids (Zhukova and Aizdaicher, 1995)

Fatty acids		**% total fatty acids**
Symbol	**Scientific Name**	
14:0	Myristic	13.0
16:0	Palmitic	18.0
16:1n7	Palmitoleic	28.8
18:1n9	Oleic	0.3
18:2n6	Linoleic	0.7
18:3n3	Linolenic	0.8
18:3n6	Calendic	0.3
18:4n3	Parinaric	0.2
20:4n6	Arachidonic	2.3
20:5n3	Eicosapentaenoic (EPA)	34.0
22:6n3	Decosahexaenoic (DHA)	1.2

Table 2.4 Composition and preparation of Walne medium (modified from Laing, 1991)

Constituents	Quantities
Solution A (at 1 ml per liter of culture)	
Ferric chloride ($FeCl_3$)	0.8 g[(a)]
Manganous chloride ($MnCl_2$, $4H_2O$)	0.4 g
Boric acid (H_3BO_3)	33.6 g
EDTA[(b)], di-sodium salt	45.0 g
Sodium di-hydrogen orthophosphate (NaH_2PO_4, $2H_2O$)	20.0 g
Sodium nitrate ($NaNO_3$)	100.0 g
Solution B	1.0 ml
Make up to 1 litre with fresh water[(c)]	Heat to dissolve
Solution B	
Zinc chloride ($ZnCl_2$)	2.1 g
Cobaltous chloride ($CoCl_2$,6 H_2O)	2.0 g
Ammonium molybdate ($(NH_4)_6Mo_7O_{24}$, $4H_2O$)	0.9 g
Cupric sulphate ($CuSO_4$, $5H_2O$)	2.0 g
Concentrated HCl	10.0 ml
Make up to 100 ml fresh water[(c)]	Heat to dissolve
Solution C (at 0.1 ml per liter of culture)	
Vitamin B_1	0.2 g
Solution E	25.0 ml
Make up to 200 ml with fresh water[(c)]	
Solution D (for culture of diatoms-used in addition to solutions A and C, at 2 ml per liter of culture)	
Sodium metasilicate (Na_2SiO_3, $5H_2O$)	40.0 g
Make up to 1 litre with fresh water[(c)]	Shake to dissolve
Solution E	
Vitamin B_{12}	0.1 g
Make up to 250 ml with fresh water[(c)]	
Solution F (for culture of *Chroomonas salina* - used in addition to solutions A and C, at 1 ml per liter of culture)	
Sodium nitrate ($NaNO_3$)	200.0 g
Make up to 1 litre with fresh water[(c)]	

(a) Use 2.0 g for culture of *Chaetoceros calcitrans* in filtered sea water;

(b) EDTA;

(c) Use distilled water if possible.

Table 2.5 Composition and preparation of standard Guillard's F/2 medium (modified from Smith et al., 1993)

Nutrients	Final concentration [mg L^{-1} sea water]	Stock solution preparations
$NaNO_3$	75	*Nitrate/Phosphate solution*
$NaH_2PO_4.H_2O$	5	*Working stock:* add 75 g $NaNO_3$ + 5 g NaH_2PO_4 to 1 L distilled water (DW)
$Na_2SiO_3.9H_2O$	30	*Silicate solution* : add 30 g $NaSiO_3$ to 1 L DW
$CoCl_2.6H_2O$	0.01	*Trace metal/EDTA solution*
$CuSO_4.5H_2O$	0.01	*Primary stocks*: make 5 separate 1 L stocks of (g L^{-1} DW) 10.0 g $CoCl_2$, 9.8 g $CuSO_4$, 180 g $MnCl_2$, 6.3 g Na_2MoO_4 , 22.0 g $ZnSO_4$
$MnCl_2.4H_2O$	0.18	
$Na_2MoO_4.2H_2O$	0.006	
$ZnSO_4.7H_2O$	0.022	*Working stock*: add 1 mL of each primary stock solution + 4.35 g Na_2EDTA + 3.15 g $FeCl_3$ to 1 L DW
Na_2EDTA	4.36	
$FeCl_3.6H_2O$	3.15	
Thiamin HCl	0.1	*Vitamin solution*
Biotin	0.0005	*Primary stocks:* add 20 g thiamin HCl + 0.1 g biotin + 0.1 g B_{12} to 1 L DW
B_{12}	0.0005	*Working stock*: add 5 mL primary stock to 1 L DW
*add 1 mL each of the four working stock solutions per liter of seawater		

Table 2.6 Prospects and limitations of various culture systems for algae (Ugwu et al, 2007)

Culture systems	Prospects	Limitations
Open ponds	Relatively economical, easy to clean up after cultivation, good for mass cultivation of algae	Little control of culture conditions, difficulty in growing algal cultures for long periods, poor productivity, occupy large land mass, limited to few strains of algae, cultures are easily contaminated
Flat-plate photobioreactors	Large illumination surface area, suitable for outdoor cultures, good for immobilization of algae, good light path, good biomass productivities, relatively cheap, easy to clean up, readily tempered, low oxygen buildup	Scale-up require many compartments and support materials, difficulty in controlling culture temperature, some degree of wall growth, possibility of hydrodynamic stress to some algal strains
Tubular photobioreactors	Large illumination surface area, suitable for outdoor cultures, fairly good biomass productivities, relatively cheap	Gradients of pH, dissolved oxygen and CO2 along the tubes, fouling, some degree of wall growth, requires large land space
Vertical-column photobioreactors	High mass transfer, good mixing with low shear stress, low energy consumption, high potentials for scalability, easy to sterilize, readily tempered, good for immobilization of algae, reduced photoinhibition and photo-oxidation	Small illumination surface area, their construction require sophisticated materials, shear stress to algal cultures, decrease of illumination surface area upon scale-up
Airlift photobioreactors	No moving parts, easy to operate, high gas adsorption efficiency, good heat transfer	Poor mixing, limited to low viscosity system, excessive foaming

Table 2.7 Literature review

Microalgae	Type of reactor	Volumn [L]	T [ºC]	Light intensity [Lux]	Medium	Cell density [x10^5 cells mL^{-1}]	μ [h^{-1}]	Remarks	Reference
Porphyridium sp.	Airlift bioreactor	35	20	-	Organic complex medium	165.6 (2 days)	-	Studies of mixing in a concentric tube airliftbioreactor with different spargers	Merchuk et al., 1998
	Bubble column	35	20	-	Organic complex medium	106.2 (2 days)	-		
Phaeodactylum tricornutum.	Airlift bioreactor	20	22	17020	Modified Ukeles medium	-	2.16	Growth and biochemical characterization of microalgal biomass produced in bubble column and airlift photobioreactors: studies in fed-batch culture	Mirón et al., 2002
	Bubble column	20	22	17020	Modified Ukeles medium	-	1.92		
Undaria pinnatifida	Airlift bioreactor	2.5	25	2960	sterilized fresh medium	-	0.12	Comparison of photobioreactors for cultivation of *Undaria pinnatifida* gametophytes	Xu et al., 2002
	Bubble column	2.5	25	2960	sterilized fresh medium	-	0.08		
Gyrodinium impudicum strain KG03	Airlift bioreactor	2	22.5	11100	F/2 medium	1.23	0.69	Optimal conditions for the production of sulfated polysaccharide bymarine microalga Gyrodinium impudicum strain KG03	Yim et al., 2003
Chaetoceros calcitrans	Airlift bioreactor	17	30	29600	modified standard F/2 medium	88.8	1.78	Optimal growth conditions and the cultivation of *Chaetoceros calcitrans* in airlift photobioreactor	Krichnavarak et al., 2005
	Bubble column	17	30	29600	modified standard F/2 medium	58.0	0.91		
Haematococcus pluvialis	Airlift bioreactor	2.2	26	12580	Bold medium	11	0.23	Haematococcus pluvialis cultivation in split-cylinder internal-loopairlift photobioreactor under aeration conditions avoiding celldamage	Vega-Estrada et al., 2005
Spirulina platensis	Open pond	65	30	4070	medium of Schl¨osser	0.5 gL^{-1}	0.17	Cultivation of *Spirulina platensis* in a combinedairlift-tubular reactor system	Converti et al., 2006
	Tubular photobioreactor	5.5	30	8880	medium of Schl¨osser	10.6 gL^{-1}	0.19		

Table 2.7 Literature review (cont.)

Microalgae	Type of reactor	Volumn [L]	T [ºC]	Light intensity [Lux]	Medium	Cell density [x10^5 cells mL^{-1}]	μ [h^{-1}]	Remarks	Reference
Haematococcus pluvialis	Airlift bioreactor	3	21	1480	F1	7.95	0.45	Photoautotrophic high-density cultivation of vegetative cellsof Haematococcus pluvialis in airlift bioreactor	Kaewpintong et al., 2006
	Bubble column	3	21	1480	F1	4.20	0.36		
Many Microalgae	Airlift bioreactor	-	20-30	13690	Inorganic medium	-	-	Biodiesel from microalgae	Chisti, 2007
Chaetoceros calcitrans	Airlift bioreactor	2.8	30	25900	F/2 medium	16.2	1.54	Enhanced productivity of Chaetoceros calcitrans in airlift photobioreactors	Krichnavarak et al., 2007
	Airlift bioreactor	12	30	25900	F/2 medium	38.6	2.22		
	Airlift bioreactor	17	30	25900	F/2 medium	88.8	1.78		
	Airlift bioreactor	170	30		Airlift bioreactor	29.6	0.62		
	Bubble column	17	30	25900	F/2 medium	49.6	0.69		
	Bubble column	170	30	25900	F/2 medium	25.1	0.59		
Spirulina platensis	Airlift bioreactor	1.5	25	4000	Zarrouk's medium	-	0.45	Comparison of two different pneumatically mixed columnphotobioreactors for the cultivation of Artrospira platensis(Spirulina platensis)	Oncel et al., 2008
	Bubble column	1.5	25	4000	Zarrouk's medium	-	0.33		

CHAPTER III

MATERIALS AND METHODS

3.1 Experimental Apparatus

Figure 3.1 illustrates the experimental setup for the determination of optimal conditions for the growth of *Chaetoceros calcitrans*. The external loop airlift photobioreactor (ELAP) with the size of 17 L used in this investigation is made of clear acrylic plastic to allow visual observation of the ongoing phenomena, and also to allow the passage of light through the column. ELAP consists of two vertical tubes both with the height (h_1) of 200 cm and diameters of riser and downcomer of 5.4 and 10.4 cm, respectively. The riser and the downcomer are connected by connection tubes at the top and bottom sections of column. These connection tubes have an inside diameter of 5.4 cm with the length of 20 cm (L_c). The riser, downcomer and connection tubes have the wall thickness of 3 mm and are designed to be assembled by several pieces to allow an alternation of configuration of the system as shown in Figure 3.2. Dimension of the various parts of the system is displayed in Table 3.1. The cultivation of *Chaetoceros calcitrans* in internal loop airlift photobioreactor (ILAP) is also carried out to compare the results with the ELAP. The internal loop with the size of 17 L (ILAP) is used where the draft tube is installed centrally in the outer column separating the downcomer from riser with the ratio between downcomer and riser cross sectional of 2.620 (see Figure 3.3 for a schematic). A calibrated rotameter was used to control the volume of gas flow supplied to the system through a porous gas sparger at the base of the column where the superficial gas velocity (u_{sg}) is controlled at 1-4 cm s^{-1}. Six 36W fluorescent lighting bulbs (120 cm long each) are provided on both sides of the column (3 bulbs on each side) and two lighting bulbs are in between the two columns. These light bulbs are placed 10 cm away from the column. The light intensity is measured by “Digicon LX-50 lux meter” around the column, and this is converted into µmol photon m^{-2} s^{-1} using Equation 3.1. The

investigation is carried out at room temperature which is around 27-35 ^{o}C. Dimension of various parts of the system is shown in Table 3.1.

3.2 Experimental Methods

3.2.1 Experimental preparation

3.2.1.1 Treatment of fresh seawater

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

1. Dilute the seawater to 30 ppt with tap water.
2. Disinfect the seawater with 50 ppm (parts per million) of chlorine (as sodium hypochloride).
3. Sparge air through the sea water for 2–3 h to remove residual chlorine in seawater. (adding sodium thiosulfate to neutralize chlorine if aeration cannot eliminate the chlorine) Check by adding potassium iodide in seawater if chlorine is not exhausted, seawater is turned to yellow.

3.2.1.2 Preparation of culture medium

The modified standard F/2 (Guillard's) medium (modified from Smith et al., 1993) is prepared according to the composition as shown in Table 3.2.

3.2.2 Cultivation in ELAP (Semi-batch culture system)

1. Sterilize ELAP with 50 ppm chlorine (as sodium hypochloride)
2. Supply air through the porous sparger located centrally at the bottom of the column for 1 day
3. Drain all the water and rinse the column with tap water to eliminate the remaining chlorine

4. Fill in the column with sterilized seawater, culture medium together with the pure culture and adjust the total volume to 17 L. Initial cell concentrations of both columns are 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 the air filter size 0.2 μm) through a porous sparger and adjusts the superficial gas velocity to1 cm s^{-1}
7. Supply both sides of column with fluorescent light bulbs, placed along the column height (10,000 ± 1,000 luxes or 135 ± 14 μmol photon $m^{-2}s^{-1}$) as shown in Figures 3.1 and 3.3
8. Take samples and count for the cell density using a Haemacytometer (mentioned in Section 3.3.2) at every 6 hours until the stationary growth is observed
9. Calculate the specific growth rate using Equation 3.5, the productivity using Equation 3.6, and the specific productivity using Equation 3.7
10. Subculture the cells and adjust its concentration to 1×10^6 cells mL^{-1} and the culture medium as necessary for the quantity of the additional seawater
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 first culture
12. Repeat Steps 1–11 again but change the superficial gas velocity in Step 6 to 2, 3 and 4 cm s^{-1}
13. Repeat Steps 1–10 again but change configuration of external loop airlift photobioreactor as shown in Figure 3.2 and dimension of the various parts of the system is displayed in Table 3.1.

3.2.3 Culture system by using mixture of air and carbon dioxide (Batch culture system)

1. Repeat Steps 1–5 in section 3.2.2

2. Sparge a mixture of air and CO_2 through the reactor bottom at superficial gas velocity of 3 cm s^{-1}
3. Adjust the flow rate of CO_2 for 1%, 3%, 5% and 10% of aeration rate
4. Supply both sides of column with fluorescent light bulbs, placed along the column height (10,000 ± 1,000 luxes or 135 ± 14 μmol photon $m^{-2}s^{-1}$)
5. Take samples and count for the cell density using a Haemacytometer (mentioned in Section 3.3.2) at every 6 hours until the stationary growth is observed
6. Calculate the specific growth rate using Equation 3.5, the productivity using Equation 3.6, and the specific productivity using Equation 3.7

3.2.4 Determination of cell dry weight

1. Take the 10 L of sample of cell culture in ELAP-LTCT at u_{sg} of 3 cm s^{-1} to centrifuge at 4500 rpm, 20 minutes, and 25°C
2. Add water into sample and centrifuge at same condition two more times
3. Dry and weigh filter membranes (47 mm in diameter and 0.45 μm in pore size)
4. Pour sample into filter membranes
5. Dry the filter membranes from (4) for 1 day
6. Weigh the sample and record the weight
7. Subtract the weight of sample in (6) with the weight of filter membrane in (3) and multiply with the volume of the harvested cells to obtain the cell dry weight in g L^{-1}

3.2.5 The analysis of nutritional value

The nutritional analysis of the sample was conducted by the Food and Testing Laboratory, Faculty of Science, Chulalongkorn University. The samples were analyzed for their protein, carbohydrate and total fat contents.

3.2.5.1 The analysis of protein

The crude protein content is obtained by multiplying the amount of nitrogen content by the factor of 6.25. The nitrogen content is calculated from

the amount of ammonia produced and is expressed as the percentage by mass or in grams per kilogram. The crude protein content, w_p, is calculated as a percentage by mass, using the following equation:

$$w_p = w_N \times F \quad \text{..... (3.1)}$$

where

w_N = the nitrogen content of the sample, expressed as a percentage by mass to four decimal places

F = the factor to convert Kjeldahl nitrogen to protein; for feedstuffs, $F = 6.25$

(References: Thai Industrial Standard, Part2: Block digestion/steam distillation method)

3.2.5.2 The analysis of total fat

The total fat content is obtained using the method as follows:

1. Extract 2 g of test portion (dry cell) on small paper in funnel with five 20 mL portion H_2O prior to drying for ether extraction
2. Use thimble with porosity permitting rapid passage of ether
3. Extraction period may vary from 4 h at a condensation rate of 5-6 drop s^{-1} to 16 h at 2-3 drop s^{-1}
4. Dry extract 30 min at 100^{o}C
5. Cool and weigh

(References: AOAC, Official methods of analysis, Method 920.39)

3.2.5.3 The analysis of carbohydrate

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

Total carbohydrates [%] = 100 – {(protein + fat + moisture + ash) [%]}..... (3.2)

3.3 Analyses

3.3.1 Determination of light intensity

The light intensity can be calculated from Equation 3.1 as follows:

$$I = \frac{E}{74} \quad \text{..... (3.3)}$$

where

I = light intensity [μmol photon $m^{-2}s^{-1}$]

E = light intensity [lux]

3.3.2 Determination of cell concentration

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

1. Clean the counting slide and cover glass
2. Fill the slide with sample
3. Cover the slide with cover glass, avoid of bubbles
4. Count the cell in 25 medium squares on the grid (25 medium squares per 1 large square)
5. Calculate the cells number, using Equation 3.2:

$$N = n \times 10^6 \quad \text{..... (3.4)}$$

where

N = cells concentration [cells mL^{-1}]

n = cell number was calculated from haemacytometer

3.3.3 Determination of specific growth rate

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

$$\mu = \frac{\ln(N_2) - \ln(N_1)}{t_2 - t_1} \quad \text{..... (3.5)}$$

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]

3.3.4 Determination of productivity

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

$$P = \frac{N_2 - N_1}{t_2 - t_1} \times \frac{V \times 1000}{3600} \quad \text{…. (3.6)}$$

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]

3.3.5 Determination of specific productivity

The specific productivity for the cultivation of the diatom in the ALPBR can be calculated from Equation 3.6:

$$SP = \frac{P}{V} \quad \text{….. (3.7)}$$

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

Table 3.1 Dimensions of ELAP (parameters as shown in Fig. 3.1)

Configurations	h_1 **[cm]**	h_2 **[cm]**	L_c **[cm]**	L_h **[cm]**
UTCT	180	10	20	120
LTCT	180	10	20	100
ITCT	180	10	20	100 (inclined)

Remarks

h_1 = total height of external loop airlift photobioreactor [cm]

h_2 = height of bottom connection tube [cm]

L_c = connection tubes length between riser and downcomer [cm]

L_h = height of riser and downcomer [cm]

Table 3.2 Composition of the modified standard F/2 (Guillard's) stock solution (modified from Smith et al., 1993)

Nutrients	Final concentration [mg L^{-1} seawater]	Stock solution preparations
$NaNO_3$	75	*Nitrate/Phosphate solution*
$NaH_2PO_4.H_2O$	10	*Working stock:* add 75 g $NaNO_3$ + 10 g NaH_2PO_4 to 1 L distilled water (DW)
$Na_2SiO_3.9H_2O$	60	*Silicate solution* : add 60 g Na_2SiO_3 to 1 L DW
$CoCl_2.6H_2O$	0.01	*Trace metal/EDTA solution*
$CuSO_4.5H_2O$	0.01	*Primary stocks*: make 5 separate 1 L stocks of [g L^{-1} DW] 10.0 g $CoCl_2$, 9.8 g $CuSO_4$, 180 g $MnCl_2$, 6.3 g Na_2MoO_4 , 22.0 g $ZnSO_4$
$MnCl_2.4H_2O$	0.18	
$Na_2MoO_4.2H_2O$	0.006	
$ZnSO_4.7H_2O$	0.022	*Working stock*: add 1 mL of each primary stock solution + 4.35 g Na_2EDTA + 3.15 g $FeCl_3$ to 1 L DW
Na_2EDTA	4.36	
$FeCl_3.6H_2O$	3.15	
Thiamin HCl	0.1	*Vitamin solution*
Biotin	0.0005	*Primary stocks:* add 20 g thiamin HCl + 0.1 g biotin + 0.1 g B_{12} to 1 L DW
B_{12}	0.0005	*Working stock*: add 5 mL primary stock to 1 L DW

*add 1 mL each of the four working stock solutions per liter of seawater

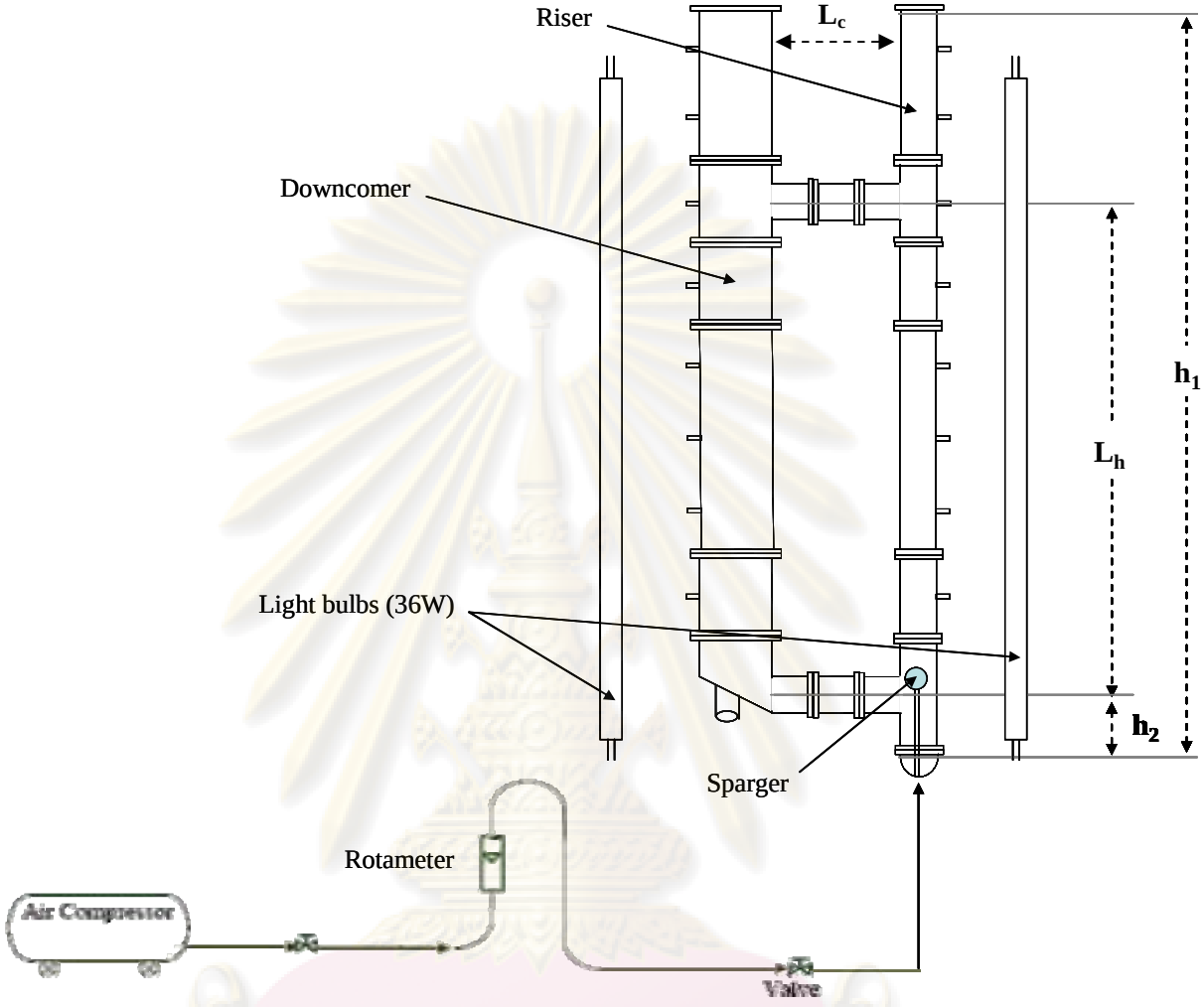


Figure 3.1 Experimental setup for the cultivation of *Chaetoceros calcitrans* in external loop airlift photobioreactor

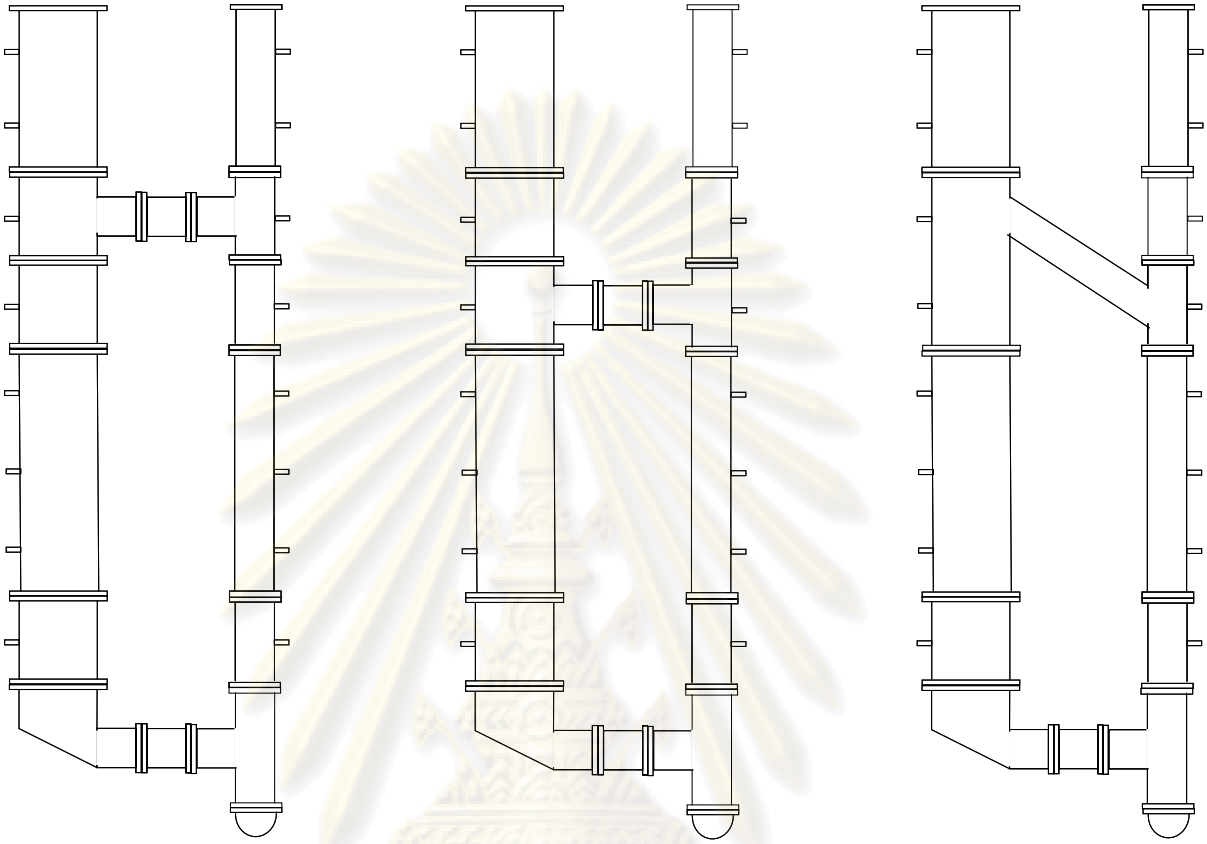


Figure 3.2 Various configurations of external loop airlift photobioreactor **(a)** UTCT **(b)** LTCT **(c)** ITCT

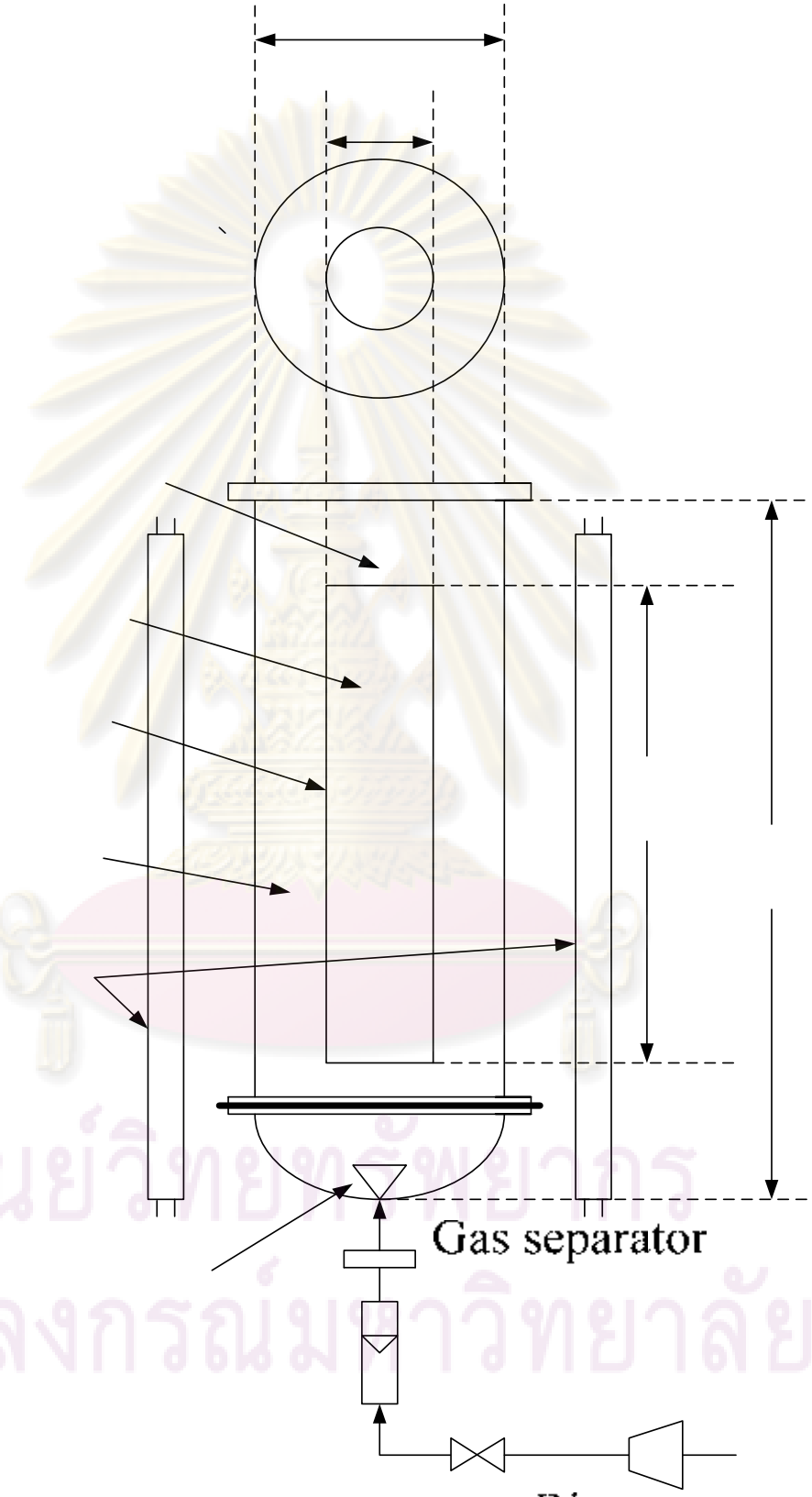


Figure 3.3 Experimental setup for the cultivation of *Chaetoceros calcitrans* in internal loop airlift photobioreactor

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Cultivation in ELAP

The experiment was carried out as a semi-batch cultivation in 17 L ELAP as described in Section 3.1 with the modified standard F/2 (Guillard's) medium at 135 μmol photon $m^{-2}s^{-1}$ and with the ratio between downcomer and riser cross sectional (A_d/A_r) of 3.72. The determination of optimal conditions for cell growth in ELAP was achieved using the method as mentioned in Section 3.2.2.

4.1.1 Effect of aeration rate

The effect of aeration rate on the growth of *C. calcitrans* was investigated using 17 L ELAPs of three different configurations (see Figure 3.2). In each configuration, the aeration rate (measured in terms of superficial velocity based on riser area, u_{sg}) was varied in the range of 1 to 4 cm s^{-1}. In this experiment, cells were harvested once the concentration almost reached the maximum. After that, the fresh medium was added and the cells were re-cultured until the next harvesting period. This was repeated three times. The resulting best growth performances are compared in Figure 4.1. Note that the maximum cell concentration and specific growth rate were selected as the best of the three subcultures. It can be observed that the best cell growth rate occurred at u_{sg} of 3 cm s^{-1} in all configurations. This agreed well with the findings of Krichnavaruk et al. (2005) and Sriouam (2007) who illustrated that *C. calcitrans* grew best in the airlift operated at u_{sg} of 3 cm s^{-1}.

At low range of aeration rate (u_{sg} = 1-3 cm s^{-1}), an increase in the aeration led to increases in both cell concentration and specific growth rate. Aeration generally induced mixing, liquid circulation and mass transfer between gas and liquid phases in the airlift systems (Krichnavaruk and Pavasant, 2002) and therefore increasing aeration could result in a better mixing in the system, (Xu et al., 2002) which then imposed positive effect on the growth. It is mentioned here that, in Figure 4.1(c), the

data at u_{sg} of 1 cm s^{-1} was not available as most of the previous results (Krichnavaruk et al., 2005 and Sriouam, 2007) indicated that the performance at u_{sg} of 1 cm s^{-1} was markedly poorer than that at u_{sg} >1 cm s^{-1}, and also the results in Figure 4.1 (a) and (b) confirmed just this. Hence, no attempt was done to repeat this condition with ITCT-ELAP.

At high aeration rate (u_{sg} > 3 cm s^{-1}), cell concentration and specific growth rate declined with an increase in aeration. This was because high aeration induced quite a number of negative effects on growth, e.g. high interaction between bubbles and cells (shear stress) caused cell disruption and, light shading due to excessive small bubbles in downcomer (Sriouam, 2007). Although light shading due to excession bubbles in ELAP was visually observed to be not as serve as that in ILAP, however, at high u_{sg}, there was quite a number of small bubbles in the downcomer and this could lead to a reduction in the light penetration.

4.1.2 Effect of configuration of ELAP

Figure 4.2 is a new arrangement of the results displayed in Figure 4.1 but was intended to illustrate the effect of reactor-configuration more clearly. This figure indicates that cells grew best in LTCT-ELAP where the connection tube was moved down the height of the airlift (20 cm lower than that of UTCT-ELAP). In this system, a decrease in the height of the column before the connection tube, L_h, reduced the contact time between bubbles and liquid, which then reduced the energy transfer, causing slower liquid velocity (Rujiruttanakul, 2007). Consequently, this configuration allowed more bubbles to remain in the riser promoting gas-liquid mass transfer and therefore CO_2 could be more easily transferred to the liquid and being consumed as a source of carbon for the growth of the alga. Lesser bubbles were found in the downcomer as liquid velocity was not enough to drag the high speed bubbles from the riser, which can be observed in Figure 4.3a and 4.3b. Moreover, liquid velocity in LTCT-ELAP was relatively slow when compared with UTCT-ELAP, and this facilitated the disengagement of bubbles from the system. Figure 4.4 illustrates that the disengagement of bubbles from LTCT-ELAP was more effective than the other two airlifts.

The ELAP with inclined connection tubes (ITCT-ELAP) was designed to promote the rising velocity of the bubbles in the riser and therefore to better separate gas bubbles from the liquid phase. This should have exerted positive influence on the growth. However, this seemed not to be the case and Figure 4.4c illustrates that the disengagement of bubbles in ITCT-ELAP was not any better than LTCT. This could be because the inclined connection tubes also allowed a longer contact time between bubbles and liquid, promoting the liquid velocity and reducing the gas-liquid mass transfer in the system. As a result, the performance of such system was not as good as that of LTCT-ELAP.

4.1.3 Effect of subculture cycle

The cultivation of *C. calcitrans* in ELAP was set in semi-batch fashion. The culture was grown from the initial cell density of 1 x 10^5 cells mL^{-1} until it reached the desired cell concentration, and this typically required approximately 3-4 days. The culture was then cultivated where the harvested volume was calculated to yield the initial cell concentration of 1x10^6 cells mL^{-1} which was ready for the next batch (Example of the calculation is provided in Appendix A). This harvesting cycle was repeated three times and the result from each cycle in terms of specific growth rate are demonstrated in Figure 4.3.

The results illustrate that the first batch of all configurations always provided the best growth rate followed by the second and third batches, respectively. It could be that the medium for the first batch (modified standard F/2) was always present at its highest concentration. After the first harvest, the fresh medium was added to replenish the harvested volume (about 15L) and therefore this medium was diluted by the spent medium in the reactor. Hence, the concentration of the medium should be lower in the second batch than in the first batch and the same occurred during the subsequent harvest. If it was assumed that the growth was a function of nutrient concentrations which is quite likely to be a viable assumption, it was expected that the growth the subsequent harvest should be lower than the previous adjacent cycle, and this was exactly what was observed during the course of this semi-batch experiments. Nevertheless, it was anticipated that, as this continues, the reduction in the growth performance would become negligible as the nutrient concentration would reach some

constant level. This exercise demonstrates, again, that the cultivation of *C. calcitrans* could be achieved successfully in a semi-batch mode. This allows an effective cultivation which minimizes the tedius washing step as required in batch operation.

4.2 Cultivation in different type of airlift photobioreactor

In this work, ELAP and the internal loop airlift photobioreactor (ILAP) were used for the investigation of the effect of different types of airlift photobioreactor on cell growth. The ILAP used in this part was with the size of 17 L, A_d/A_r of 2.62, whereas the LTCT-ELAP was the regular ELAP with L_h = 100 cm because it provided the best growth performance as illustrated in the previous section. The nutrient used in both types of airlift photobioreactor was with the modified standard F/2 (Guillard's) medium and the light intensity was set at 135 μmol photon $m^{-2}s^{-1}$. The results on the maximum cell concentration and maximum specific growth rate from both systems are shown in Table 4.1. Both systems performed best at u_{sg} of 3-4 cm s^{-1} and it was quite clear that LTCT-ELAP provided a better cell growth than ILAP at all aeration rates.

Figure 4.4 illustrates the results from the cultivation of the diatom in LTCT-ELAP and ILAP at u_{sg} of 3 and 4 cm s^{-1} in the semi-batch cultivation. Figure 4.4 (a) demonstrates that, at u_{sg} of 3 cm s^{-1}, both cell concentration and specific growth rate obtained from LTCT-ELAP were always higher than those from ILAP at all culture cycles. The maximum specific growth rate was 0.0846 h^{-1} in LTCT-ELAP and 0.0702 h^{-1} in ILAP. At u_{sg} of 4 cm s^{-1}, the maximum specific growth rate from LTCT-ELAP (0.0796 h^{-1}) was still higher, but only slightly, than ILAP (0.0775 h^{-1}). This was because LTCT-ELAP naturally reduced the number of bubbles in the downcomer, therefore reducing the bubble shading effect and promoting a better light penetration to the reactor. In other words, more light should be available for the culture in LTCT-ELAP than in ILAP, and so the light utilization efficiency of cell should also be better in LTCT-ELAP. From previous section, it became clear that the airlift operated best at u_{sg} of 3 cm s^{-1}. However, this section also presents the results from the cases with u_{sg} of 4 cm s^{-1} as the performance at this u_{sg} was, in many cases, comparable to that of u_{sg} = 3 cm s^{-1}. At the aeration of u_{sg} = 4 cm s^{-1}, both types of airlift photobioreactor could

see more small bubbles in both riser and downcomer due to a higher dragging force induced by the higher liquid velocity. It should be mentioned that the quantity of bubbles in ILAP was always higher than that in ELAP. These bubbles in the downcomer might block the light penetration and reduced the light utilization efficiency as reported in Sriouam (2007) which then reduced the light utilization efficiency. In addition, larger bubbles generally observed in the system could indicate that the flow regime in the reactor (riser in particular) became closer to slug flow which might not be suitable for the growth. It is concluded therefore that the performance was best at u_{sg} of 3 cm s^{-1} regardless of the reactor configuration.

4.3 Effect of CO_2 concentration

The effect of CO_2 concentration on the growth of *C. calcitrans* was investigated in the 17 L LTCT-ELAP as batch cultivation with the initial cell density of $1x10^5$ cells mL^{-1} at 135 μmol photon $m^{-2}s^{-1}$. The culture was aerated at u_{sg} of 3 cm s^{-1} with a mixture of air and CO_2 at 1%, 3% and 5% by volume. In fact, the growth at 10% CO_2 was also examined but it appeared that cells could not survive at such conditions (results not shown here). This finding agreed with the conclusion from Chiu et al. (2009). Figure 4.5 indicates that the performance of the system without CO_2 was slightly lower than those cases with CO_2 particularly at the first 50 hours of cultivation. However, after the first 50 hours, the culture without CO_2 started to have better growth and reached the maximum cell density of $11.9x10^6$ cells mL^{-1} (see details in Table 4.2). The CO_2 fixation was related directly to cell density as cells need carbon for their growth and the only source of carbon in this system was from CO_2. Hence, it was expected that the culture aerated with CO_2 which was enriched with more carbon source should exert positive effect to cell growth rate. However, this benefit was only observed in a very early stage of growth as stated above. The effect of pH might be of significance as the acidity of the medium seemed to be affected considerably by the addition of CO_2. This could adversely affect the growth as cells seemed to reach stationary level at a much lower density, i.e. $9.25 x10^6$, $7.80 x10^6$, and $6.96 x10^6$ cells mL^{-1}, for cases with 1% CO_2 (pH = 5.9), 3% CO_2 (pH = 5.59) and 5% CO_2 (pH = 5.37), respectively.

The evaluation of CO_2 utilization by *C. calcitrans* is illustrated in Table 4.3. The results indicated that, for the 17 L LTCT-ELAP, cells consumed about 10.7 g of CO_2 as a carbon source in the culture without CO_2, while these figures reduced to 8.27, 6.96 and 6.20 g in the cultures with 1, 3 and 5% CO_2 in the inlet air, respectively. These were equivalent the CO_2 conversion of 89.1-96.7, 2.4, 0.7, and 0.4%. This revealed the fact that, with the conditions used in this work, only very limited quantity of CO_2 could be utilized, and even with the natural level (0.035-0.040%) could be adequate for such cultivation. In other words, the addition of CO_2 to the air inlet did not have any potential positive effect on the productivity of the diatom. It could observe from the CO_2 conversion in the last column of Table 4.3. Cells seemed to be highly effective in utilizing CO_2 when the system was operated with normal air, however, this CO_2 conversion reduced drastically when the system was operated with CO_2 enriched air. This suggested that *C. calcitrans* could not utilize CO_2 as much as expected. Although the exact reason for this has not been investigated as this was only a preliminary test, the rising level of pH was expected to be the main cause for this. It is noted that the pH range of the medium in the case with normal air varied in the range of 8.2 to 8.7, and this dropped to only 5.37-5.9 when extra CO_2 was added.

4.4 Nutritional value of *C. calcitrans*

For this experiment, diatom was grown in 17 L of LTCT-ELAP at the superficial gas velocity of 3 cm s^{-1} with light intensity of 135 μmol photon $m^{-2}s^{-1}$ and with the modified standard F/2 (Guillard's) medium as reported in Krichnavaruk et al. (2005, 2007) and Sriouam (2007). The analysis of the sample determined the level of protein, carbohydrate and total fat content, in the diatom. Protein was determined by Kjeldahl technique (King and Wooton, 1956), while carbohydrate was by the calculation from humidity, and ash, and total fat was extracted by AOAC method (ACOA, 1995). Expressed as percentage of dry weight, the compositions of carbohydrate, protein and total fat are 31.25 %, 16.38 % and 22.90%, respectively.

Many species characteristics are thought to influence the nutritional value of microalgae, such as cell wall digestibility (Epifanio et al., 1981), biochemical

composition and cell size (Fernandez-Reiriz et al., 1989), temperature, salinity and carbon dioxide addition (Raghavan et al., 2008). Various microalgal species have become a natural feed for many aquaculture organisms (De Pauw and Persoone, 1988). Due to its high protein content as listed in Table 4.4, microalgae might also serve as a possible replacement to animal protein for a direct consumption by humans (Shelef and Soeder, 1980).

When compared to the biochemical composition of other microalgal species, *C. calcitrans* exhibited a reasonably high content of protein, carbohydrate and total fat. This is similar to the biochemical composition of other strains of *Cheatoceros* which was reported to be in the range of 31.3-57.6% (protein), 9.7-16.4% (carbohydrate) and 13.6-27.1% (total fat), respectively.

Table 4.1 Maximum cell concentration and maximum specific growth rate in different types of airlift photobioreactor for the cultivation of *C. calcitrans*

u_{sg} [cm s^{-1}]	ELAP		ILAP	
	Maximum cell concentration [cells mL^{-1}]	**Maximum specific growth rate [h^{-1}]**	**Maximum cell concentration [cells mL^{-1}]**	**Maximum specific growth rate [h^{-1}]**
1	6,640,000	0.0572	4,650,000	0.0447
2	9,230,000	0.0787	7,400,000	0.0700
3	11,930,000	0.0846	9,150,000	0.0702
4	10,750,000	0.0796	8,505,000	0.0775

Table 4.2 Maximum cell concentration and maximum specific growth rate of *C. calcitrans* under various CO_2 concentrations

CO_2 concentration (% of air)	**pH**	**Cell concentration for 50 h. of cultivation [cells mL^{-1}]**	**Maximum cell concentration [cells mL^{-1}]**	**Specific growth rate for 50 h. of cultivation [h^{-1}]**	**Maximum specific growth rate [h^{-1}]**
0	8.22	4,720,000	11,930,000	0.0831	0.0846
1	5.90	6,565,000	9,250,000	0.1004	0.0803
3	5.59	6,985,000	7,800,000	0.0965	0.0660
5	5.37	6,040,000	6,960,000	0.0868	0.0508
10	4.86	-	-	-	-

Table 4.3 Analysis of CO_2 utilization by *C. calcitrans* in 17L LCTC-ELAP

CO_2 concentration* [%]	**Final cell mass*** [g]**	**pH**	**Total CO_2 inlet [g]**	**CO_2 utilization [g]**	**CO_2 conversion [%]**
0.035-0.040*	5.83	8.22	11.06-12.01	10.69	84.6-96.7
0.1**	5.83	8.22	31.59	10.69	33.8
1	4.51	5.90	345.10	8.27	2.4
3	3.80	5.59	947.80	6.96	0.7
5	3.38	5.37	1603.96	6.20	0.4
10	-	4.86	-	-	-

* Concentration in the air inlet (Hayashi et al., 1995 and Izumo et al., 2007)

** CO_2 level in air without the addition of CO_2

*** Final cell mass were achieve from 17L ELAP-LTCT when cells reached in stationary phase

Table 4.4 Proximate composition of different species of microalgae

Species	Protein	Carbohydrates	Total fat	Source
Chaetoceros calcitrans	31.3	16.4	22.9	This work
Chaetoceros calcitrans	57.6	10.2	27.1	Coutteau (1996)
Chaetoceros calcitrans	40.1	9.7	13.6	Lora Vilchis and Doktor (2001)
Chaetoceros gracilis (Chaetoceros muelleri)	48.2	18.9	28.9	Coutteau (1996)
Chaetoceros muelleri	40.6–68.4	-	-	Lango-Alemán (1999)
Chaetoceros muelleri	21.1–44.9	5.8–11.0	6.9–13.6	Cuevas-Rocha (2001)
Chaetoceros muelleri	33.3	10.8	44.8	Lora Vilchis and Doktor (2001)
Chaetoceros muelleri	44.4	11.5	20.3	Lora-Vilchis et al. (2004)
Chaetoceros muelleri	37.4–63.6	11.0–18.3	19.8–24.4	Teniza-Guillén (2004)
Chaetoceros muelleri	38.6–71.7	14.7–22.8	13.6–35.1	López-Elías et al. (2005)
Chaetoceros sp.	60.3	16.0	23.7	Cordero-Esquivel and Voltolina (1994)
Chaetoceros sp.	41.2–49.5	12.7–30.6	16.7–31.3	Sánchez-Saavedra and Voltolina, (1996a,b)
Chlorella ellipsoidea	10.6	-	1.13	Cabrera and Bum Hur (2009)
Chlorella vulgaris	55.0	19.3	7.6	Morris et al. (2008)
Nitzchia closterium	26	9.8	13	Brown (1991)
Skeletonema costatum	25	4.6	10	Brown (1991)
Skeletonema costatum	31	21.5	1.3	Monkunsit (2008)
Spirulina platensis	54.2	-	26.6	Li et al. (2003)
Spirulina platensis	74.0	11.9	6.8	Jaime-Ceballos (2006)
Spirulina sp.	67.0	25.5	30.1	Hernández and Olguín (2002)

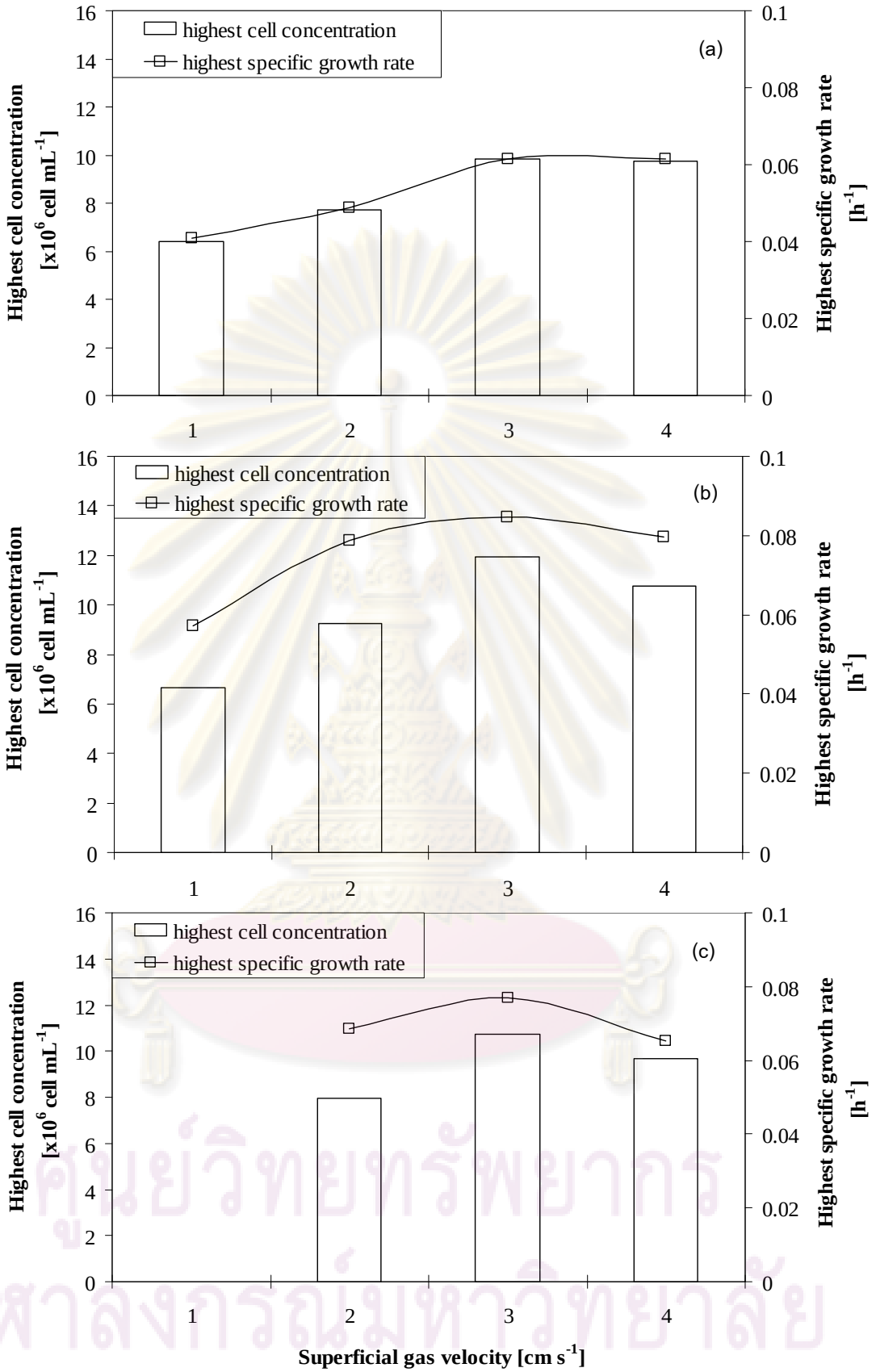


Figure 4.1 Effect of aeration rates on the highest cell concentration and the highest specific growth rates (μ) of first batch in ELAPs of three different configurations **(a)** UTCT **(b)** LTCT **(c)** ITCT

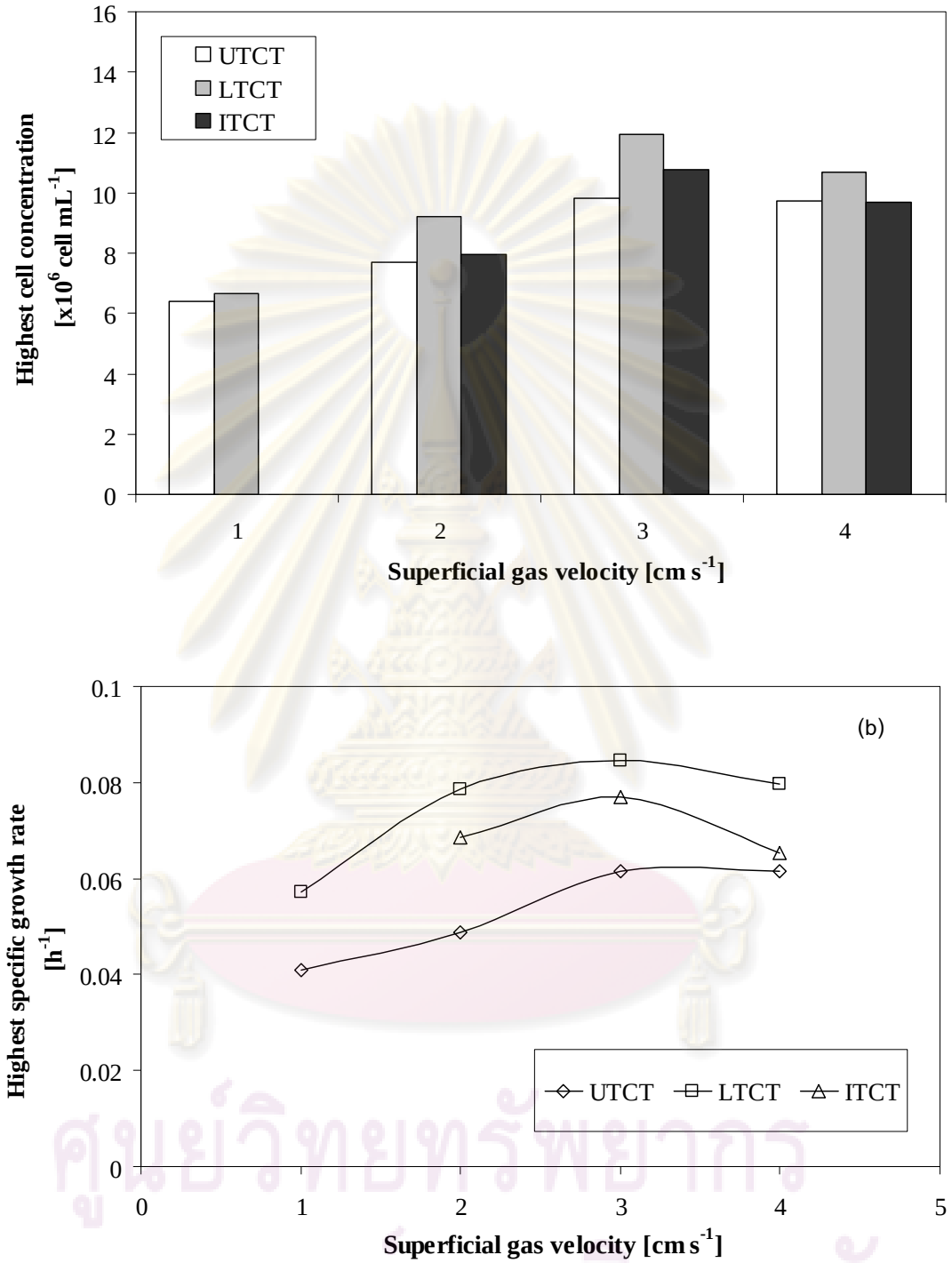
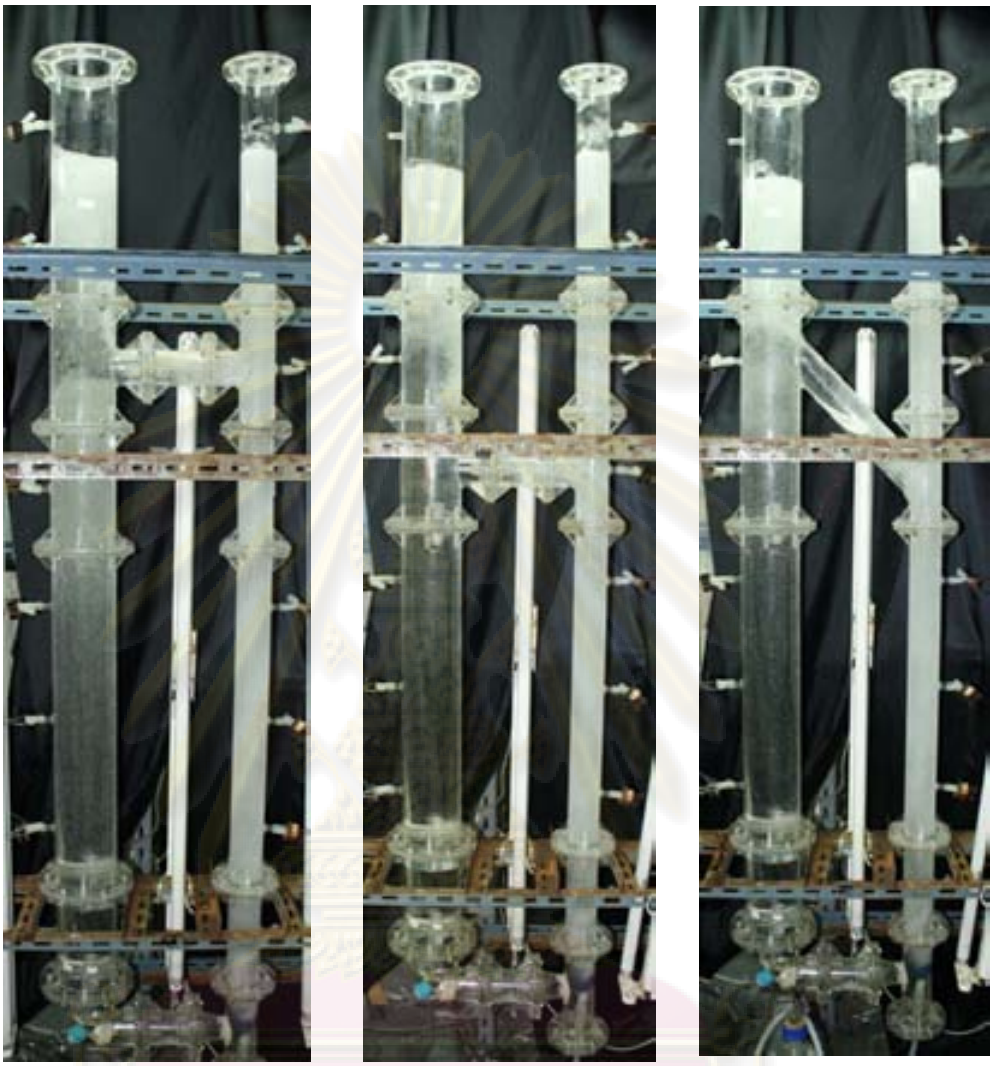


Figure 4.2 Effect of configurations of first batch in ELAPs on; **(a)** the highest cell concentration; **(b)** specific growth rate



(a) **(b)** **(c)**

Figure 4.3 The quality of bubbles in ELAPs of three different configurations **(a)** UTCT **(b)** LTCT **(c)** ITCT

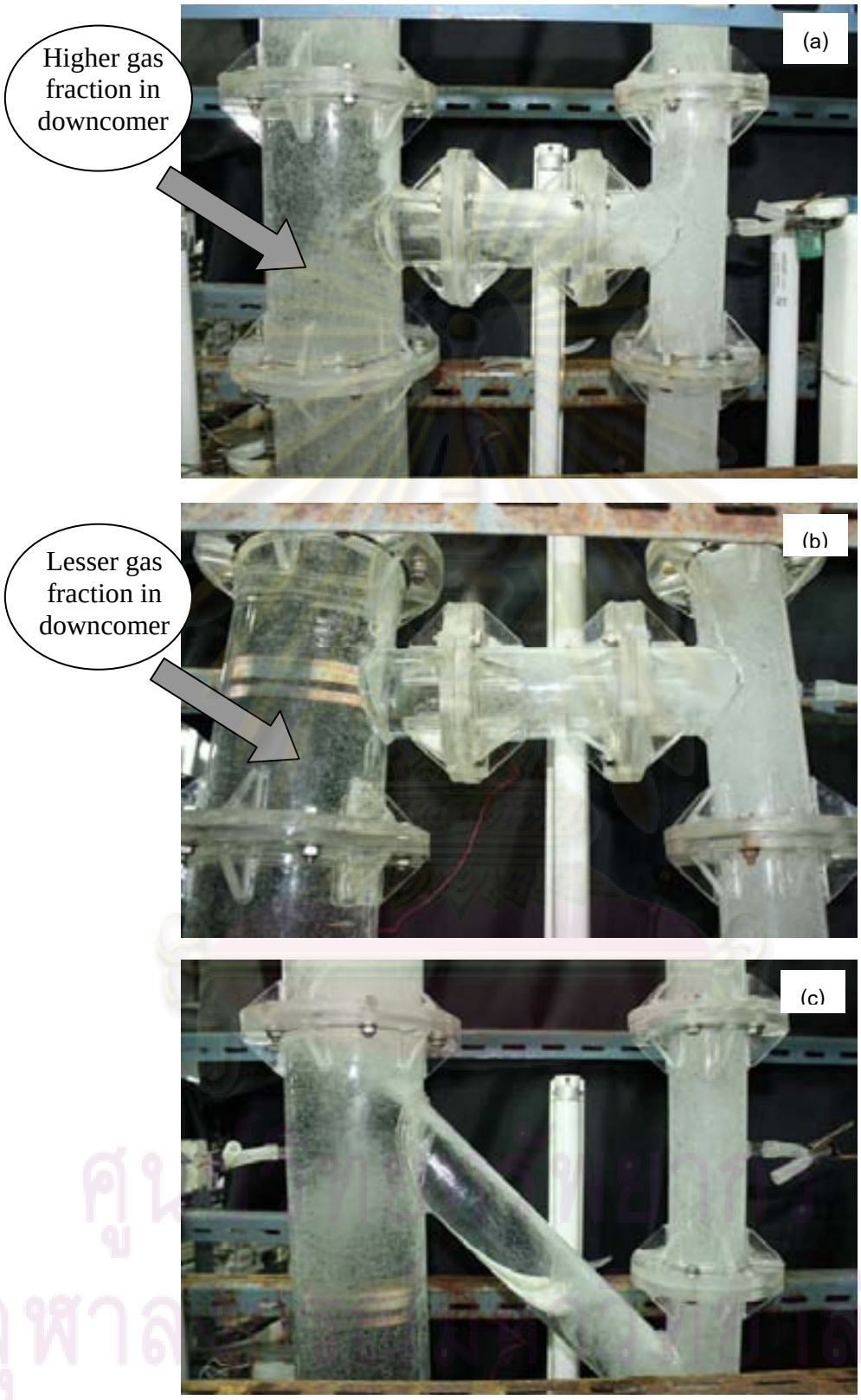


Figure 4.4 Rheology of bubbles ELAPs of three different configurations **(a)** UTCT **(b)** LTCT **(c)** ITCT

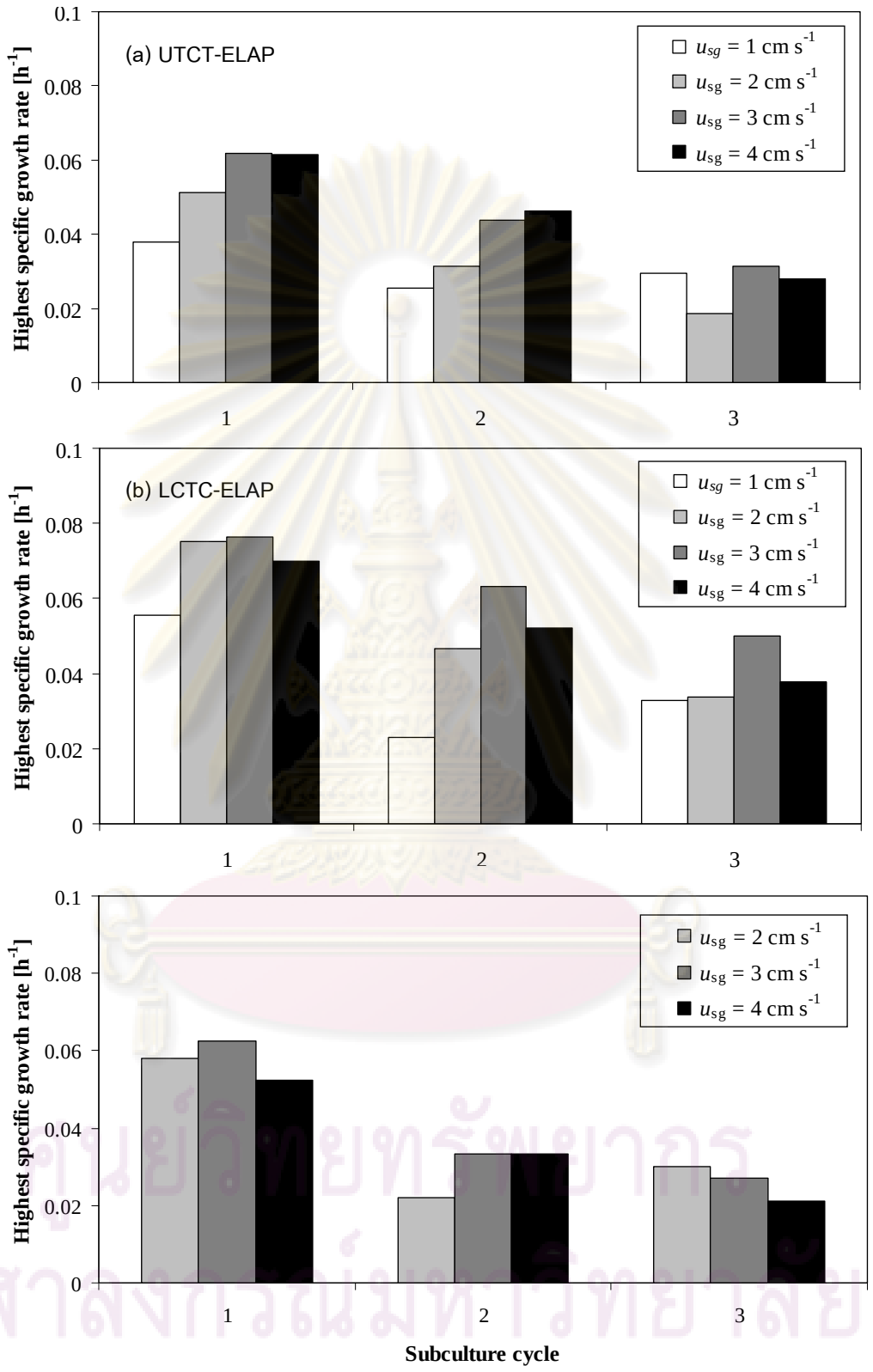


Figure 4.5 Effect of subculture cycle on the highest specific growth rates in various configurations of ELAP

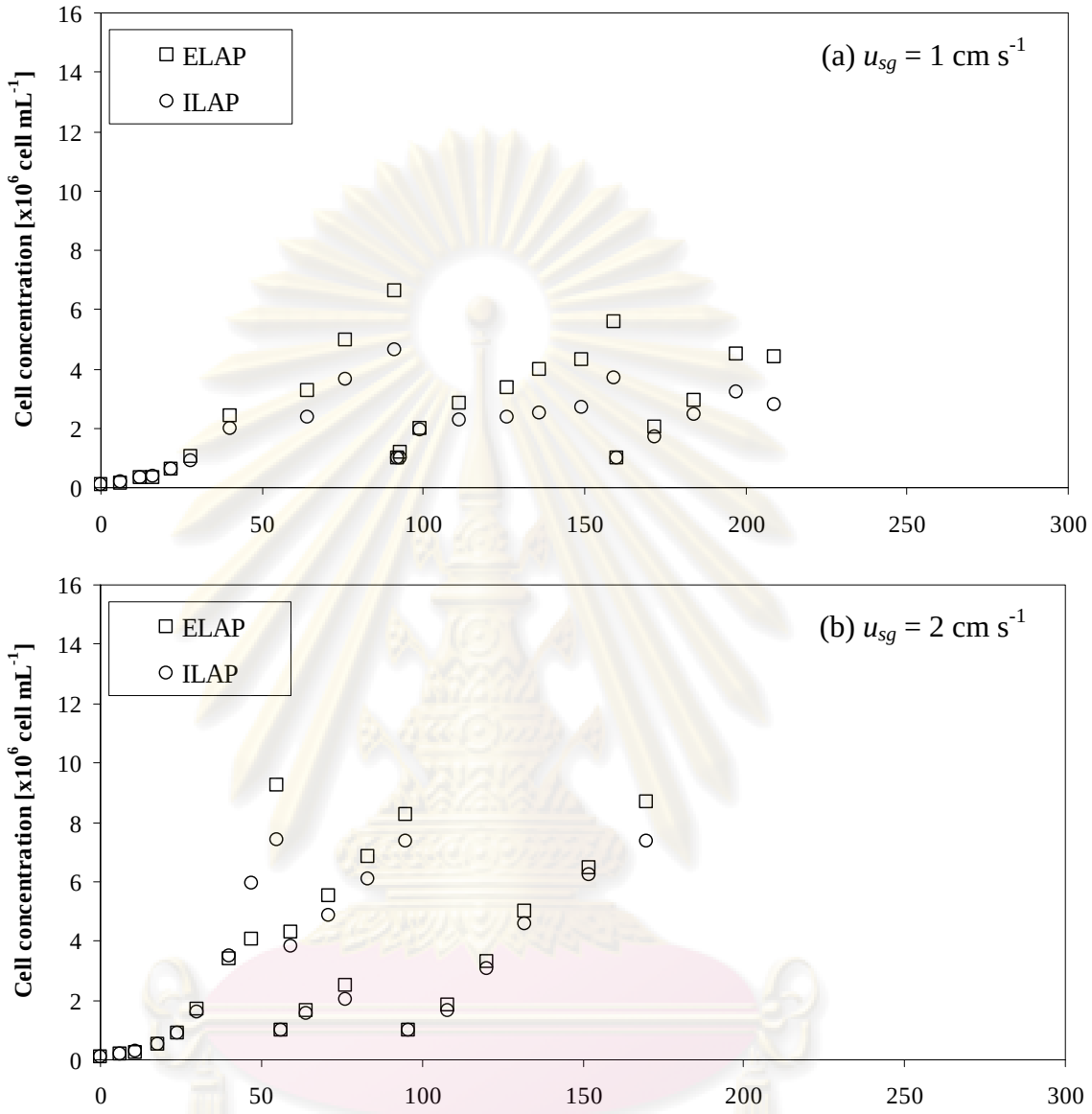


Figure 4.6 Comparison between growth rate of *C. calcitrans* in LTCT-ELAP and ILAP

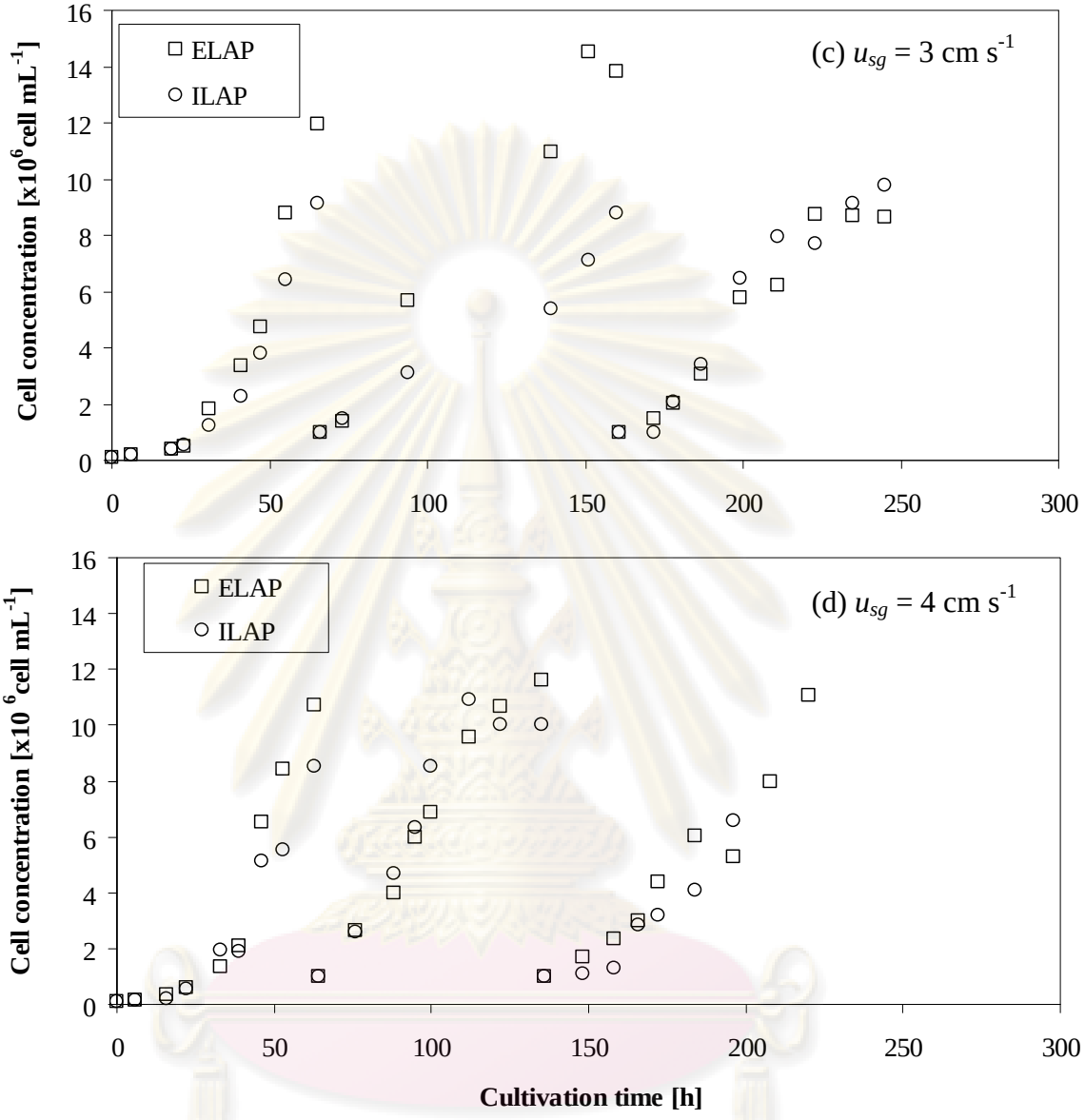


Figure 4.6 (Cont.) Comparison between growth rate of *C. calcitrans* in LTCT-ELAP and ILAP

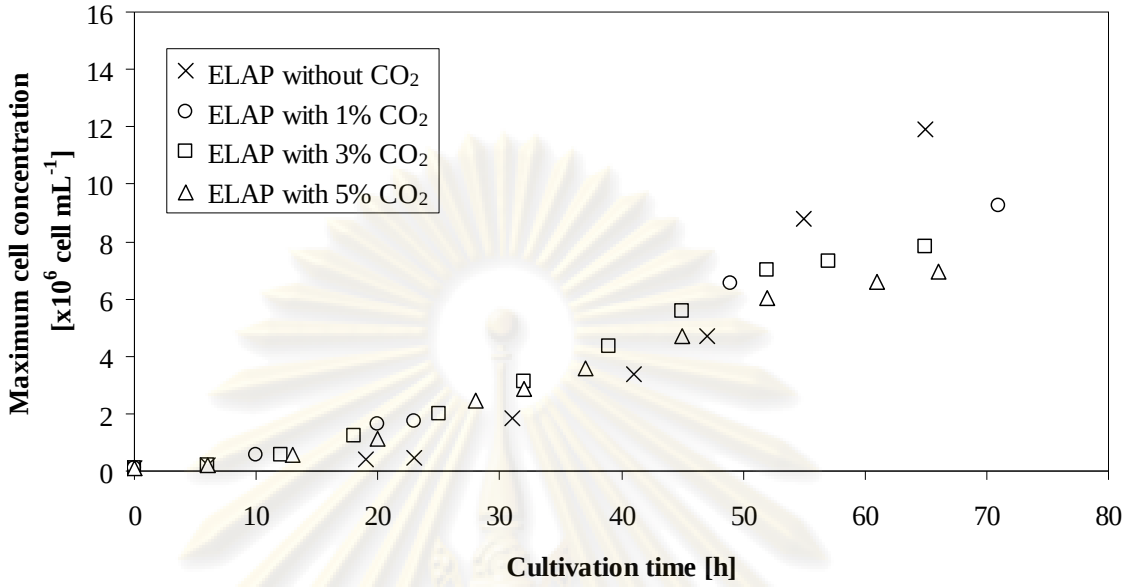


Figure 4.7 Effect of the concentrations of CO_2 aeration on the growth of *C. calcitrans* with air and the mixture of air and CO_2 at 1%, 3% and 5%

CHAPTER V

CONCLUSIONS, CONTRIBUTIONS AND RECOMMENTDATIONS

5.1 Conclusions

This thesis aims to determine growth conditions and the effects of design configurations and operating conditions on the cultivation of *C. calcitrans* in the different designs of the external loop airlift photobioreactor (ELAP). Table 5.1 summarizes the growth performance from the various airlift systems. The optimal algorithm for the growth of the diatom in the 17L ELAP with A_d:A_r of 3.717 was the semi-batch cultivation in LTCT-ELAP at u_{sg} of 3 cm s^{-1} where the maximum cell density was found to be 11.9×10^6 cell mL^{-1} and the maximum specific growth rate of 0.0846 h^{-1}.

The cultivation in LTCT-ELAP was proven to be superior to that of ILAP as LTCT-ELAP helped reduce the number of bubbles in the downcomer, lessening the bubble shading phenomena, and promoting a better light penetration to cells.

Carbon dioxide is one of the major factors controlling the growth of the diatom. This work provides the results on the pre-evaluation of the effect of CO_2 for the cultivation of *C. calcitrans* in the LTCT-ELAP. Although cells of *C. calcitrans* could grow in the system with as much as 5% CO_2, the growth was not as good as when the cells were cultivated in normal air (without CO_2). This could be attributed to the high acidity level imposed by CO_2.

5.2 Contributions

Our previous work has demonstrated the success in applying airlift photobioreactors for the cultivation of *Chaetoceros calcitrans* which is a diatom essential as shrimp larvae feed. The airlift system employed in these previous works is the internal loop type, and the investigation illustrates the design and operating conditions which are appropriate to the growth of such diatom. The limitation particularly on the shading of light intensity was analyzed. This work can be viewed as a continue of such investigation. External loop airlift photobioreactor (ELAP) was selected as the model airlift system as the configuration with two columns could practically allow a more effective control of light intensity.

With the results as presented in this work, a success in the cultivation with ELAP was elucidated. In fact, ELAP explicitly outperformed ILAP in achieving both higher specific growth rate and higher maximum cell concentration (11.93×10^6 cells mL^{-1} and 0.0846 h^{-1}, respectively). However, to exchange for better light utilization, ELAPs suffer from its sophisticating structure (when compared with the internal loop type). Therefore it is difficult to ultimately conclude whether which system is better and the choice will depend on several other factors such as the flexibility of the reactor and the area requirement, etc. Moreover, the effect of CO_2 on the growth was pre-evaluated to examine the potential of using this system for the uptake of CO_2 from the atmosphere which is essential for the global reduction target of the various greenhouse gases. However, this issue needs to have further evaluation as suggested in the following section.

5.3 Recommendations

- The investigation on the effect of CO_2 was still not complete as CO_2 reduced the medium pH and exerted negative effect on the growth of the diatom. Future work should be designed to further evaluate this issue. The use of buffer solution could provide some viable answer for this and if this is not enough, perhaps an advanced controlling system has to be used.

- Scaling up is always an engineering problem which often gains interests from both academic and industrial researchers. However, the configuration of such external loop airlift does not easily allow the scale up, and how to increase the size without or with minimal level of disturbance to the system performance therefore offer some challenging research outreach.

Table 5.1 Summary of the growth performance of *C. calcitrans*

u_{sg} [cm s^{-1}]	Maximum cell concentration [cells mL^{-1}]			Maximum specific growth rate [h^{-1}]		
	UTCT-ELAP	**LTCT-ELAP**	**ITCT-ELAP**	**UTCT-ELAP**	**LTCT-ELAP**	**ITCT-ELAP**
1	6,400,000	6,640,000	-	0.0409	0.0572	-
2	7,710,000	9,230,000	7,960,000	0.0487	0.0787	0.0686
3	9,815,000	11,930,000	10,750,000	0.0614	0.0846	0.0770
4	9,750,000	10,750,000	9,690,000	0.0616	0.0796	0.0652

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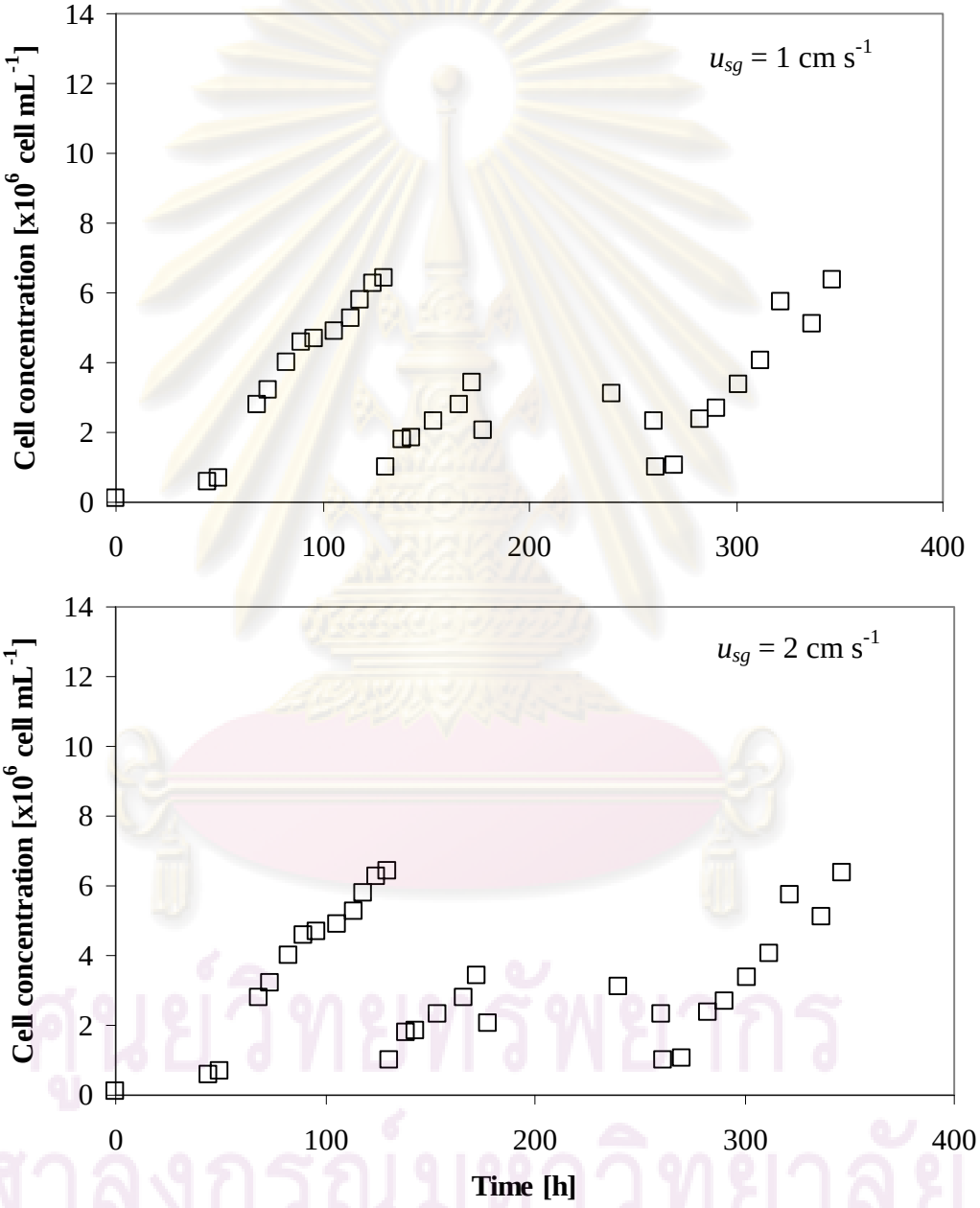
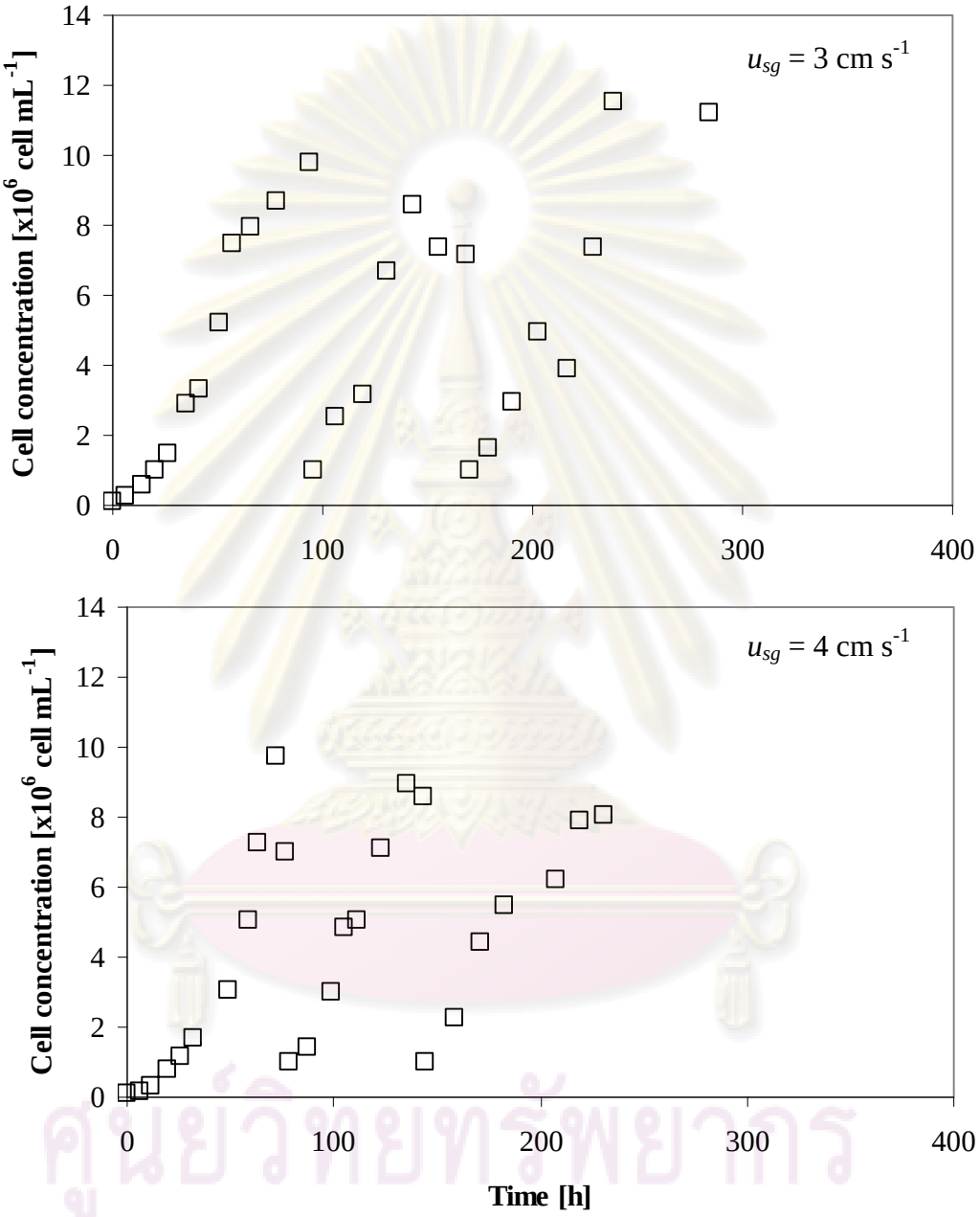
APPENDICES

Appendix A: The growth curve of *C. calcitrans* different configurations in various superficial gas velocity

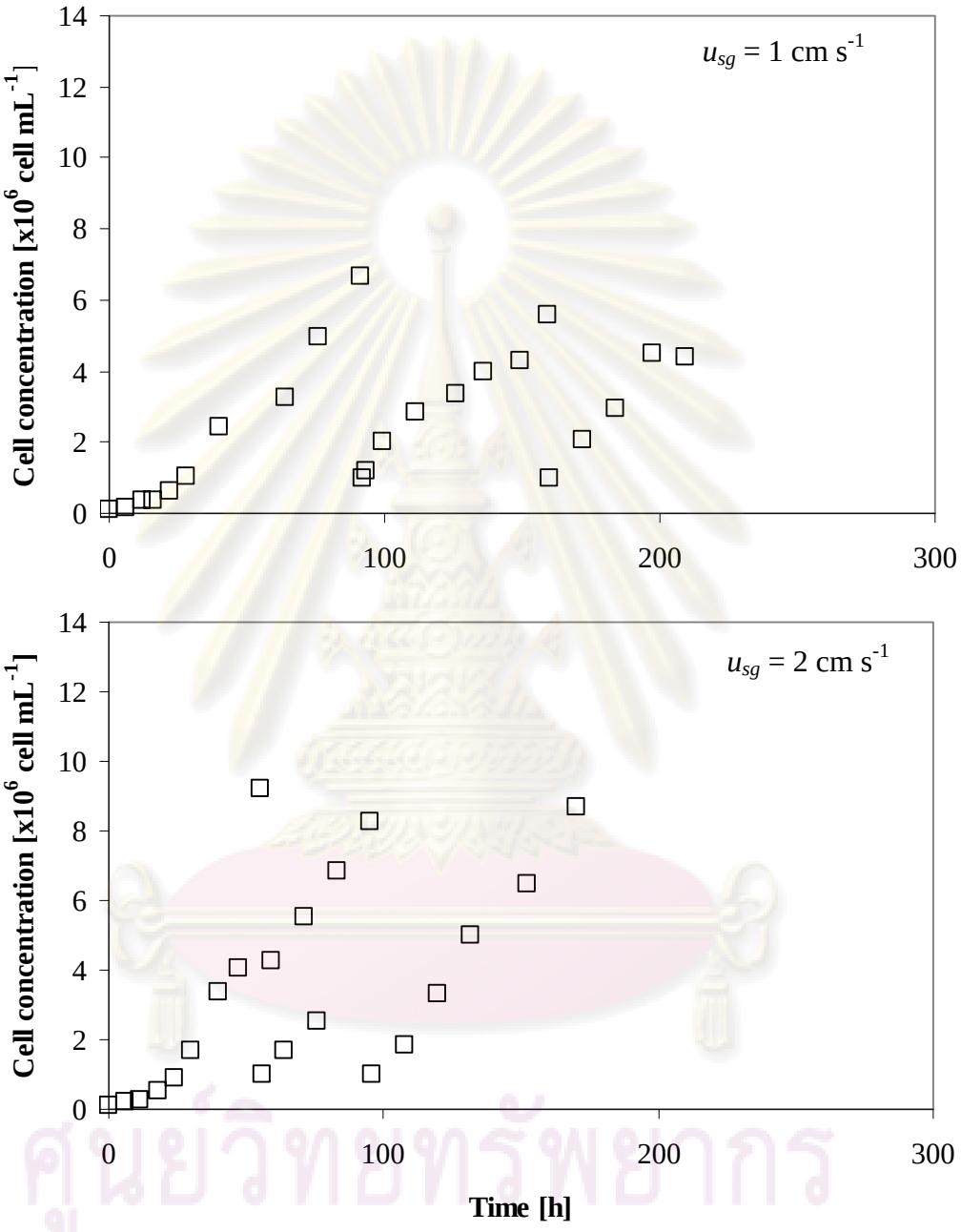
ELAP-UTCT



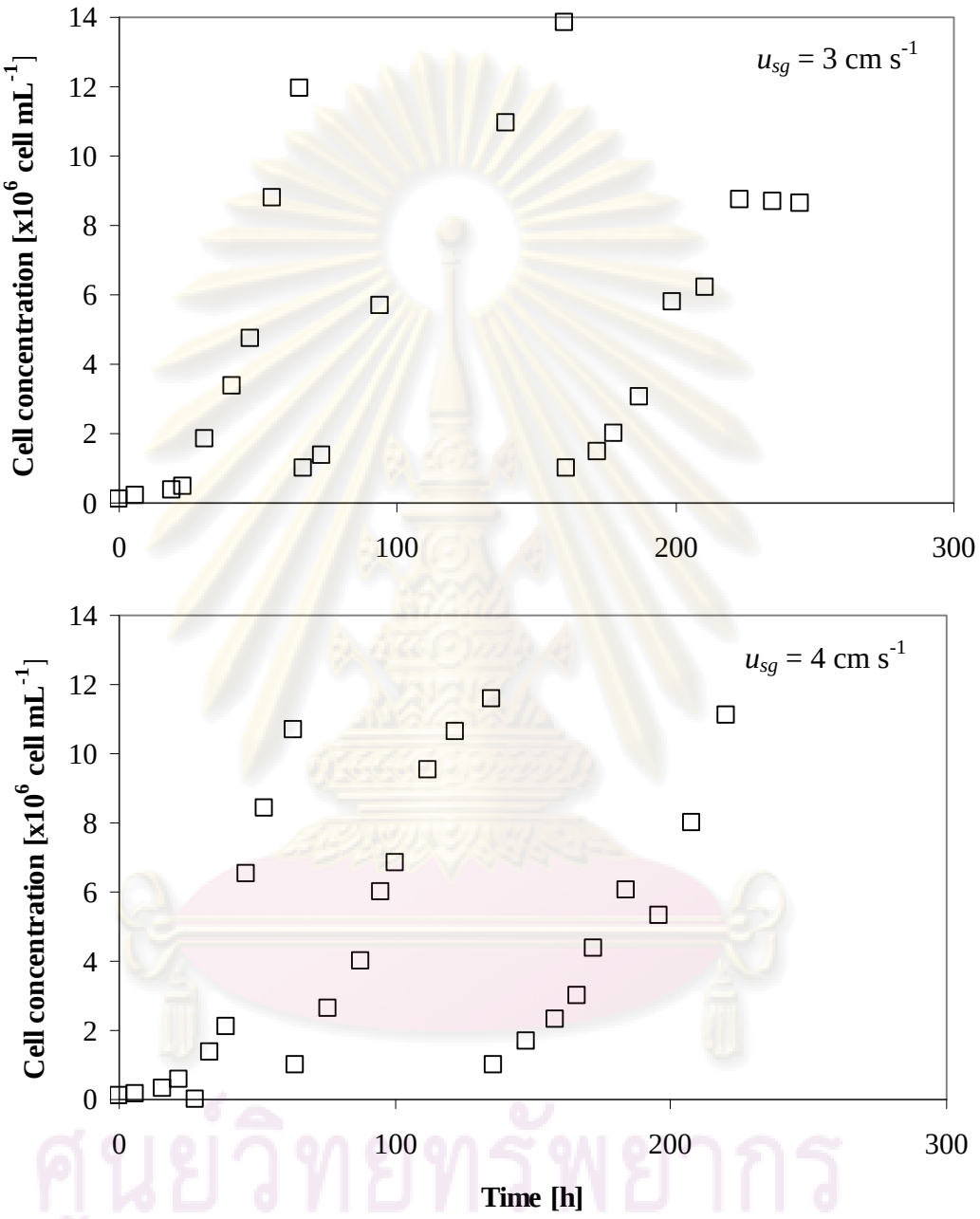
ELAP-UTCT



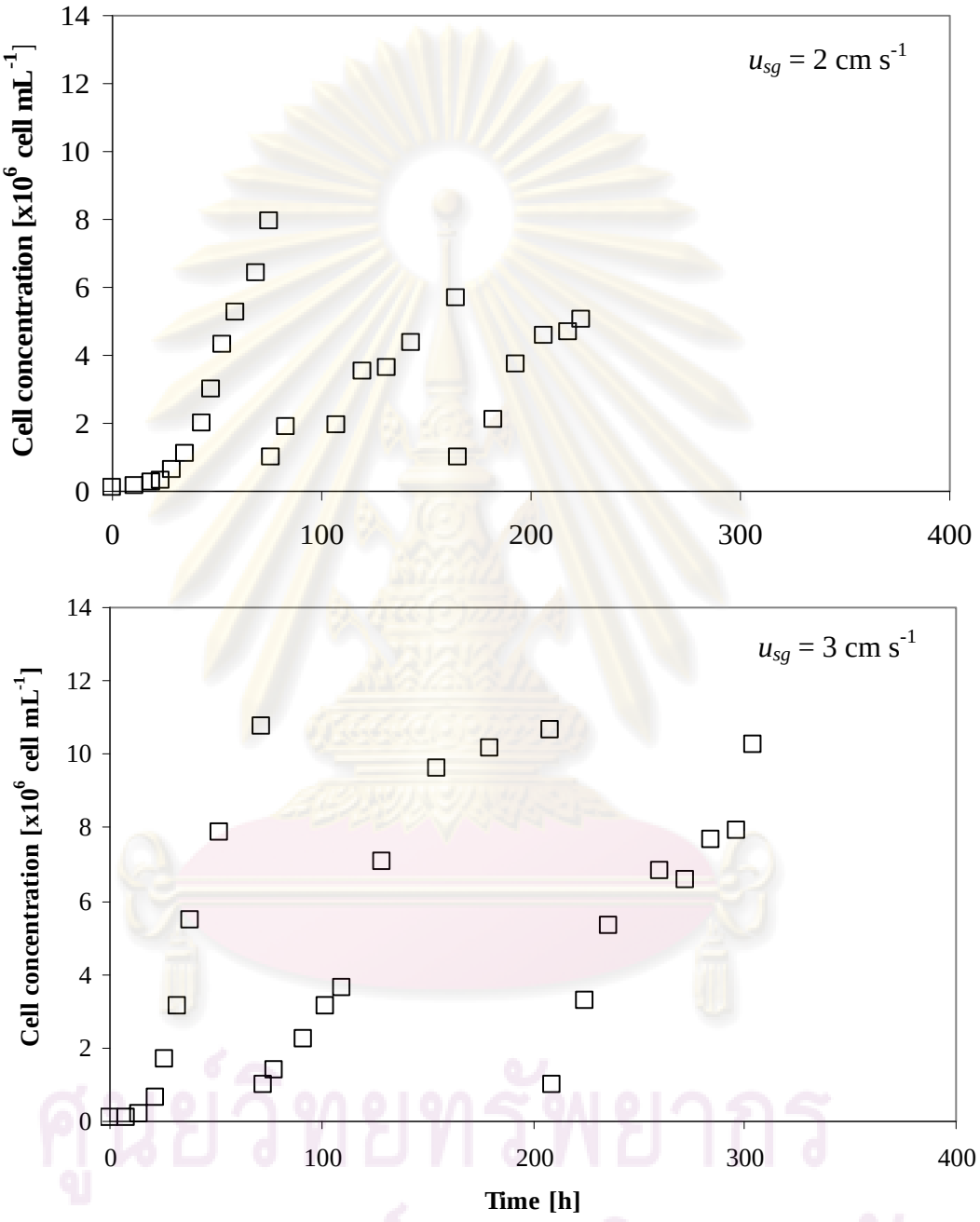
ELAP-LTCT



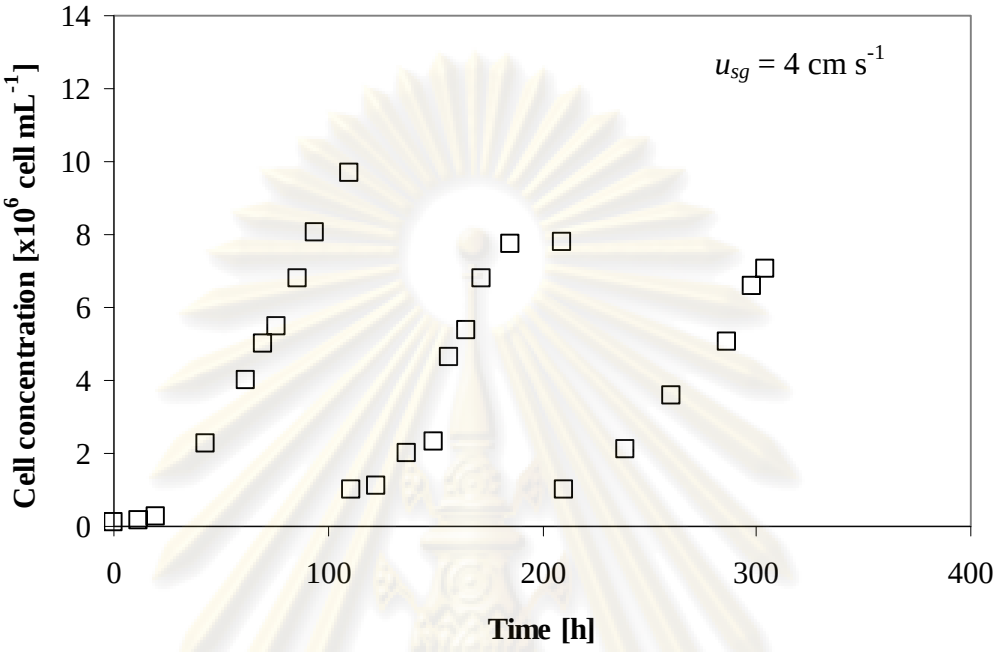
ELAP-LTCT



ELAP-ITCT



ELAP-ITCT



Appendix B: The productivity and specific productivity in various u_{sg} all congurations

For UCTC-ELAP

u_{sg} [cm s^{-1}]	Productivity of subculture cycle [cells s^{-1}]			Specific productivity of subculture cycle [cells $L^{-1}s^{-1}$]		
	1	2	3	1	2	3
1	$2.90x10^5$	$4.17x10^5$	$3.11x10^5$	$0.17x10^5$	$0.024x10^5$	$0.18x10^5$
2	$4.33x10^5$	$5.08x10^5$	$1.80x10^5$	$0.26x10^5$	$0.30x10^5$	$0.10x10^5$
3	$5.62x10^5$	$4.65x10^5$	$8.30\ x10^5$	$0.33x10^5$	$0.27\ x10^5$	$0.49x10^5$
4	$8.80x10^5$	$7.79x10^5$	$3.79x10^5$	$0.52x10^5$	$0.46x10^5$	$0.22x10^5$

For LCTC-ELAP

u_{sg} [cm s^{-1}]	Productivity of subculture cycle [cells s^{-1}]			Specific productivity of subculture cycle [cells $L^{-1}s^{-1}$]		
	1	2	3	1	2	3
1	$3.87x10^5$	$2.83x10^5$	$4.65x10^5$	$0.23x10^5$	$0.17x10^5$	$0.27x10^5$
2	$14.26x10^5$	$14.25x10^5$	$5.09x10^5$	$0.84x10^5$	$0.84x10^5$	$0.29x10^5$
3	$14.03x10^5$	$7.34x10^5$	$7.06x10^5$	$0.83x10^5$	$0.43x10^5$	$0.42x10^5$
4	$1.47\ x10^5$	$7.17x10^5$	$6.66x10^5$	$0.86x10^5$	$0.42\ x10^5$	$0.39x10^5$

For ICTC-ELAP

u_{sg} [cm s^{-1}]	Productivity of subculture cycle [cells s^{-1}]			Specific productivity of subculture cycle [cells $L^{-1}s^{-1}$]		
	1	**2**	**3**	**1**	**2**	**3**
1	-	-	-	-	-	-
2	$6.50x10^5$	$2.33x10^5$	$3.32x10^5$	$0.38x10^5$	$0.14x10^5$	$0.20x10^5$
3	$5.62x10^5$	$4.65x10^5$	$8.30\ x10^5$	$0.33x10^5$	$0.27\ x10^5$	$0.49x10^5$
4	$0.95x10^5$	$0.34x10^5$	$0.42x10^5$	$0.56x10^5$	$0.20x10^5$	$0.24x10^5$

Appendix C: The calculation of cell concentration for semi-batch cultivation

The cell concentration in the culture can be determined using equation as follows:

- Take the sample from ELAP
- Count the cell density using Haemacytometer at every 6 hours
- Calculate the initial cell concentration to $1x10^6$ cells mL^{-1} for the second batch of subculturing using Equation (For example, the data of the system in LTCT-ELAP at u_{sg} of 3 cm s^{-1})

$$C_1V_1 = C_2V_2$$
$$(11.93x10^6 \text{ cells mL}^{-1})(V_1) = (1x10^6 \text{ cells mL}^{-1})(17 \text{ L})$$
$$V_1 = 1.42 \text{ L}$$

where

C_1 = the final cell concentration before subculturing [cells mL^{-1}]

C_2 = the initial cell concentration for the second batch of subcultureing [cells mL^{-1}]

V_1 = the volume of cell is added into the second batch [L]

V_2 = the volume of cell in ELAP [L]

- Add 1.42 L of the culture into ELAP for the second batch of subcultureing
- Fill in the column with 15.58 L of sterilized seawater to replenish the harvested volume
- Add 15.58 mL of fresh medium into ELAP

Appendix D: The calculation of conversion of CO_2 by *C. calcitrans*

Example case: Cultivation of *C. calcitrans* with air (0.04% CO_2)

Mass of CO_2 feed into reactor

Conditions: Flow rate (Q) = 0.03 m s^{-1}

Diameter of riser (d_r) = 0.054 m

Time to input air to reactor (t) = 65 h

Composition of CO_2 in air (CO_2 conc.) = 4% = 0.0004 v/v

Molecular weight of CO_2 (MW CO_2) = 44 g mol^{-1} CO_2.

The amount of CO_2 in the input air can be calculated from:

$$CO_2 = Q \times area\ riser \times CO_2\ conc. \times t \times MW\ CO_2 \times \frac{1\ mol}{22.4\ L}$$

$$CO_2 = \frac{0.03 \times \pi \times 0.054^2 \times 65 \times 3600 \times 0.0004 \times 1000 \times 44}{4 \times 22.4} = 12.64\ \text{g } CO_2$$

CO_2 uptake to cell mass

Conditions: Cell initial (X_0) = 100,000 cell mL^{-1}

Cell maximum (X_m) = 11,930,000 cell mL^{-1}

Cell density ($X_{density}$) = 2.9x10^{-11} g $cell^{-1}$

Volume of reactor (V) = 17 L

Ratio of molecule of cell ($CH_{1.2}O_{0.5}N_{0.2}$) per carbon (C) (ratio) = 0.5

Molecular weight of CO_2 (MW CO_2) = 44 g mol^{-1} CO_2

Molecular weight of C (MW C)= 12 g mol^{-1} C

The amount of CO_2 uptake can be calculated from:

$$CO_2 = \frac{(X_m - X_0) \times X_{density} \times V \times ratio \times MW\ CO_2}{MW\ C}$$

$$CO_2 = \frac{(11{,}930{,}000 - 100{,}000) \times 2.9 \times 10^{-11} \times 17 \times 1000 \times 0.5 \times 44}{12} = 10.69\ \text{g } CO_2$$

% conversion of CO_2

Conversion = CO2 uptake to cell mass / Mass of CO2 feed into reactor

$$= \frac{10.69}{12.64} \times 100$$

$$= 84.6\ \%$$

BIOGRAPHY

Ms. Piyanate Nakseedee was born on 14th December, 1984 in Bangkok. She finished his higher secondary course from Surasakmontree School in March, 2002. After that, she studied in the major of Chemical Engineering in Faculty of Engineering at Thammasat University. She continued her further study for Master's degree in Chemical Engineering (Biochemical Engineering research group) at Chulalongkorn University in 2006.