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CULTIVATION OF A DIATOM *Chaetoceros calcitrans* IN AIRLIFT BIOREACTOR.

Miss Worapannee Loataweesup.

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วรพรรณี เหล่าทวีทรัพย์ : การเพาะเลี้ยงไดอะตอมคีโตเซอลอส แคลซิแทรนซในถังปฏิกรณ์ชีวภาพแบบอากาศยก (CULTIVATION OF A DIATOM *Chaetoceros calcitrans* IN AIRLIFT BIOREACTOR) อาจารย์ที่ปรึกษาวิทยานิพนธ์ : ผู้ช่วยศาสตราจารย์ ดร.ประเสริฐ ภวสันต์, อาจารย์ที่ปรึกษาวิทยานิพนธ์ร่วม : ดร. สรวิศ เผ่าทองศุข, 130 หน้า. ISBN 974-172-407-1.

สภาวะที่เหมาะสมในการเจริญเติบโตของไดอะตอมคีโตเซอลอส แคลซิแทรนซที่สามารถหาได้จากการเลี้ยงในถังสัมผัสแบบธรรมดาที่ทำจากแก้วใสขนาด 2.5 ลิตร ได้ผลสรุปดังนี้คือ ความเข้มแสง 200 ไมโครโมล โฟตอน ต่อตารางเมตร ต่อวินาที โดยให้ระยะเวลาการให้แสงสว่างตลอดทั้งวัน รวมทั้งเพิ่มความเข้มข้นของซิลิกาและฟอสฟอรัสเป็นสองเท่าของปริมาณที่ใช้ในสูตรอาหารมาตรฐานเอฟ/2ของกิลลาร์ด

การใช้ถังปฏิกรณ์ชีวภาพแบบอากาศยกเพาะเลี้ยงไดอะตอมคีโตเซอลอส แคลซิแทรนซให้อัตราการเจริญเติบโตจำเพาะสูงกว่าเมื่อเลี้ยงในถังสัมผัสแบบธรรมดา ทั้งนี้เนื่องจากการไหลของของไหลในถังปฏิกรณ์ชีวภาพแบบอากาศยกเป็นการไหลอย่างมีรูปแบบ ที่ส่งผลให้ไดอะตอมไหลวนเข้าหาแสงได้อย่างมีประสิทธิภาพ โดยความเร็วของอากาศที่ให้กับถังปฏิกรณ์ชีวภาพมีผลต่ออัตราการเจริญเติบโตของไดอะตอมค่อนข้างมาก ซึ่งอัตราการเจริญเติบโตจำเพาะสูงสุดของไดอะตอมนี้เมื่อเพาะเลี้ยงแบบกะในระบบที่มีความเร็วของการให้อากาศที่ 3 เซนติเมตรต่อวินาที มีค่าเท่ากับ 0.074 ต่อชั่วโมง และความเข้มข้นเซลล์สูงสุดคือ 20.15×10^{6} เซลล์ต่อมิลลิลิตร การเพาะเลี้ยงไดอะตอมแบบกึ่งต่อเนื่องในถังปฏิกรณ์ชีวภาพแบบอากาศยกแสดงให้เห็นศักยภาพของถังปฏิกรณ์ชีวภาพชนิดนี้ในการเพาะเลี้ยงไดอะตอมคีโตเซอลอส แคลซิแทรนซอย่างต่อเนื่อง โดยที่เมื่อทำการเก็บเกี่ยวผลได้ของเซลล์ทุก12 ชั่วโมง ด้วยปริมาตร 60% ของปริมาตรทั้งหมดในการเพาะเลี้ยง ได้อัตราการเจริญเติบโตจำเพาะสูงสุดถึง 0.0854 ต่อชั่วโมง และผลผลิตของเซลล์ 4.138×10^{11} เซลล์ต่อวัน (11.84 มิลลิกรัม ต่อลิตร ต่อวัน)

ภาควิชา วิศวกรรมเคมี ลายมือชื่อนิสิต.....

สาขาวิชา วิศวกรรมเคมี ลายมือชื่ออาจารย์ที่ปรึกษา.....

ปีการศึกษา 2545 ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

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KEY WORD: *Chaetoceros calcitrans* / AIRLIFT BIOREACTOR / MASS PRODUCTION / HIGH CELLS DENSITY / OPTIMAL CONDITION

WORAPANNEE LOATAWEESUP : CULTIVATION OF A DIATOM *Chaetoceros calcitrans* IN AIRLIFT BIOREACTOR. THESIS ADVISOR : ASSIST. PROF. PRASERT PAVASANT, Ph.D. THESIS CO-ADVISER : SORAWIT POWTONGSOOK, Ph.D. 130 pp. ISBN 974-172-407-1.

Optimal conditions for the growth of a diatom *Chaetoceros calcitrans* were investigated in the small glass bubble column of 2.5 L. The light intensity for the highest growth rate was found to be at 200 □mol photon $m^{-2}s^{-1}$. At this light intensity, the diatom required no dark period for it growth. A suitable nutrient for the growth of this diatom was found to be a modified standard F/2 medium with a two fold of silica and phosphorus concentrations.

The cultivation of *C.calcitrans* in the ALBR was superior to that in the BC. This was due primarily to the well defined flow pattern in the ALBR which led to a more effective light utilization of the algae. The optimal aeration rate of 3 cm s^{-1} was found to yield a maximum specific growth rate of 0.074 h^{-1} with a cell concentration of $20.15x10^{6}$ cells mL^{-1} in batch culture. A semi-continuous culture could be achieved where the harvest was performed at every 12 hours and with a harvesting volume of 60% of the total culture volume. In this case, the maximum specific growth rate (μ) achievable in this semi-continuous culture was 0.0854 h^{-1} and the productivity of *C.calcitrans* was $4.138x10^{11}$ cells d^{-1} (11.84 mg $L^{-1}d^{-1}$).

Department Chemical Engineering Student's signature…..……...

Field of study Chemical Engineering Advisor signature……...……..

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NOMENCLATURE

ALBR	airlift bioreactor	
A_d	apparent contracted cross sectional area at downcomer	[m^2]
A_r	apparent contracted cross sectional area at riser	[m^2]
ATP	adenosine triphosphate	
BC	bubble column	
BIOCOIL	helical tubular photobioreactor	
B_{12}	α-5,6-dimethylbenzimidazolyl cyanocobalamin	
C	Chlorophyll concentration	[mgChl. mL^{-1}]
C.calcitrans	*Chaetoceros calcitrans*	
CoA	coenzyme A	
d	dilution factor	
DO	dissolved oxygen	[mgO_2]
DO_s	Slope of the curve of dissolved oxygen Concentration versus time	[mgO_2 min^{-1}]
DNA	deoxyribonucleic acid	
DW	dry weight per algal cell	[g $cell^{-1}$]
DW_A	average dry weight retained on algal filter	[g]
DW_C	average dry weight retained on control filter	[g]
EMP	Embden-Meyerhoff-Parnas pathway	
HMP	Hexose-monophosphate pathway	
I_k	saturating light intensity	[μmol $m^{-2}s^{-1}$]
LD cycle	light/dark cycle	
Mot.	motile	
N	algal concentration	[cells mL^{-1}]
$NADPH_2$	nicotinamide adenine dinucleotide phosphate	
NH_4^+	ammonium	
N-M	Nonmotile	
n_1	number of cells counted upper	[cells]

NOMENCLATURE(Cont.)

n_2	number of cells counted rafter	[cells]
P	phosphorus	
PFD	photon flux density	[μmol $m^{-2}s^{-1}$]
PI curve	photosynthetic oxygen evolution rate versus light intensity curve	
P_{max}	maximal photosynthesis oxygen evolution rate	
POER	photosynthetic oxygen evolution rate	
PS I	photosystem I	
PS II	photosystem II	
PUFAs	polyunsaturated fatty acids	
RuBisCO	1,5-bisphosphate carboxylase/oxygenase	
RuBP	1,5-bisphosphate	
Si	Silicon	
TCA	Tricarboxylic acid cycle or Krebs cycle	
V	volume of algal culture	[L]
VAP	vertical alveolar panel	

Greek symbols

μ	specific growth rate	[h^{-1}]
λ	wavelength of light	[nm]

CHAPTER 1

Introduction

1.1 Rationale

The wild catch of shrimp from the world's oceans reached maximum yield of about 1.6 million metric tons per year by the late 1970s. At the same time, the demand and prices for shrimp continued to increase. The increasing demand can only be satisfied by the culture of shrimp through aquaculture practices. Shrimp culture has undergone dramatic changes, which were made possible by technological breakthroughs in reproduction, nutrient supply and culture methods.

One of the most important factors in successful shrimp culture is to have a good food supply. Algae are among a few photosynthetic microorganisms that directly produce and accumulate lipids in great quantities (Engel *et al*., 2000). The use of algae as food for shrimp larvae is a basic component of hatchery operations because some polyunsaturated fatty acids (PUFAs) synthesized by algae are essential for growth and survival of marine larvae such as shrimp (de Pauw and Parsoone, 1988).

Note that the increasing demand of PUFAs have led to growing efforts in the production of raw materials containing PUFAs from conventional agriculture (*e.g.* nuts) as well as from some unconventional sources (*e.g.* beef). Algae, hence, may be served as one alternative natural resource for PUFAs (Chiou *et al*., 2001).

The history of the commercial scale algal cultures spans about 30 years with applications to aquaculture. Typical cultivation ponds can usually be categorized into open and close systems. A very-large scale algal culture is usually an open system which is easy to operate and consumes less resource than close systems. However, this type of system can only reach low

cell densities resulting in a large area requirement, expensive harvesting procedures, and unfavorable economics. The main reason for these drawbacks of the open system is the uncertainly in the availability of light source (usually sunlight) which is one of the most crucial factors for the growth of algae. The development of closed bioreactor for mass cultivation of algae enables the adjustment of the light intensity which leads to a successful production of ultrahigh cell density of algae. However, the existing closed systems, *e.g.* flat plate or tubular bioreactors, suffer serious drawbacks from poor mixing and gas-liquid mass transfer.

The airlift bioreactors (ALBR) have become an attractive alternative for biotechnology applications (Merchuk *et al*., 1996). The main advantages of ALBR include good mixing, well-defined fluid flow pattern, and relatively high gas-liquid mass transfer rate. It is worth noting that the mixing in the ALBR could be obtained without causing too much shear force in the liquid phase which could inhibit the growth of the algae.

This research therefore focuses on the optimization of an ALBR for a production of ultrahigh cell density of *Chaetoceros calcitrans* which, due to its high PUFAs cell content, plays a major role as a food chain component in many mariculture hatcheries. In addition, the technical potentiality of combining the ALBR and the conventional open system for a mass production of *Chaetoceros calcitrans* will be investigated.

1.2 Objective

The objective of this work is to develop an airlift bioreactor (ALBR) for a cultivation of high cells density of *Chaetoceros calcitrans*.

1.3 Working scopes

A cultivation of *Chaetoceros calcitrans* in this work will be subjected to the following factors:

1.3.1 light intensities at approximate 40 – 600 μmol photon $m^{-2}s^{-1}$

1.3.2 light/dark cycles at cycle times of 16/8 – 24/0 h/cycle

1.3.3 nutrient requirements for maximum growth per liter of F/2 (Guillard's) medium

1.3.3.1 Silica concentration 0-4.8 mg

1.3.3.2 Phosphorus concentration 0-3.6 mg

1.3.3.3 Nitrogen concentration 0-42 mg

1.3.3.4 Vitamin B_{12} concentration 0-3 μg

CHAPTER 2

Backgrounds and Literature Review

2.1 Introduction to algae

2.1.1 What are algae?

Algae are highly diverse group of organisms that have important functions in aquatic habitats. Algae can be autotroph or heterotroph (Berner, 1993). Usually algae are photoautotrophic organisms with chlorophyll and unicellular reproductive structures. The number of recognized species probably greatly underestimates the actual number of species because many habitats and regions have not been extensively sampled and many algae are very small and hard to distinguish from each other. The divisions of algae are distinguished by variety of chemical and morphological differences. The summary of the algal divisions are shown in Table 2.1 (Bold and Wynne, 1985; Lee, 1989).

All divisions have chlorophyll *a*, but different divisions can also have chlorophylls *b*, *c* or *d*. Distinctive accessory pigments, such as phycobillins and fucoxanthin, are characteristics of different algal divisions. These pigments may color the algae red, blue or golden-brown, if they are not green with chlorophyll as the dominant pigment.

The different divisions also have chemically different cell walls and storaged products, or they have distinctive forms of motility or number of flagella. Ultrastructural features, such as the number of membranes around chloroplasts, also distinguish the different divisions and indicate that the algae have many ancestors and are an evolutionarily diverse group (Cavalier-Smith, 1986).

2.1.1.1 Algal Morphology

The plant body of an alga is called thallus. Algal thalli range from small solitary cells to large, complex multicellular structures. The size of the algae varies from unicells less than 1 μm to attain a length of more than 50 m (Nelson and Brand, 1979). Even though these groups have great evolutionary, genetic and chemical differences, they share many of the same growth forms. The blue-green algae, green algae and diatoms have the greatest morphological diversity with unicellular, colonial and filamentous forms. Most motile diatoms are unicellular and move by means of raphae. Nonmotile algae such as diatom may be attached to substrate or entangled in the matrix of other organisms to which they are attached. The morphological diversity of algae is elucidated in Table 2.2

2.1.1.2 What is diatom?

Algal can be unicellular or multicellular organism. The most numerous of the unicellular forms are the diatoms. Diatoms are in a class of *Bacillariophyceae* (*Diatomaphyceae*). The characteristic feature of the *Bacillariophyceae* is their ability to secrete an external wall composed of silica, so called the “frustule” (a rigid two part boxlike cell wall). Diatoms are immediately recognizable by their cases or frustules. The chloroplasts contain chlorophylls *a*, c_1 and c_2 with the major carotenoid being fucoxanthin, which gives the cells their characteristic colors as yellowish or brownish (Lewin *et al*., 1984).

Usually diatoms occur singly but some species form chains, as in some species of *Chaetoceros, Thalassiosira* or *Lauderia*. In freshwater, most colonial diatoms are encased in a low density gelatineous sac which aids the flotation of the organisms (Schmaljohann and Rottger, 1978). There is a central mass of cytoplasm, containing the nucleus, joined by cytoplasmic strand to the wall of the cell. A feature unique to diatoms is that the frustule is in two parts or valves; one fitting snugly into the other rather likes a box into

its lid. Diatoms range in size from a few microns to about 1 mm but many of them can unite to form long chains, often several centimeters in length.

Diatoms normally store oil or fatty acids as the end product of photosynthetic activity. That is the main cause for using diatoms as a food for aquatic larvae. Diatoms widely used in hatcheries are *Skeletonema, Chaetoceros*, *etc* (Marasigan, 1989). *Chaetoceros* was among the first diatoms to be utilized effectively as a live, natural food for shrimp larvae (Berner, 1993).

2.1.1.3 Morphology of Chaetoceros

Chaetoceros has more than 160 species, the largest number of any planktonic diatom. The genus is more or less widespread in warm and cold waters. The cells of *Chaetoceros* are usually oval in a cross-sectional plane and have nearly flat ends. A pair of long thin spines is found at each end of the frustule, and these, by fusion with those of neighboring cells, unite them into chains. Chloroplasts are variable in size and number. This large and variable genus is truly planktonic but some species are more typical of inshore waters than others (Hargraves, 1972).

Chaetoceros calcitrans

Chaetoceros calcitrans is a unicellular diatom, with cell united by their long setae to form filaments. They range in length from 8-12 μm. 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 percentages of biochemical composition and total fatty acids of *Chaetoceros calcitrans* are shown in Tables 2.3 and 2.4, respectively.

2.1.2 Photosynthesis and Respiration

2.1.2.1 Photosynthesis

Oxygenic photosynthesis is a coupled oxidation/reduction reaction, whereby water is photooxidized, the protons and electrons produced reduce carbon dioxide to organic carbon. The photosynthetic apparatus consists of pigment proteins, two types of reaction centers, a series of electron pigment proteins, electron carriers and enzymes (Davison, 1991).

In photosystem II (PS II) oxidation of water leads to the evolution of oxygen, and a weak reductant is formed. In photosystem I (PS I) a strong reductant is formed, which is ultimately used to reduce CO_2 or NO_3^-. The actual transformation of the energy of the photons to chemical energy occurs in the reaction center associated with PS II and PS I respectively. The pigment-proteins harvest light and transfer the energy to the reaction center. The enzymes responsible for reducing carbon dioxide to organic carbon, is 1,5-bisphosphate carboxylase/oxygenase (RuBisCO) (Sukenik *et al*., 1987).

The schematic diagram for the light reactions in photosynthesis is shown in Figure 2.1.

2.1.2.2 Respiration

Whereas photosynthesis consumers CO_2 and produces O_2, respiration uses O_2 and releases CO_2.

(a) Dark respiration

Considerable amounts of organic carbon provided by photosynthesis are reconverted to carbondioxide (CO_2) through the Embden-Meyerhoff-Parnas (EMP) pathway of glycolysis and aerobic degradation. The EMP pathway functions can be affecting only the fate of the initially formed pyruvate. Part of the pyruvate enters the Tricarboxylic acid (TCA or Krebs

cycle) cycle are transferred to oxygen by the respiratory electron transport chain, thereby producing more adenosine triphosphate (ATP). The reactions of the EMP pathway in aerobic conditions are the following:

$$2CH_3COCO_2H + 6H_2O + 20NAD \rightarrow 6CO_2 + 20NADH \quad (2.1)$$

$$24NADH \rightarrow 24NAD + 36ATP \quad (2.2)$$

The Hexose-monophosphate (HMP) pathway and EMP pathway share the initial formation of glucose-6-phosphate. In HMP pathway, this is dehydrogenated to 6-phosphogluconolactone and then oxidative decarboxylated to carbondioxide and ribose phosphate. Under aerobic conditions the ribose is eventually oxidized to CO_2. The summary is as follows:

$$C_6H_{12}O_6 + 6H_2O + ATP \rightarrow 6CO_2 + 24NADH \quad (2.3)$$

$$24NADH \rightarrow 24NAD + 36ATP \quad (2.4)$$

(b) Photorespiration

Photorespiration is defined as a light dependent oxygen (O_2) uptake and CO_2 release that occurs in photosynthetic tissues. Photorespiration arises because 1,5-bisphosphate (RuBP) carboxylase can also act as an oxygenase, binding O_2 rather than CO_2 to RuBP. The carboxylase activity is competitively inhibited by O_2, so that photorespiration is more pronounced at high O_2 or low CO_2 concentrations. The summary of algal metabolism is shown in Figure 2.2 (Raven *et al*., 1992, Vymazal, 1995).

2.1.3 Factors controlling algal growth

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

2.1.3.1 Nutrients

Essential elements are usually divided into macronutrients and micronutrients. Macronutrients are required in a relatively large quantity and are used generally as building materials. Examples of macronutrients are C, H, O, N, P, S, K, and Mg. Micronutrients are required in small amounts as cofactors in enzyme systems or are used in electron transport systems. Fe, Cu, Zn, Mo. are examples of micronutrients. Generally, a deficiency in any nutrient is reflected in a greatly decreased cellular content. In that way, nutrients are important to complete the cells and the reproductive stages during cell's life cycle (Reitan *et al*., 1994). Roles of some essential elements in algal metabolism are shown in Table 2.5.

2.1.3.2 Temperature

Temperature is one of the major factors controlling the rate of photosynthesis in all algae. In general, growth rate increases exponentially with temperature up to an optimum temperature, then declines rapidly as temperature exceeds this optimum. Note that, the temperature at which an organism is most abundant in nature does not necessarily correspond to its optimum temperature as determined in the laboratory.

2.1.3.3 Light intensities

Light is considered to be the principal factor limiting algal productivity. Photosynthesis in the algae occurs mainly in the visible range of spectrum between 400 and 700 nm. Irradiance may be measured and expressed energetically in units of watts meter^{-2}. Irradiance measurements are given in SI units as micromoles meter^{-2} second^{-1} whereas a non-SI unit of microeinsteins meter^{-2} second^{-1} is also frequently used.

Irradiance and photon flux density (PFD) may be readily interconverted as: (Luning, 1981)

$$1\,\mu\text{mol}\,(\text{m}^{-2}\text{s}^{-1}) \;=\; 1\;\;\mu\text{E}\,\text{m}^{-2}\text{s}^{-1} = \frac{119.7}{\lambda}\;\;\text{W}\,\text{m}^{-2} \;=\; 50\;\;\text{lux}$$

where λ = the wavelength of the light in nanometer.

Different algal classes have significantly different light requirements for growth and photosynthesis. Light intensity level is positively associated with algal biomass and can influence community structure and succession (Richardson *et al*., 1983). At high light intensity, when cells are transferred to a light regime; the photoinhibition response that commonly occurs is attributable to a reduced capacity to dissipate excess excitation resulting in an increase in photosystem II (PS II) damage and decrease in photosynthesis (Huang and Rorrer, 2002).

In contrast, under low light intensity conditions eventually results in a steady state response where an abundance of cellular energy (ATP and NADPH) is available to compensate for costly protein synthesis, primarily associated with damage to putative D1 protein of PS II and pigment production. In continual light intensity, the rate of damage equals the rate of repair and there is no apparent photoinhibition of photosynthesis.

2.1.3.4 Photoperiod

Most algae require a photoperiod of alternating light and dark (Rebolloso Fuentes *et al.,* 1999, Lunning, 1981). The photoperiod is defined on a light/dark (LD) cycle of 24 hours duration. By the suggestion of Grima *et al*, (1996), this was representing the factor of the LD cycles due to three elements occurring simultaneously. These are: (a) photon capture starting the chain of biochemical reactions and leading to biomass synthesis; (b) reversible loss of photon trap activity due to high light intensity; and (c) photon trap recovery, which also occurs in the dark.

The photosynthetic apparatus within the chloroplast of an algal cell contains a complex array of proteins and enzymes that are sensitive to excess light. In particular, the reaction center of PS II is damaged at light saturation conditions and net photodamage occurs if the rate of D1 protein damaged exceeds the rate of its repair processes.

2.1.3.5 Carbon dioxide and Aeration

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.

Aeration is necessary as a means of mixing to prevent sedimentation of algae, to ensure that all cells of the population are equally exposed to the light and nutrients, 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 (air contains about 0.03% CO_2 by volume) where the rate at which CO_2 is transferred to water can effectively limit the algal growth.

A supply of CO_2 enriched air may be necessary to maintain exponential growth in dense cultures but high concentrations may have inhibitory effects. The excessive addition of CO_2 will move the pH to the acid range (as a result of the CO_2/HCO_3^- balance) and this may bring the growth to an end.

Table 2.6 summarizes some of the factors which have either beneficial or detrimental effects to the algal growth.

2.1.4 Photosynthetic and Light Intensity

Photosynthetic rate increase linearly with light intensity until it approaches a plateau at saturating light intensities. Very high light intensities

are often inhibitory. High light intensities may also inhibit respiration of active photosynthesizing cells. The curvilinear plot relating photosynthesis to light intensity (Figure 2.3) contains considerable information about the photosynthetic apparatus. At low light intensity, photosynthetic rate is limited by the light reactions of photosynthesis *i.e.* by chemical reactions involving the number of photon traps of the cells and is linearly related to incident light.

At saturating light intensities (I_k), the maximum photosynthetic rate (P_{max}), is a function of dark reaction of photosynthesis and becomes independent of light. Because the reactions are enzymatic in nature, temperature as well as other factors which influence cell mechanisms (*i.g*. nutrient levels) well affect P_{max} (Raven and Johnson, 1992). The PI curve response to photoinhibition; shown by POER after P_{max} with increasing light intensity. Hence the maximum efficiency of the photosynthetic can be obtained by point of the P_{max} of this curve (Huang and Rorrer, 2002).

2.1.5 Utilization of Algae

Man's use of algae is far more diverse and economically important than generally realized. There are many species of algae which are of economical for pharmaceutical purposes or for industrial uses. Some typical utilization of algae is illustrated in Table 2.7.

Generally, algae are the main suppliers of organic matter and energy in marine ecosystems, a source of essential fatty acids, and a food for sea animals. It was shown that some polyunsaturated fatty acids (PUFAs) synthesized by algae are essential for the growth and survival of marine fish, shrimp and molluses (de Pauw and Persoone, 1988, Pantastico, 1989). The diatom, *Chaetoceros calcitrans* are widely used in prawn larvae aquaculture in Thailand since prawn larvae do not have an ability to synthesize PUFAs themselves; rather they acquire them from *Chaetoceros calcitrans* that they consume.

2.2 Bioreactors

2.2.1 Introduction to Culture Systems

Algal culture is one of the modern biotechnologies. There are several considerations as to which choice of culture system to be selected (Baquerisse *et al*., 1999). Factors to be considered include: the biology of the algae, the cost of land, labor, energy, water, nutrients, climate and the type of final product (Borowitzka, 1992).

The various large-scale culture systems also need to be compared on their basic performance such as their light utilization efficiency, ability to maintain the unialgal culture and how easy they are to scale up from laboratory to large scales. The final choice of system is almost always a compromise between all of these considerations to achieve an economically feasible technology.

A common feature of most algal species currently produced commercially is that they grow in highly selective environments such as high salinity (*Dunaliella)* or high alkalinity *(Spirulina)* which means that they can be grown in open air ponds and still remain relatively free of contamination by other algae and protozoa (Lee, 2001). Other species without this selective advantage must be grown in closed systems.

A literature review of each type of culture systems will be provided below:

2.2.2 Open Pond Systems

All very large commercial systems used today are shallow open-air systems. The culture is agitated or circulated by some mechanical means such as paddle wheel or rotating scraper (Lee, 1997). The major types of open-air systems currently used are tanks, shallow ponds, circular ponds and raceway ponds (Figure 2.4). The success of this open-air system depends significantly on the climate factor. In most case, the ambient temperature in

the vicinity of the culture pond was appropriate for the growth of algae. Examples of this type of systems are the open pond system developed in Czech Republic by Setlik *et al*. (1970) for cultivation of *Chlorella sp.* The culture dept was less than 10 cm and achieved a high cell density of 10 g L^{-1}, and the areal productivity of 25 g $m^{-2}d^{-1}$. In Israel, about 0.5 g L^{-1} of *Dunaliella* could be maintained in the open race-way pond and the productivity of about 27 g $m^{-2}d^{-1}$ was reported (Richmond *et al*., 1990). *Chaetoceros calcitrans* was produced at the Aquaculture Department of the Southeast Asian Fisheries Development Center. A final cell density of $2.65x10^6$ cells mL^{-1} was obtained from 1 ton outdoor fiber glass tank after 4 days of culture (Samonte *et al*., 1993).

Mass culture of *Nodularia harveyana* was studied in open pond with paddle wheel by Pushparaj *et al*. (1994). The culture depth was maintained at 8 cm. The maximum yield obtained was 12.0 g dry weight $m^{-2}d^{-1}$. Raceway ponds were also employed for the culture of *Dunaliella* in the USA (Ben-Amotz, 1995), whilst the Betatene Ltd used a very large 250 ha pond in producing *Dunaliella salina* at Whyalla and Hutt Lagoon where the mixing was induced only by natural convection (wind) (Borowitzka, 1997). More examples of the use of different pond systems for the cultivation of algae are summarized in Table 2.8.

The major problem of open pond systems is that the productivity achieved is less than theoretically possible and it is difficult to control the culture environment. The pond depth is compromised to be small enough to provide adequate light to the algal cells and to give an adequate water depth for mixing, and to be large enough to avoid large changes in ionic composition due to evaporation and to have a high volume per unit area.

2.2.3 New Innovative Systems

Despite the success of open systems, future advance in algal mass culture will require closed systems. The conceptual objectives of closed systems are to reduce the light path, to increase the amount of light available

to each cell, to have high cell concentration and to maintain monoculture in culture conditions (Lee, 1986).

In recent years, there have been several major advances in the design and operation of closed bioreactors for algal culture and several systems are likely to be commercial realities in the near future. These systems can guarantee increased volumetric yield and higher quality biomass, but due to its technical complexity, calls for expert personnel are required and additional energy input for cooling the culture is still the major drawback. The advantages and disadvantages of open and closed systems are summarized in Table 2.9.

The major developments of bioreactors for mass algal cultivation are classified by their physical appearance into 2 types. The descriptions follow:

2.2.3.1 Flat plat bioreactor and column bioreactor

This type of bioreactors is a high column which may be cylinder or flat in shape with effective mixing and oxygen removal achieved by air bubbling. A unique advantage of these bioreactors is that they may be tilted towards the sun, ensuring higher adsorption of incident energy and areal productivity (Richmond and Cheng-Wu, 2001). The narrow light path in column and flat plate bioreactors allows high cell concentration. Table 2.8 summarizes the types of culture systems including the flat plate and column bioreactors which were used to grow various types of algae species and some of these are detailed below.

Tredici *et al*. (1991) developed a rigid panel with alveoli and referred to as 'vertical alveolar panel (VAP)' (Figure 2.5). These VAP reactors with a surface area of 0.5 m^2 were constructed from Plexiglas alveolar sheets 1.6 cm in thickness. Principle of this system is inclination of area of reactor with the angle of illuminated light. This system was employed for the cultivation of *Anabaena azollae* and *Spirulina platensis* and it was shown that the productivity of these two strains were 11 and 15.8 g $m^{-2}d^{-1}$, respectively. Merchuk *et al*. (1998) employed a bubble column bioreactor for the culture of

Porphyridium sp. and reported that the maximum cell number of $22x10^6$ cells $L^{-1}d^{-1}$ could be achieved. Recently, 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 $L^{-1}d^{-1}$.

2.2.3.2 Tubular bioreactor and Airlift bioreactor

These bioreactors 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. This results in an increase in the incident light energy input per unit volume and a reduction in the self-shading phenomenon. Nevertheless, mixing problems could be challenging when filamentous instead of spherical microorganisms are grown in dense cultures in bioreactors made with small diameter tubes. Indeed, microorganisms can produce a relatively highly viscous suspension, even at a low biomass concentration (Torzillo *et al*., 1993).

Lee and Low (1991) reported net biomass productivities of 3.64 g $L^{-1}d^{-1}$ for *Chlorella pyrenonoidosa* in a 1.2 cm diameter tubular reactor in Singapore. Pushparaj *et al*. (1994) reported the productivity of *Nodularia harveyana* in tubular bioreactor and paddle wheel open pond. The higher productivity obtained in tubular bioreactor was 14.0 g dry weight $m^{-2}d^{-1}$ compared with paddle wheel open pond. Grima *et al*. (1996) reported productivities of 2.7 g $L^{-1}d^{-1}$ for *Phaeodactylum tricornutum* in a 3.0 cm diameter tubular reactor in Elmira, Spain. Borowitzka *et al*. (1996, 1997 and 1999) achieved sustainable productivities of 1.2 g $L^{-1}d^{-1}$ and more than 1.0 g $L^{-1}d^{-1}$ with *Tetraselmis chuii* and *Isochrysis galbana,* respectively, in a 700 L pilot scale helical tubular photobioreactor (the BIOCOIL™) (Figure 2.6) with 2.4 cm diameter tubing, located outdoors in Perth, Australia.

Many bioprocesses have been studied in ALBR with successful results such as a 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 ALBR when compared with bubble column (Figure 2.7).

Reviews of applications of bioreactors for algae production are given in Table 2.8.

Table 2.1 : Summary of divisions and classes of algae (Gilmour and Gale, 1988).

Kingdom	Division (Phylum)	Class
EUBACTERIA	Cyanophyta (Cyanobacteria)	Cyanophyceae
	Prochlorophyta (chloroxybacteria)	Prochlorophyceae
	Glaucophyta	Glaucophyceae
	Rhodophyta	1) Bangiophyceae
		2) Florideophyceae
	Heterokontophyta	1) Chrysophyceae
		2) Parmophyceae
		3) Sarcinochysidophyce
		4) Xanthophyceae
		5) Eustigmatophyceae
		6) Bacillariophyceae
		7) Raphidophyceae
		8) Dictyochophyceae
		9) Phaeophyceae
		10) Oomycetes
	Haptophyta	Haptophyceae
	Haptophyceae	Cryptophyceae
	Dinophyta	Dinophyceae
	Euglenophyta	Euglenophyceae
	Chlororachniophyta	Chlorarachniophyceae
	Chlorophyta	1) Prasinophyceae
		2) Chlorophyceae
		3) Ulvophyceae
		4) Cladophorophyceae
		5) Brypsidophyceae
		6) Zygnematophyceae
		7) Trentepohliophyceae
		8) Klebsormidiophyceae
		9) Clarophyceae

Table 2.2 : Morphological Variability in the Divisions of motile and nonmotile algae (Stevenson *et al*., 1996).

Taxon	Morphology						Means of motility
	Unicellar		Colonial		Filamentous		
	Mot	N-M	Mot	N-M	Mot	N-M	
Cyanophyta (blue-green algae)		✓		✓	✓	✓	Sheaths
Chlorophyta (green algae)	✓	✓	✓	✓		✓	Flagella and pectin
Bacillariophyta (diatoms)	✓	✓		✓	1	✓	Raphae
Rhodaphyta (red algae)	✓	✓	✓	✓		✓	
Chrysophyta (chrysophytes)	✓	✓	✓	✓		✓	Flagella and pseudopods
Xanthophyta (xanthophytes)						✓	
Euglenophyta (euglenoids)		✓					Flagella
Pyrrophyta (dinoflagellates)	✓	✓		✓			Flagella
Crytophyta (crytomonads)	✓						Flagella

Mot. = Motile
N-M = Nonmotile
1 = *Bacillaria*

Table 2.3 : Biochemical composition of *Chaetoceros calcitrans* expressed as a percentage of dry weight (Zhukova and Aizdaicher, 1995).

Biochemical composition	% dry weight
Protein	27.21
Nucleic acid	10.00
Total lipids	11.80
Polyunsaturated fatty acids (PUFAs)	0.90

Table 2.4 : 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 2.5 : Role of inorganic nutrients in algal metabolism (de Boer, 1981).

Element	Probable Function	Examples of compounds
Nitrogen	Major metabolic importance as compounds	Amino acids, purines, pyrimidines, porphyrins, amino, sugars, amines
Phosphorus	Structure, energy transfer	ATP, GTP, nucleic acids, phospholipids, coenzymes, coenzyme A, phosphoenolpyruvate
Potassium	Osmotic regulation, pH control, protein conformation and stability	Probably occures predominantly in ionic form
Calcium	Structural, enzyme activation, ion transport	Calcium alginate, $CaCO_3$
Magnesium	Photosynthetic pigments, enzyme activation, ion transport, ribosome stability	Chlorophyll
Sulfer	Active groups in enzymes and coenzymes, structural	Methionine, cysteine, glutathione agar, carrageenan,sulfolipids, coenzyme
Iron	Active groups of porphyrin molecules and enzymes	Ferredoxin, cytochromes, nitrate and nitrite reductases, catalase
Manganese	Electron transport in photosystem , maintenance of chloroplast membrane structure	Manganin
Copper	Electron transport in photosynthesis enzymes	Plastocyanin, amine oxidase
Zinc	Enzyme, ribosome structure	Carbonic anhydrase
Molybdenum	Nitrate reduction, ion absorption	Nitrate reductase
Silicon	Structural	Selicon
Chlorine	Photosystem II	Terpenes
Boron	Regulation and utilization RNA metabolism	Phosphogluconates
Cobalt	Component of vitamin B_{12}	Vitamin B_{12}

Table 2.6 : Effect of factors controlling algal growth. [1/3]

Factor	Condition	Genus or species of alga	Effect	Reference
Nutrients	Sodium Chloride 25%	*Dunalielle salina*	grow fast	Belay, 1997
	Sodium Chloride 0.5%	*Marchantia polymorpha*	growth inhibition	Belay, 1997
	nitrogen concentration below 4%	*Microcystis aeruginosa*	yield diminished	Gerloff and Skoog, 1954
	0.8 mM phosphat	*Phaeodactylum tricornutum*	grow fast	Laws *et al*., 1983
	silica concentration below 25 mg L^{-1}	*Fragilaria crotonensia*	death	Paasche, 1980
	silica concentration below 25 mg L^{-1}	*Nitzschia palea*	death	Paasche, 1980
	Vitamin B_{12} was added to medium	*Volvox globator*	grow fast	Pintner et al., 1963
	0.005 g L^{-1} Fe	*Nodularia harveyana*	grow fast	Pushparaj *et al*., 1994
	$NaNo_3$ 5 g L^{-1}	*Spirulina platensis*	grow fast	Qiang and Richmond, 1996
Light	200 μE $m^{-2}s^{-1}$	*Phacodactylum tricornutum*		Acien Fernandez *et al*., 1997
	200 μE $m^{-2}s^{-1}$	*Scytonema sp.*		Chetsumon *et al*., 1994
	200 μE $m^{-2}s^{-1}$	*Muriellopsis sp.*		Del Campo *et al*., 2001
	170 μE $m^{-2}s^{-1}$	*Anabaena siamensis*	maximum growth	Janssen *et al*., 1999
	200 μE $m^{-2}s^{-1}$	*Marchantia polymorpha*		Katoh *et al*., 1980
	980 μE $m^{-2}s^{-1}$	*Chlorella sp. Strain HA-1*		Morita *et al*., 2000
	240 μE $m^{-2}s^{-1}$	*Chlamydomonas reinhardtii*		Qiang and Richmond, 1996

Table 2.6 : Effect of factors controlling algal growth. [2/3]

Factor	Condition	Genus or species of alga	Effect	Reference
Light	50 μE $m^{-2}s^{-1}$	*Nodularia harveyana*	maximum growth	Pushparaj *et al*., 1994
	200 μE $m^{-2}s^{-1}$	*Chaetoceros calcitrans*		Samonte *et al*., 1993
	1500 μE $m^{-2}s^{-1}$	*Spirulina platensis*		Vonshak and Guy, 1988
Temperature	25 ^{o}C	*Scenedesmus sp.*	maximum growth	Ahlgren, 1987
	30 ^{o}C	*Anabaena azollae*		Belkin and Bussiba, 1991
	25 ^{o}C	*Porphyridium purpureum*		Dermoun, 1987
	42 ^{o}C	*Achnanthes exigua*		Fairchild and Sheriden, 1974
	25 ^{o}C	*Marchantia polymorpha*		Katoh *et al*., 1980
	28 ^{o}C	*Monodus subterraneus*		Qiang and Richmond, 1996
	20 ^{o}C	*Cladophora glomerata*		Simpson and Eaton, 1986
	18-30 ^{o}C	*Chaetoceros calcitrans*		Samonte *et al*., 1993
	36 ^{o}C	*Spirulina platensis*		Tomaselli *et al*., 1987
	38 ^{o}C	*Anabaena siamensis*		Vonshak *et al*., 1982
	23 ^{o}C	*Nannochloropsis sp.*		Zittelli *et al*., 1999

Table 2.6 : Effect of factors controlling algal growth. [3/3]

Factor	Condition	Genus or species of alga	Effect	Reference
Light / Dark cycle	12/12 h/h	*Chaetoceros gracilis*	maximum growth	Hazzard *et al*., 1997
	18/6 h/h	*Porphyridium sp*		Merchuk *et al*., 1998
	12/12 h/h	*Chlorella sp. Strain HA-1*		Morita *et al*., 2000
	16/8 h/h	*Chlorella sorokiniana*		Pirt, 1986
	12/12 h/h	*Agardhiella subulata*		Rorrer *et al*., 1995
	16/8 h/h	*Laminaria saccharina*		Rorrer *et al*., 1995
	16/8 h/h	*Porphyra umbilicalis*		Rorrer *et al*., 1995
	10/14 h/h	*Porphyra linearis*		Rorrer *et al*., 1995
	12/12 h/h	*Porphyra sp.*		Rorrer *et al*., 1995
	12/12 h/h	*Pterocladia capillacea*		Rorrer *et al*., 1995
	12/12 h/h	*Chaetoceros calcitrans*		Samonte *et al*., 1993
	16/8 h/h	*Phaeodactylum tricornutum*		Yongmanitchai and Ward, 1991

Table 2.7 : Utilization of algae. [1/2]

Utilization	Genus or species of alga	Product (used as)	Reference
Food	*Euchema*	gilin cai (China)	Berner, 1993
	Porphyra		Kennard and Janekeh, 1991
	Gracilaria		Redfield, 1963
Health food	*Spirulina*	Phycocyanin	Borowitzka, 1999
	Haematococcus	Astaxanthin	Bubrick, 1991
	Dunaliella	β-carotene	Gudin and Therpenier, 1986
	Chlorella	High protein	Schoutens et al., 1986
Fertilizer	*Anabaena azollae*	rice fields	Boussiba, 1988
	Anabaena siamensis		Qiang and Richmond, 1996
	Tylopothrix tenuis		Vymazal, 1995
Phycological extracts	Brown algae	Alginic acid	Becker, 1994
	Delesseria sanguinea	Blood-anticoagulant compound	Berner, 1993
	Red Algae	Agar	Burlew, 1953
	Gelidium	Agar	Goldman, 1979
	Euchemia spp.	Carrageenan	Vymazal, 1995
	Furcellaria lumbricalis	Danish agar	Vymazal, 1995
	Gloiopetis sp.	Funoran agar	Vymazal, 1995

Table 2.7 : Utilization of algae. [2/2]

Utilization	Genus or species of alga	Product (used as)	Reference
Medicine	*Scytonema hofmanni*	Antibiotics	Becker, 1994
	Chlorella	Antibiotic effect on different bacteria	Benz-Amotz and Avron, 1989
	Brown algae	Against a high blood pressure	Berner, 1993
Fodder	Seaweeds	feed for livestock	Berner, 1993
Fisheries	*Fragilaria oceania*	feed for fish	Burlew, 1953
Chemical Industry	*Laminaria*	Sodium and Potassium alginate used in the production of soap glass, porcelain and alum source of iodine	Gudin and Therpenier, 1986 Kennard and Janekeh, 1991 Redfield, 1963
Energy	*Clostridium themosacharolytieum*	ethanol	Berner, 1993
	Clostridium sp.	isopropanol and butanol	Burlew, 1953
	Seaweeds	methane	Vymazal, 1995
Algal aquaculture	*Enteromorpha*	fish feed	Becker, 1994
	Euchemia	fish feed	Borowitzka, 1999
	Gelidium	fish feed	Goldman *et al*., 1979
	Chaetoceros	shrimp and rotifer feed	Grima *et al*., 1993
	Macrocystis	fish feed	Kyle *et al*., 1998
	Laminaria	fish feed	Redfield, 1963
	Chaetoceros calcitrans	shrimp feed	Samonte *et al*., 1993

Table 2.8 : Example of applications of bioreactor for algae production. [1/4]

Reactor type	Genus or Species of algae	Biomass or Productivities	volume or surface area	References
Open ponds system				
Paddle-wheel raceway ponds	*Dunaliella sp.*	0.5 g dry weight L^{-1}	250 ha	Ben-Amotz,1995
Open ponds	*Anabaena azollae*	17 g $m^{-2}d^{-1}$	106 m^3	Boussiba, 1988
Open ponds	*Anabaena variabilis*	22 g $m^{-2}d^{-1}$	surf / vol 25-125 m^{-1}	Fontes *et al*., 1989
Raceway ponds	*Spirulina platensis*	14.5 g $m^{-2}d^{-1}$	300 L	Pushparaj *et al*., 1997
Open raceway ponds	*Anabaena siamensis*	0.086 g $L^{-1}d^{-1}$	300 L	Richmond *et al*., 1993
Fiber glass tanks	*Chaetoceros calcitrans*	$2.65x10^6$ cells mL^{-1} (4 days)	1 m^3	Samonte *et al*., 1993
Paddle-wheel raceway ponds	*Spirulina platensis*	20.8 g $m^{-2}d^{-1}$	$3x10^4$ L	Vonshak and Guy, 1992
Flat plat and Column bioreactor				
Columns	*Scytonema sp.*	4 g $L^{-1}d^{-1}$	2.3 L	Chetsumon *et al*., 1994
Bubble column	*Porphyridium sp.*	$1.062x10^{10}$ cells L^{-1} (2 days)	35 L	Merchuk *et al*., 1998
Alveolar panel	*Spirulina platensis*	24.3 g $m^{-2}d^{-1}$	18 L	Pushparaj *et al*., 1997
Vertical glass column	*Isochrysis galbana*	1.6 g $L^{-1}d^{-1}$	25.5 L	Qiang and Richmond, 1994
Flat plate inclined	*Spirulina platensis*	9 g $L^{-1}d^{-1}$	7.2 L	Qiang *et al*., 1996
Flat plate inclined	*Anabaena siamensis*	1.43 g $L^{-1}d^{-1}$	50 L	Qiang *et al*., 1996
Flat plate inclined	*Spirulina platensis*	51 g $m^{-2}d^{-1}$	50 L	Qiang *et al*., 1996
Vertical Flat plate glass	*Nannochloropsis sp.*	12.1 g $m^{-2}d^{-1}$	500 L	Richmond and Cheng-Wu, 2001

Table 2.8 : Example of applications of bioreactor for algae production. [2/4]

Reactor type	Genus or Species of algae	Biomass or Productivities	volume or surface area	References
Flat plat and Column bioreactor				
Flat plate glass	*Nannochloropsis sp.*	22.4 g $m^{-2}d^{-1}$	0.52 m^2	Richmond and Zou, 1999
Glass bubble column	*Laminaria saccharina*	0.18 g $L^{-1}d^{-1}$	0.28 L	Rorrer *et al*., 1995
Vertical Alveolar Panel (VAP)	*Anabaena azollae*	11 g $m^{-2}d^{-1}$	0.5 m^2	Tredici *et al*., 1991
Vertical Alveolar Panel (VAP)	*Spirulina platensis*	15.8 g $m^{-2}d^{-1}$	0.5 m^2	Tredici *et al*., 1991
Sun-oriented panel	*Spirulina platensis*	24 g $m^{-2}d^{-1}$	1000 L	Tredici and Materassi, 1992
Curved chamber	*Spirulina platensis*	1.64 g $L^{-1}d^{-1}$	6.4 L	Tredici and Zittelli, 1998
Flat chamber	*Spirulina platensis*	1.93 g $L^{-1}d^{-1}$	4.8 L	Tredici and Zittelli, 1998
Horizontal flat panel	*Spirulina platensis*	1.09 g $L^{-1}d^{-1}$	32.3 L	Tredici and Zittelli, 1998
Modular flat panel	*Nannochloropsis sp.*	1.45 g $L^{-1}d^{-1}$	20.5 L	Zittelli *et al*., 1999
Tubular and Airlift bioreactor				
Helical tubular	*Phaeodactylum tricornutum*	0.133 g $L^{-1}d^{-1}$	30 L	Chrismadha and Borowitzka, 1994
Outdoor tubular	*Muriellopsis sp.*	40 g $m^{-2}d^{-1}$	55 L	Del Campo *et al*., 2001
Tubular	*Isochrysis galbana*	0.32 g $L^{-1}d^{-1}$	50 L	Grima *et al*., 1994
Tubular	*Phaeodactylum tricornutum*	2.76 g $L^{-1}d^{-1}$	0.22 m^3	Grima *et al*., 1996
Tubular	*Porphyridium cruentum*	17-23 g $m^{-2}d^{-1}$	6 m^3	Gudin and Chaumont, 1991

Table 2.8 : Example of applications of bioreactor for algae production. [3/4]

Reactor type	Genus or Species of algae	Biomass or Productivities	volume or surface area	References
Tubular and Airlift bioreactor				
Tubular	*Scenedesmus acutus*	20.9 g $m^{-2}d^{-1}$	6 m^3	Gudin and Chaumont, 1991
Vertical tubular airlift	*Nannochloropsis sp.*	10.12 g $m^{-2}d^{-1}$	12.1 L	James and Al Khars, 1990
Airlift	*Chlamydomonas reinhardtii*	0.22 g L^{-1}	0.6 L	Janssen *et al*., 1999
Tubular loop	*Chlorella pyrenoidosa*	130 g $m^{-2}d^{-1}$	10 L	Lee and Low, 1991
α-type tubular	*Chlorella pyrenoidosa*	72.5 g $m^{-2}d^{-1}$	300 L	Lee *et al*., 1995
Tubular	*Chlorella sorokiniana*	127 g $m^{-2}d^{-1}$	10 L	Lee *et al*., 1996
Helical tubular	*Monodus subterraneus*	1.7 g $L^{-1}d^{-1}$	4.5 L	Lu *et al*., 2001
Airlift	*Porphyridium sp.*	$1.656x10^{10}$ cells L^{-1} (2 days)	35 L	Merchuk *et al*., 1998
Conical helical	*Chlorella sp.*	28.3 g $m^{-2}d^{-1}$	14 L	Morita *et al*., 2000
Tubular	*Nodularia harveyana*	14 g $m^{-2}d^{-1}$	51 L	Pushparaj *et al*., 1994
Tubular	*Anabaena siamensis*	0.55 g $L^{-1}d^{-1}$	300 L	Richmond *et al*., 1993
Tubular	*Spirulina platensis*	27.0 g $m^{-2}d^{-1}$	300 L	Richmond *et al*., 1993
Airlift	*Synechococcus sp.*	1.4 g $L^{-1}d^{-1}$	7 L	Suh and Lee, 2001
Tubular airlift	*Spirulina platensis*	27.8 g $m^{-2}d^{-1}$	145 L	Torzillo *et al*., 1993
Tubular	*Spirulina sp.*	27.8 g $m^{-2}d^{-1}$	51 L	Torzillo *et al*., 1996

Table 2.8 : Example of applications of bioreactor for algae production. [4/4]

Reactor type	Genus or Species of algae	Biomass or Productivities	volume or surface area	References
Tubular and Airlift bioreactor				
Tubular	*Spirulina platensis*	27.9 g $m^{-2}d^{-1}$	51 L	Torzillo *et al*., 1996
Coiled tubular	*Spirulina platensis*	0.9 g $L^{-1}d^{-1}$	111 L	Tredici and Zittelli, 1998
Horizontal tubular	*Spirulina platensis*	1.26 g $L^{-1}d^{-1}$	32.3 L	Tredici and Zittelli, 1998
Helical tubular	*Spirulina platensis*	0.51 g $m^{-2}d^{-1}$	14.7 L	Watanabe and Hall, 1995; 1996
Tubular	*Chlorococcum sp.*	13.5 g $L^{-1}d^{-1}$	10 L	Zhang *et al*., 1997

Table 2.9 : Advantages and disadvantages of open pond systems and closed systems.

Advantages	Disadvantages
Open pond systems	
- low operating cost as sunlight impinging on the surface is the main energy source for warming the pond and for algal growth	- the productivity achieved is less than that possible theoretically - difficult to control the culture environment - high day and night culture temperature changing especially in temperature zones - low surface-to-volume ratio that obliges maintenance of a low volumetric productivity and low cell density that leads to high harvesting costs
Closed systems	
- minimal risk of overgrowth by other algae - attain a better thermal profile - permit operation at high population densities - achieve higher productivity - can be operated over a much wider climatic range	-an expensive cooling is required in order to maintain optimum temperature - many of the new algae and algal products must be grown free of potential contaminants such as heavy metals and micro-organisms

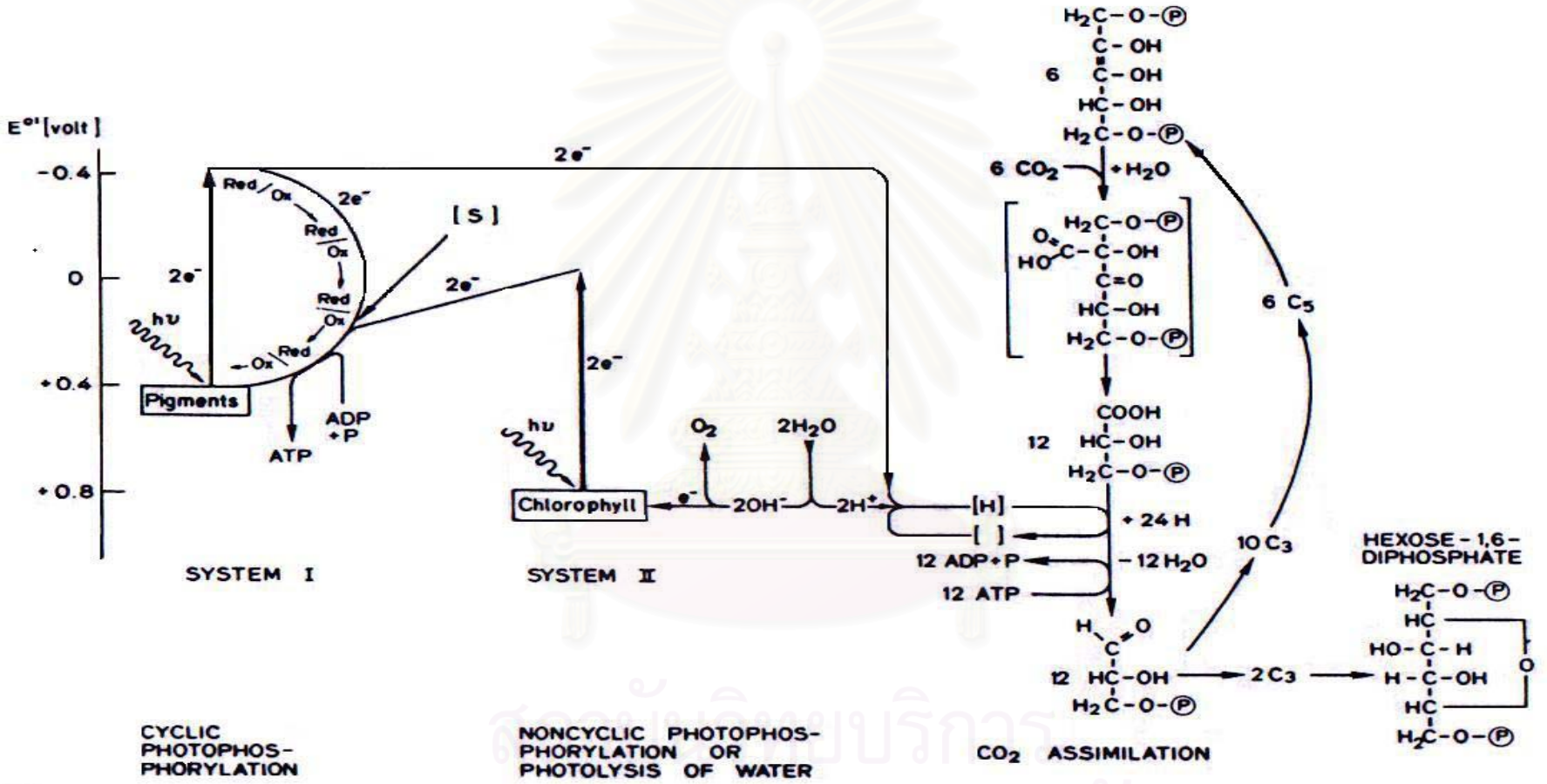


Figure 2.1 : Schematic diagram for the light reactions in photosynthesis (Vymazal, 1995).

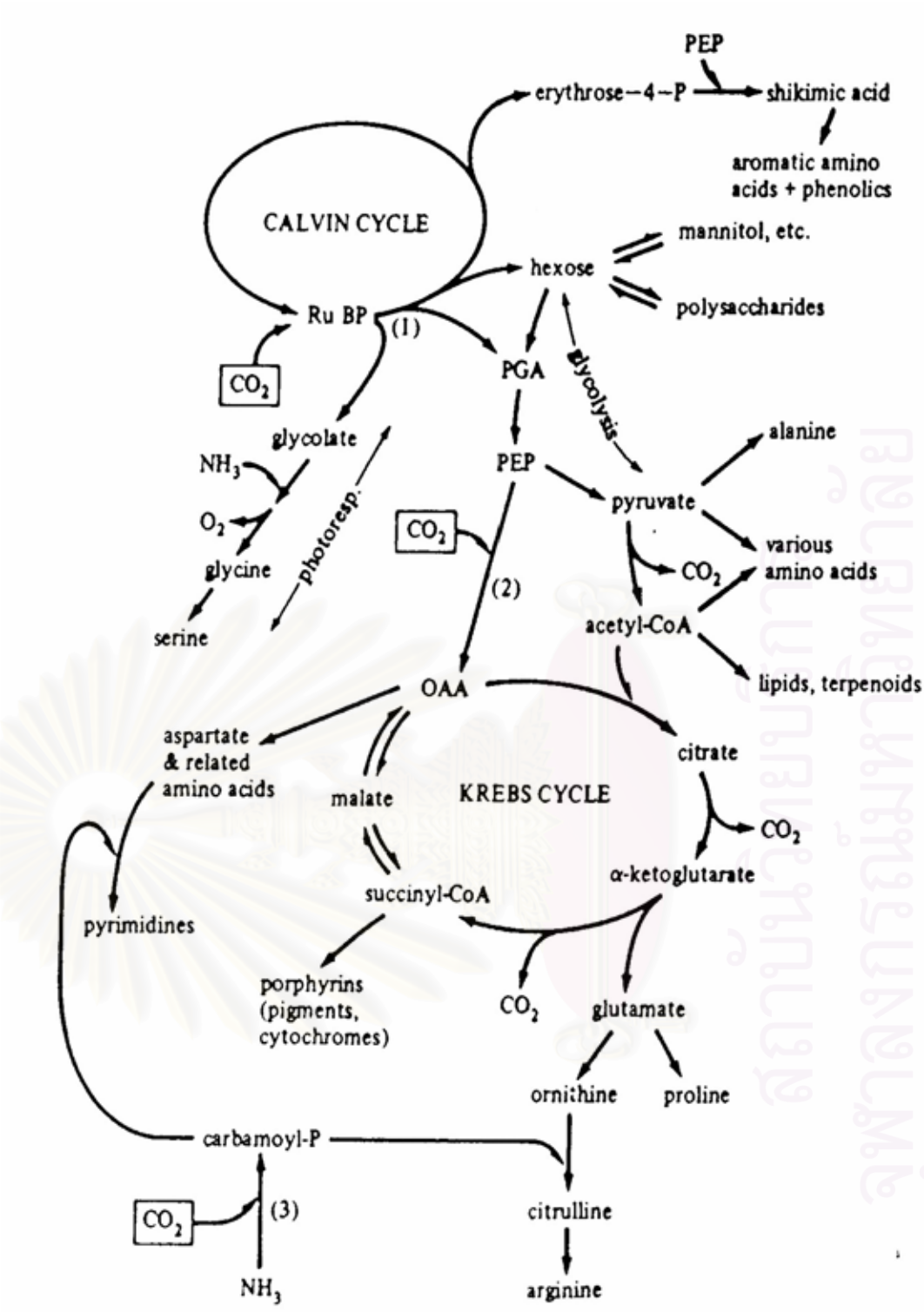


Figure 2.2 : The summary of algal metabolism (Vymazal, 1995).

Where (1) RuBP carboxylase
(2) PEP carboxylase or carboxykinase
(3) carbamoyl-phosphate-synthetase

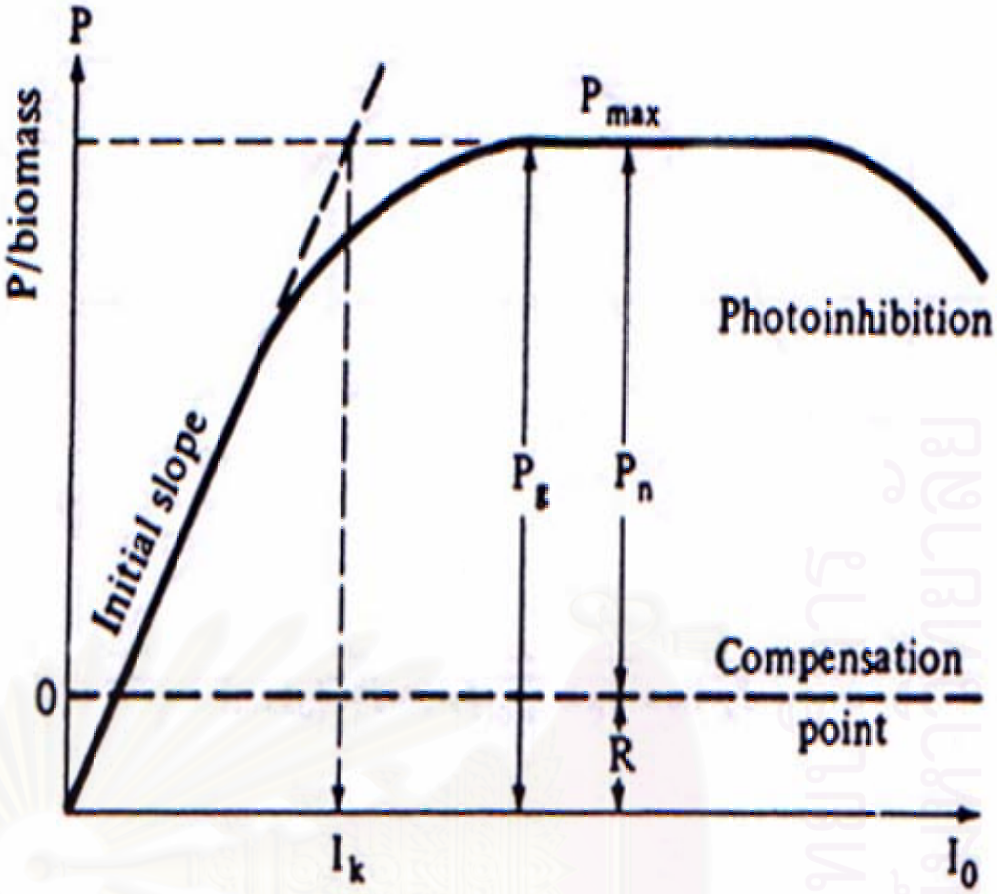


Figure 2.3 : The classic curvilinear plot relating photosynthesis to light intensity (Vymazal, 1995).

Where P = photosynthesis
P_{max} = maximum photosynthesis
P_g = gross photosynthesis
P_n = net photosynthesis
R = respiration
I_o = incident PFD
I_k = saturating PFD

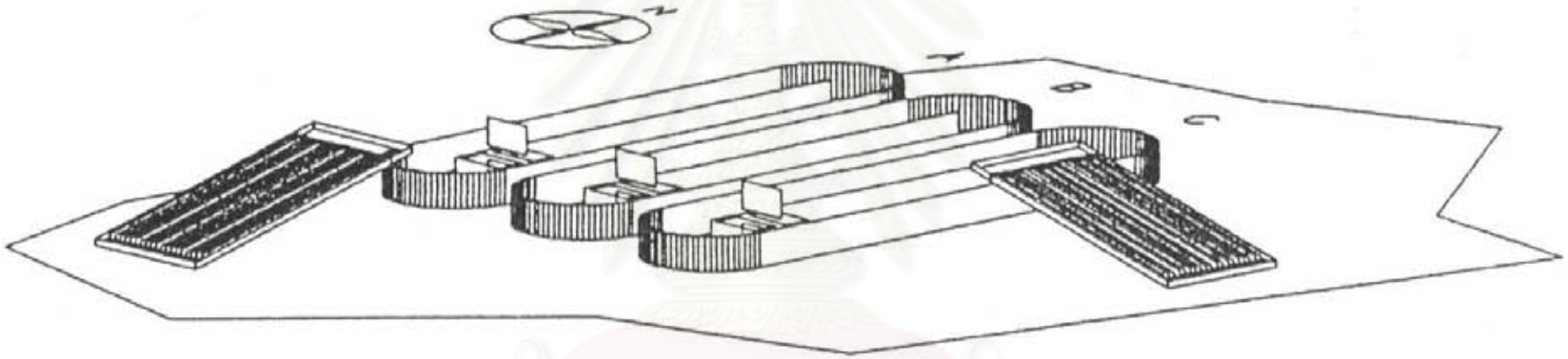


Figure 2.4 : General view of integrated raceway pond (Pushparaj, 1997).

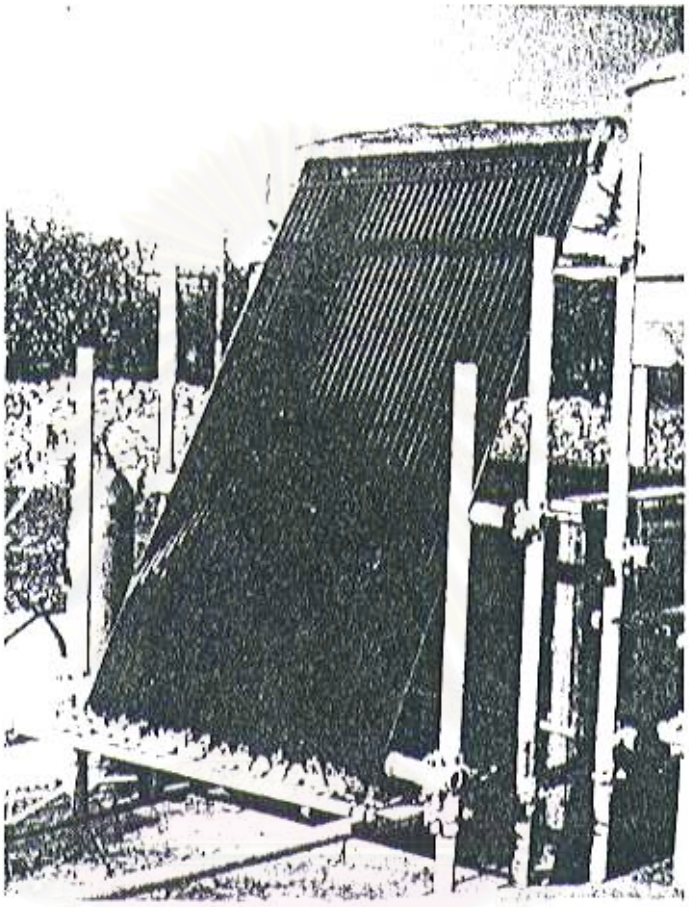


Figure 2.5 : A vertical alveolar panel (VAP) of 2.2 m^2 surface area used for the outdoor mass cultivation of *S. platensis* in winter (Tredici *et al*., 1991).

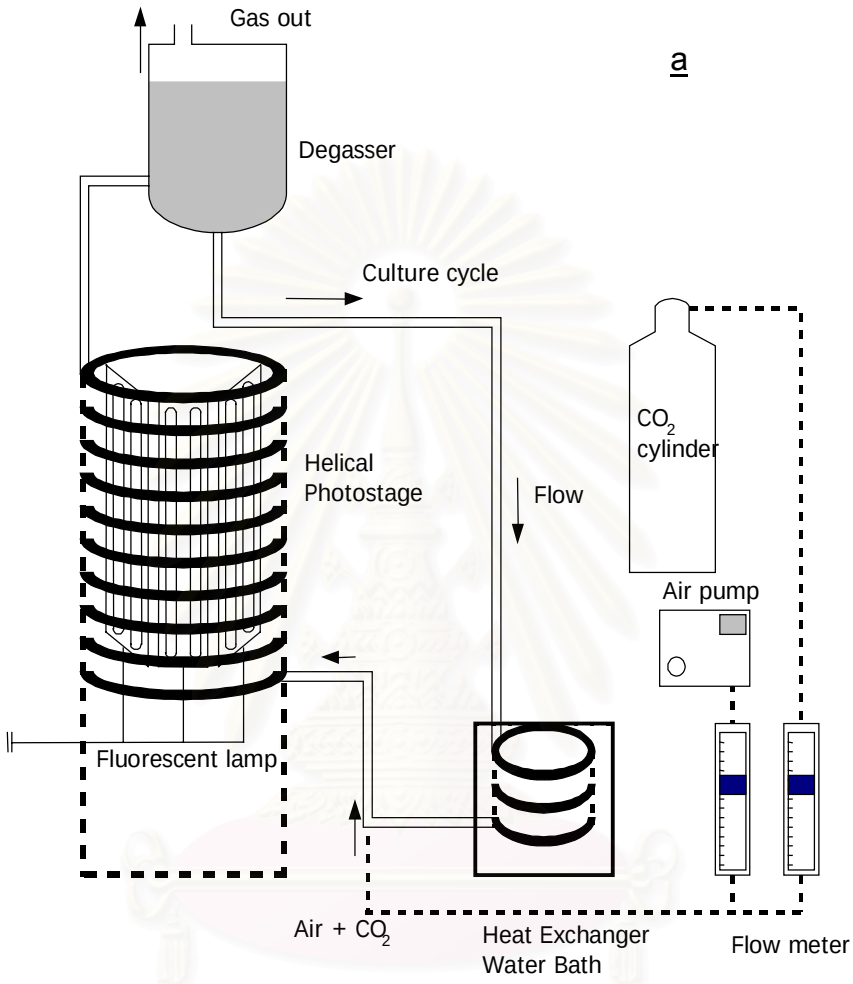


Figure 2.6 : A schematic diagram of helical tubular photobioreactor system and two systems of operation. (a) The airlift system in which the gas stream moves within the helical photostage and gas flow results in the cycling of the culture medium (Watanabe *et al*., 1995). [1/2]

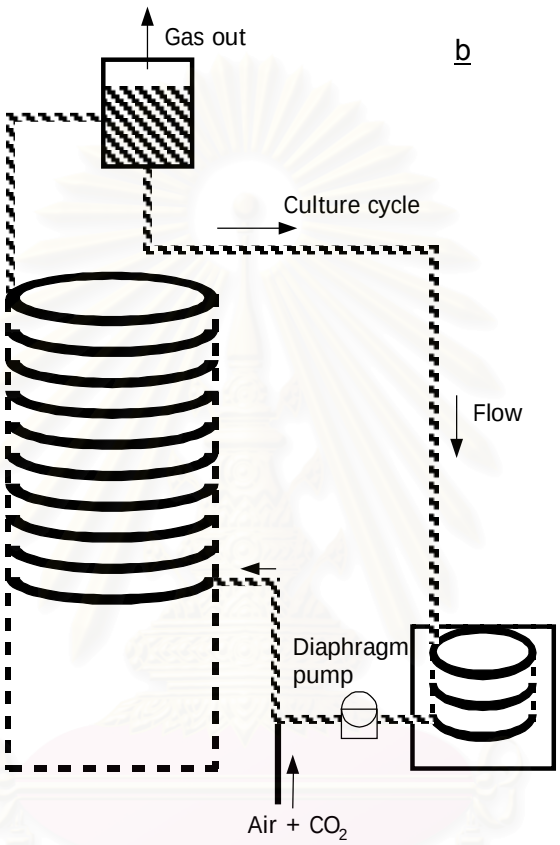
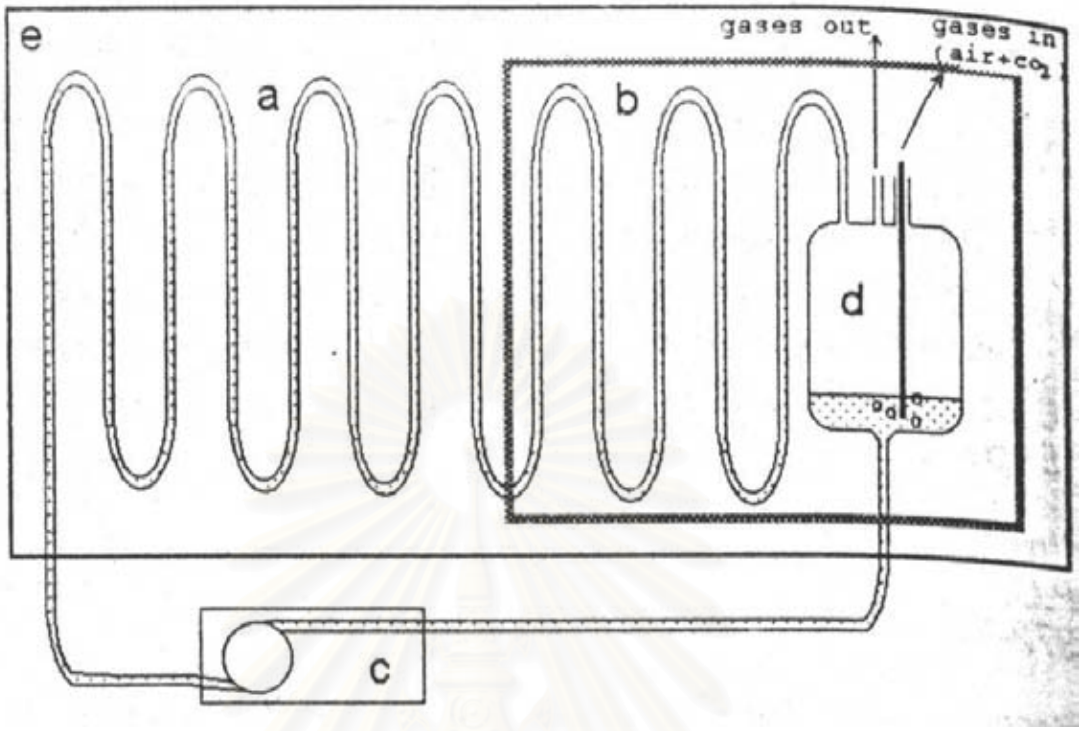
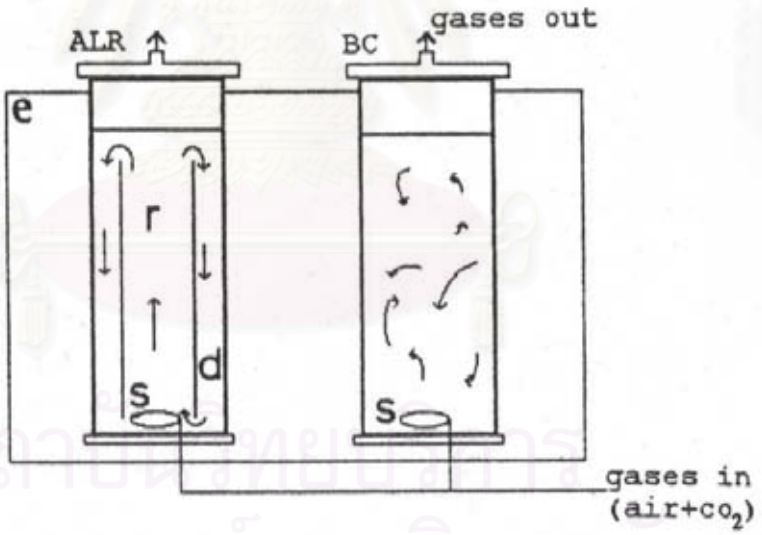


Figure 2.6 : (b) The pumped cycle system in which the diaphragm pump cycles the medium and the gas stream moves within the helical photostage. The height of photobioreactor constructed is 1.8 m and that of the helical photostage is 0.9 m. Total number of coils is 37. Outer width of the helical photostage is 56.5 cm and inner width is 54 cm (Watanabe *et al*., 1995). [2/2]



(i)



(ii)

Figure 2.7 : (i) Tubular reactor for kinetic determinations:
a-illuminated volume; b-dark volume; c-peristaltic pump;
d--aeration flask; e-light bank.
(ii) Column reactor: ALR-air-lift reactor; BC-bubble column;
s-gas spargers; r-riser of the air-lift reactor;
d-downcomer of the air-lift reactor (Merchuk *et al.*, 1998).

CHAPTER 3

Materials and Methods

3.1 Experiment Apparatus

Figure 3.1A illustrates the experimental setup for the determination of optimal conditions for the growth of *Chaetoceros calcitrans (C.calcitrans)*. A clear glass bubble column with an inside diameter of 9.5 cm and a height of 40 cm was used as a culture vessel where an air distributor was placed centrally at the bottom of the column. Figure 3.1B and C shown schematic diagram of the airlift bioreactor (ALBR) and bubble column (BC) used for the production of ultrahigh density of *C.calcitrans*. These were built with external tubes of the same diameter and were supplied with air at the flow rates indicated in Table 3.1, which also given the main dimensions of the bioreactors.

The ALBR was composed of the draft tube installed centrally inside the outer column. The reactor column and the draft tube were made of a clear acrylic plastic. The ratio between the downcomer and riser cross sectional areas (A_d/A_r) was set at approximately 1.79. To prevent cell sedimentation in the bottom area of the bioreactor (due to poor mixing, the bottom of the outer tube was designed with a concave shape. Air was sparged into the bioreactor through a sparger in the base of the draft tube. Both experimental setups were operated in temperature room at the range of 25-35 ^{o}C.

3.2 Experimental Methods

The experiment in this work can be divided into 2 parts:

(i) the determination of optimal condition (light intensity, light/dark cycle and nutrients)

(ii) the *C.calcitrans* culture at high cells density

As part of this research, efforts to maximize output of cell concentration from the diatom *C.calcitrans*. In the first section, data obtained with glass bubble column and dimensions have been presented in Table 3.1. Studied the effect of light intensity (measured with LUX-meter around the culture column and converted to the unit of μmol photon $m^{-2}s^{-1}$ which presented in section 2.1.3.3), photoperiod and nutrients concentration, which has been varied on the middle range of F/2 (Guillard's) medium (Table 3.3). The second section, the ability to achieve high cells density were studied in the BC and ALBR under different aeration rates indicated in Table 3.1, which also given the main dimensions of the reactors. Finally, bioreactor in which given very high concentration was chosen as the most appropriate system for growing *C.calcitrans* and results from semi-continuous culture of *C.calcitrans* were discussed.

In these works, both reactors were held at room temperature. The liquid movement was driven by the injection of air into the system. However, the only difference between two bioreactors was that a draft tube was located coaxially in the ALBR (Figure 3.1). This central tube had the main role of transforming flow of the liquid, which was approximately random in the BC, into a more ordered pattern. The liquid were ascended through the central riser due to the difference in hydrostatic pressure, and also to the energy transferred from the gas input (kinetic energy and work for volume expansion) descends through the annular downcomer.

3.2.1 Experimental preparation

3.2.1.1 Sea water treatment

Sea water used for algal culture should be free of organisms that may compete with the unicellular algae. Sterilization of sea water by chlorination was required.

1) added 1-2 mg L^{-1} of chlorine and incubation for 24 h without aeration to purify sea water
2) sparged air through the sea water for 2–3 h to remove residual chlorine in sea water (addition of sodium thiosulfate to neutralize chlorine if aeration fails to eliminate the chlorine)

3.2.1.2 Preparation of the culture media

Prepared F/2 (Guillard's) medium with chemical compositions, this was shown in Table 3.2.

3.2.1.3 Isolation of pure algal strain

1) prepared 1.5-2% agar medium by weighing out 15 g of agar powder and placing it into about 2 L Erlenmeyer flask to which 1 L of sea water was added
2) heated the flask on a hot plate and let it boil twice (*i.e.* heat until it boils, let it cool and let it boil a second time)
3) added nutrients (see Table 3.2) before autoclaving
4) covered the flask with aluminum foil
5) autoclaved at 125 ^{o}C , 1 atm for 30 minutes
6) sterilized petri-dishes by incubation for 30 minutes at 150 ^{o}C
7) prepared agar plates aseptically by pouring the warm autoclaving agar into the sterile petri-dishes (25–30 mL for a petri-dish) and cool for about 2 h
8) placed the petri-dishes upside-down
9) when agar plates were cooled, streak the algal sample onto the agar surface with a sterile loop (previously heated to red-hot and cooled)
10) placed the petri-dishes upside-down on an illuminated rack
11) observed cell colonies which should grow on the surface after 5–21 days

3.2.1.4 Upscale of cell culture

1) selected the colony and transfered them with a sterile loop into a test tube filled with 5–10 mL of sterilized culture medium and shake it regularity during incubation on an illuminated rack
2) when a color of the culture become greenish, check the isolated algal strain under the microscope
3) inoculated cells from test tube into 50 mL sterilized culture medium and shake it regularity during incubation on an illuminated rack
4) repeat 2) and 3) but upscale to 500 mL of sterilized culture medium (used 10-15% volume of inoculum)

3.2.1.5 Preparation of varied cell concentration

1) separated the cultural cell (300 mL) in the exponential phase at about $2x10^6$ cells mL^{-1} and centrifuged cell with centrifugal apparatus “KUBOTA 7820”
2) decanted supernatant and filled with the range of fresh medium at 50-600 mL for concentration level about 1-12 ($x10^6$) cells mL^{-1} (100 mL per concentration)

3.2.2 Determination of optimal condition

1) prepared seawater and nutrient according to 3.2.1.1 and 3.2.1.2 for the culture in a clear glass bubble column. 10-15% by volume of inoculum was added into the column at room temperature and mixing was induced by sparging air bubbles at the base of the column at the rate of approximately 0.8 cm s^{-1} with various light intensity indicated in Table 3.3
2) illuminated the culture with a fluorescent lamp or other types that provide adequate intensity
3) measure cell growth (in section 3.3)

4) determined Photosynthetic Oxygen Evolution Rate (POER) in 3.3.6 where the cell concentrations were varied in range of 1-12 ($x10^6$ cells mL^{-1}) as indicated in 3.2.1.5.
5) determined the optimal light intensity
6) repeated experiment with optimal light intensity from (5), but change the light/dark cycle as indicated in Table 3.3
7) repeated experiment with optimal light intensity from (5) and light/dark cycle from (6), but change the nutrient concentrations as indicated in Table 3.3

3.2.3 High culture of C.calcitrans

3.2.3.1 Culture of C.calcitrans in bubble column and airlift bioreactor

1) prepared seawater and nutrient according to 3.2.1.1 and 3.2.1.2 for the culture in a glass bubble column.
2) prepared the inoculum for the airlift bioreactor and bubble column where the volume of inoculum was set at 10% of the total volume of both bioreactors
3) illuminated with the fluorescent lamp or other types that provided adequate intensity
4) cultured cells (triplicate) in both bioreactors with optimal conditions determined from Experiment in 3.2.2 where air was sparged through the bioreactors at the aeration rate of 3 cm s^{-1}
5) measured cell growth (Section 3.3.1)

3.2.3.2 Determination of the effect of aeration rate

Repeat experiment in 3.2.3.1 with aeration rate as indicated in Table 3.1 and measure cell growth (Section 3.3.1).

3.2.3.3 Determination of semi-continuous production

1) determined growth curve of the experiment in 3.2.3.2 at an aeration rate that provide maximum cell growth and repeat that experiment
2) cell concentration at an exponential phase was diluted with percent dilution as indicated in Table 3.1

The summary of the work on the cultivation of *C.calcitrans* in this work was provided in the flow chart in Table 3.4.

3.3 Determination of growth

3.3.1 Determination of cell concentrations by Haemacytometer

A counting chamber (normally used for blood cell counts) was used for cell counting. Haemacytometer, it had 2 rafters allowing for 2 sub-samples to be examined.

1) diluted sample if needed and clean slide and cover glass with tissue paper
2) pressed cover glass onto the slide
3) filled both slides of the counting chamber under the cover-glass with a single smooth flow of suspension (avoid air bubbles)
4) counted cells in 20 small squares of each grid under a microscope (objective 40x). Count cells which touch the upper and left border but not those which touch the lower and rights borders (see Figure 3.2)
5) counted the sub-sample on the other slide of the chamber in the same way
6) calculate cells number as followed :

$$\text{cells number (N; cells mL}^{-1}) = \frac{n_1 + n_2}{160} \times 10^6 \times d \qquad (3.1)$$

where n_1 and n_2 = number of cells counted in upper and rafter

d = dilution factor

3.3.2 Determination of specific growth rate

The specific growth rate was calculated by the following equation:

$$\text{specific growth rate } (\mu, \text{h}^{-1}) = \frac{\text{Ln}(N_2) - \text{Ln}(N_1)}{t_2 - t_1} \qquad (3.2)$$

where N_1 and N_2 (cells mL^{-1}) = cells concentration at t_1 and t_2 respectively

t_1 and t_2 (hour) = time at first time and next time

3.3.3 Cellular dry weight estimation of algae

Dry weight of algal cells can be determined by filtering and drying algae.

1) determined accurately (3 duplicate counts, the concentration of the algal culture to be sampled for dry weight analysis)
2) filtered an exact volume of culture on glass fiber filters (1μm pore size) using a Buchner setup connected to a vacuum pump
3) washed the filter with a solution of ammonium formate (0.5 M) to remove salts
4) followed the same procedure with control filters on which an equal volume of seawater was filtered (triplicate). The strength of the applied vacuum will determine the amount of salts retained on the control filters.

5) dried the filters at 100 ^{o}C for 4
6) calculated the dry weight per algal cell according to the formula:

$$\text{DW } (\text{g cell}^{-1}) = \frac{(\text{DW}_\text{A} - \text{DW}_\text{C})}{(\text{N} \times \text{V})} \qquad (3.3)$$

with DW_A = average dry weight retained on algal filter (g)
DW_C = average dry weight retained on control filter (g)
N = algal concentration (cells mL^{-1})
V = volume of algal culture (mL)

3.3.4 Determination of oxygen production rate

The photosynthetic activity of the cultures was measured by determining the O_2 evolution. The basic concept of the procedure was described by Guterman *et al.* (1989; 1990). The oxygen concentration in the culture system was brought under air saturation value (at about 2-3 mg L^{-1}) by bubbling pure N_2 in the culture. The whole system was closed and the O_2 measurements were performed using microprocessor logging D.O. meter HI 964400.

The oxygen production rate in the culture was described by slope of the curve of dissolved oxygen (*DO*) concentration versus time, DO_s.

3.3.5 Determination of Chlorophyll concentration

1) sampling 5 mL of the algal suspension (triplicate) from the medium by filtration using GF/C filter paper
2) Re-suspend the filtrates algal pellet including GF/C filter paper in 5 mL of absolute methanol and then incubate in the water bath at 70 ^{o}C for 2 minutes, leaved it cool
3) Then, centrifuge the sample at 3500 rpm
4) Measure the clear supernatant at the wavelength of 665 nm
5) Calculation:

$$C\ \left(mg\ L^{-1}\right) \quad = \quad OD_{665\ nm}\ x\ 13.6 \qquad (3.4)$$

where C = Chlorophyll *a* concentration (mg L^{-1})

$OD_{665\ nm}$ = optical density at 665 nm

13.6 = conversion factor (Morakot, 2001)

3.3.6 Photosynthetic Oxygen Evolution Rate

Photosynthetic oxygen evolution rate (POER) can be calculated by the following equation:

$$POER\ \left(mgO_2\ mgChl.^{-1}h^{-1}\right) \quad = \quad \frac{DO_s\ x\ 60}{C\ x\ V} \qquad (3.5)$$

where POER = Photosynthetic Oxygen Evolution Rate (mgO_2 $mgChl.^{-1}h^{-1}$)

DO_s = slope of the curve of dissolved oxygen (*DO*) concentration versus time (mgO_2 min^{-1})

C = chlorophyll concentration (mg chlorophyll mL^{-1})

V = volume of algal culture (mL)

Table 3.1: Main dimensions of the bioreactors

		Bubble Column		Airlift bioreactor
		small	large	
Column height (cm)		43	120	120
Column diameter (cm) :	outer tube	9.5	13.7	13.7
	inner tube	-	-	9.3
Liquid volume (L)		2.5	16	16
Aeration rate (cm s^{-1})		0.8	1-4	1-4
Harvested volume (%)		-	-	50-70

Table 3.2: F/2 (Guillard's) medium (Guillard, 1975).

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
$MnCl_2.4H_2O$	0.18	1 L stocks of (g L^{-1} DW) 10.0 g $CoCl_2$, 9.8 g $CuSO_4$,
$Na_2MoO_4.2H_2O$	0.006	180 g $MnCl_2$, 6.3 g Na_2MoO_4 , 22.0 g $ZnSO_4$
$ZnSO_4.7H_2O$	0.022	*Working stock*:
Na_2EDTA	4.36	add 1 mL of each primary stock solution +
$FeCl_3.6H_2O$	3.15	4.35 g Na_2EDTA + 3.15 g $FeCl_3$ to 1 L DW
Thiamin HCl	0.1	*Vitamin solution*
Biotin	0.0005	*Primary stocks:*
B_{12}	0.0005	add 20 g thiamin HCl + 0.1 g biotin + 0.1 g B_{12} to 1 L DW
		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 3.3 : Variation of growth factors in the determination of optimal condition growth

Culture Conditions	Variation growth factor
Light intensity (μmol photon $m^{-2}s^{-1}$)	40-600
Light/dark cycles (h/h)	16/8-24/0
Silica concentration (mg L^{-1})	0-4.8
Phosphorus concentration (mg L^{-1})	0-3.6
Nitrogen Concentration (mg L^{-1})	0-42
Vitamin B_{12} concentration (μg L^{-1})	0-3

Table 3.4 : Cell Cultivation Methods

Culture Conditions	Primary Stock Cultures	Secondary Stock Cultures		Large-scale Cultures	
Volume	Petri-dish	Erlenmeyer flask		Glass bubble column	Airlift bioreactor/Bubble column
	20 mL	100 mL	500 mL	Table 3.1	
Illumination (Light Intensity)	1-2 units 36 watt Fluorescent lamps (Daylight type)			Table 3.3	
Aeration/Agitation	None	Shaking	Shaking		
Temperature (^{o}C)	Room temperature (control equivalent to ambient)				
Volume of inoculum	1 loop full	10-15 mL	50-75 mL	300 mL	12% culture volume
Culture media	Table 3.2				Modified F/2 medium
Media treatment	Autoclaved			treated with chlorine	
Culture period	7-10 days	5-7 days	1-4 days	to maximum cell density	

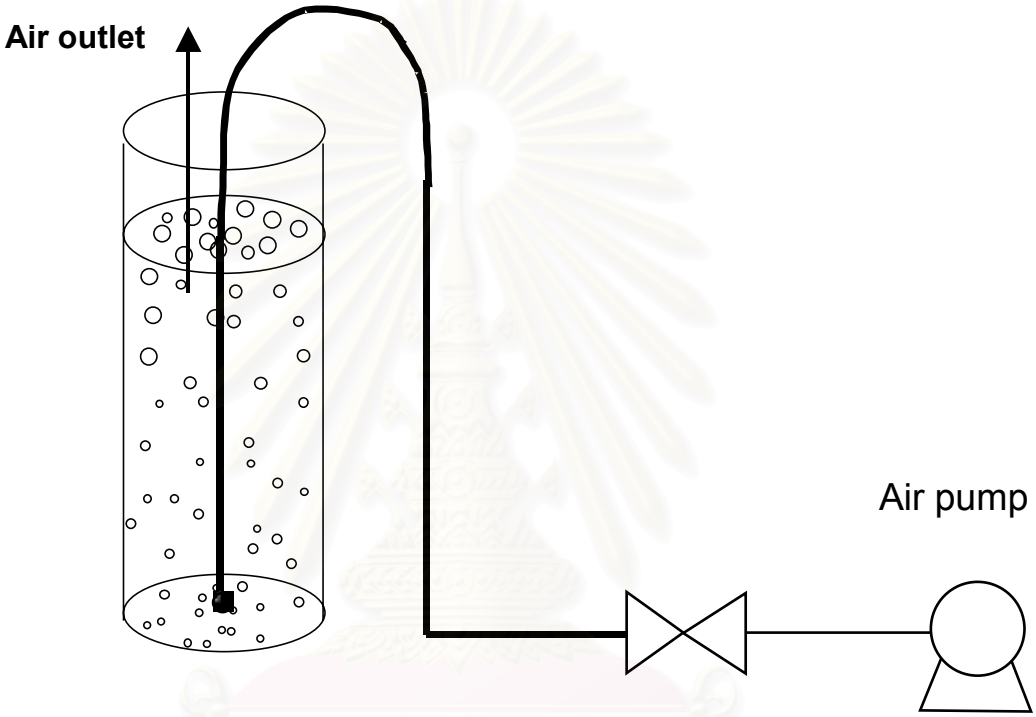


Figure 3.1A : Experimental setup of a glass bubble column for the determination of optimal condition growth of *Chaetoceros calcitrans*. [1/3]

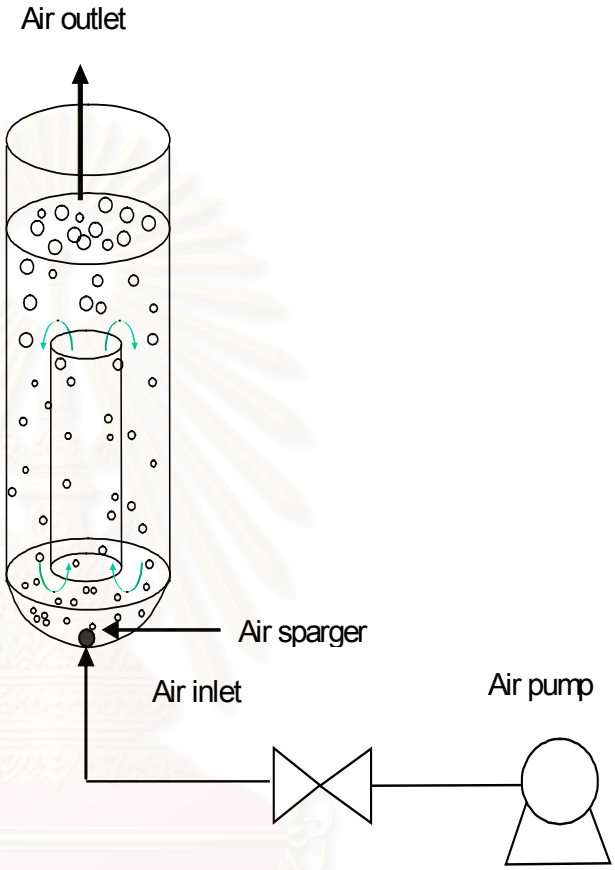


Figure 3.1B : Experimental setup for an airlift bioreactor. [2/3]

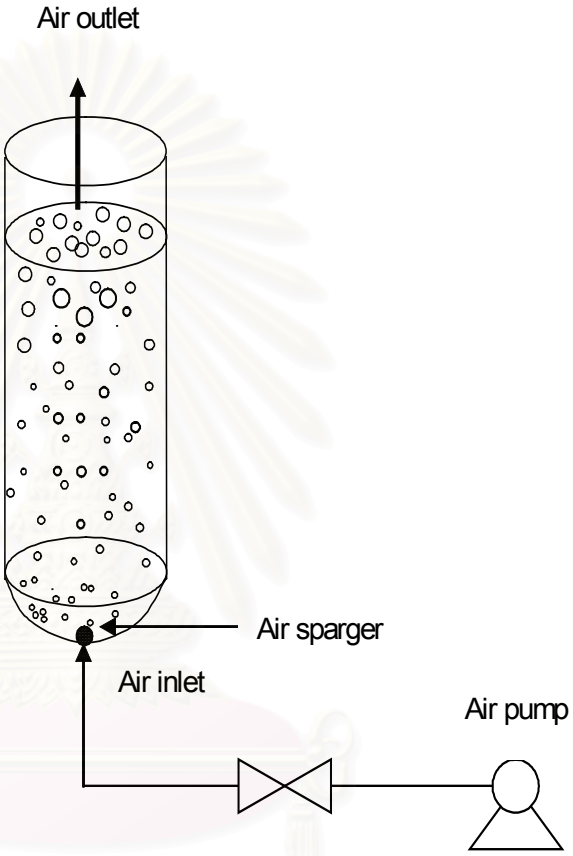


Figure 3.1C : Experimental setup for a bubble column. [3/3]

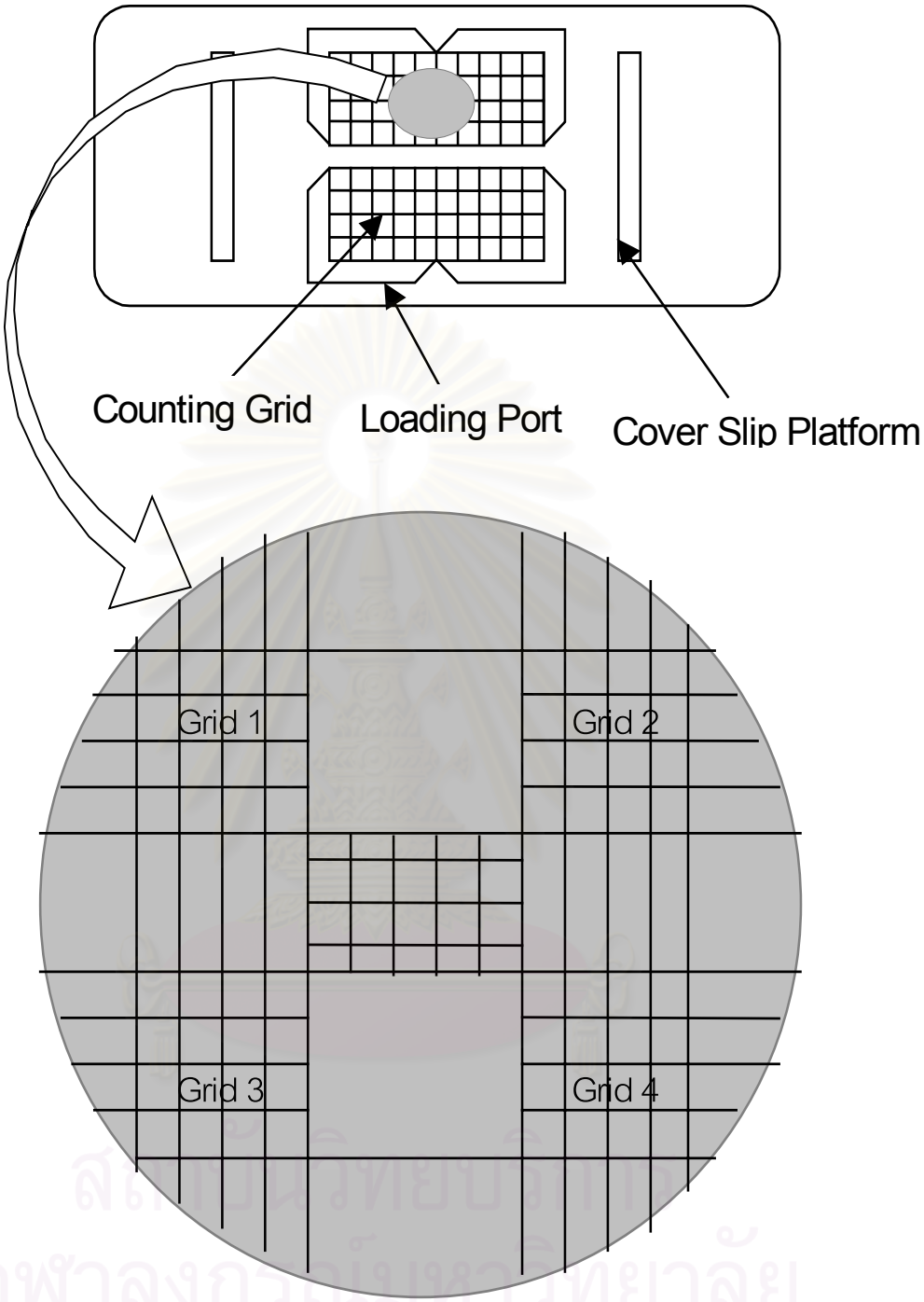
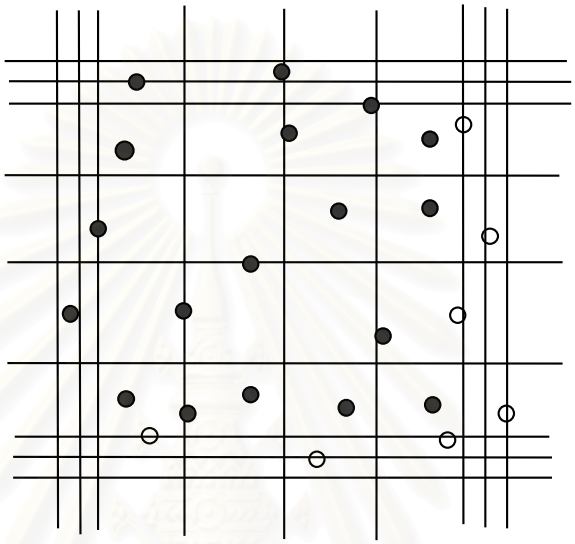


Figure 3.2A : Schematic diagram of Burker (Fox, 1983). [1/2]



Count the cells in the square and those with touch the top and left border (•). Do not count the ones touching the right and lower border (o).

Figure 3.2B : Schematic diagram of Burker (Fox, 1983). [2/2]

CHAPTER 4

Results and Discussion

4.1 Determination of optimal conditions

4.1.1 Effect of light intensity

The effect of light intensity on maximum cell concentration were shown in Figure 4.1.1A which indicated that cell growth were influenced by the amount of light intensity applied to the glass bubble column. It revealed that excessive light could be wasted and even reduced the cell growth. The experimental data indicated that the light intensity higher than 200 μmol photon $m^{-2}s^{-1}$ induced photoinhibition.

Figure 4.1.1B shown specific growth rates determined during the exponential growth phase at each light intensity which were calculated from the linear portion of the semi-log plots of the growth data. Although it was generally expected that increasing in light intensity would induce an efficient cell growth, the data shows that light intensity above 200 μmol photon $m^{-2}s^{-1}$ led to a decline in the specific growth rate which indicated the effect of photoinhibition.

This finding could be explained by the explanation of *e.g.* Sukenik *et al*. (1987), Davison (1991), Grima *et al*. (1996), Vymazal (1995), Huang and Rorrer (2002), based on the known fact that the oversaturation of light causes damages to the photosystem II (PS II) D1 protein that carries the binding site of the electron carrier. The photosystem damage was a function of light intensity, and a simultaneously repair mechanism exists which produces new D1 molecules to replace damaged ones. The rate of this repair mechanism was independent of light. In other words, at low light intensity, all damaged D1 protein molecules were replaced almost immediately, and the net damage to the photosynthetic was negligible. At high light intensity, on the other hand,

a part of the energy absorbed results in oversaturation of PS II and impairment of the activity of the photon traps. Repair occurred simultaneously with the damage, but at a lower rate. Hence, lower growth rate was observed in this high light intensity region.

In this experiment, photosynthetic rate was represented by the rate of oxygen released from the culture sample and called photosynthetic oxygen evolution rate (POER). The procedures indicated in 3.3.3 to 3.3.5 were used to generate a curve between POER and light intensity, or PI curve. A representative PI curve for *Chaetoceros calcitrans (C.calcitrans)* is presented in Figure 4.1.1C where the maximal photosynthesis (P_{max}) was taken as the maximum value of POER. This element had been recognized in the literature review (Section 2.1) as responsible in photosynthetic activity of algal cultures.

Figure 4.1.1C illustrated that a higher POER could be obtained at low cell concentrations (lower than $7x10^6$ cells mL^{-1}). In this region, the photosynthetic rate increased steadily with the cell density and the most active cell density was found to be at about $7x10^6$ cells mL^{-1}. At cell concentration higher than $7x10^6$ cells mL^{-1}, the activity of the diatom was observed to vary adversely with the cell density. Self shading effect where the light blockage occurred due to a dense cell population played a role in regulating the POER.

To determine the optimal light intensity for *C.*calcitrans, the procedure explained in Sukenik *et al*. (1987), Davison (1991), Grima *et al*. (1996), Vymazal (1995), Huang and Rorrer (2002), was applied, where the optimal light intensity in this experiment was around 200 μmol photon $m^{-2}s^{-1}$ (see Appendix C for the results of this determination).

4.1.2 Effect of photoperiod

The effect of the illumination photoperiod on the growth of *C.calcitrans* was assessed in the glass bubble column, which was operated at conditions provided in Table 3.4. The average light intensity was set at 200 μmol photon $m^{-2}s^{-1}$ and then exposed to a step change in photoperiod. Figure 4.1.2 shown maximum cell concentration and specific growth rate (μ)

for *C.calcitrans* over a 5 days cultivation time at photoperiods of 16:8, 20:4 and 24:0 light/dark (LD) cycle.

It was expected that the growth of diatom would be enhanced if some dark period was introduced into the system as it would provide time for the repairing mechanism of the photon traps. However, the results in Figure 4.1.2 did not support this school of thoughts. The reason might be that this experiment was performed with the light intensity of 200 μmol photon $m^{-2}s^{-1}$ where the repairing activity took place at a sufficient rate when compared to the rate of the damage of D1 protein. Hence, the introduction of dark period did not show appreciable effect on cell growth. Rather, the introduction of dark period seemed to adversely affect the growth. Decreasing the lighting hours from 24 to 20 caused the maximum cell concentration and specific growth rate (μ) to decrease from $5.06x10^6$ to $4.56x10^6$ cells mL^{-1} and 0.6 to 0.45 h^{-1} while further decrease from 20 to 16 hours only reduced the cell concentration and specific growth rate (μ) from $4.56x10^6$ to $3.77x10^6$ cells mL^{-1} and 0.45 to 0.37 h^{-1}. It should be noted here that there existed a nonlinear relationship between the fresh mass ratio and the photoperiod. However, the reason for this still could not be explained at the time of this work.

4.1.3 Effect of nutrient concentration

Generally F/2 (Guillard's) medium was used for cultivation of the diatom but it was not especially designed for the growth of *C.calcitrans*. This investigation attempted to identify nutritional factors that controlled the growth of the culture of *C.calcitrans*. To achieve this goal, the culture were started with a cell concentration of $0.5x10^6$ cells mL^{-1} in a modified F/2 (Guillard's) medium where the composition of silica (as sodium silicate), phosphorus (as phosphate), nitrogen (as nitrate), and vitamins (thiamine, biotin, B_{12}) were varied. Detail of this standard F/2 (Guillard's) medium was shown in Table 3.3.

(a) Effect of Silica Concentration

The maximum cell concentration and specific growth rate (μ) at various silica concentrations were shown in Figure 4.1.3A. It was found that the addition of silica at 3.2 mg L^{-1} led to the highest growth rate. This value was found to be twice as much that recommended in the standard F/2 (Guillard's) medium of 1.6 mg L^{-1}. The absence of silica (0 mg L^{-1}) caused a declining cell concentration where the maximum cell concentration felt below initial concentration.

It was generally accepted that diatom cannot survive with inadequate supply of silica (Si) because Si was not only needed in the cell-wall formation, but Si was also required for deoxyribonucleic acid (DNA) synthesis. According to the observations of Guillard *et al*., (1975), a culture medium with less than 1 μM of silicate could greatly decrease the growth rate of algae and caused morphological changes. Also it was stated that the assimilation of silica into cell is directly connected with the formation of new walls, therefore there was a definite low concentration of silicate below which a population could not live. This explanation could explain the finding in this work that increasing silica concentration in the medium resulted in the increase in the growth rate of the diatom *C.calcitrans*.

Interestingly, it was found here also that increasing silica above 3.2 mg L^{-1} led to a decrease in the growth rate. This be might a result of inhibition effect caused by the overdose of silica. However, the effect of the overdose of silica on the metabolism of *C.calcitrans* needs to be verified further.

(b) Effect of Phosphorus Concentration

The effects of phosphorus concentration on the growth of *C.calcitrans* were shown in Figure 4.1.3B. The suitable phosphorus concentration for the growth was found to be around 2.4 mg L^{-1} where the highest growth rate was

observed. This concentration was two times higher than that recommended in the standard F/2 (Guillard's) medium (1.2 mg L^{-1})

The most important role of phosphorus (P) was in energy transfers through energy carrying agents *e.g*. adenosine triphosphate (ATP), NADPH, *etc*. When external phosphate concentrations were high, the ability of cells in the assimilation of phosphorus compound was repressed and the growth was inhibited. On the other hand, an inadequate level of external phosphorus reduces the cell capacity in authorizing ATP and other energy compounds which also led to a limited cell growth. According to Nelson *et al*. (1979), a phosphorus limited algae had an enzymatic activity of 25 times less than algae with sufficient available phosphorus.

(c) Effect of Nitrogen Concentration

As shown in Figure 4.1.3C, a nitrogen concentration as stated in the standard F/2 (Guillard's) medium (14 mg L^{-1}) was found to be most suitable for the growth of *C.calcitrans*. Concentrations above and below this optimal 14 mg L^{-1} led to a low growth rate.

Considering the effect of nitrogen on cellular metabolism, the availability of fixed nitrogen was a major factor influencing the growth and chemical composition of algae. The major problem associated with nitrogen starvation was a rapid reduction in photosynthetic performance. On the other hand, overdose of nitrogen leads to an overall reduction in pigmentation and a decreasing in cellular concentration. Both nitrogen starvation and overdose led to a dramatic decrease in the efficiency of energy transfer from harvesting complexes to photosystem II (PS II) reaction center (Vymazal, 1995).

(d) Effect of Vitamins Concentration

The variation of the mixed vitamin concentrations did not seem to influence the growth of *C.calcitrans* (Figure 4.1.3D). However, transferring cells to a medium lacking these nutrients would result in a low cell growth as seen in Figure 4.1.3D. This result indicates that vitamin was essential for

growth but only a tiny amount would suffice the growth of the cell. In this case, even the smallest amount used in the medium (3 $\mu g\ L^{-1}$) was adequate for the growth. The optimal amount of mixed vitamin was not determined from this experiment.

4.2 Production of high cells density

4.2.1 Cultivation in bioreactors

In this work, the ability to achieve high cell density of *C.calcitrans* in different bioreactors under an aeration rate of 1 cm s^{-1} was examined. The selected bioreactor types included the bubble column (BC) and the airlift bioreactor (ALBR). Configurations of these two bioreactors were described in Figure 3.1B and C. The bioreactors were originally planned to work at the optimal light intensity of 200 μmol photon $m^{-2}s^{-1}$; however there was an experimental constraint in setting up the light source for a large scale system without generating excessive heat. Therefore it was not possible to achieve this optimal light requirement. Rather, the bioreactors were operated at a light intensity of 120 μmol photon $m^{-2}s^{-1}$ which could be obtained from 20 fluorescent tubes each having a power of 36 W.

The growth curves of the cell concentration in both reactors were presented in Figure 4.2.1. It was clear that the performance of the ALBR was superior to that of the BC as higher biomass was obtained in the ALBR than in the BC. This difference in the performance of the two bioreactors must be derived from the difference in the behavior of the two systems, *i.e.* the liquid circulation rate. The configuration of ALBR with riser and downcomer caused uneven densities of fluid in the two sections and induced a certain pattern of liquid movement: liquid moved up in riser and down in downcomer as shown in Figure 4.2.2A (Merchuk, 1986). This well defined flow pattern was not apparent in the BC, where the aeration only superimposed random movement with no net movement of the liquid (Merchuk *et al*., 1998).

The well defined flow pattern in the ALBR meant that the diatom in the riser would, after a certain time period, flow to the downcomer where the light

was applied. Hence, most diatoms were exposed to similar light intensity. This phenomenon was not found with the random movement in the BC. Moreover, since cells were not properly recirculated in the BC, some cells were exposed to high light density at the region adjacent to the wall of the column where the light source was located (zone 2 in Figure 4.2.2B) and photoinhibition might affect those cells. On the other hand, some cells were exposed to low light density at the core of the column (zone 1 in the same figure). The cell in this zone might not have effective photosynthesis because the light intensity was relatively lower than the cell's requirement. All these reasons led to a less reproduction of the cell in BC than in the ALBR.

In addition, the operation of ALBR reduced the dead zone at the bottom of the column (zone 1) due to a higher liquid circulation rate (Merchuk *et al*., 1996). Hence, there would be less cell accumulation at the bottom of the column when compared to the BC. This accumulation of cells might cause starvation, death, and easy contamination of the whole culture which reduced the overall growth rate of the algal culture.

In brief, it could then be concluded at this point that algal cells in the ALBR could utilize light source more effectively than cells in the BC and this resulted in a higher growth rate. This finding agreed well with that of Merchuk *et al*., (1998) who indicated that the growth of the red microalga *Porphyridium sp.* in the ALBR was much better than that in the BC.

4.2.2 Effect of aeration rate

The effect of aeration rate on the productivity of *C.calcitrans* was investigated using both the ALBR and BC. The effect of aeration rates on mass production in both bioreactors were presented in Table 4.1. Increasing aeration rate (measured in terms of velocity) in the range of 1-3 cm s^{-1} was found to positively influence the growth rate of the algae (Figure 4.2.3). The maximum cell growth rate occurred at the aeration rate of 3 cm s^{-1} for the ALBR above which the growth rate dropped. In the BC, on the other hand, the growth rate was found to gradually increase with the aeration rate for the whole range of aeration rate employed in this experiment. However, the

maximum cell concentration in the BC was slightly lower than that obtained in the ALBR.

At a lower range of the aeration (0-1 cm s^{-1}), increasing aeration rate greatly induced mixing, liquid circulation rate and also the mass transfer between gas and liquid phases in both types of bioreactors (Merchuk and Stein, 1981; Gavrilescu and Tudose, 1998). The mixing of liquid caused a more homogeneity between the medium and diatom which resulted in a more efficient utilization of nutrients. A higher mass transfer might also facilitate the removal of metabolic gases such as oxygen preventing the accumulation of these gases which might adversely affect the growth rate (Tung *et al*., 1998). This led to a higher maximum cell concentration and specific growth rate (μ) as illustrated in Figure 4.2.3. In the ALBR however, a slightly better growth was observed when compared to the BC due to a well defined liquid circulation as discussed earlier.

At a high aeration rate (4 cm s^{-1}), the growth rate of *C.calcitrans* was declined in the ALBR. As the aeration only took place in the draft tube of the ALBR where the cross sectional area was only half of the outer column and therefore the aeration rate of 4 cm s^{-1} in the ALBR was equivalent to 8 cm s^{-1} in the aeration section in the ALBR alone. This high aeration rate converted the flow regime in the ALBR to the slug flow condition where small bubbles coalesced and formed very large bubbles. This reduced the liquid circulation rate because there appeared more gas fraction in the aeration section than the liquid fraction which resulted in a low liquid flow rate. Also the mass transfer rate between phases decreased as a result of decreasing specific mass transfer area (of large bubbles). A low growth rate was apparent at this aeration rate. Unlike the ALBR, the flow regime in the BC still was not converted to the slug flow condition as the aeration was supplied to the whole column without partitioning. Hence, a better growth rate was still found at this 4 cm s^{-1} aeration rate. It should be mentioned at this point that the rate of increase in the growth rate in the BC was only slightly affected by the aeration rate.

It is interesting to note that the culture systems employed in this work here could achieve a very high productivity and also concentration of

C.calcitrans. The productivity was calculated as a product between the cell concentration and the rate of harvesting of the culture. Assumed that the harvest was performed during the exponential growth of the culture (this will be described in detail later on in this chapter) which was equal to about 12 hours where the minimum to maximum cell concentration obtained from this exponential growth period was 5.58×10^6 to 15.36×10^6 cell mL^{-1} respectively. At this exponential growth phase, the specific growth rate was 0.0843 h^{-1} (3.11×10^{10} cells $L^{-1}d^{-1}$). The harvest volume was assumed to be about half of the total volume which was about 9 L. The productivity of the diatom could then be calculated as:

Cell concentration = 15.36×10^6 cells mL^{-1} (4.1)

Cell productivity = $15.36 \times 10^6 \times 10^3 \times 0.0843 \times 24$

= 3.11×10^{10} cells $L^{-1}d^{-1}$ (4.2)

This was found to be very high in comparison with the results from Samonte *et al*., (1993) who only could achieve cell concentration at 2.56×10^6 cells mL^{-1} in 4 days of the cultivation diatom *C.calcitrans* with 1 m^3 fiber glass tank (or equivalent to a merely 6.4×10^8 cells $L^{-1}d^{-1}$).

4.2.3 Semi-continuous production

The growth curve of *C.calcitrans* in the batch culture ALBR at an aeration rate of 3 cm s^{-1} was shown in Figure 4.2.4. From this curve, a maximum cell concentration occurred after 48 h of operation. A lag phase was observed during the initial period of the culture (the first 18 hours) followed by exponential growth during the next 12 h. This was followed by the stationary phase where the cell concentration reached a maximum of 20×10^6 cells mL^{-1}. As the cell harvest in the semi-continuous culture should be performed while the cell activity was still at its highest level (at the exponential phase), it was therefore decided that the semi-continuous culture should be harvested at every 12 hours to make sure that the cell activity did not enter the stationary zone. In addition, it was observed that cell concentration at the end of the

exponential phase was approximately 2-3 times that obtained at the end of the lag phase as shown in Figure 4.2.4. The culture was therefore harvested just before the end of the exponential growth phase such that the final concentration of the cell in the culture dropped to the level closed to that at the very beginning of the exponential phase. In other words, about 50-70% of the culture should be harvested at every 12 hours of operation.

Figure 4.2.5 was shown the results from the operation of the ALBR in a semi-continuous culture. It was found that the ALBR could be operated safely for quite a long period of time, and in this case, it was operated continuously for 192 hours or 8 days. After this, contamination by other microorganisms was observed and the operation was terminated for the cleaning.

In the operation of the ALBR as shown in Figure 4.2.5, after about 48 hours, the culture was harvested for the first time. According to the discussion above, the harvest was achieved by replacing 70% or about 11 L of the culture volume with fresh medium seawater. At this point, the culture was diluted where the cell concentration decreased from 20.24×10^6 to 6.51×10^6 cells mL^{-1} with a specific growth rate of 0.069 h^{-1}. Then, sub-cultured cells were cultivated for the next 12 hours at which point the next harvesting was performed. At this time, cells concentration was linearly increased to 17.58×10^6 cells mL^{-1} with specific growth rate of 0.0828 h^{-1}. The final cell concentration obtained from this very first harvesting time illustrates that the growth of cell was not enough to substitute the cells taken out during the harvest (as the final concentration dropped from 20.24×10^6 to 17.58×10^6 cell mL^{-1}. Hence, the culture was harvested at a 12 hour interval with about 60% of culture taken out for the next 5 harvesting points, and 50% for another 6 harvesting points. During the 60% by volume harvesting period, the results indicated that a slightly higher maximum cell concentration could be obtained as the number of harvest increased (from 7.37×10^6 to 9.22×10^6 cells mL^{-1}). This implies that the cell could grow at a faster rate than the dilution rate by the harvest.

Therefore changing the harvesting volume from 60% to 50% should result in an increase in the maximum cell concentration at the end of each culture period (more cell growth than cell dilution). However, the growth rate

obtained during the 50% harvest was found to be around 0.05-0.06 h^{-1} which was lower than the initial growth rate of 0.082 h^{-1} and also lower than the growth rate of 0.07-0.08 during the 60% harvesting period. This result might be attributed to the significance of the shading effect by a concentrated cell density and also the competition for nutrients by the diatom. During the 60% harvesting period, the final diluted cell concentration was found to be about 7.37×10^6 to 9.22×10^6 cells mL^{-1} whereas the final cell concentration after the 50% harvest was found to be higher at 10.81×10^6 to 11.81×10^6 cells mL^{-1}. The higher cell density could lead to a competition for nutrients, and also a shading effect which was the blocking of light path by the dense cell could occur. Hence, lower growth rates were apparent for the case of 50% by volume harvest than the rates from other harvesting conditions. As the cell became less active, the final cell concentration obtained from the 50% harvesting case was also found to be rather low when compared with the 60% harvesting case.

In short, the results in Figure 4.2.5 illustrated that *C.calcitrans* was able to maintain the exponential growth rate with the selected harvesting condition where the specific growth rate was in the range between 0.0767 to 0.0854 h^{-1} (1.84 to 2.05 d^{-1}). This corresponded to the productivity of 3.7×10^{11} to 4.1×10^{11} cells d^{-1} (or 1.5×10^{10} to 1.7×10^{10} cells L^{-1} d^{-1}), respectively. The highest cell concentration for these cases was 23.13×10^6 cells mL^{-1}. This information was summarized in Table 4.2. The maximum productivity of *C.calcitrans* occurred when the harvest was performed with 60% substitution of medium seawater to the bioreactor.

Note that if dry cell weight was to be determined, one could simply employ the following expression:

$$\text{cell mass}\,(\text{g L}^{-1}) = \left[\frac{(1.1026 \times \text{cell concentration}\,(\text{cells mL}^{-1})) + 69.7727}{16}\right] \times 10^{-3} \quad (4.3)$$

which will give the cell concentration in "g L^{-1}" (see Figure 4.2.6 for the calibration curve between cell count in cells mL^{-1} and cell mass in g L^{-1}).

Table 4.1 : Effect of aeration rate on *C.calcitrans* in bubble column and airlift bioreactor under light intensity of 120 μmol photon $m^{-2}s^{-1}$

Aeration rate	Specific growth rate (μ) [h^{-1}]		maximum cell concentration ($x10^6$) [cells mL^{-1}]	
[cm s^{-1}]	ALBR	BC	ALBR	BC
1	0.033	0.024	5.47	4.11
2	0.051	0.029	16.49	15.94
3	0.074	0.062	20.15	17.42
4	0.063	0.063	18.63	17.93

Table 4.2 : Influence of initial biomass concentration on net biomass productivity of *C.calcitrans* in airlift bioreactor

Interval culturing time	Concentration interval ($x10^6$) cells mL^{-1}		Specific growth rate (μ)	Net productivity		Sub-culture volume (V_h)	% harvest
	initial	final	h^{-1}	($x10^9$) cells d^{-1}	($x10^{-3}$) g d^{-1}	(L)	
0-48	0.74	20.24	0.0690	516.67 *	35.61	-	-
48-60	6.51	17.58	0.0828	390.12	26.88	10.85	68
60-72	7.37	20.55	0.0854	413.77 *	28.51	9.30	58
72-84	8.00	20.38	0.0779	370.33	25.52	9.81	61
84-96	8.45	21.45	0.0776	387.33	26.69	9.37	59
96-108	9.12	22.92	0.0768	406.93	28.04	9.20	57
108-120	9.22	23.13	0.0767	409.46 *	28.22	9.56	60
120-132	11.81	21.93	0.0515	200.28	13.80	7.83	49
132-144	10.81	23.20	0.0637	302.92	20.87	8.11	51
144-156	11.66	22.43	0.0545	225.32	15.53	7.96	50
156-168	11.16	22.65	0.0590	260.31	17.94	8.04	50
168-180	11.34	22.52	0.0572	245.54	16.92	7.99	50
180-192	11.59	22.60	0.0556	235.23	16.21	7.77	49

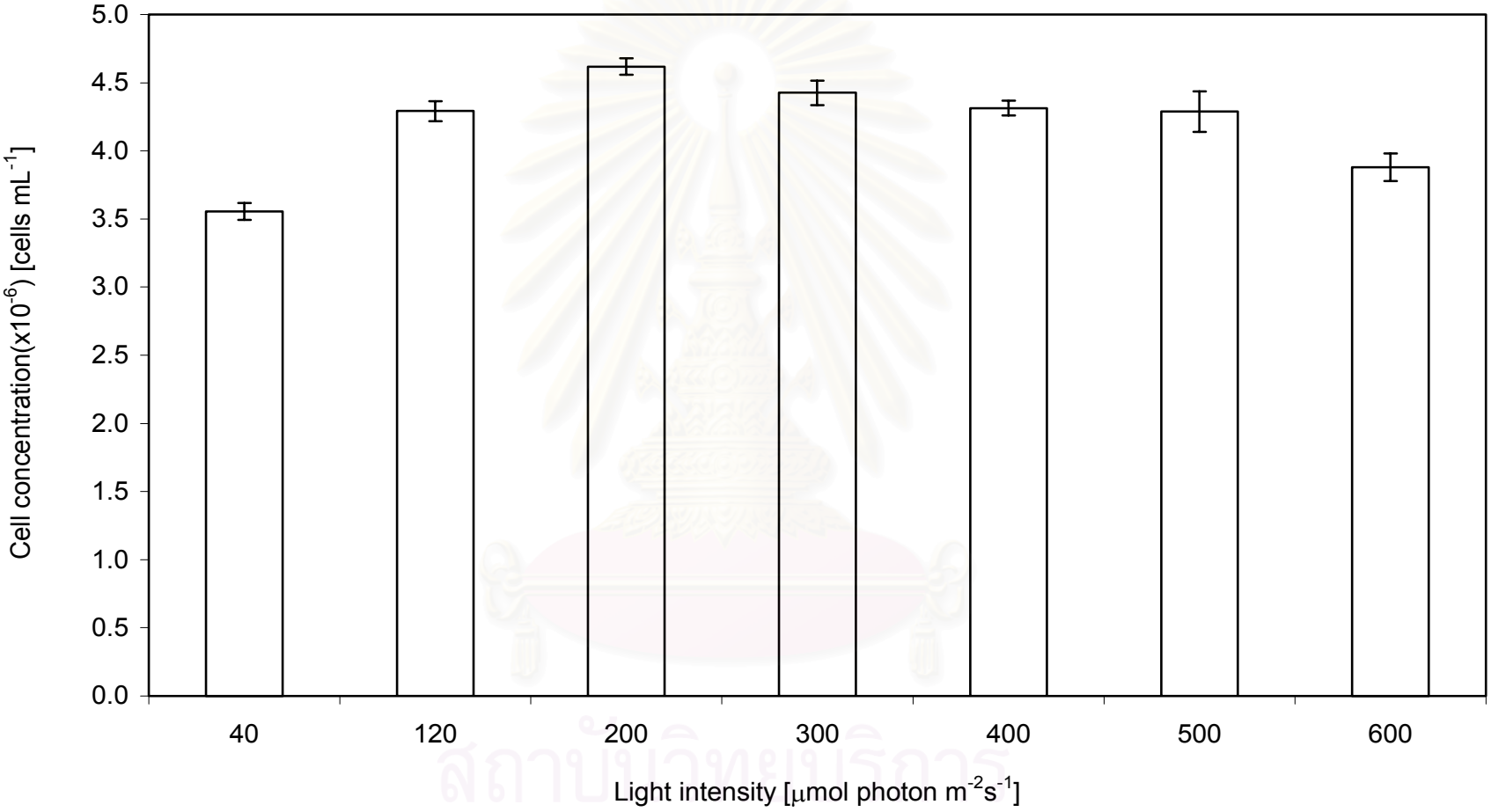


Figure 4.1.1A : Effect of light intensity on maximum cell concentration

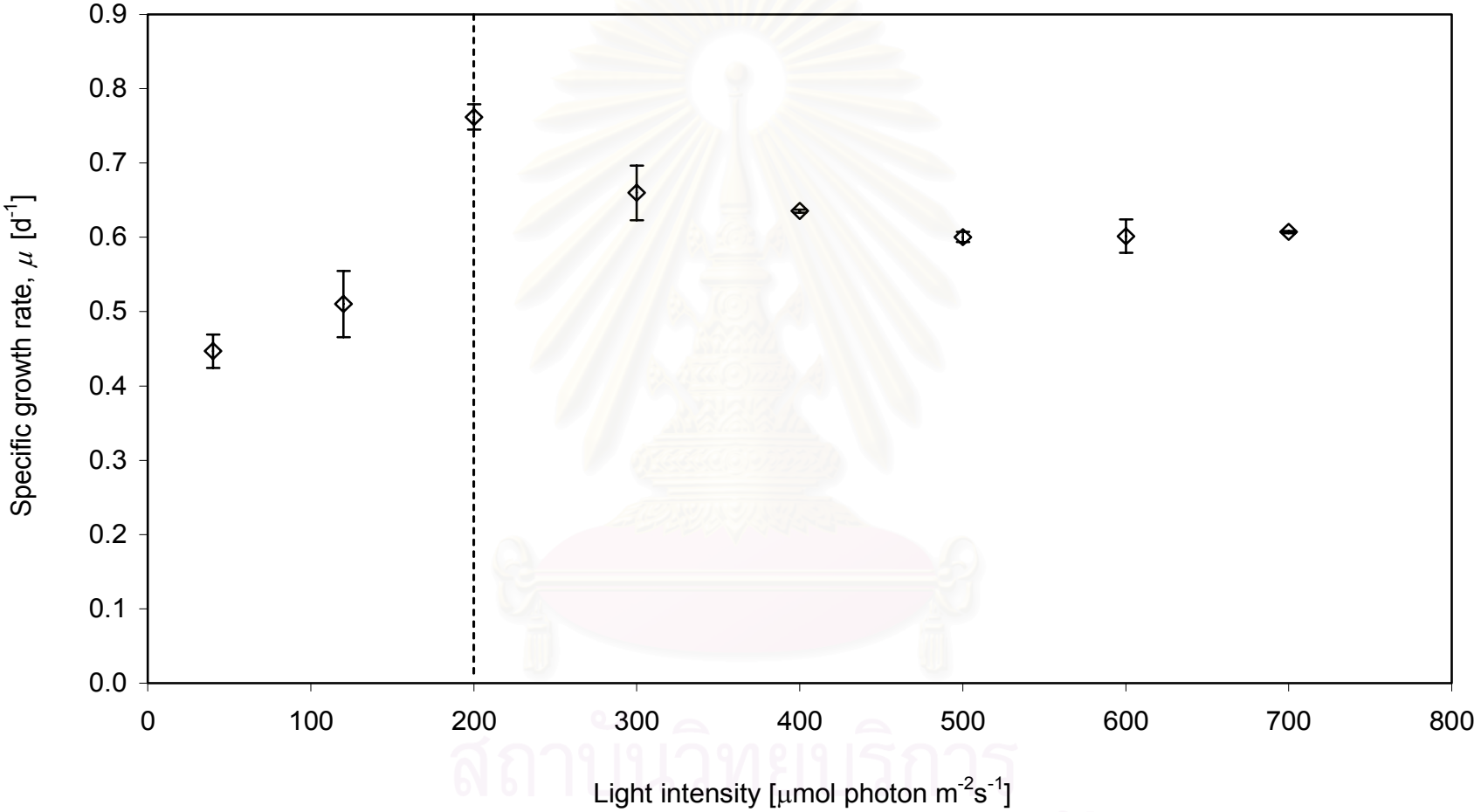


Figure 4.1.1B : Effect of light intensity on specific growth rate.

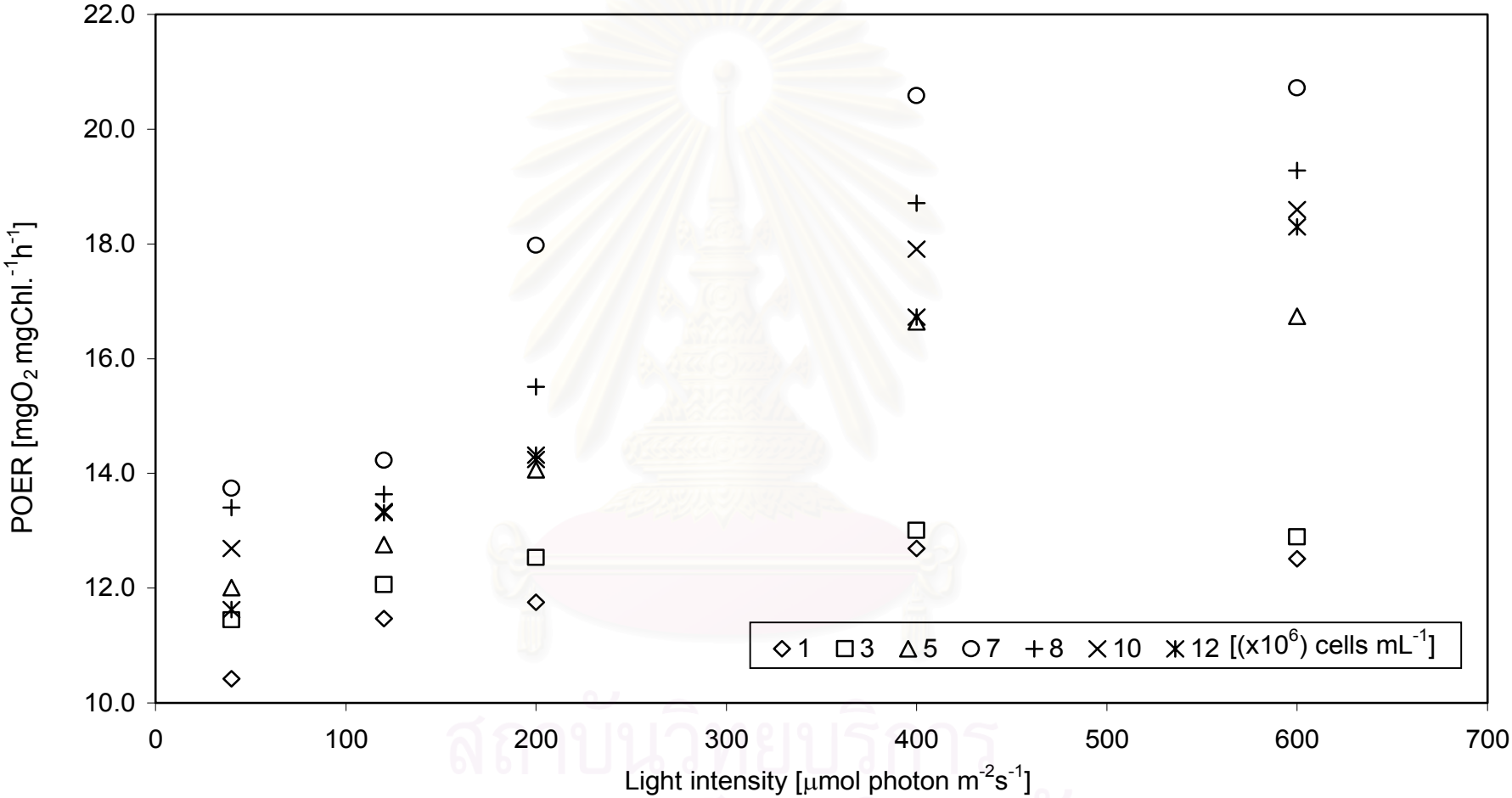


Figure 4.1.1C : Effect of light intensity on Photosynthesis Oxygen Evolution Rate (POER)

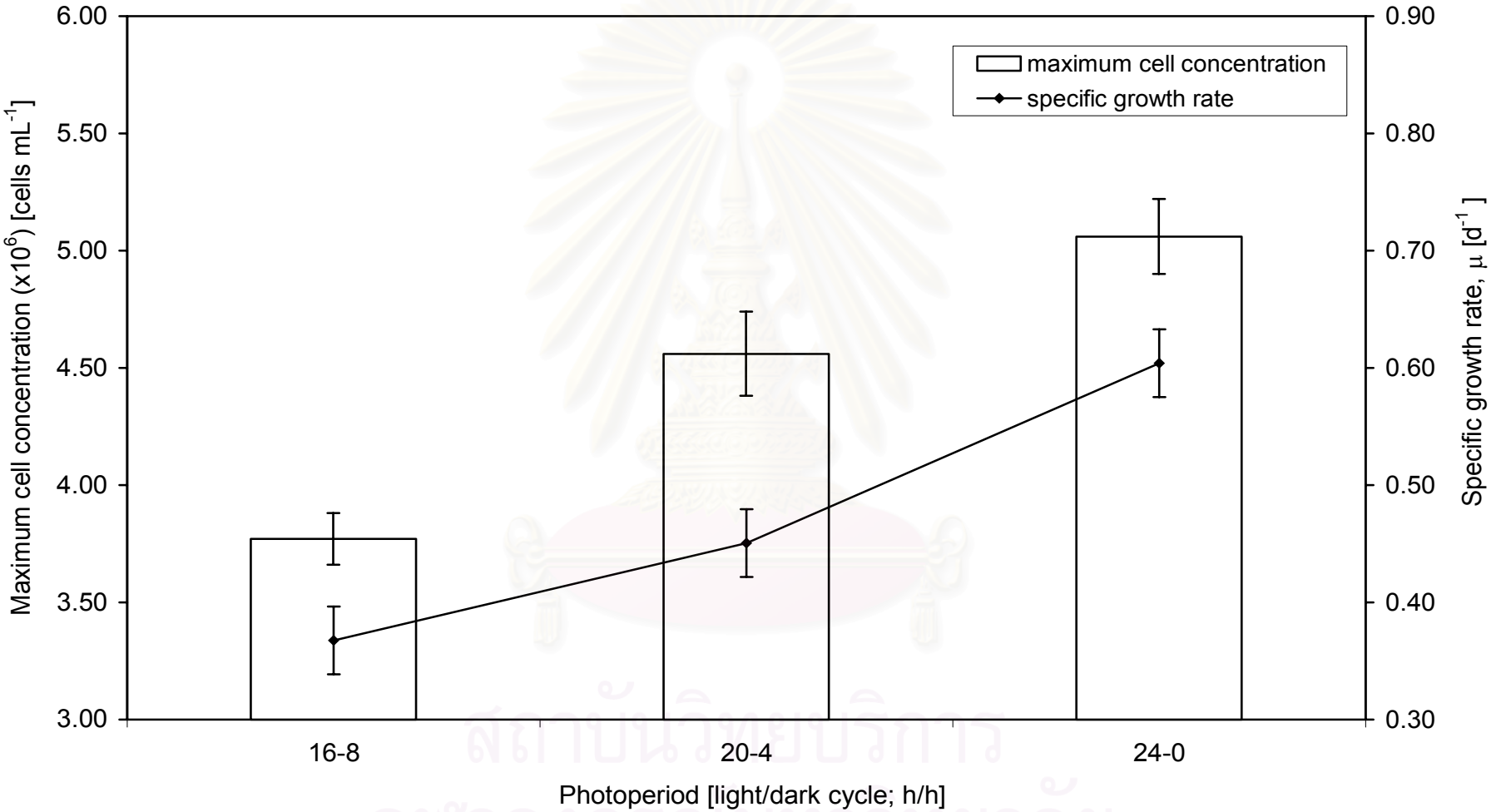


Figure 4.1.2 : Maximum cell concentration and specific growth rate at different photoperiod

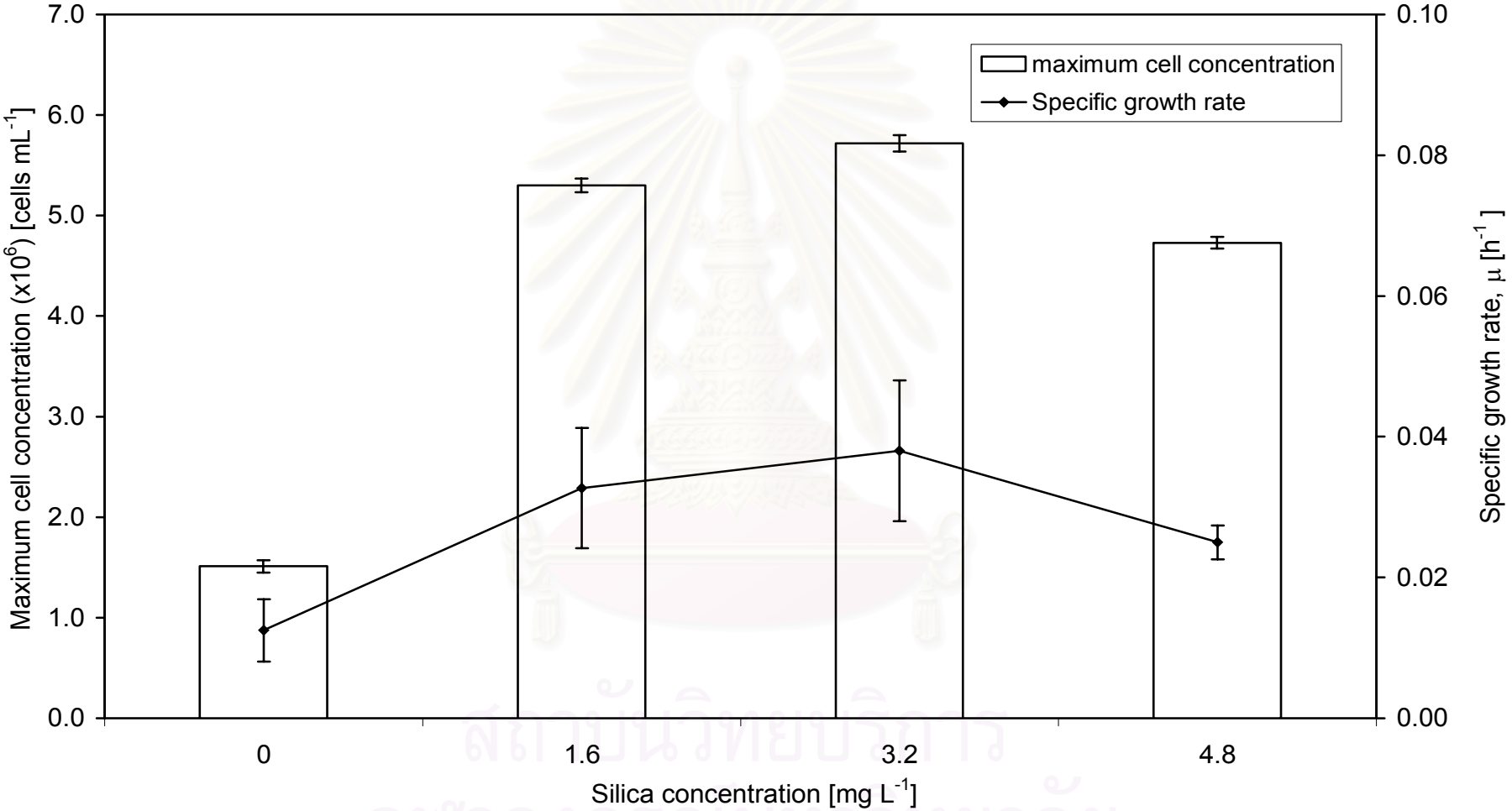


Figure 4.1.3A : Effect of silica concentration

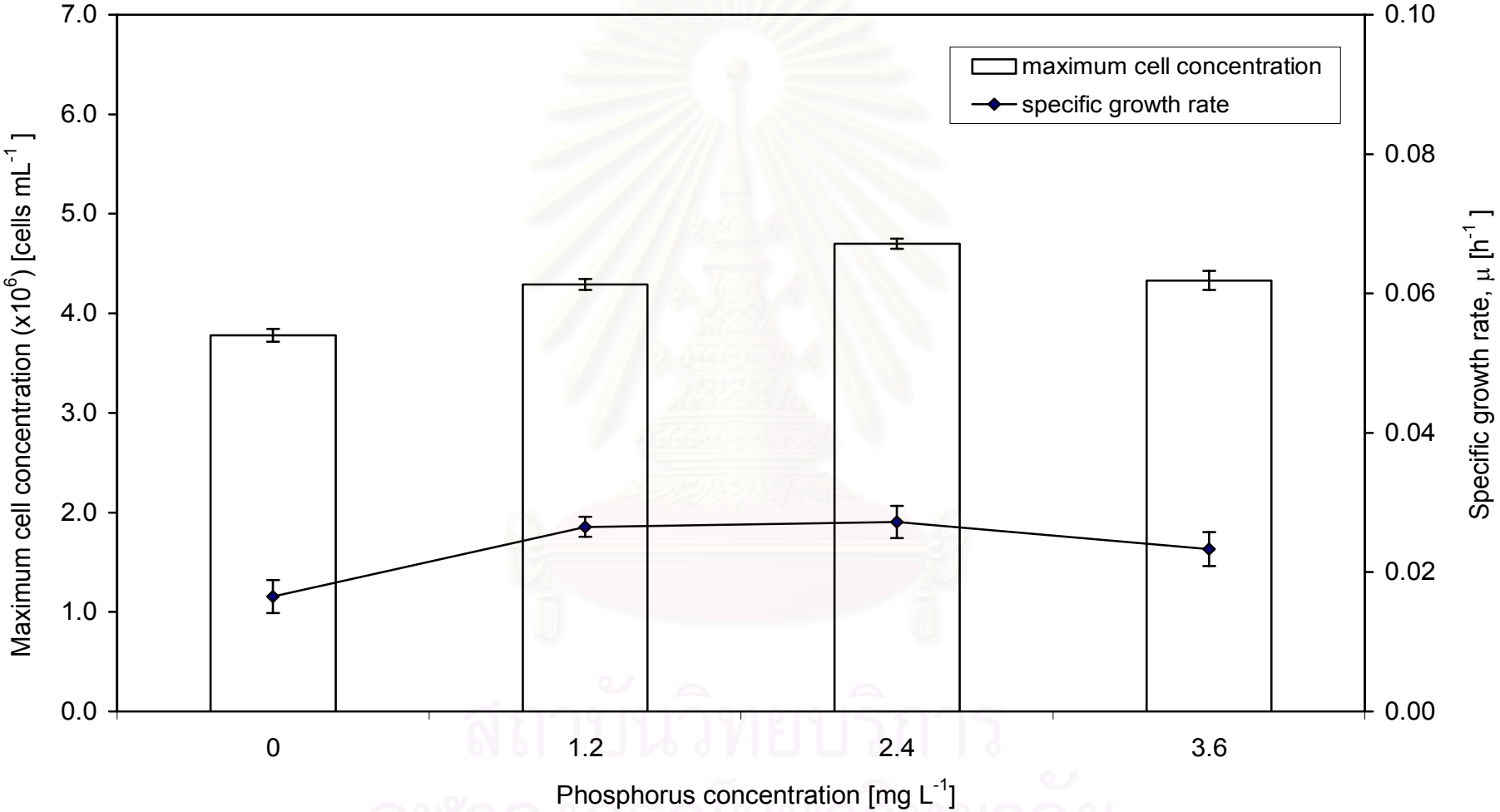


Figure 4.1.3B : Effect of phosphorus concentration

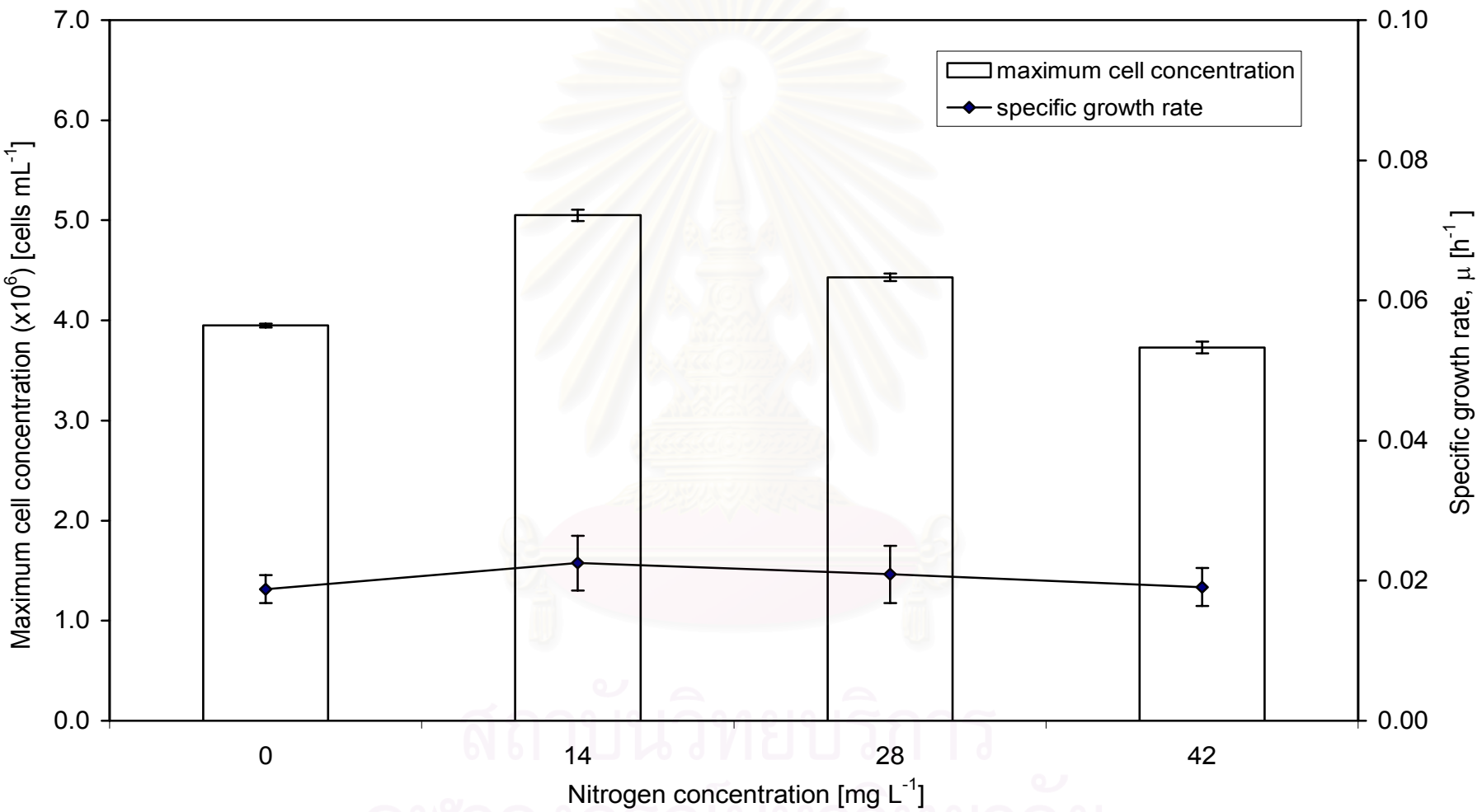


Figure 4.1.3C : Effect of nitrogen concentration

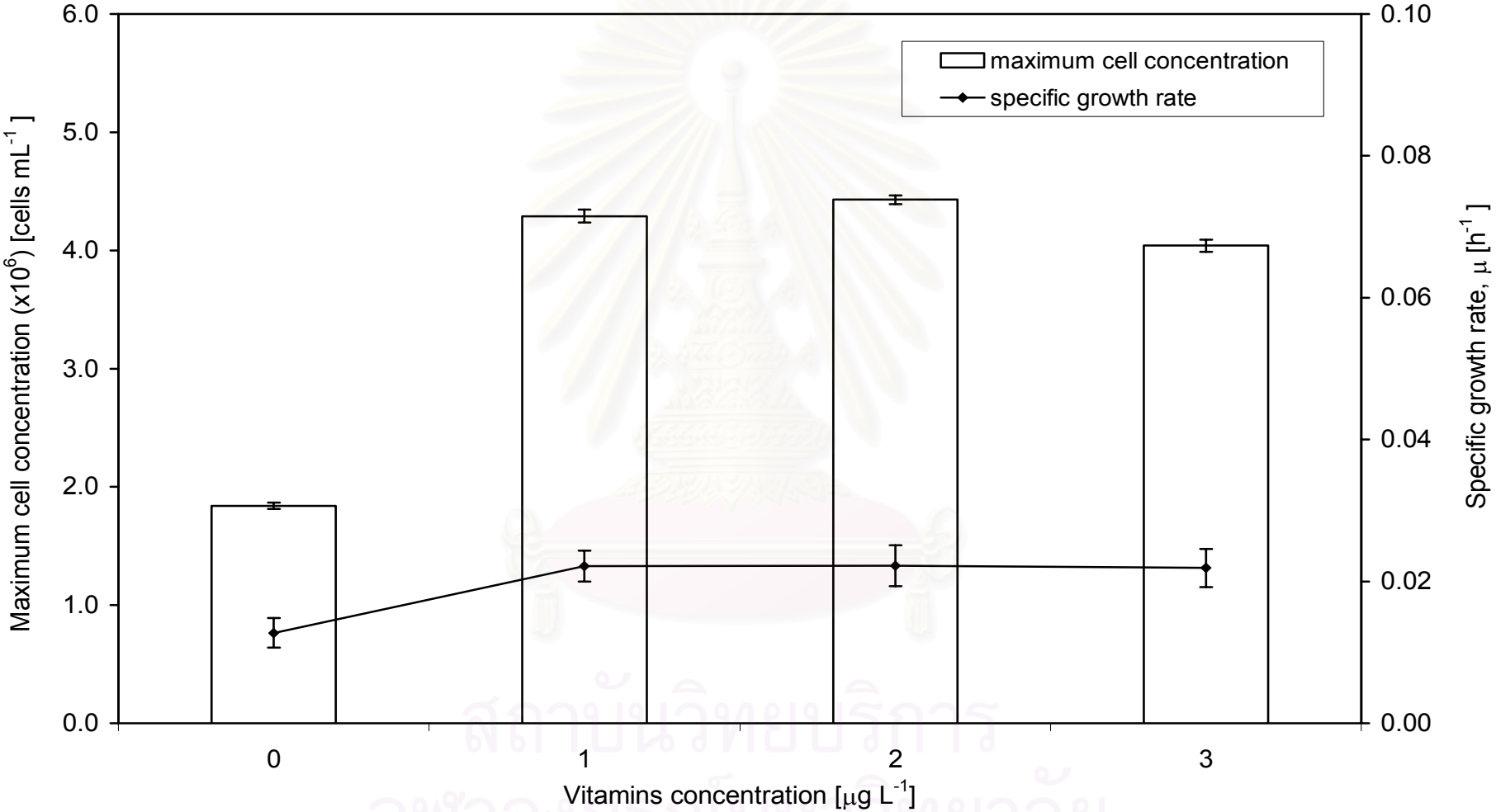


Figure 4.1.3D : Effect of vitamins concentration

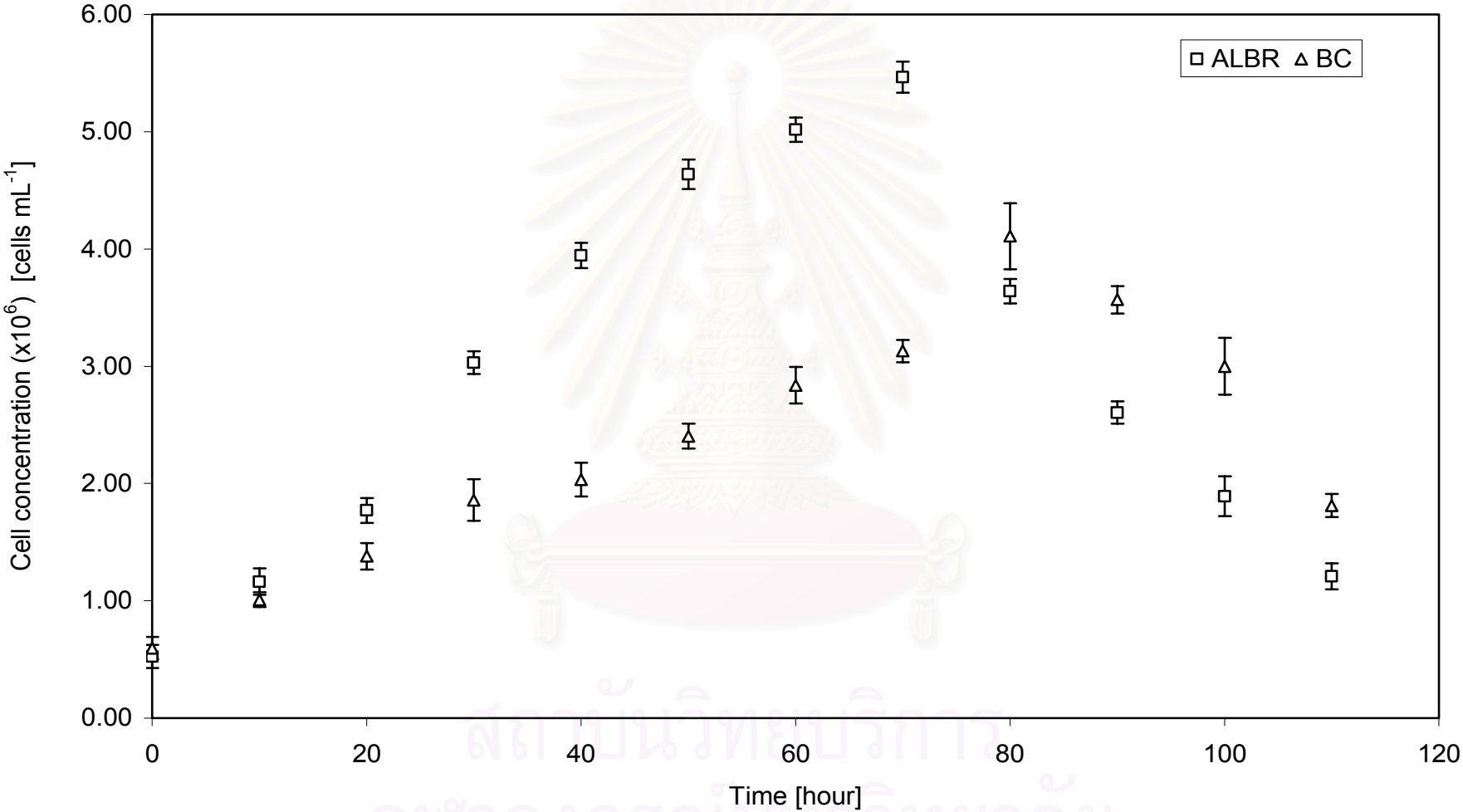


Figure 4.2.1 : Comparing growth curve of C.calcitrans in ALBR and BC at aeration rate of 1 cm s^{-1}

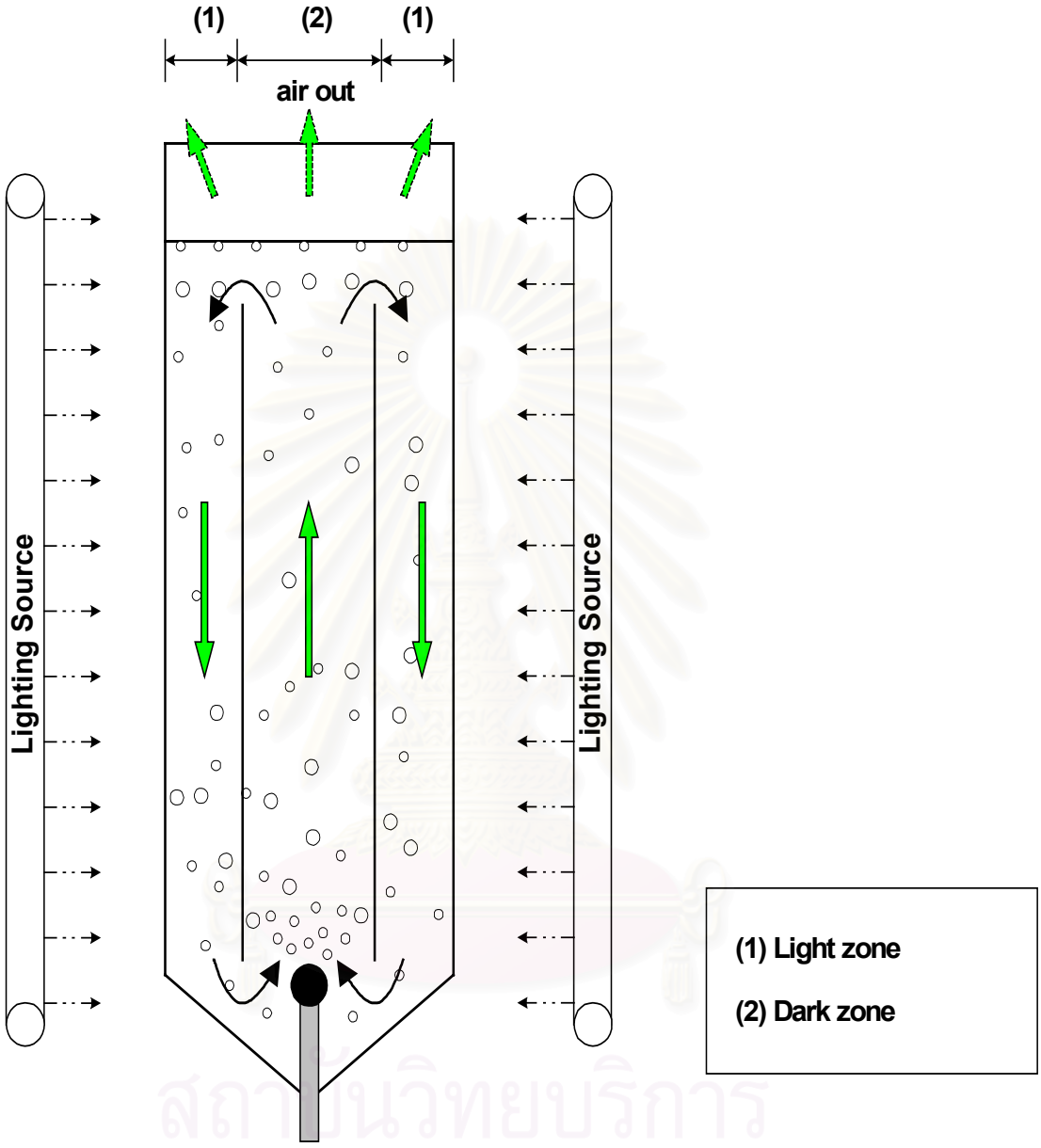


Figure 4.2.2A : Characterize flow pattern of ALBR

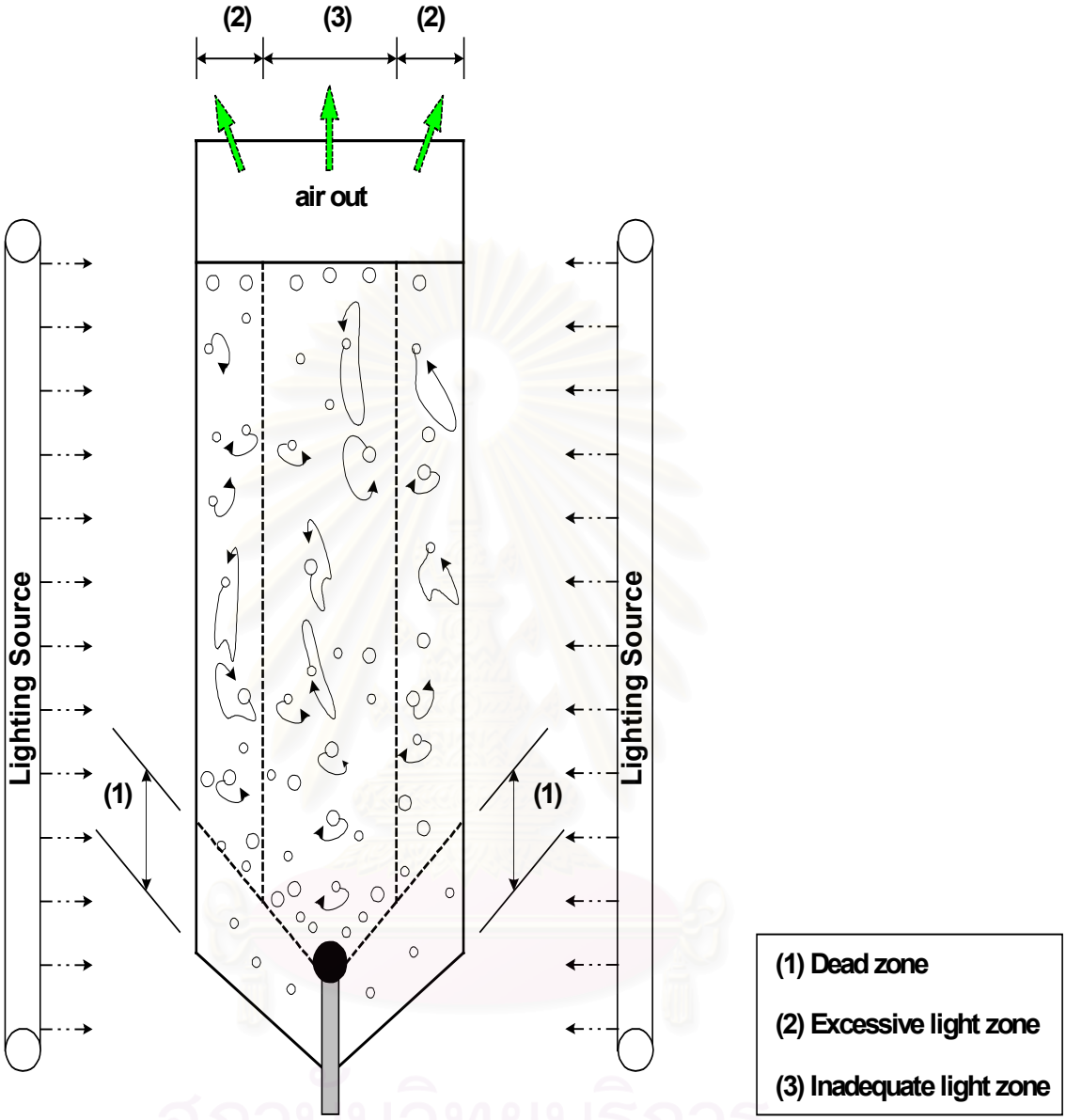


Figure 4.2.2B : Characterize flow pattern of BC

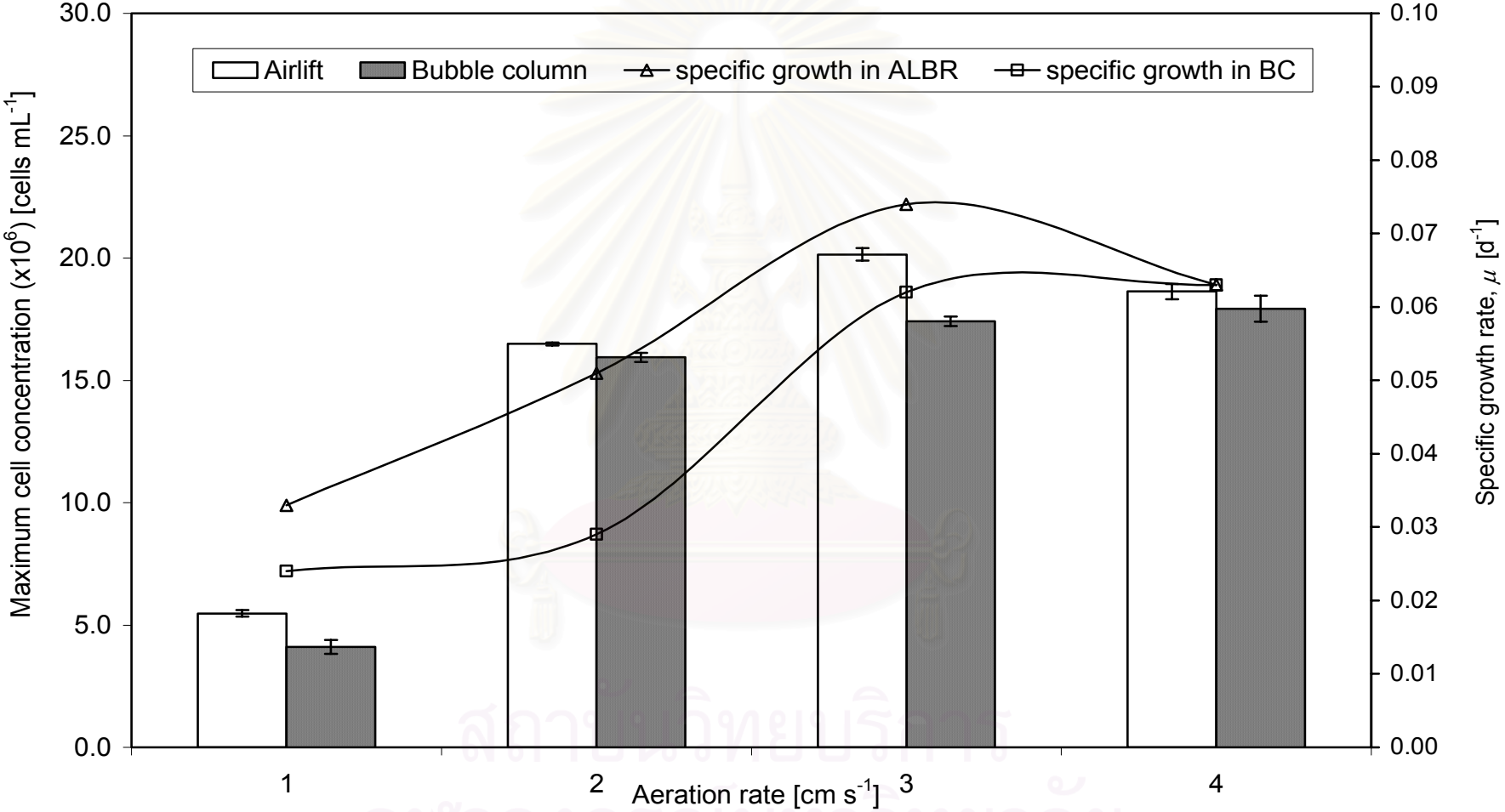


Figure 4.2.3 : Effect of aeration rate in bioreactors

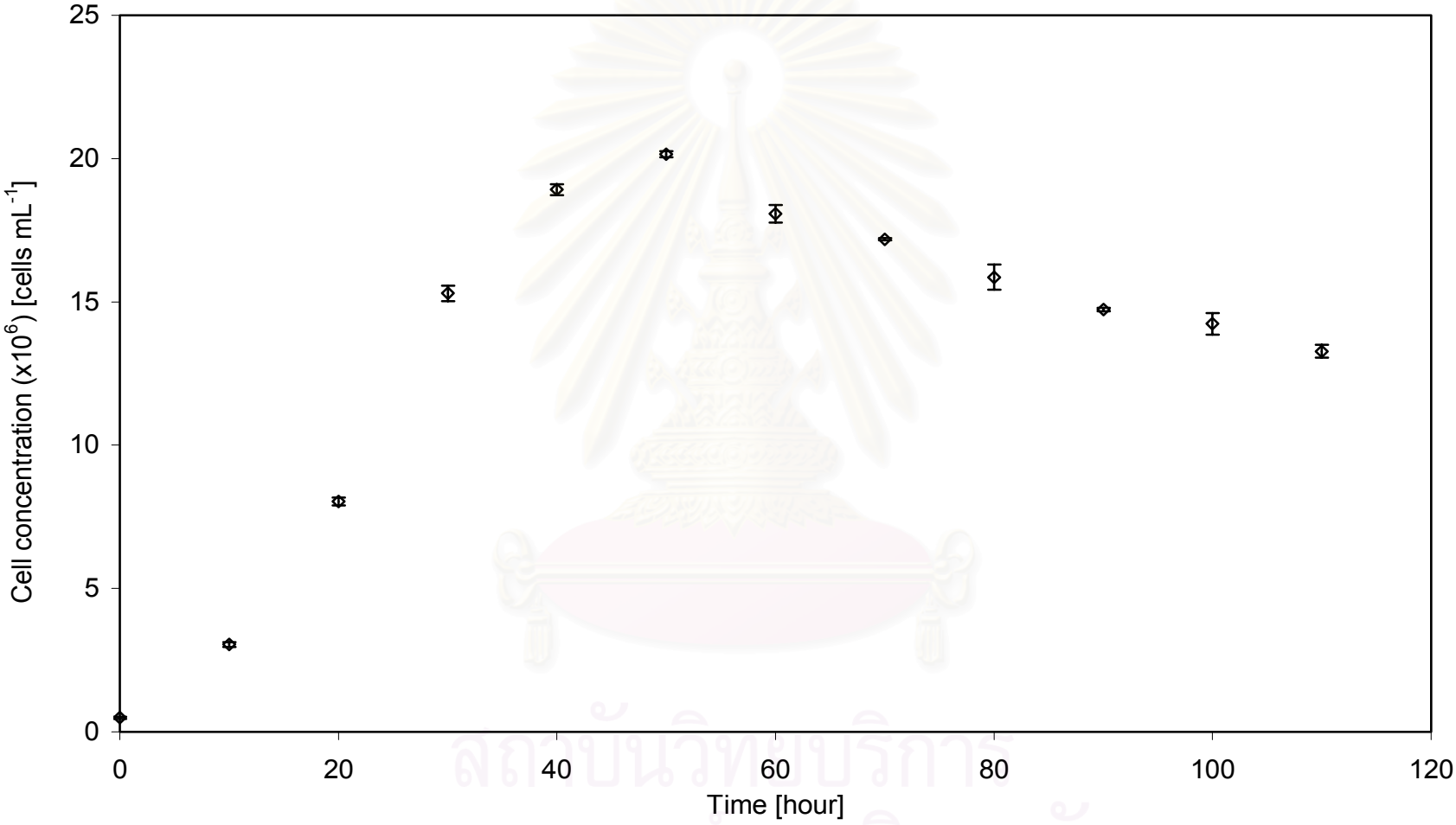


Figure 4.2.4 : Growth Curve of *C.calcitrans* in ALBR at 3 cm s^{-1}

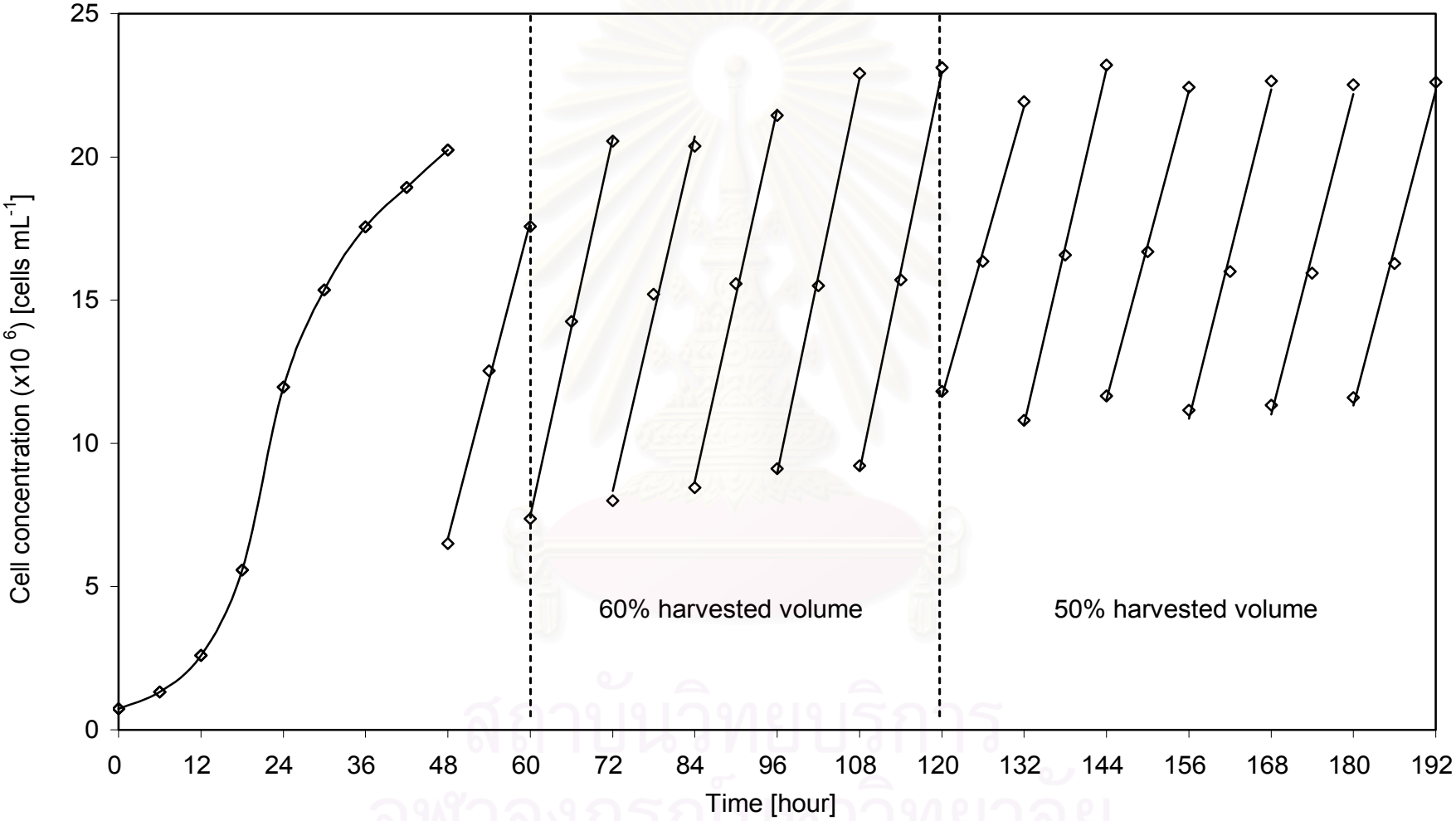


Figure 4.2.5 : Time variation of biomass concentration

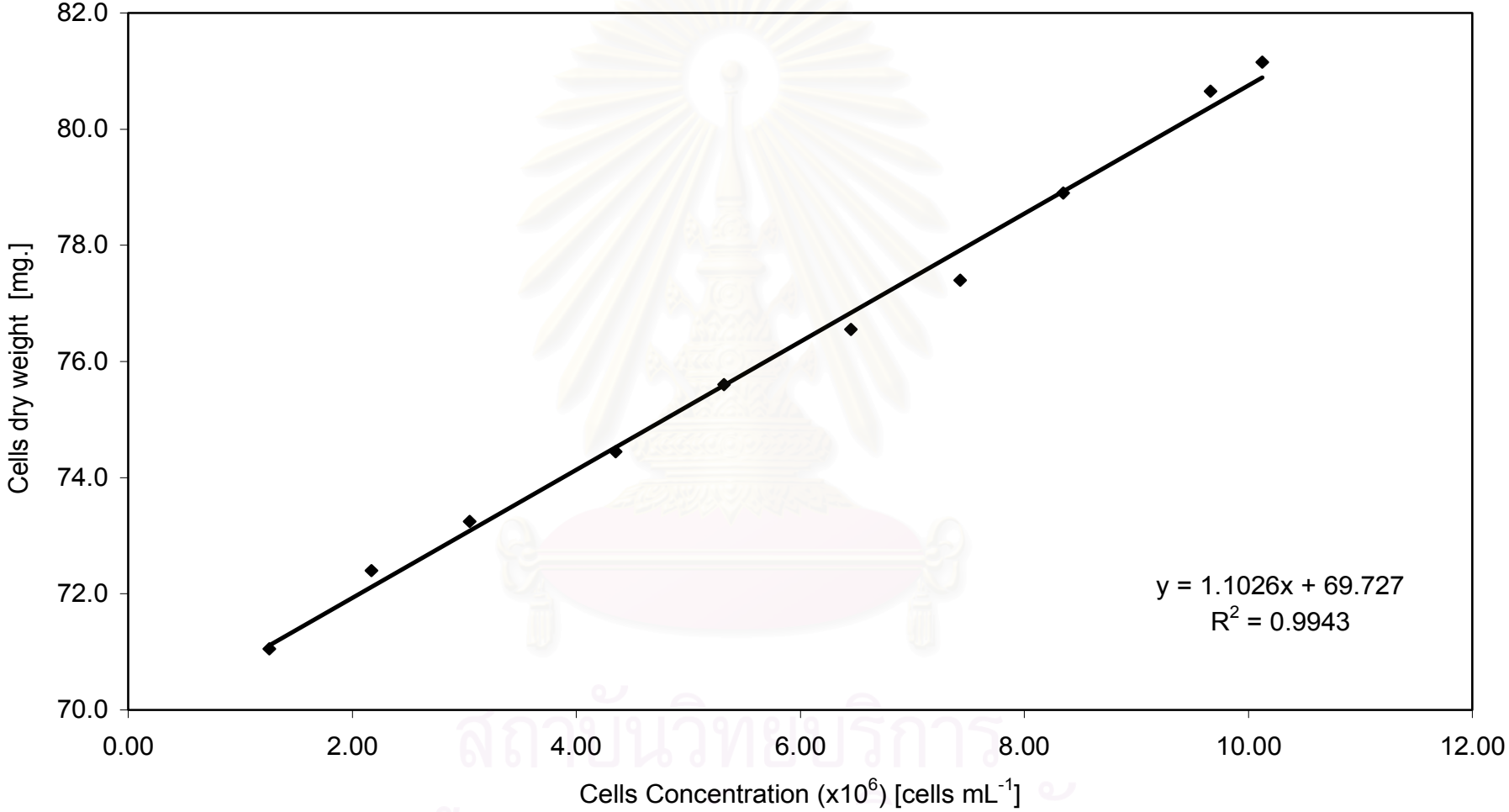


Figure 4.2.6 : Calibration curve of cell concentration versus cell dry weight

CHAPTER 5

Conclusions and Recommendations

5.1 Conclusions

The optimal condition requirements for the growth of a diatom *Chaetoceros calcitrans* were investigated in this work and summarized below:

Parameter	Optimum
Light intensity	200 μmol photon $m^{-2}s^{-1}$.
Photoperiod (LD cycle)	24:0
Nutrient concentration	Standard F/2 (Guillard's) medium with 2 fold silica and phosphorus concentrations

In addition, this work revealed that the cultivation of *C.calcitrans* in the ALBR was superior to the BC. This was due primarily to the well defined flow pattern in the ALBR which led to a more effective light utilization of the algae. The optimal aeration rate of 3 cm s^{-1} was found to yield the specific growth rate of 0.074 h^{-1} with a maximum cell concentration of $20.15x10^6$ cells mL^{-1} in batch culture system. The results obtained show that the ALBR system in this work was more efficient than those reported in other works.

A long term semi-continuous operation and optimization of the sub-culture operation was successfully determined from the experiment in this work. In this case, the maximum specific growth rate (μ) achievable was 0.0854 h^{-1}or $4.138x10^{11}$ cells d^{-1}, and the harvest of cell should be performed at every 12 hours of operation (at the aeration velocity of 3 cm s^{-1}).

5.2 Contributions

The major contribution of this research is twofold. Firstly, it shows significant data on the optimal growth conditions for the diatom *Chaetoceros calcitrans*. This diatom is widely used as a feed in aquaculture industry particularly prawn larvae cultivation and therefore the optimization of the growth condition of this diatom is important for the effective mass production. In addition, data from this research is significant as a starting point for the research in the up-scale of the culture of this diatom species.

The experiment reported here is also one of the first experiences in the culture of *C.calcitrans* in the airlift bioreactor and constitutes the initial step in testing the potential of this strain for mass production. Diatom *C.calcitrans* has demonstrated suitable growth in batch culture of ALBR, for with the net specific growth rate of 0.074 h^{-1} in the exponential growth phase at an aeration velocity of 3 cm s^{-1}. In addition, semi-continuous mode has been successfully implemented where a high cells density of *C.calcitrans* can be obtained.

5.3 Recommendations

There are still other aspects of research that should be conducted to enhance the basic knowledge in the cultivation of *C.calcitrans* and also to complete the fundamentals on the airlift bioreactor design. These are:

1. the influence of CO_2 on the growth of this diatom;
2. maximum cell concentration for desire light intensity
3. the effect of design parameters for the airlift bioreactor system (such as the ratio between the downcomer and riser cross sectional area) on the culture of *C.calcitrans*;
4. the upscale of the ALBR; and
5. the usage of ALBR in the diatom *C.calcitrans* outdoor culture.

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APPENDICES

Appendix A : Experimental data on effect of light intensity

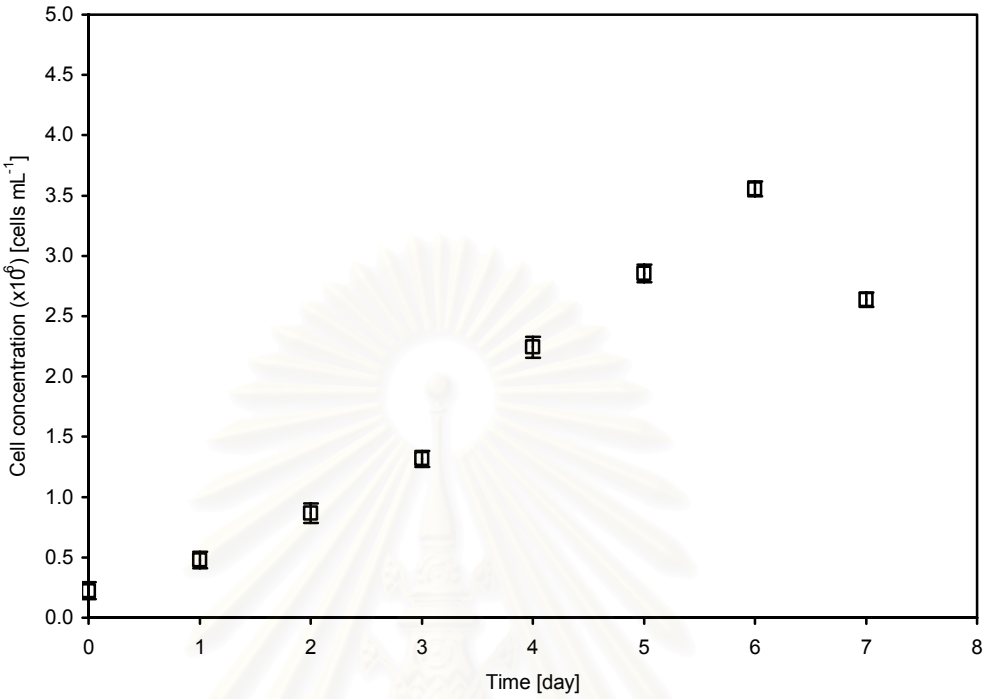

Figure A-1 : Effect of light intensity at 40 μmol photon $m^{-2}s^{-1}$ on cell concentration

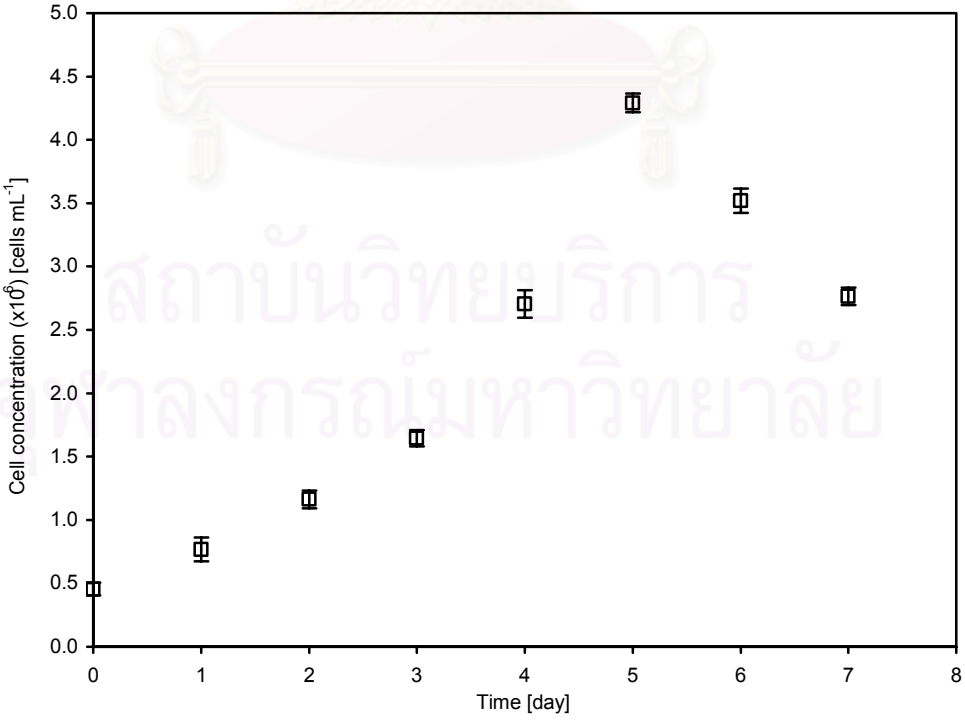

Figure A-2 : Effect of light intensity at 120 μmol photon $m^{-2}s^{-1}$ on cell concentration

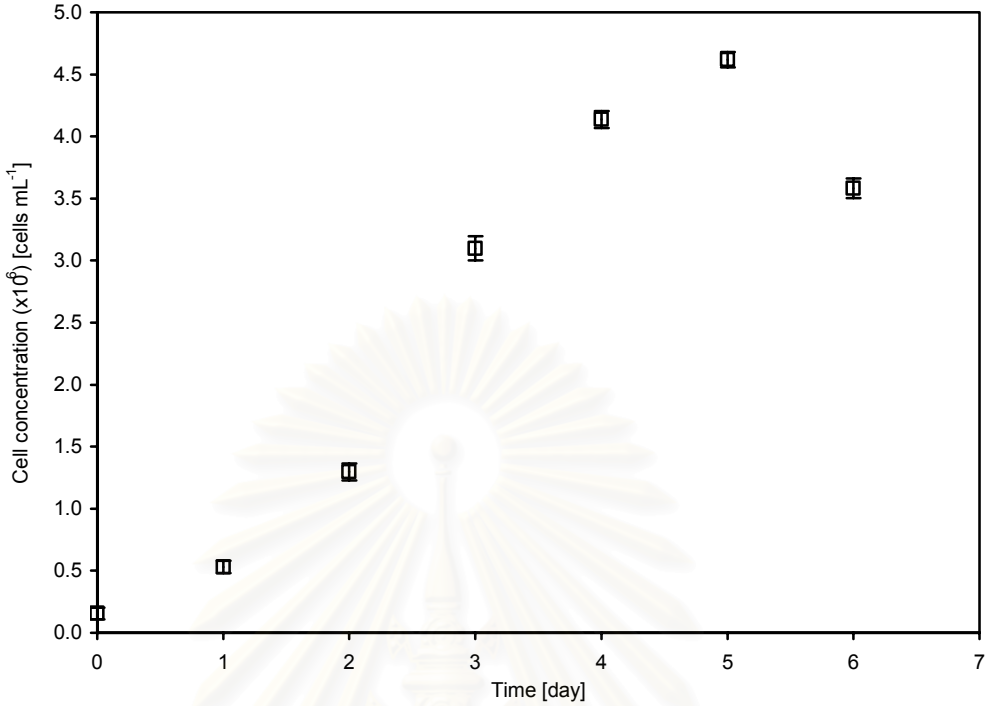

Figure A-3 : Effect of light intensity at 200 μmol photon $m^{-2}s^{-1}$ on cell concentration

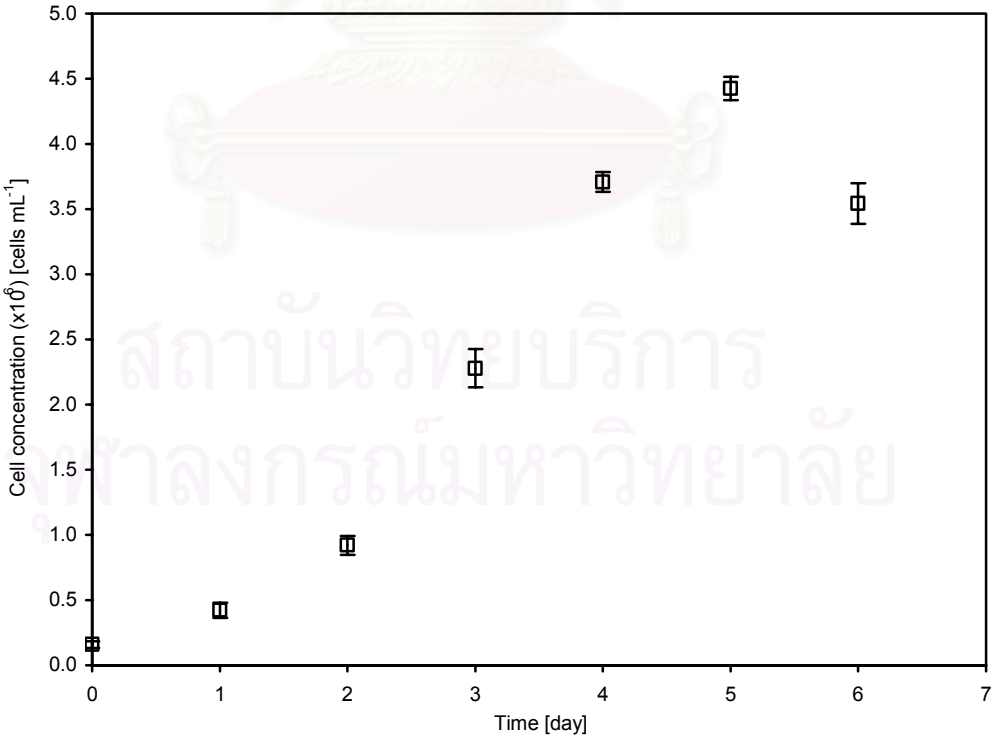

Figure A-4 : Effect of light intensity at 300 μmol photon $m^{-2}s^{-1}$ on cell concentration

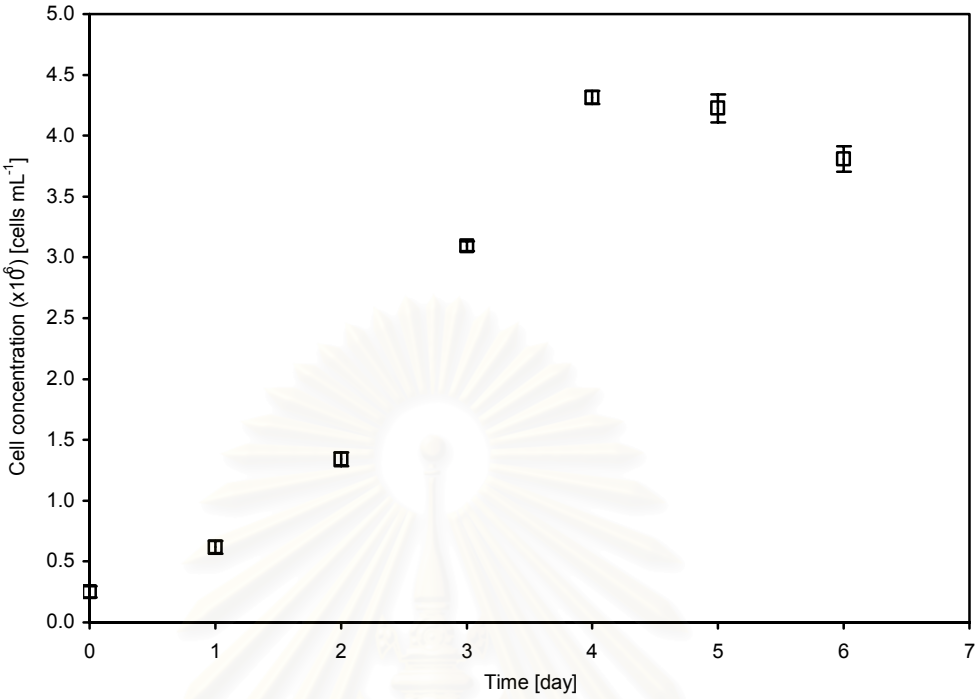

Figure A-5 : Effect of light intensity at 400 μmol photon $m^{-2}s^{-1}$ on cell concentration

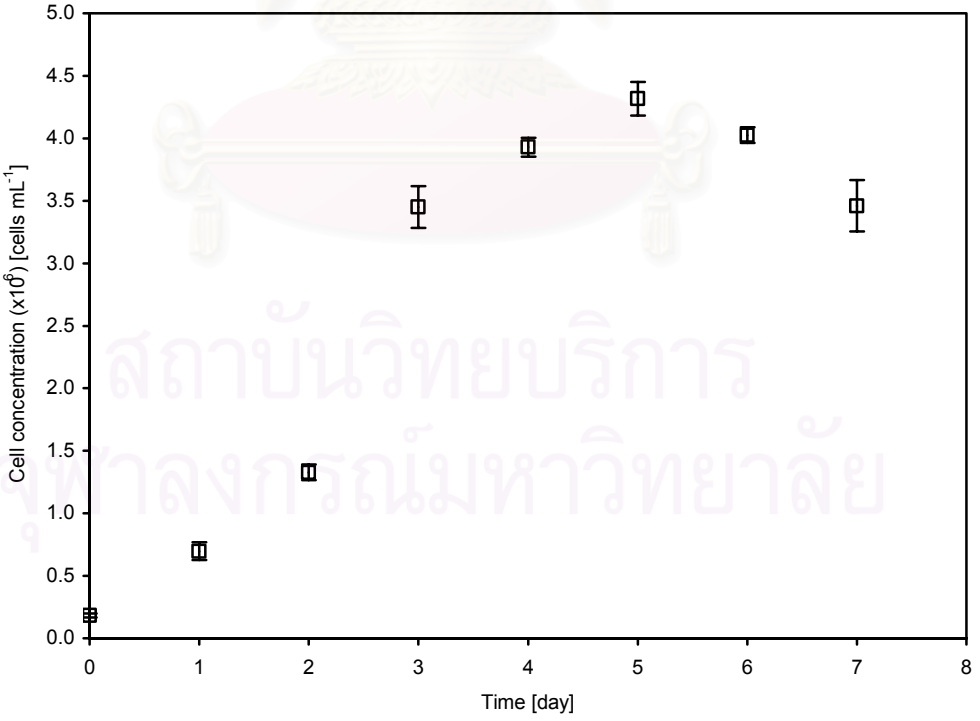

Figure A-6 : Effect of light intensity at 500 μmol photon $m^{-2}s^{-1}$ on cell concentration

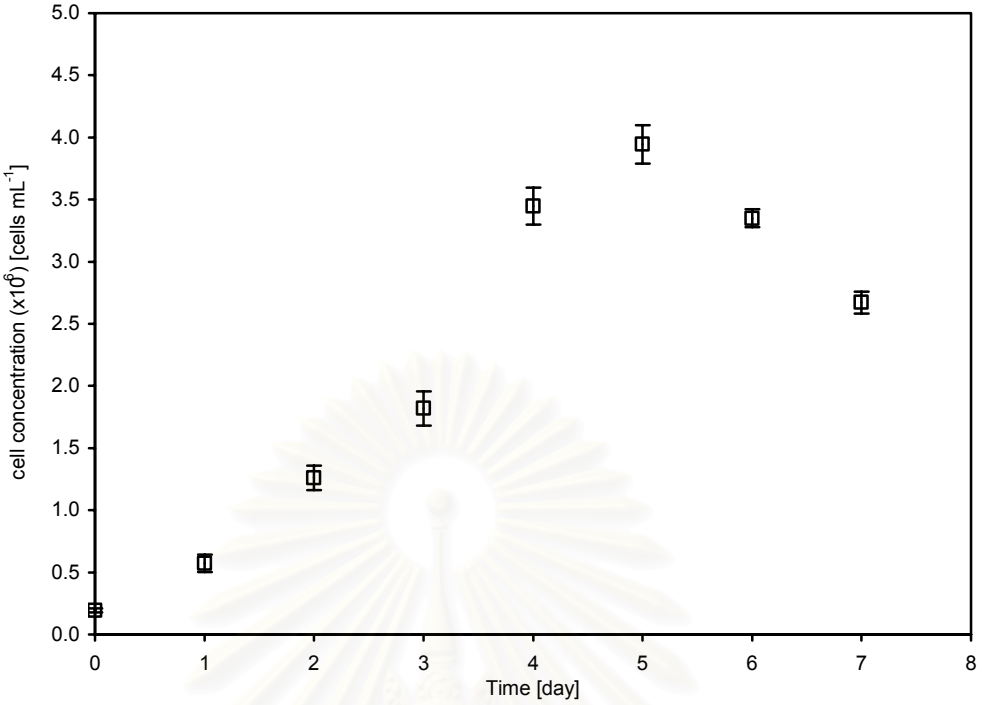

Figure A-7 : Effect of light intensity at 600 μmol photon $m^{-2}s^{-1}$ on cell concentration

Appendix B : Experimental data on POER versus light intensity

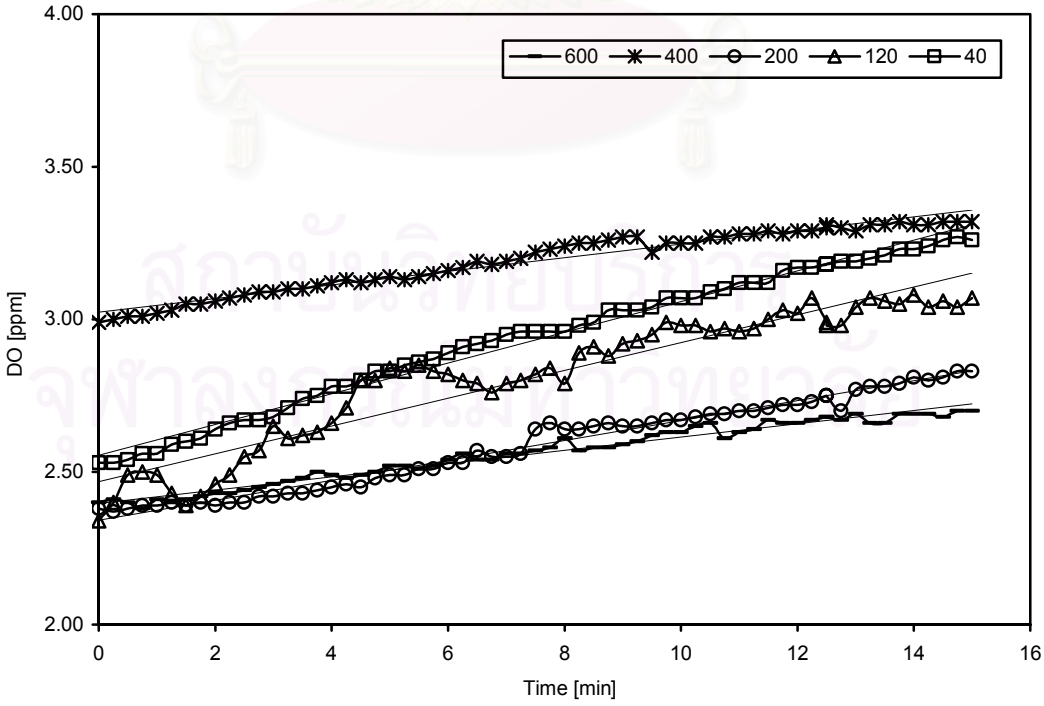

Figure B-1 : Dissolved oxygen variation on light intensity at $1x10^6$ cells mL^{-1}

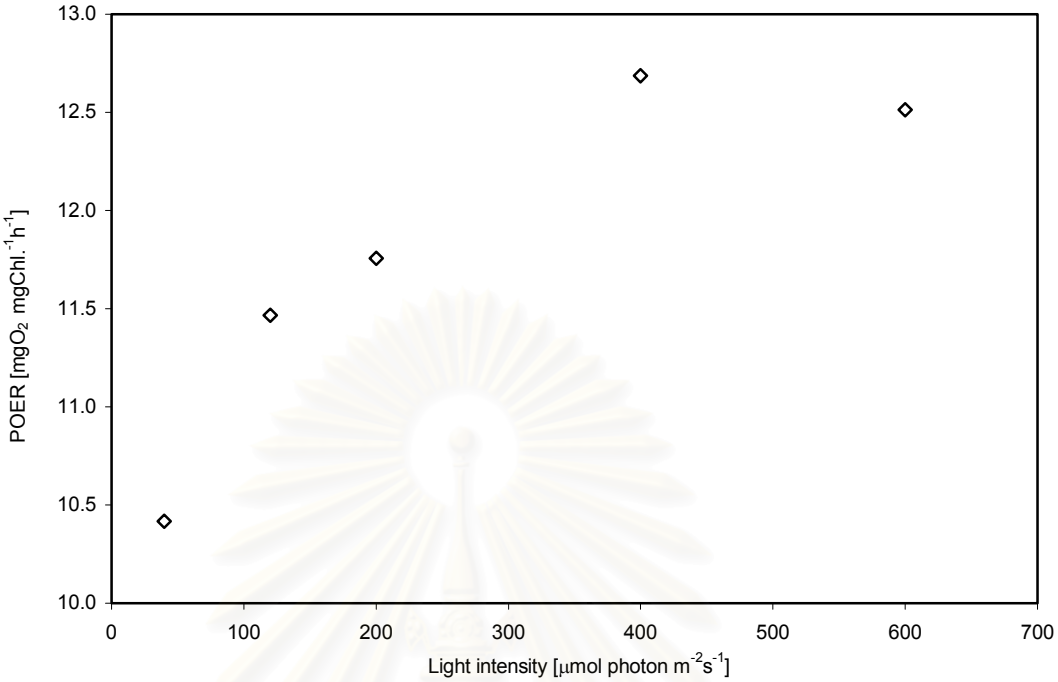

Figure B-2 : Effect of light intensity on POER at $1x10^6$ cells mL^{-1}

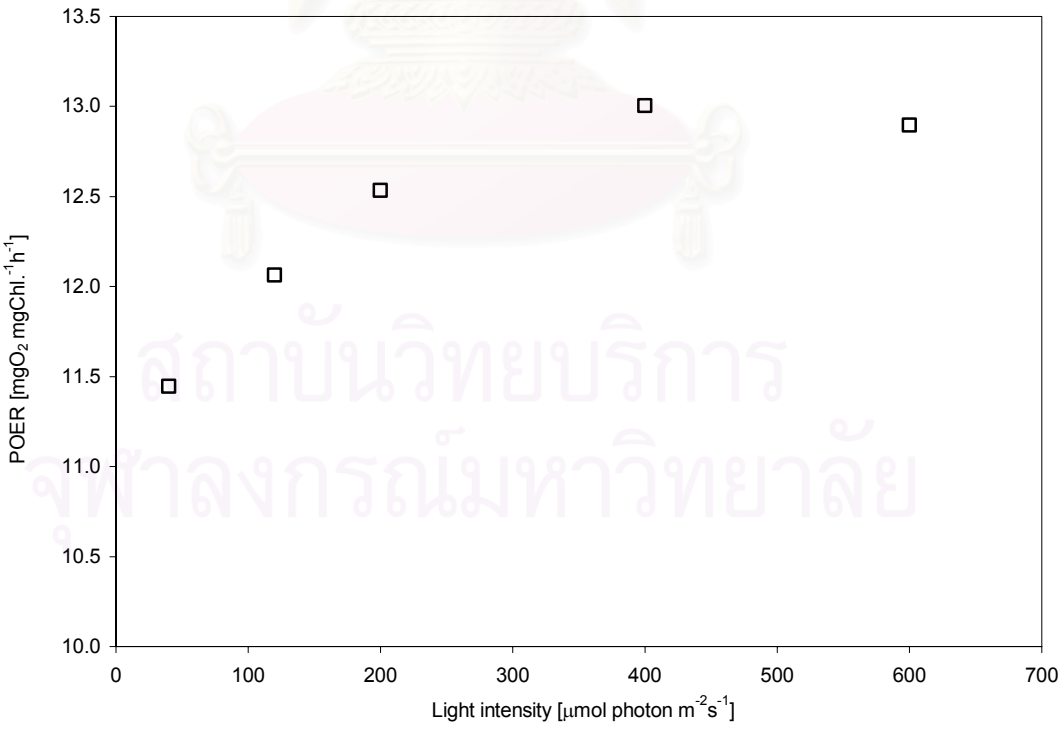

Figure B-3 : Effect of light intensity on POER at $3x10^6$ cells mL^{-1}

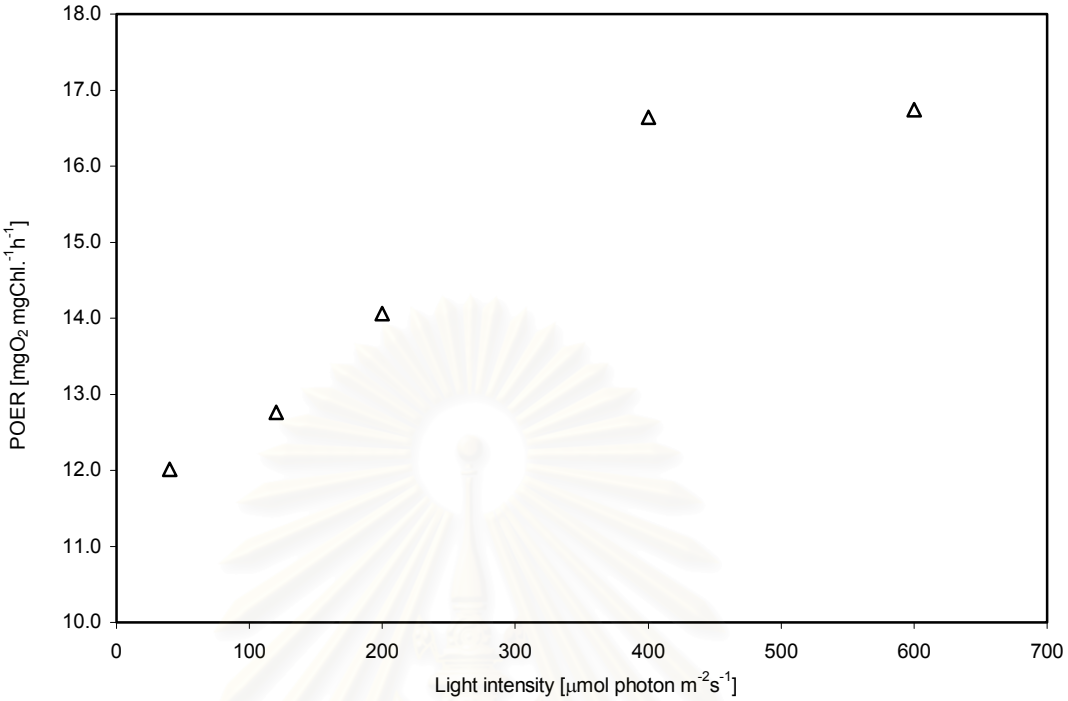

Figure B-4 : Effect of light intensity on POER at $5x10^6$ cells mL^{-1}

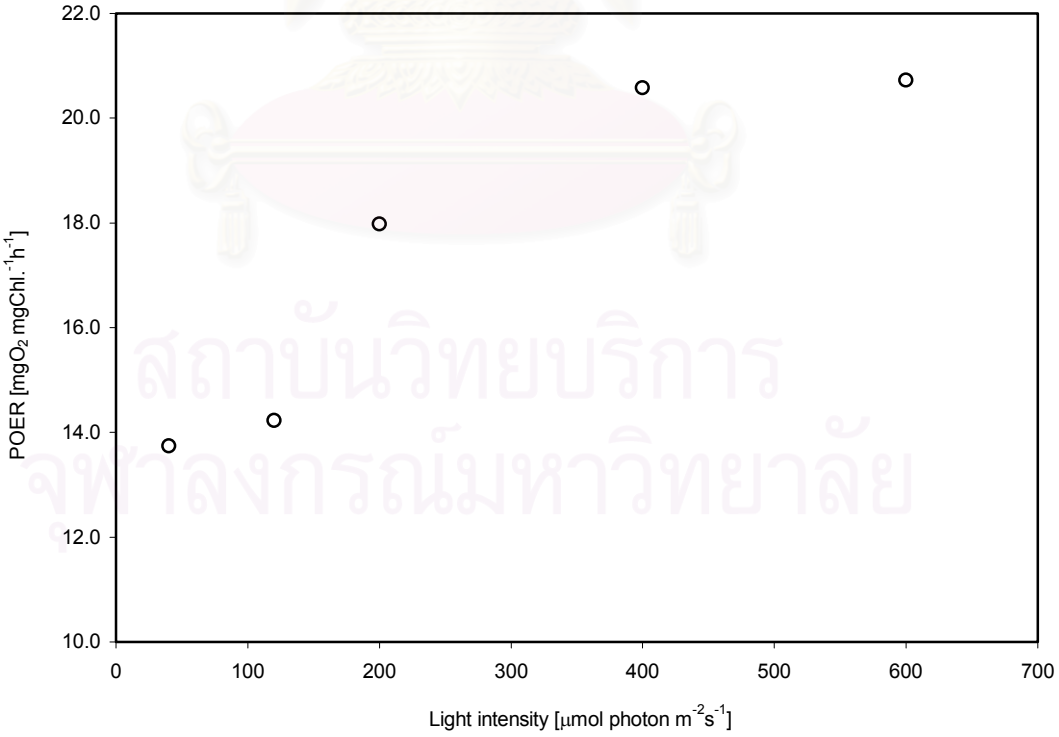

Figure B-5 : Effect of light intensity on POER at $7x10^6$ cells mL^{-1}

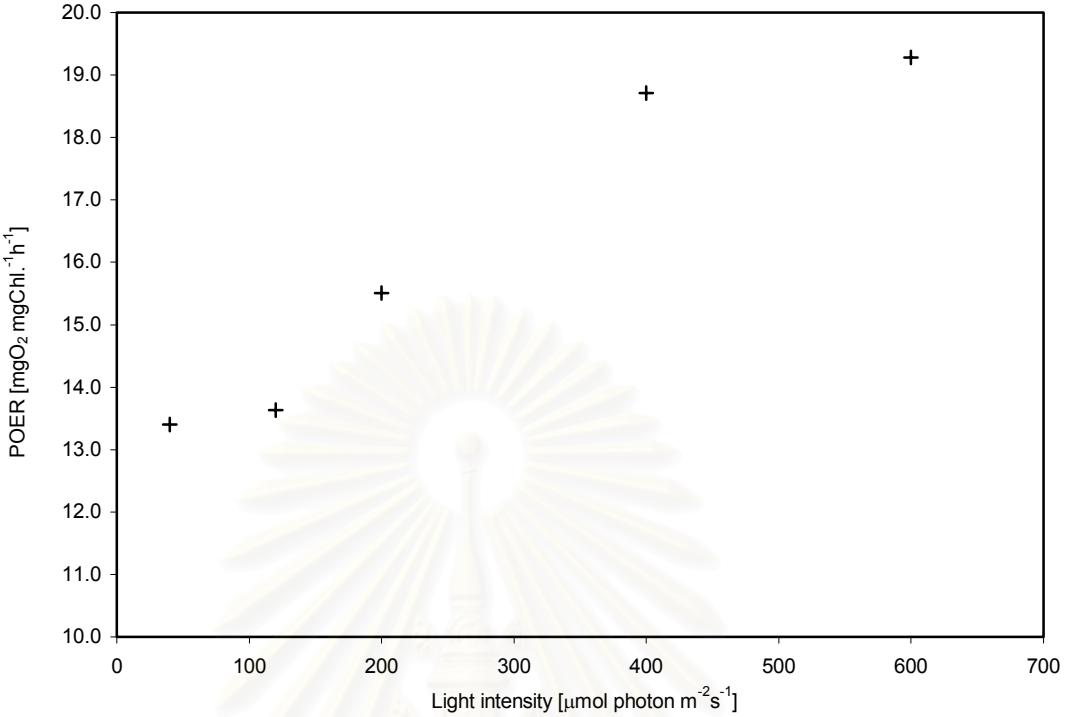

Figure B-6 : Effect of light intensity on POER at $8x10^6$ cells mL^{-1}

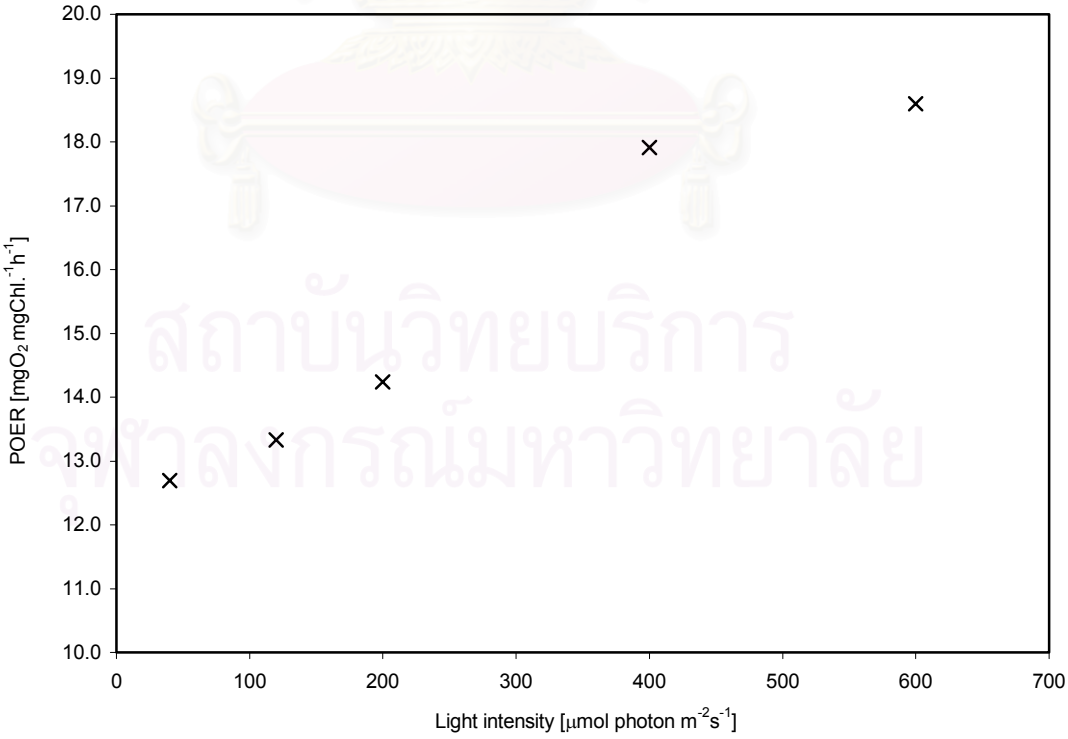

Figure B-7 : Effect of light intensity on POER at $10x10^6$ cells mL^{-1}

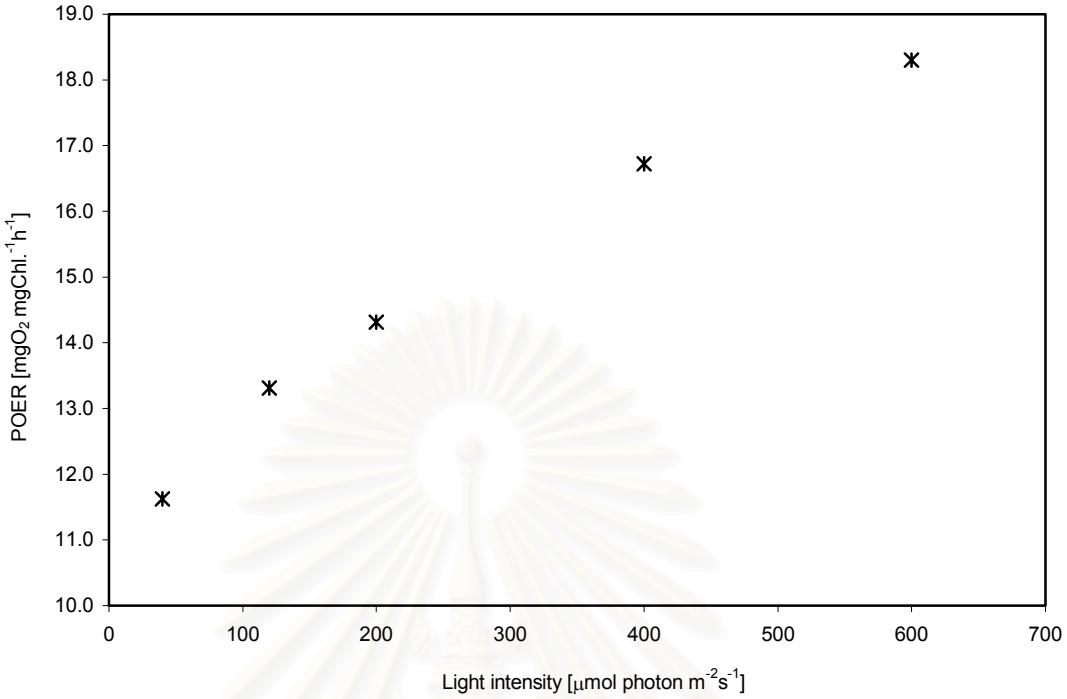

Figure B-8 : Effect of light intensity on POER at $12x10^6$ cells mL^{-1}

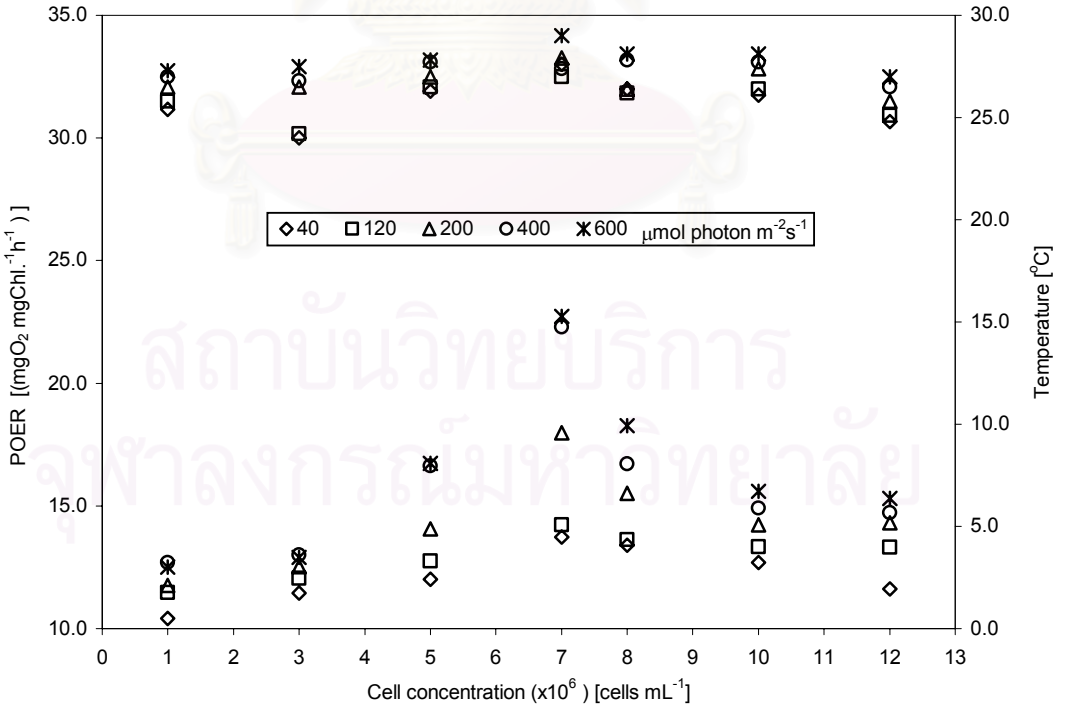

Figure B-9 : Cell concentrate versus POER and temperature at variation light intensity

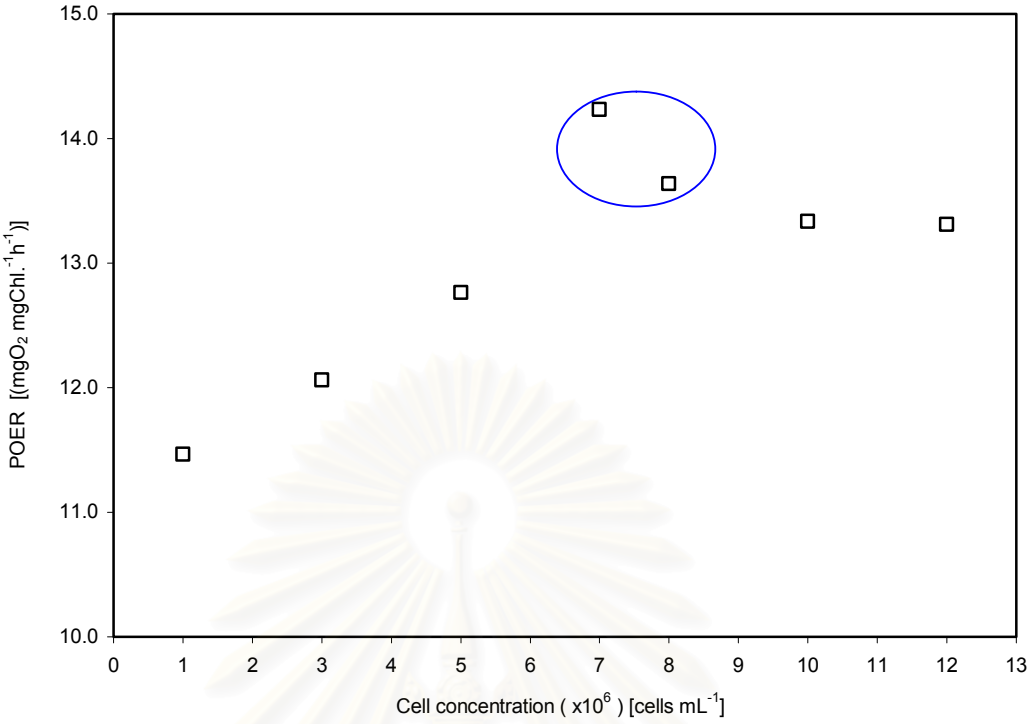

Figure B-10 : Cell concentrate variation on POER at 120 μmol photon $m^{-2}s^{-1}$

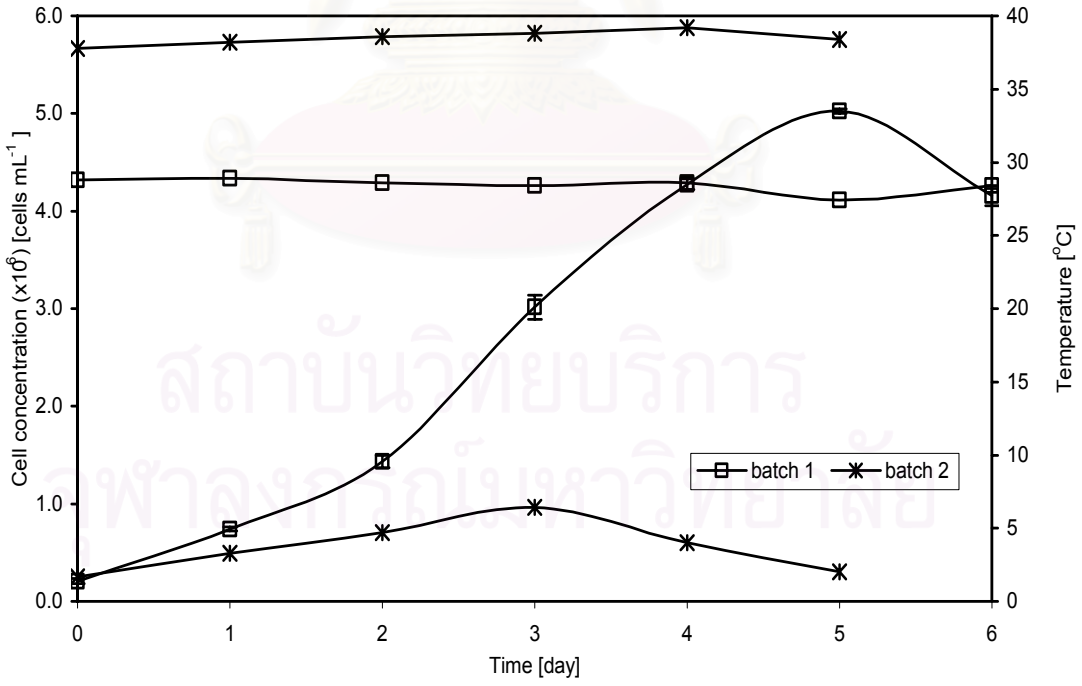

Figure B-11: Growth curve variation on culture temperature at 400 μmol photon $m^{-2}s^{-1}$

Appendix C : Experimental data on effect of photoperiod

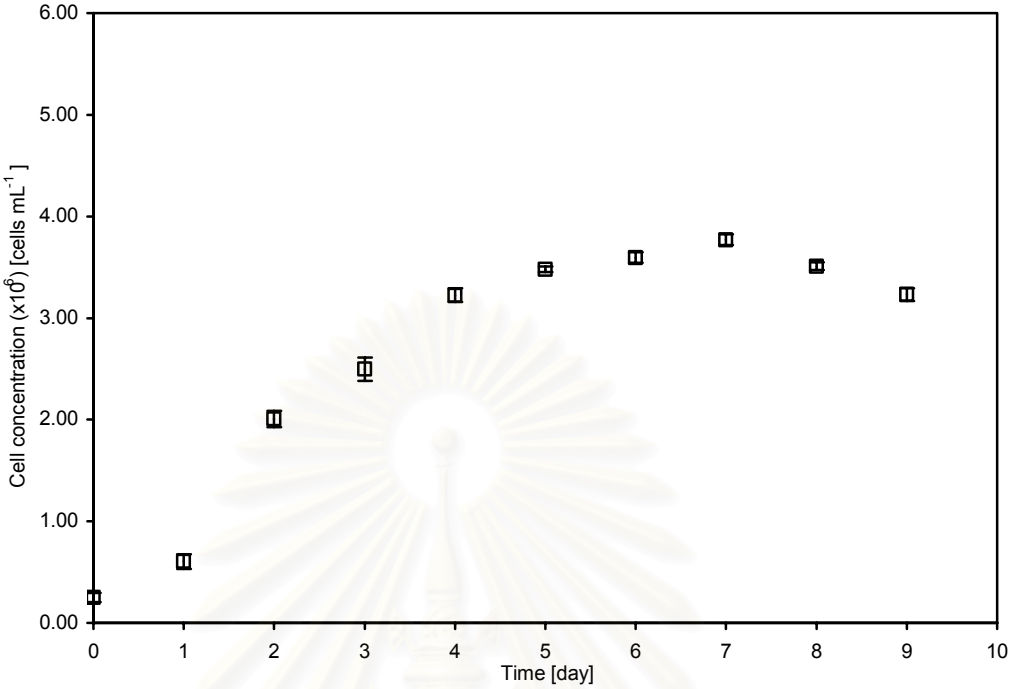

Figure C-1 : Effect of photoperiod on cell concentrationat 16:8 LD

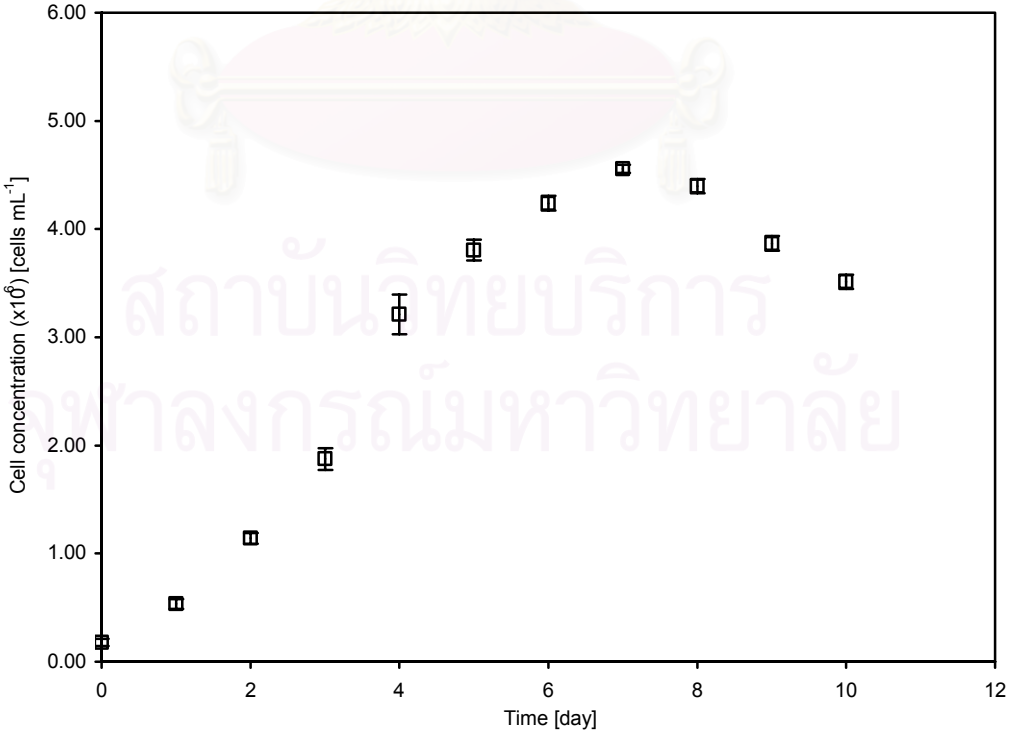

Figure C-2 : Effect of photoperiod on cell concentration at 20:4 LD

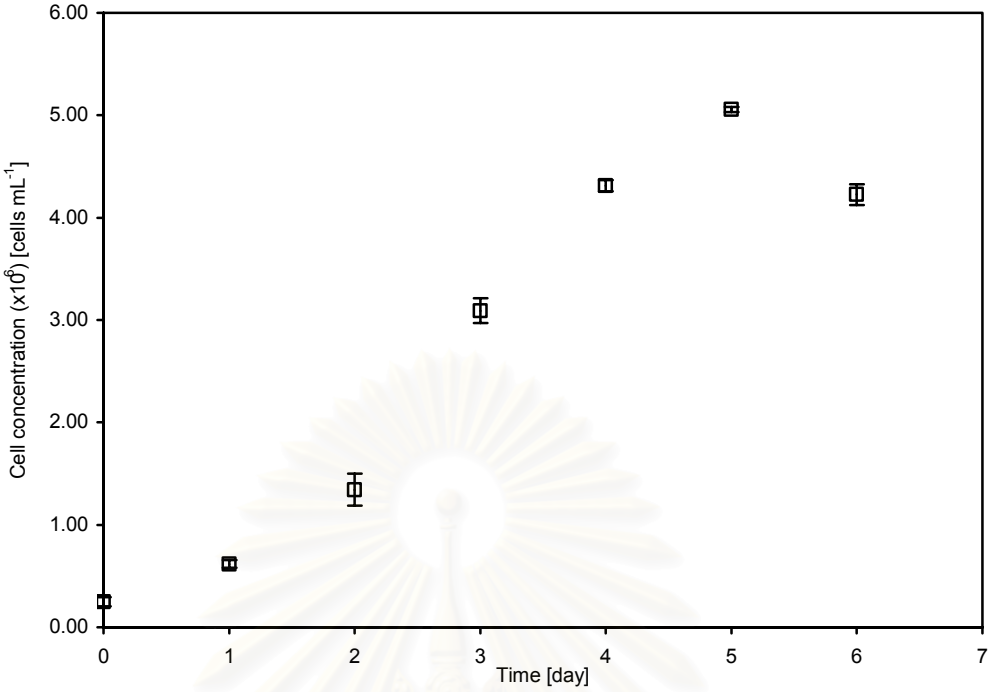

Figure C-3 : Effect of photoperiod on cell concentration at 24:0 LD

Appendix D : Experimental data on effect of nutrient concentration

Silica concentration

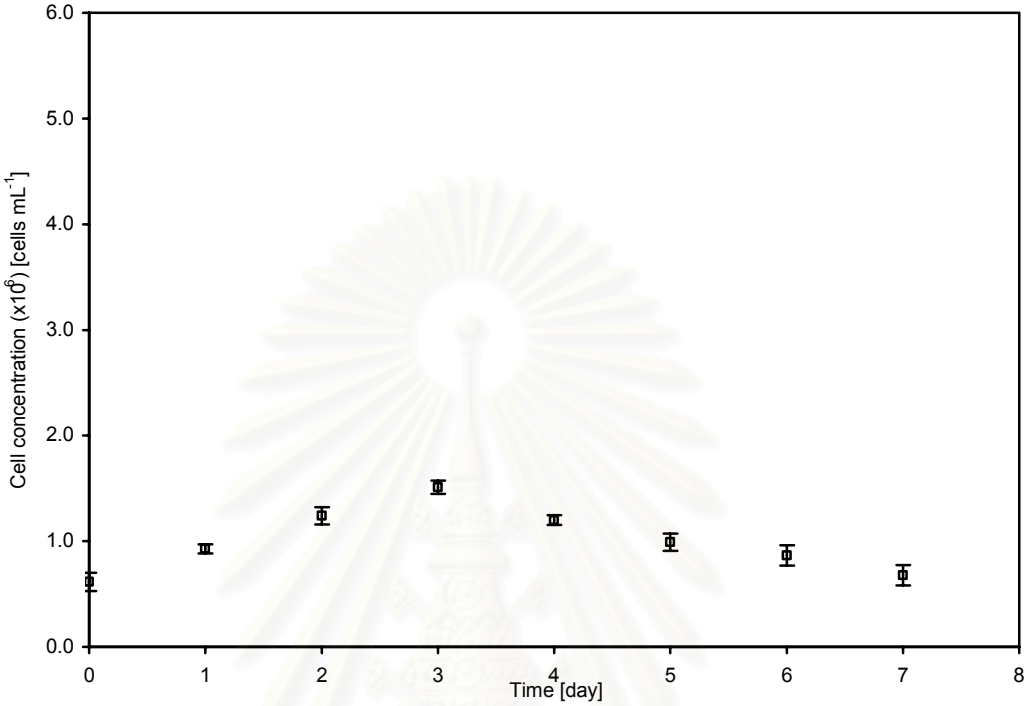

Figure D-1 : Effect of Silica Concentration at 0 mg.

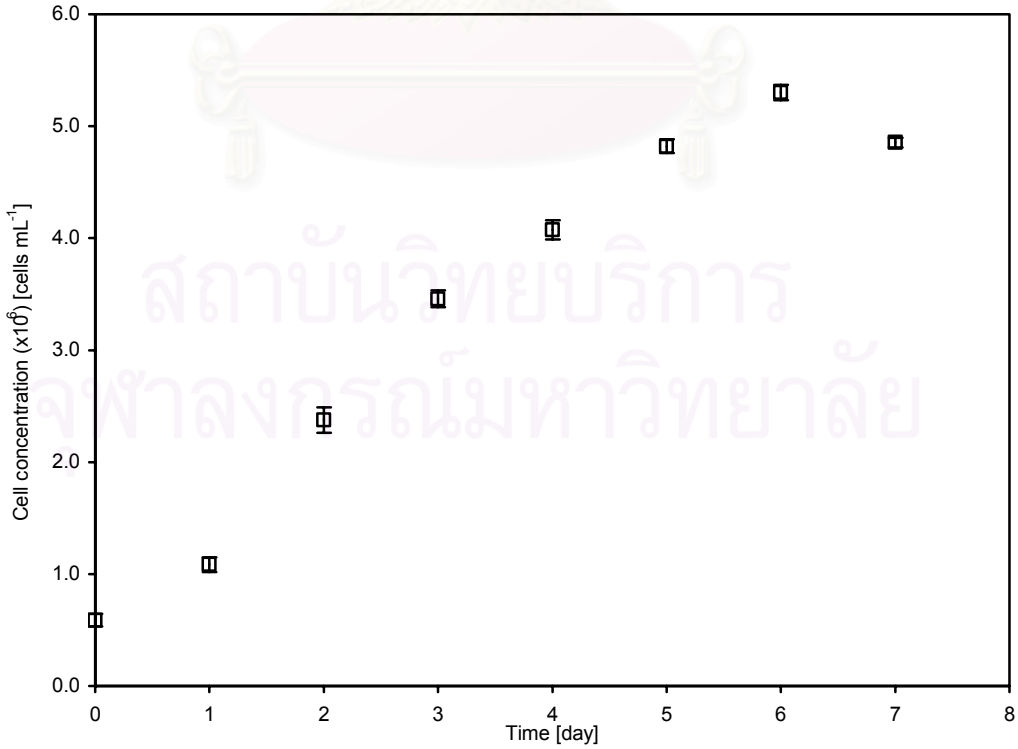

Figure D-2 : Effect of Silica Concentration at 1.6 mg.

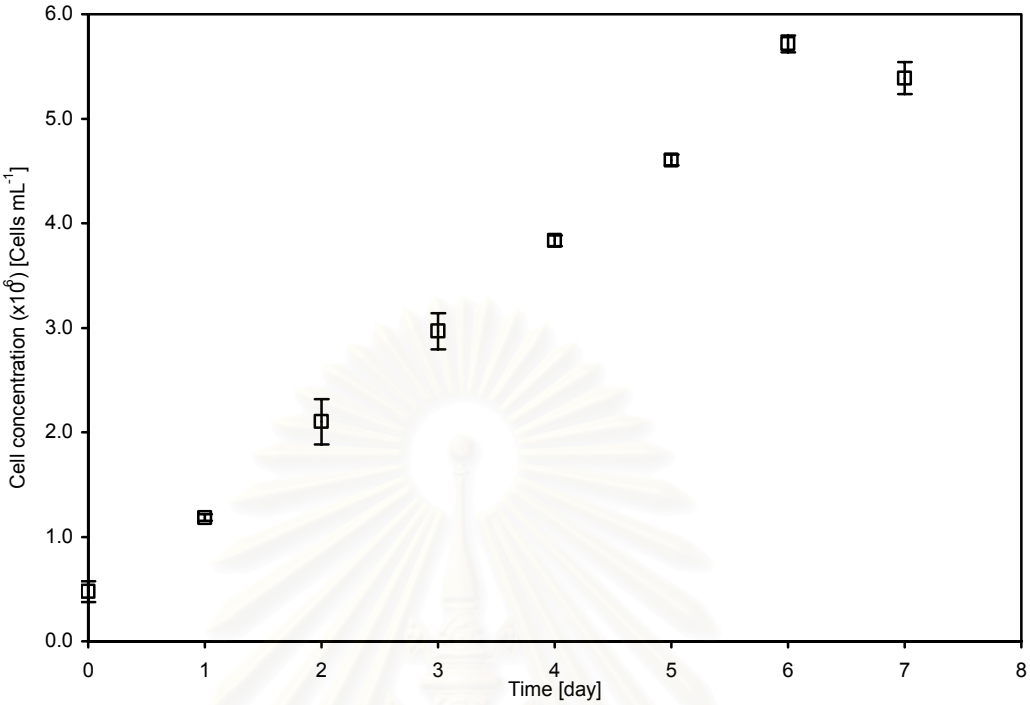

Figure D-3 : Effect of Silica Concentration at 3.2 mg.

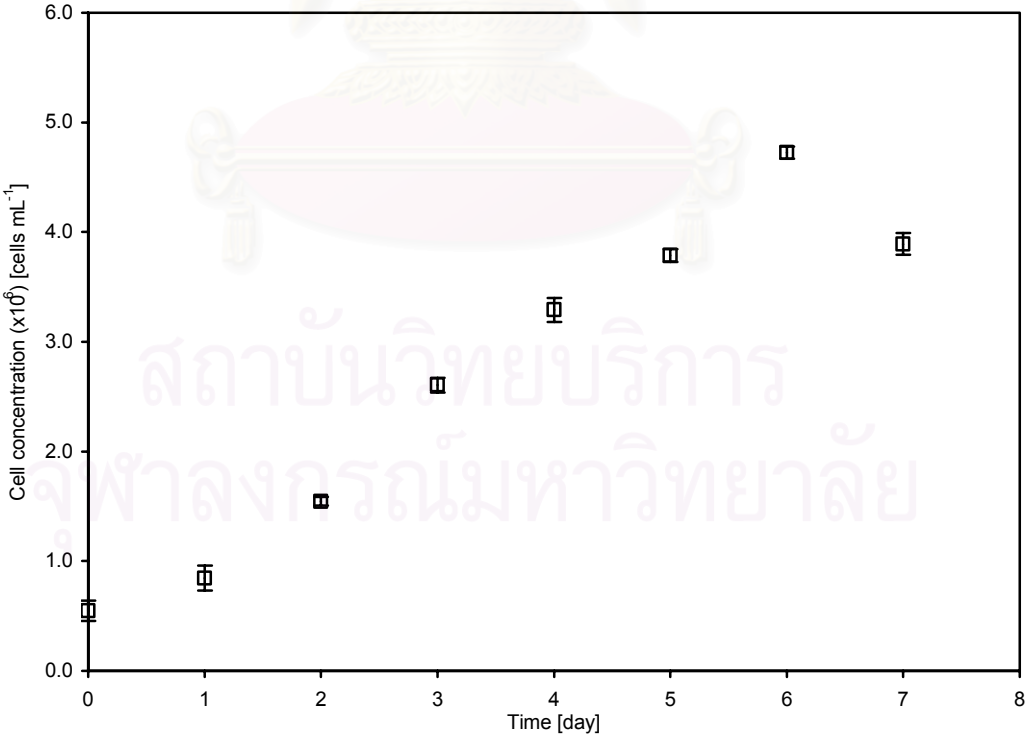

Figure D-4 : Effect of Silica Concentration at 4.8 mg.

Phosphorus concentration

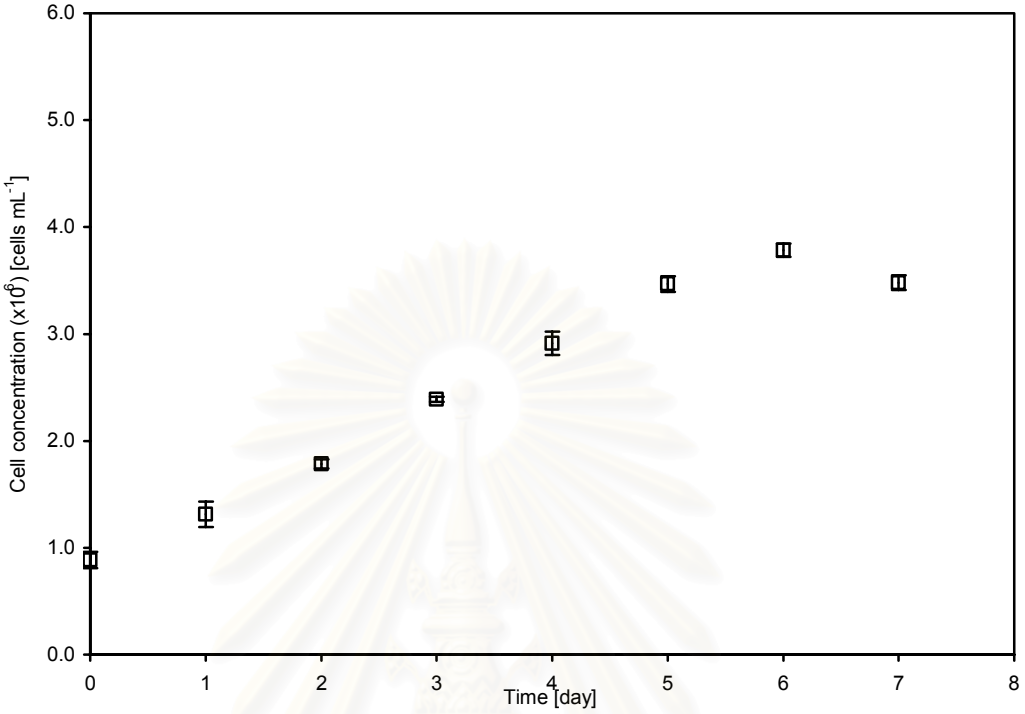

Figure D-5 : Effect of Phosphorus Concentration at 0 mg.

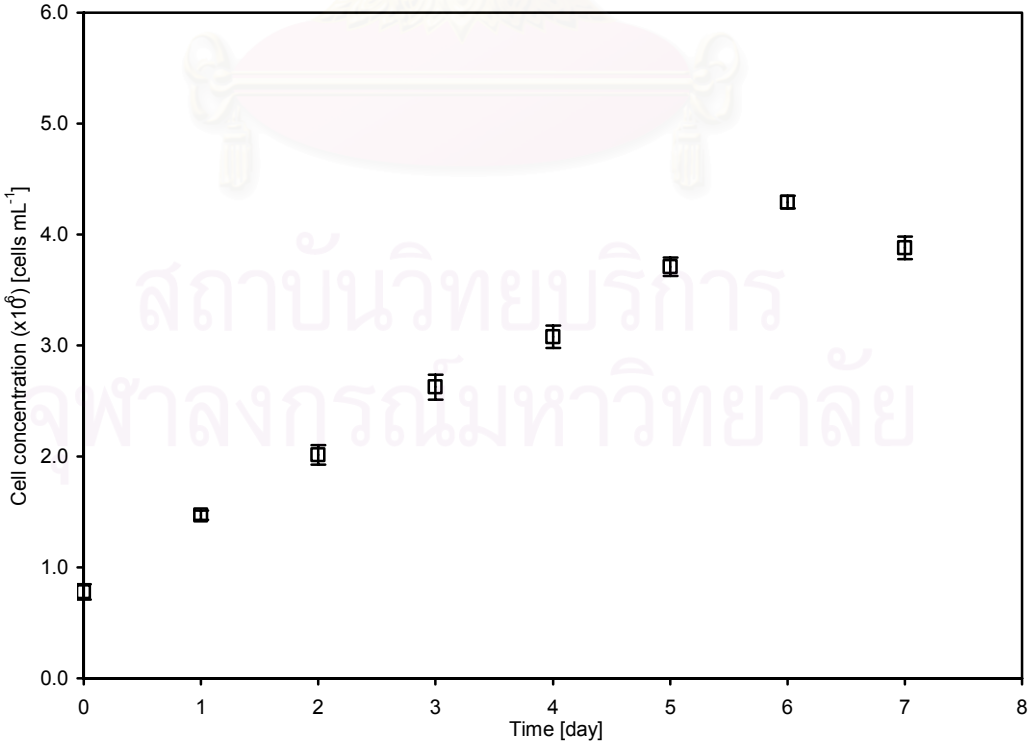

Figure D-6 : Effect of Phosphorus Concentration at 1.2 mg.

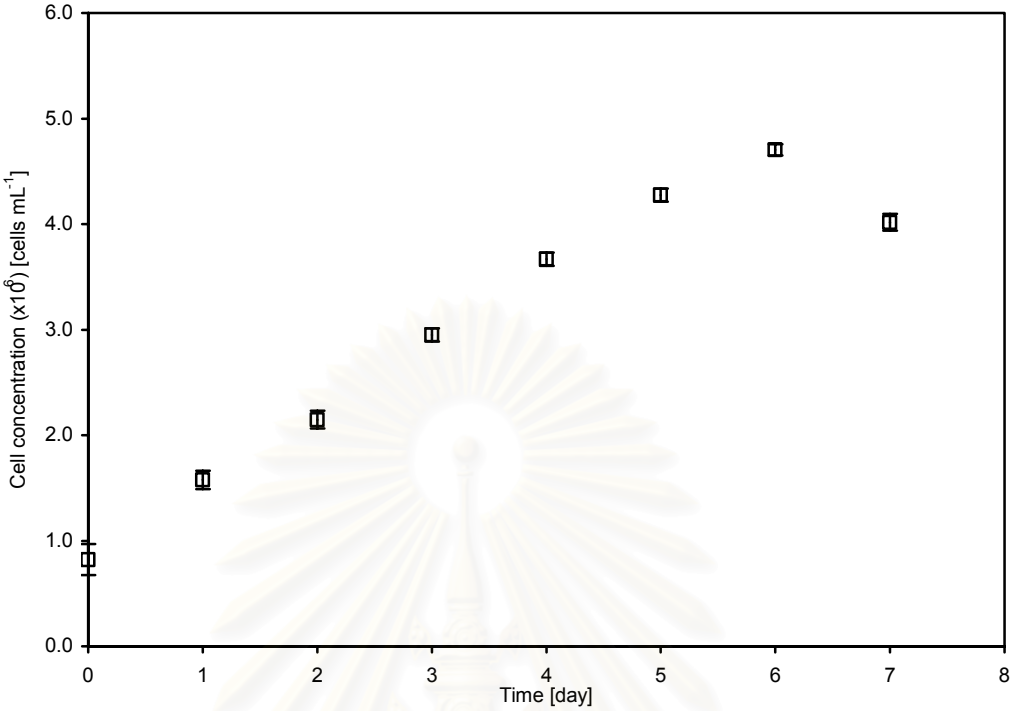

Figure D-7 : Effect of Phosphorus Concentration at 2.4 mg.

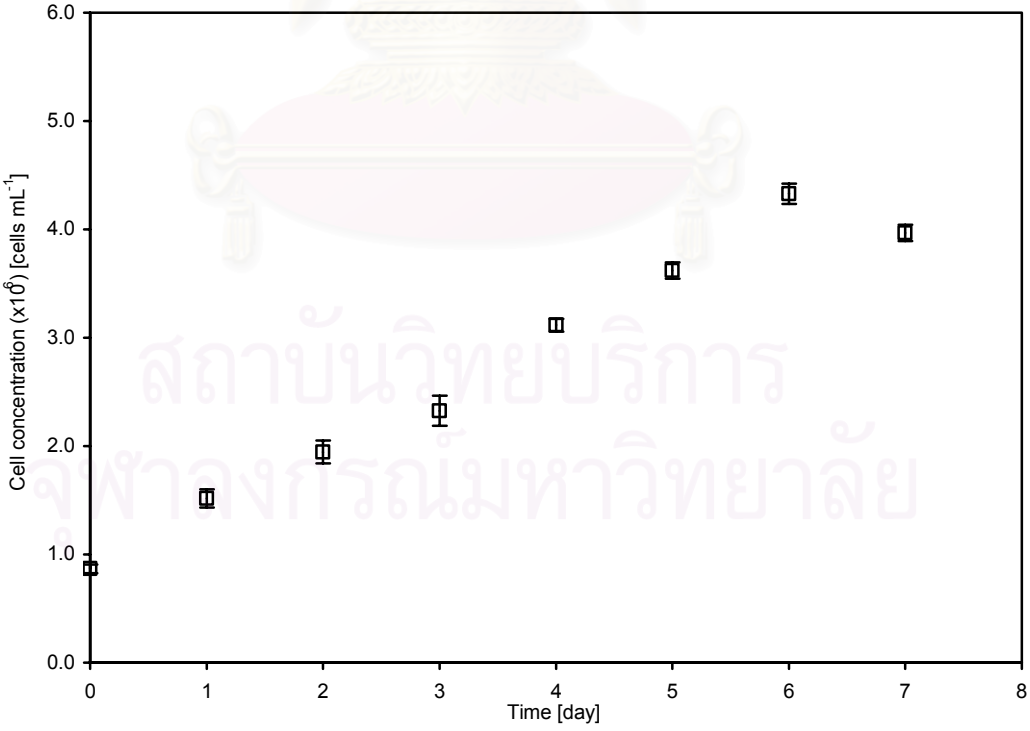

Figure D-8 : Effect of Phosphorus Concentration at 3.6 mg.

Nitrogen concentration

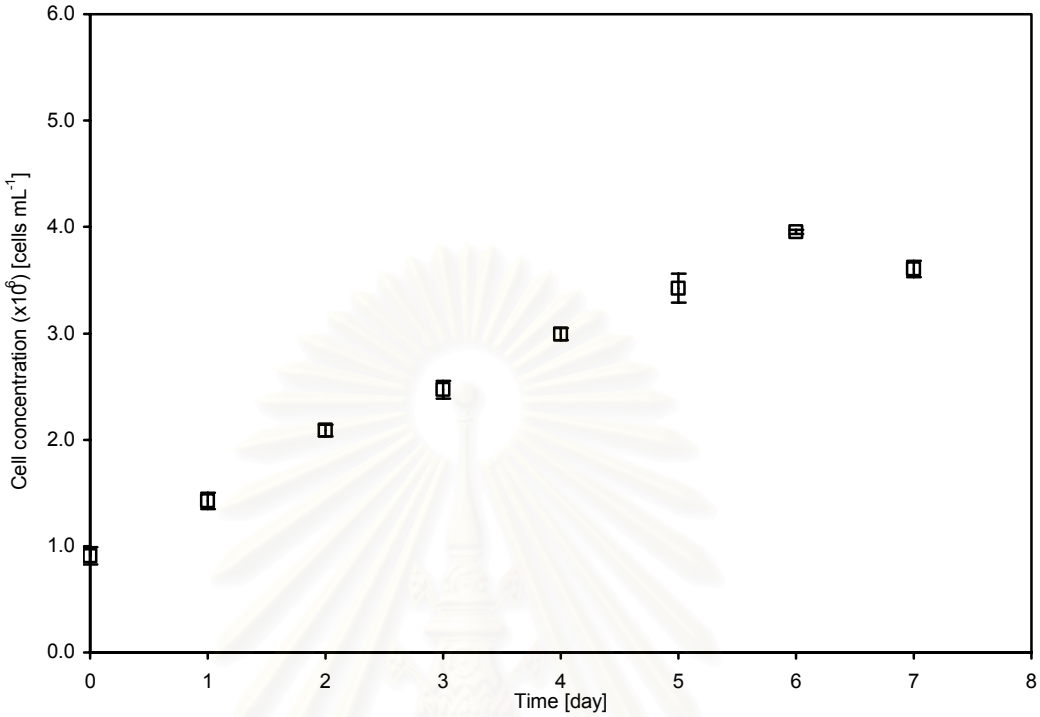

Figure D-9 : Effect of Nitrogen Concentration at 0 mg.

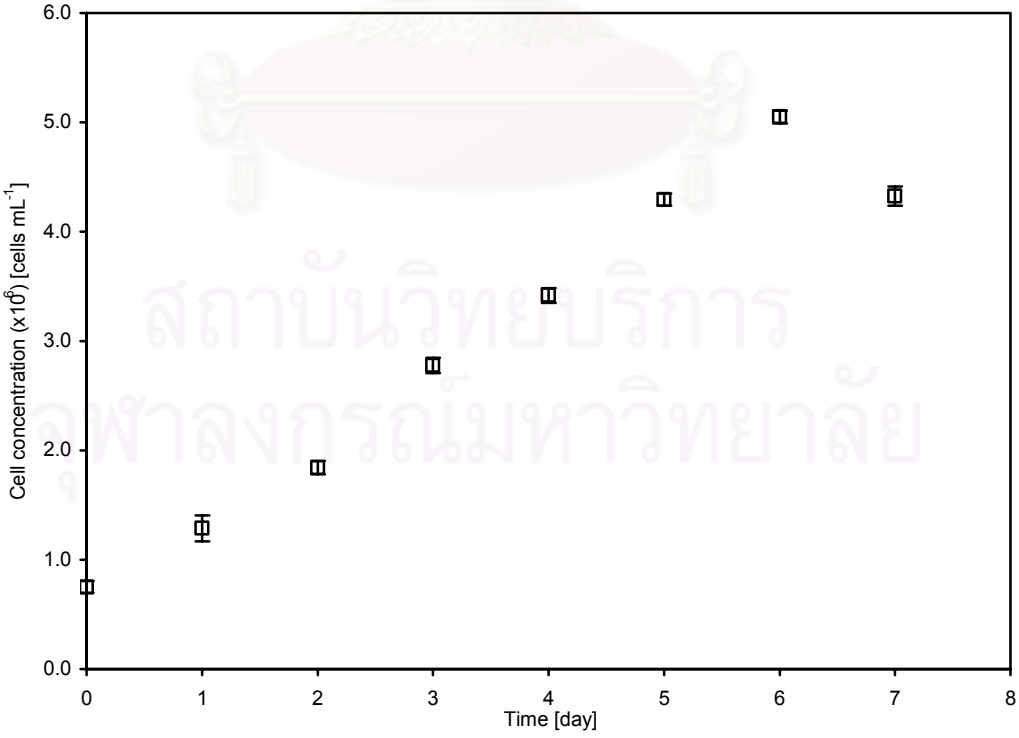

Figure D-10 : Effect of Nitrogen Concentration at 14 mg.

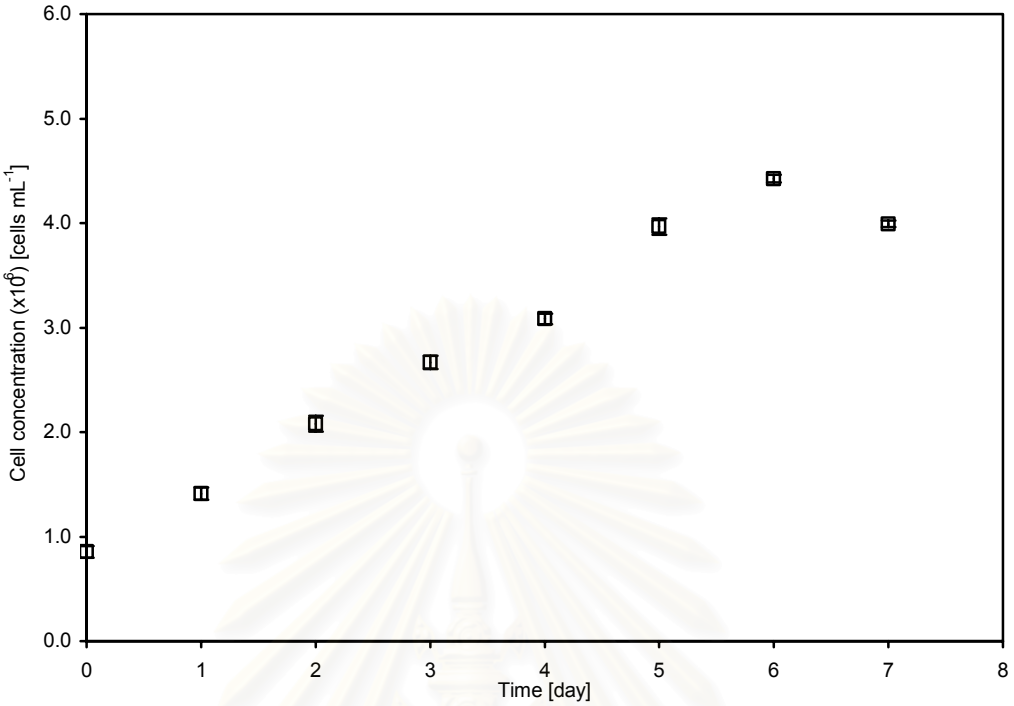

Figure D-11 : Effect of Nitrogen Concentration at 28 mg.

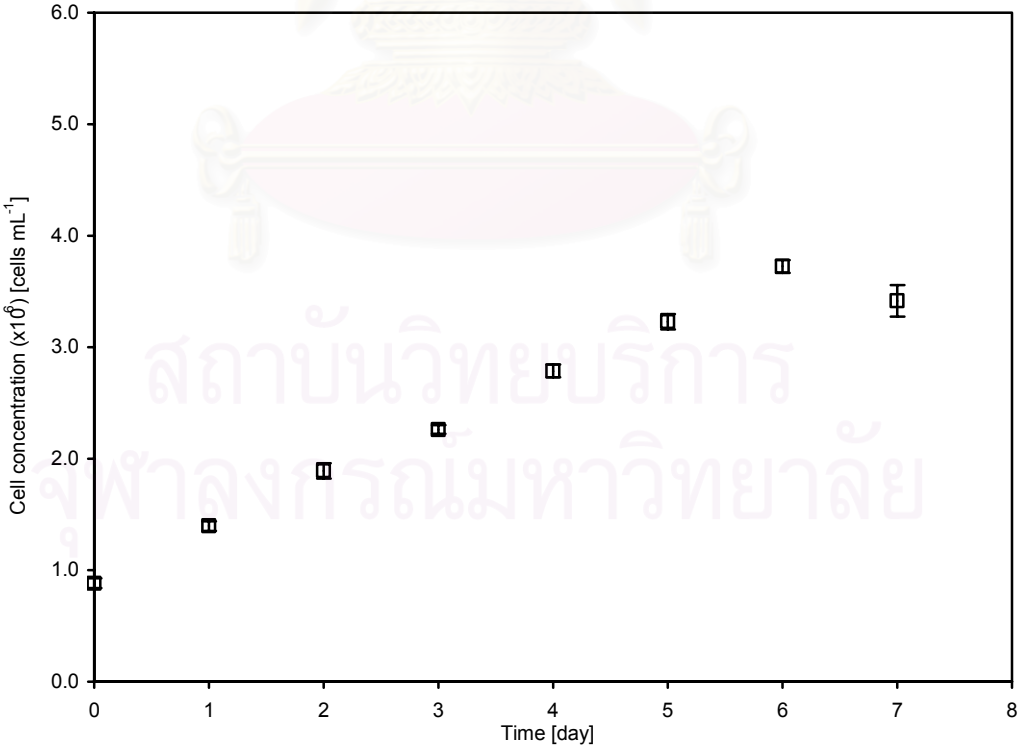

Figure D-12 : Effect of Nitrogen Concentration at 42 mg.

Vitamins concentration

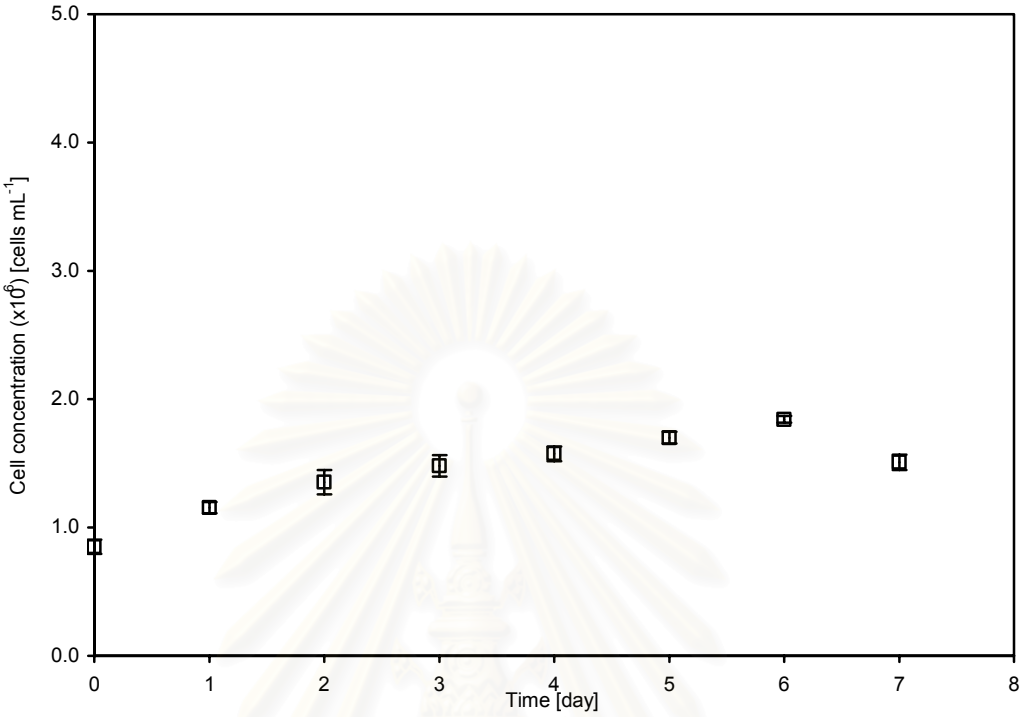

Figure D-13 : Effect of Vitamins Concentration at 0 μg.

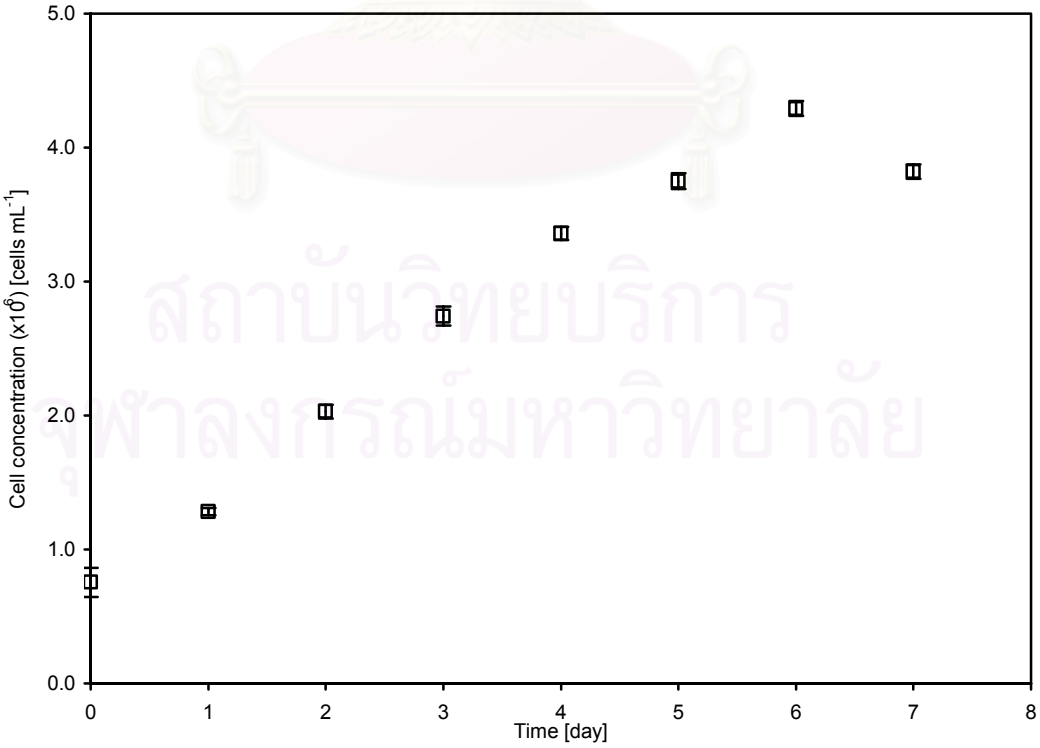

Figure D-14 : Effect of Vitamins Concentration at 1 μg.

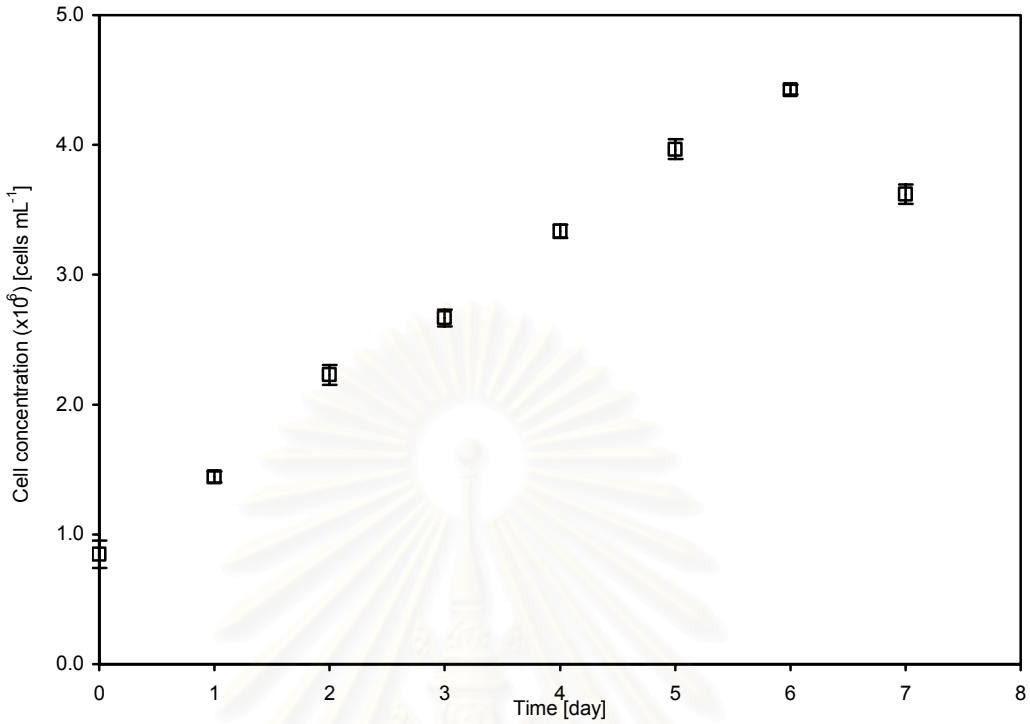

Figure D-15 : Effect of Vitamins Concentration at 2 μg.

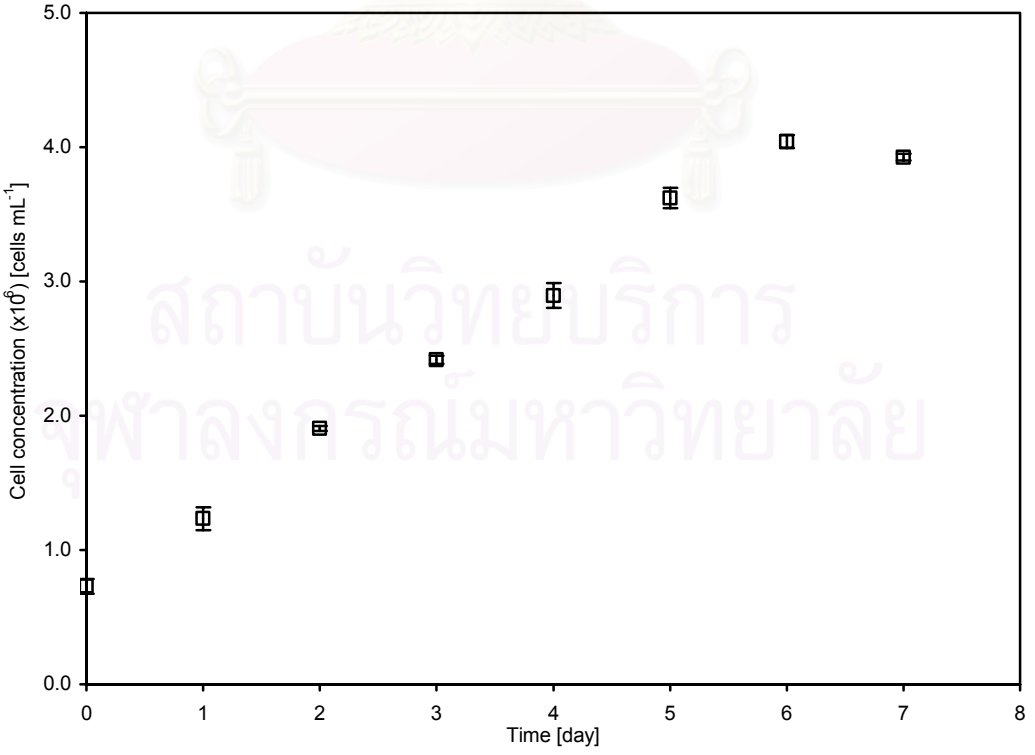

Figure D-16 : Effect of Vitamins Concentration at 3 μg.

Appendix E : Experimental data on cultivation in bioreactors

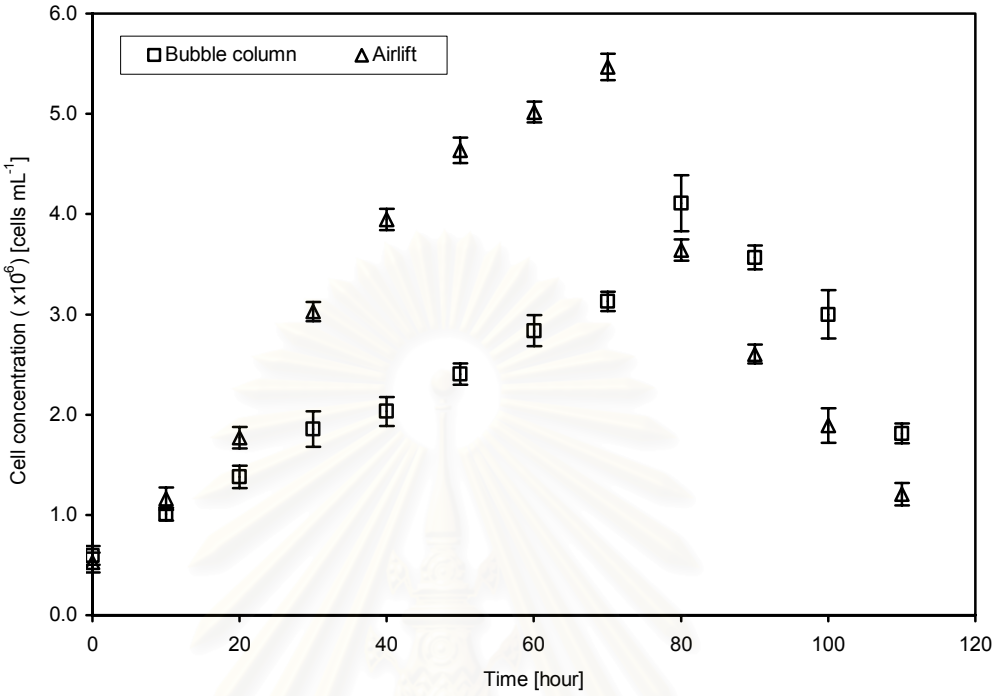

Figure E-1 : Cultivation cell in airlift bioreactor and bubble column at 1 cm s^{-1}

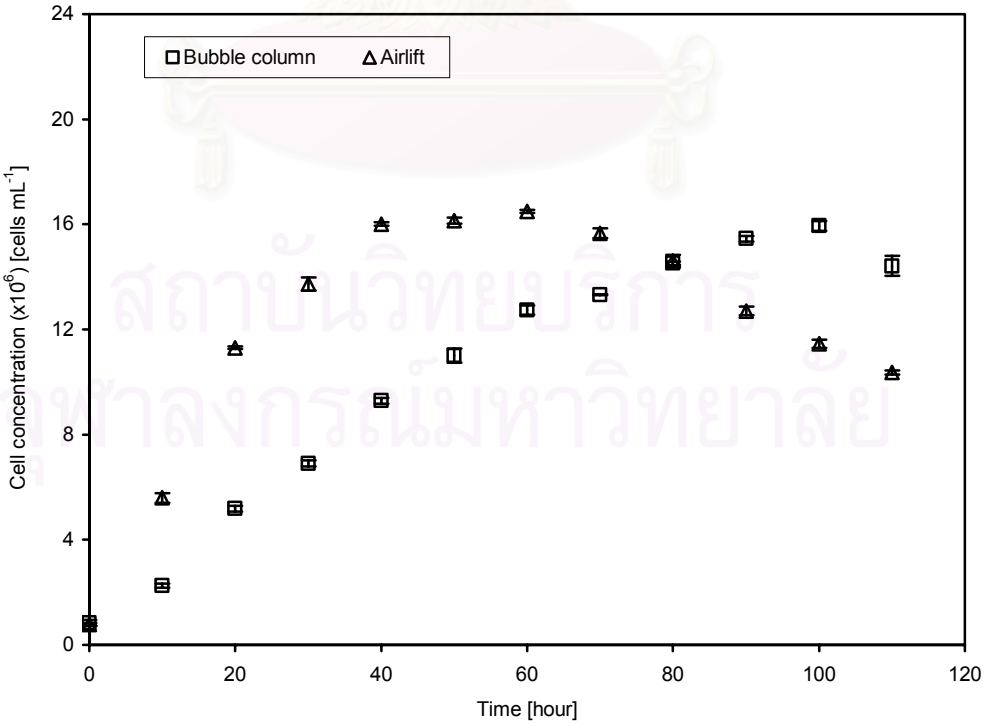

Figure E-2 : Cultivation cell in airlift bioreactor and bubble column at 2 cm s^{-1}

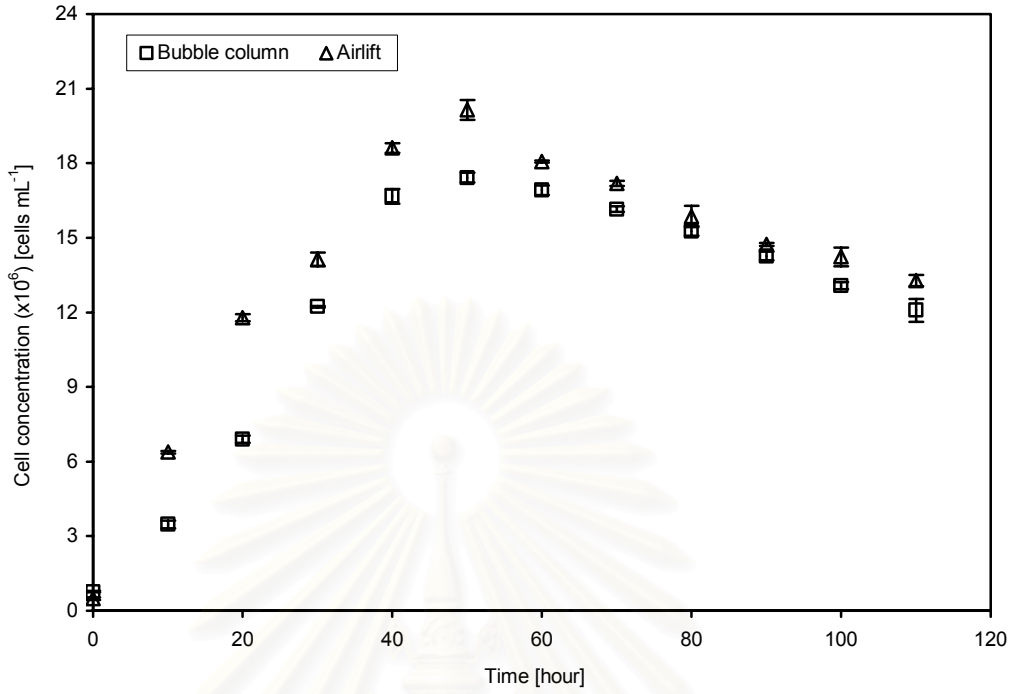

Figure E-3 : Cultivation cell in airlift bioreactor and bubble column at 3 cm s^{-1}

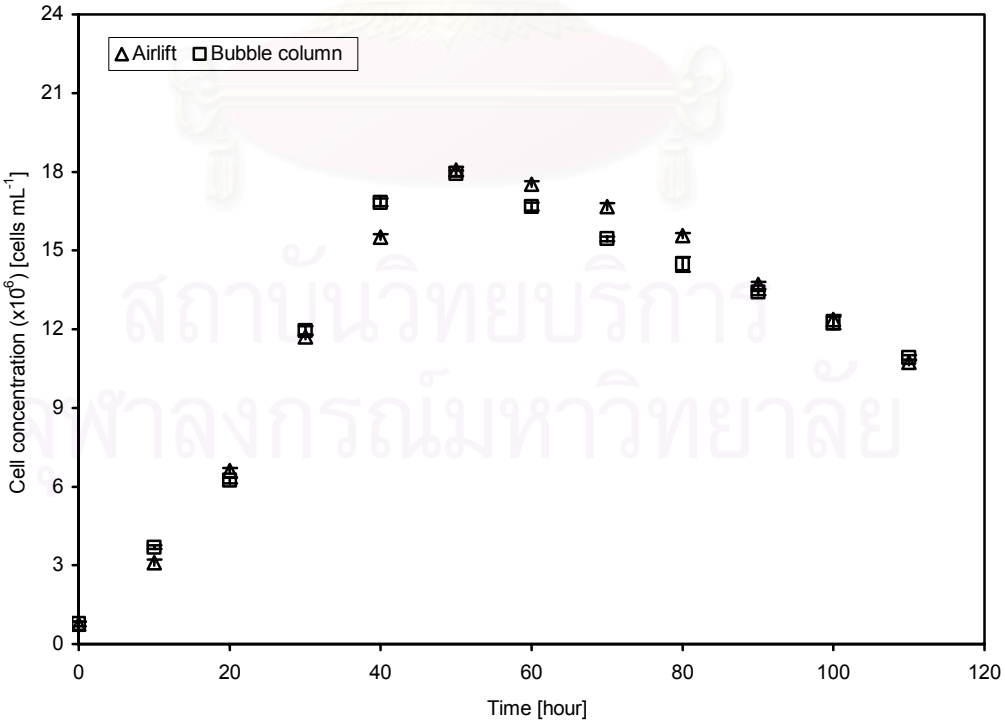

Figure E-4 : Cultivation cell in airlift bioreactor and bubble column at 4 cm s^{-1}

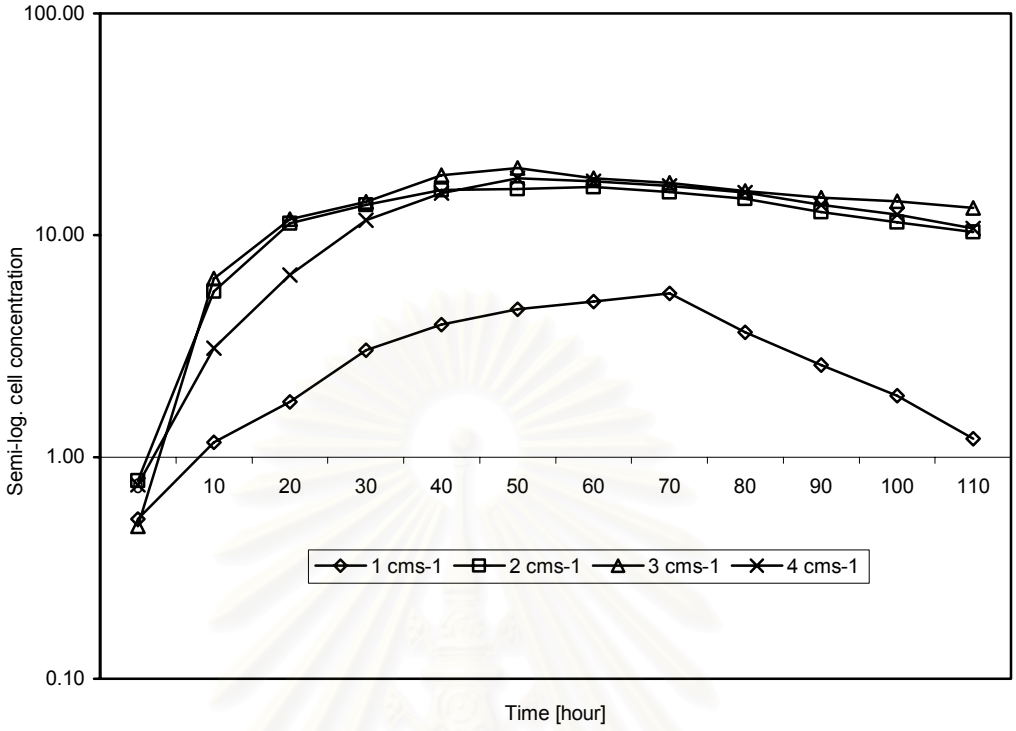

Figure E-5 : Growth variation on aeration rate in ALBR at 200 μmol photon $m^{-2}s^{-1}$

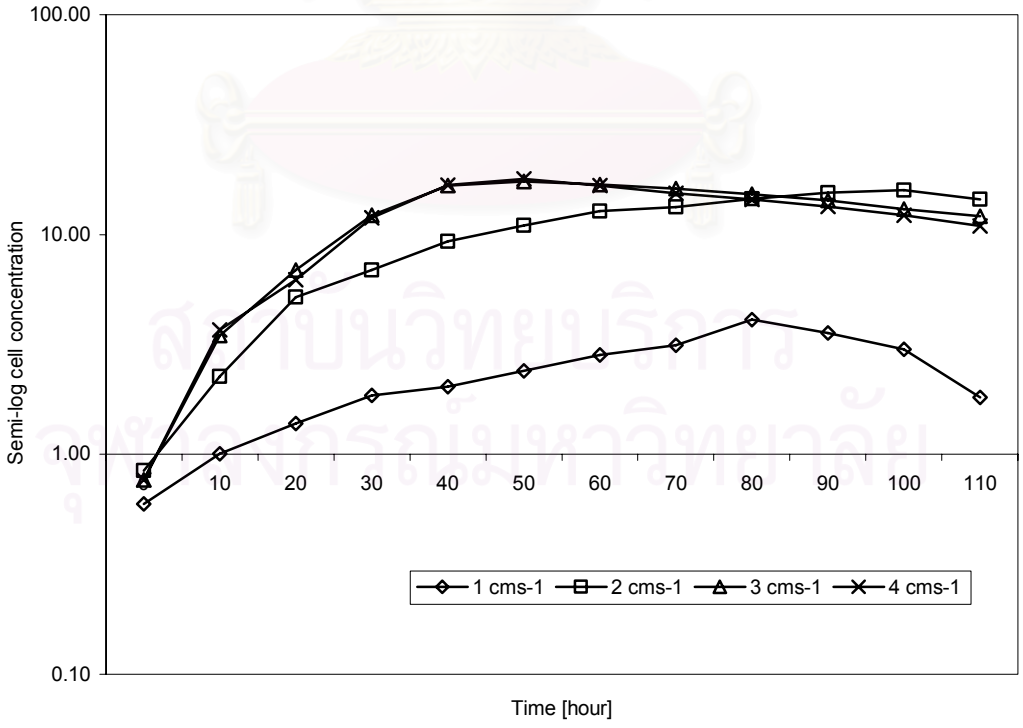

Figure E-6 : Growth variation on aeration rate at 200 μmol photon $m^{-2}s^{-1}$ in BC

Appendix F : Experimental data on time variation of biomass production

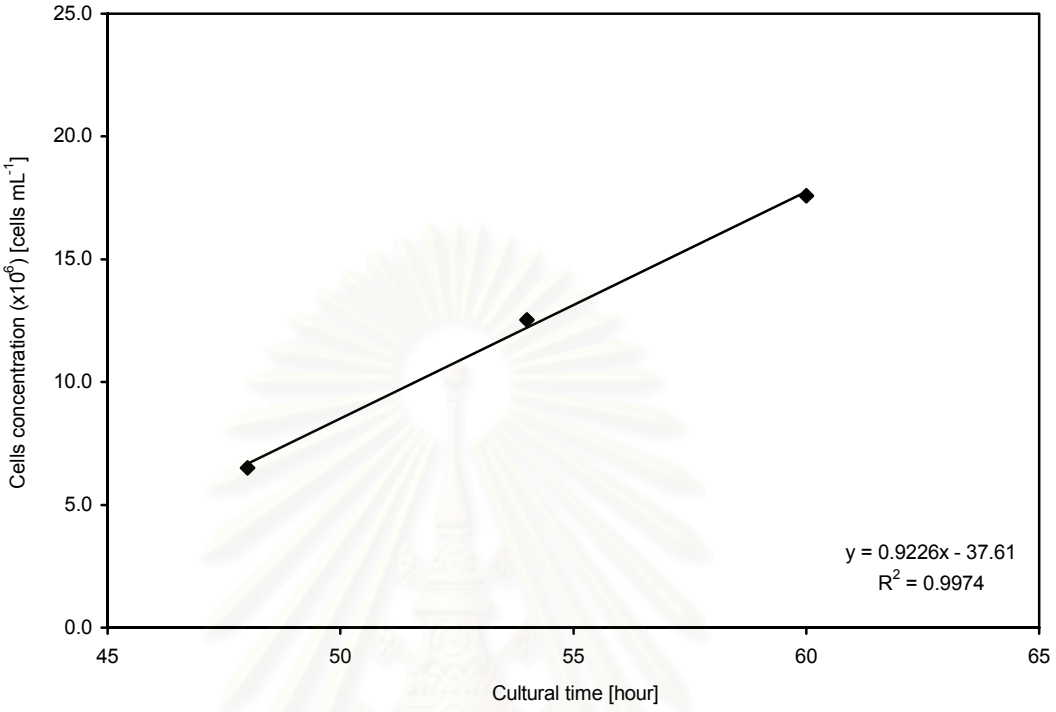

Figure F-1 : Biomass production versus time around 48-60 h.

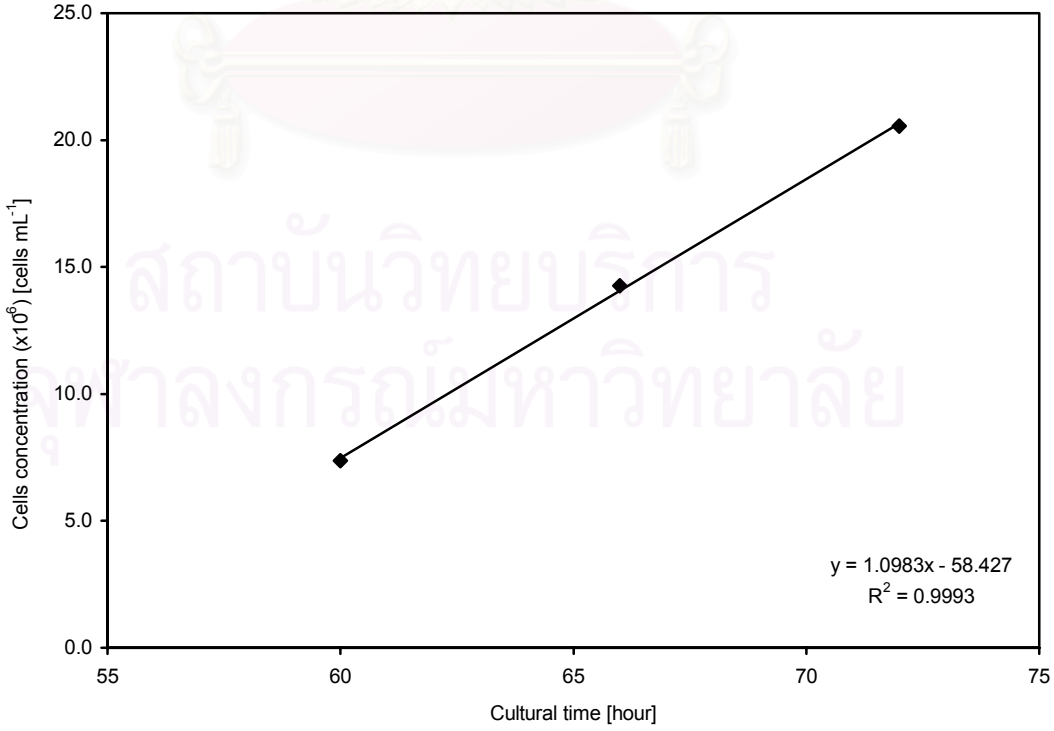

Figure F-2 : Biomass production versus time around 60-72 h.

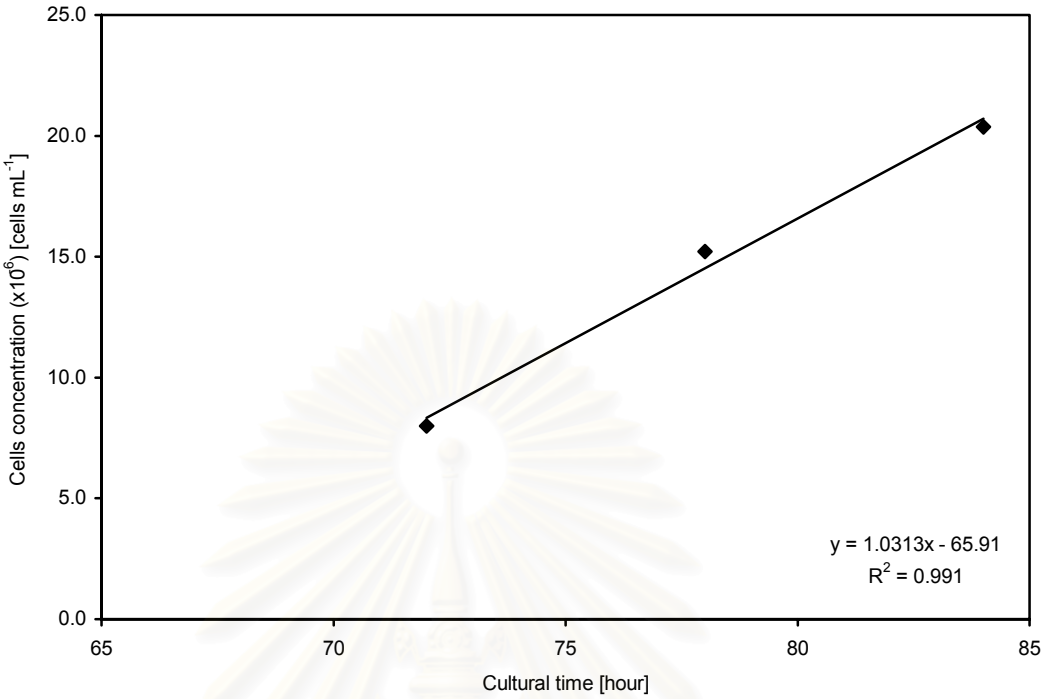

Figure F-3 : Biomass production versus time around 72-84 h.

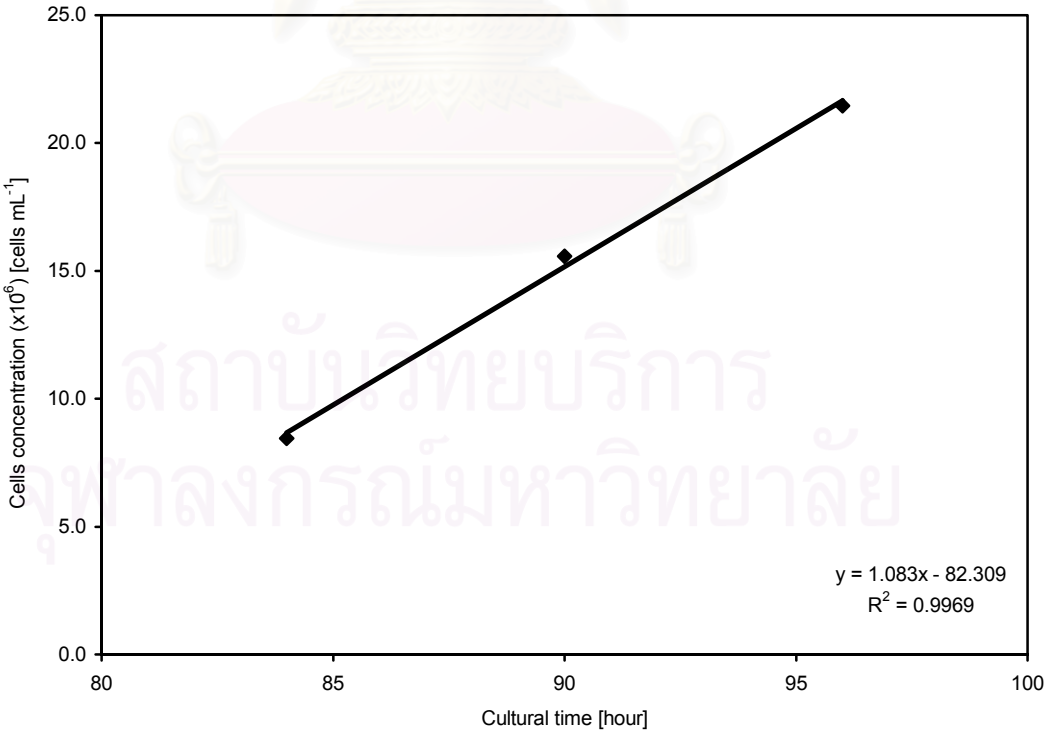

Figure F-4 : Biomass production versus time around 84-96 h.

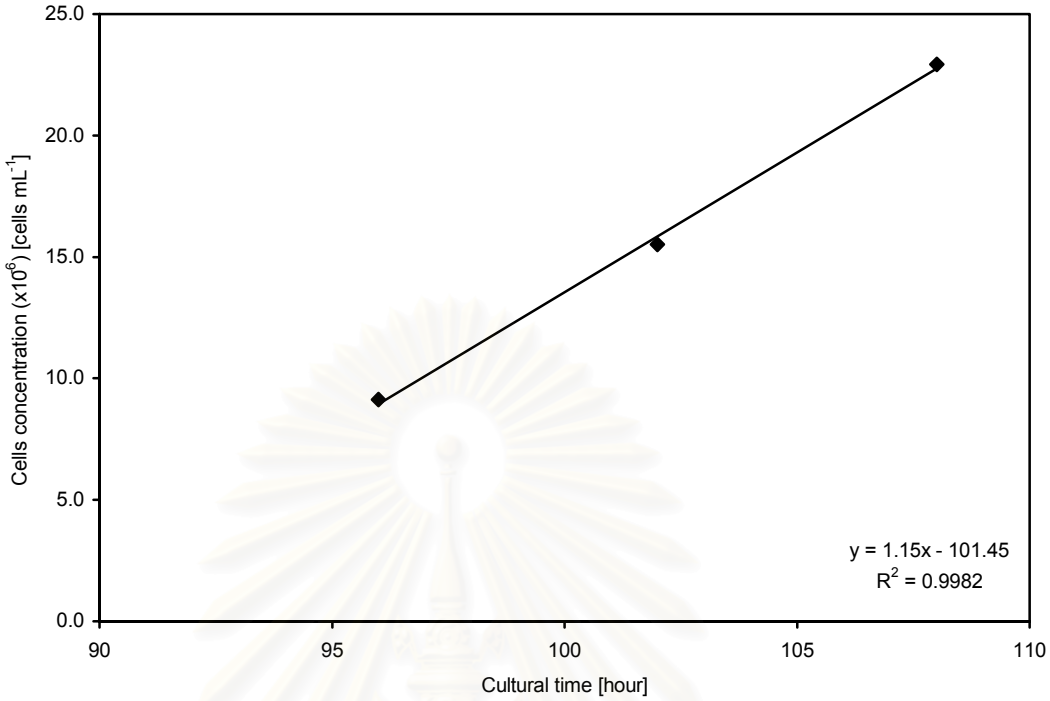

Figure F-5 : Biomass production versus time around 96-108 h.

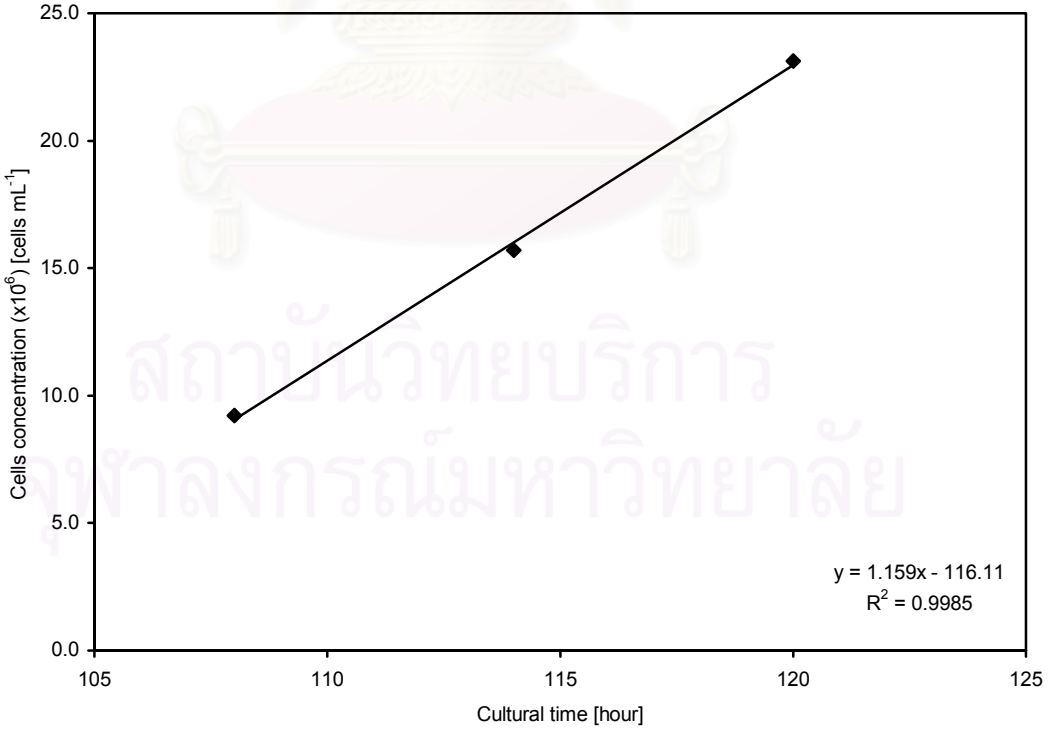

Figure F-6 : Biomass production versus time around 108-120 h.

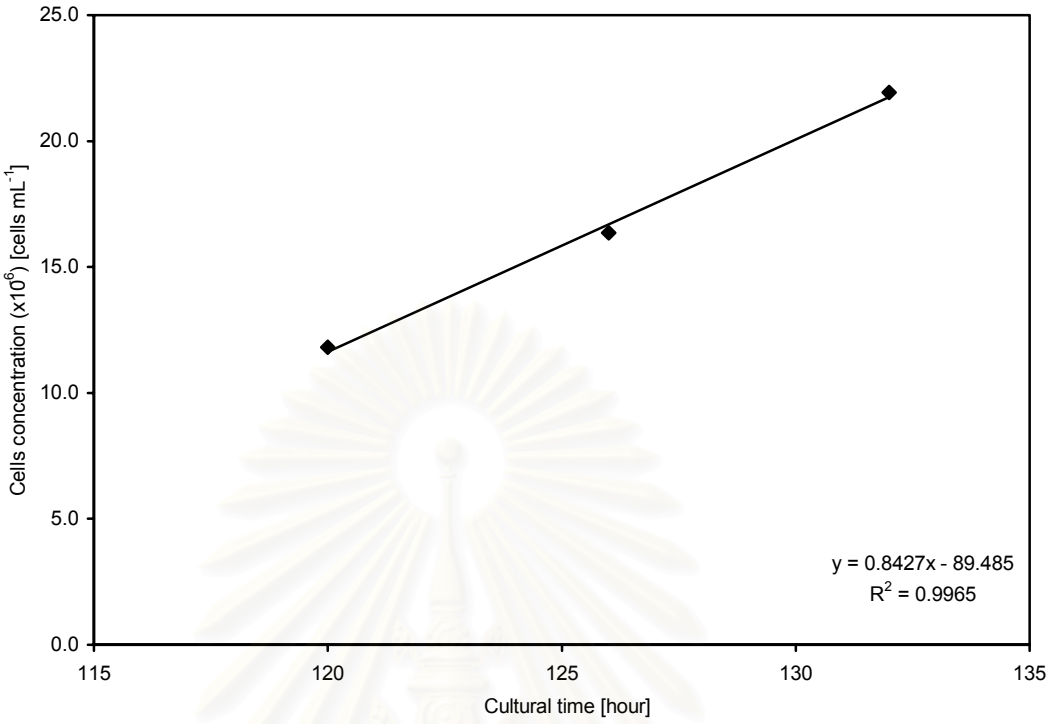

Figure F-7 : Biomass production versus time arounf 120-132 h.

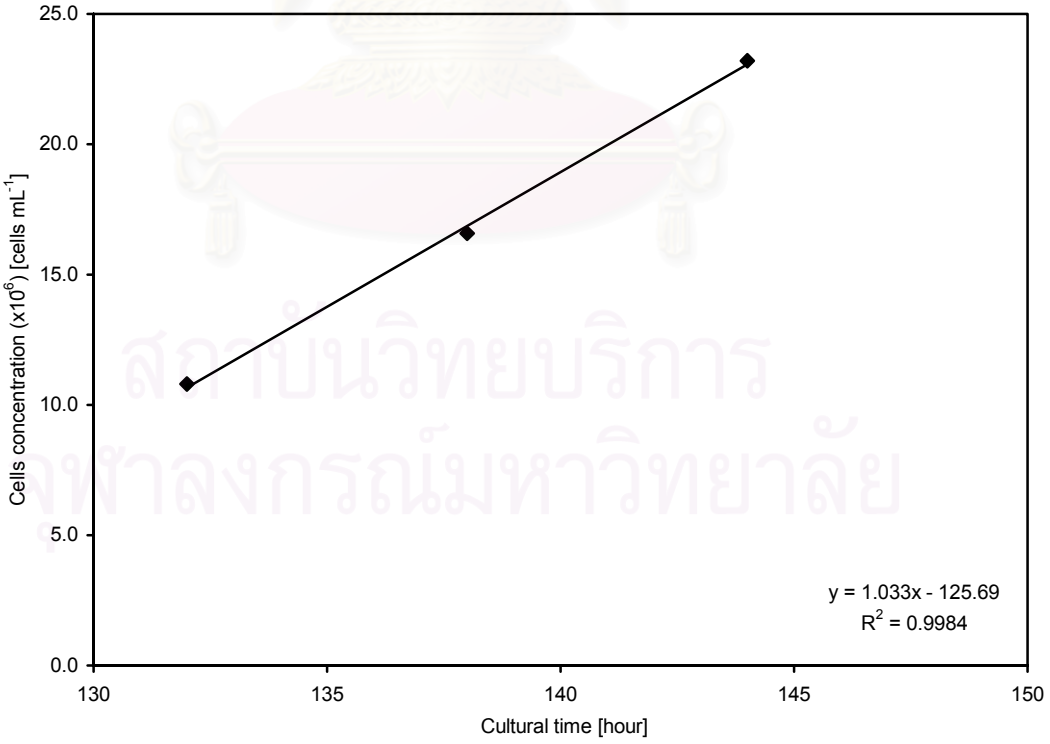

Figure F-8 : Biomass production versus time around 132-144 h.

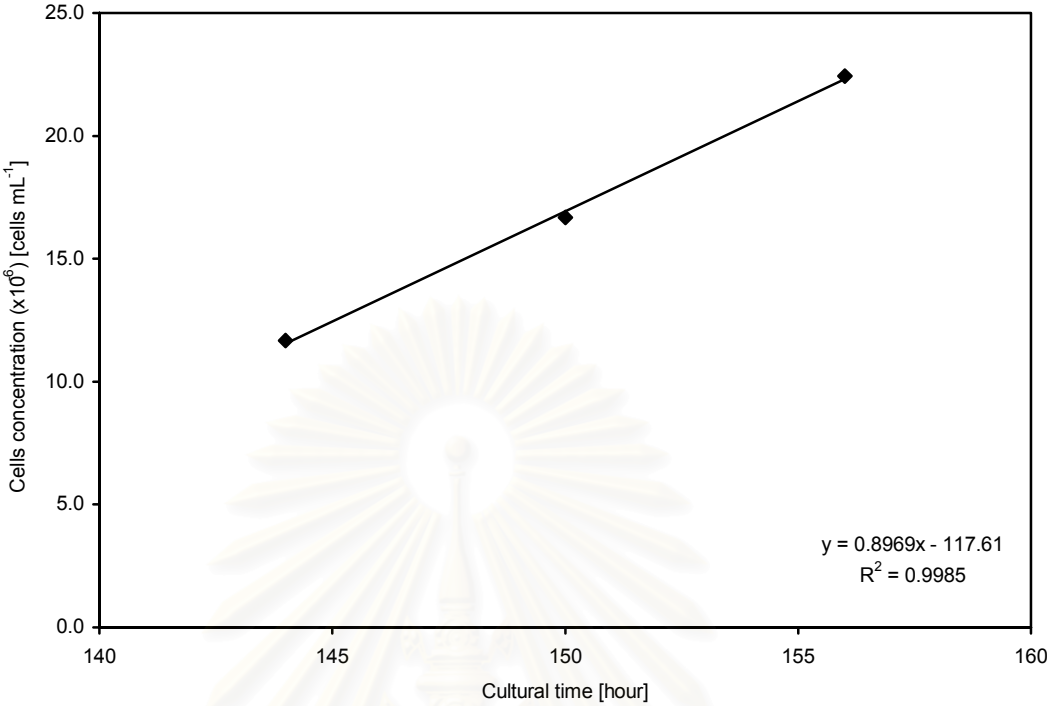

Figure F-9 : Biomass production versus time around 144-156 h.

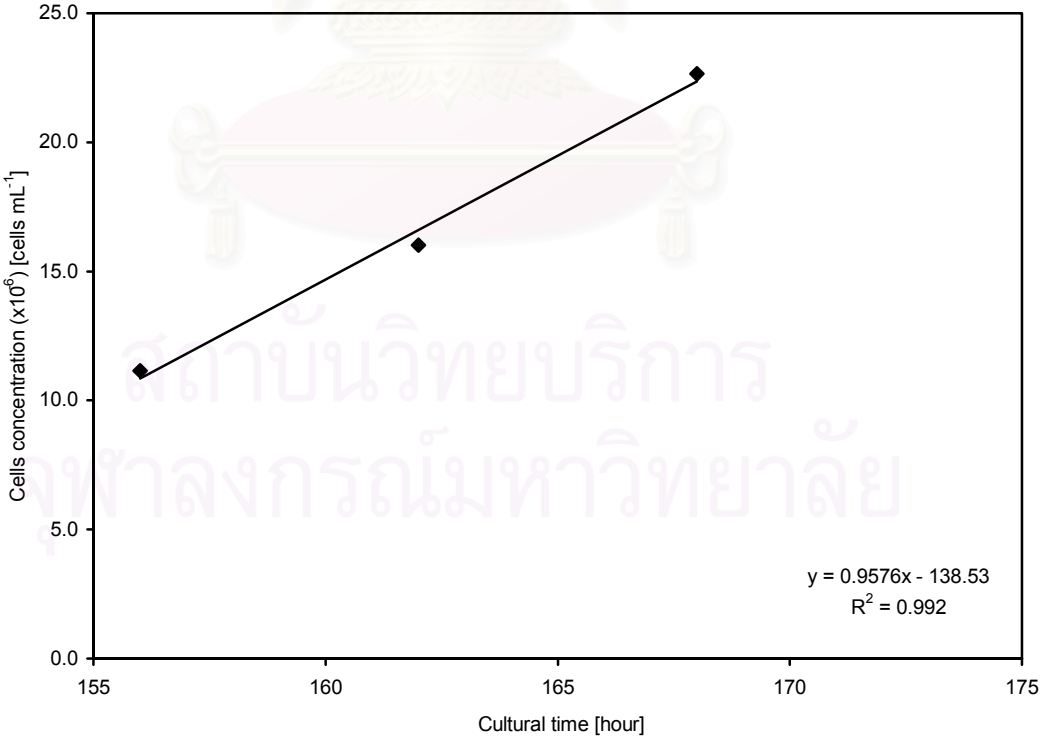

Figure F-10 : Biomass production versus time around 156-168 h.

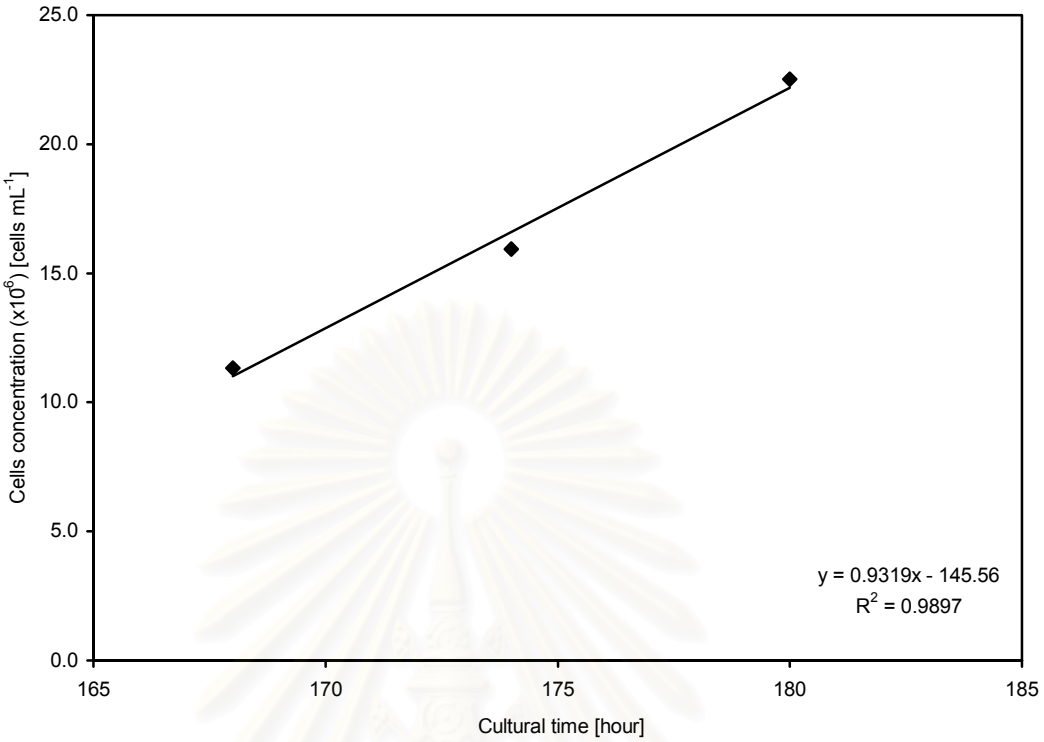

Figure F-11 : Biomass production versus time around 168-180 h.

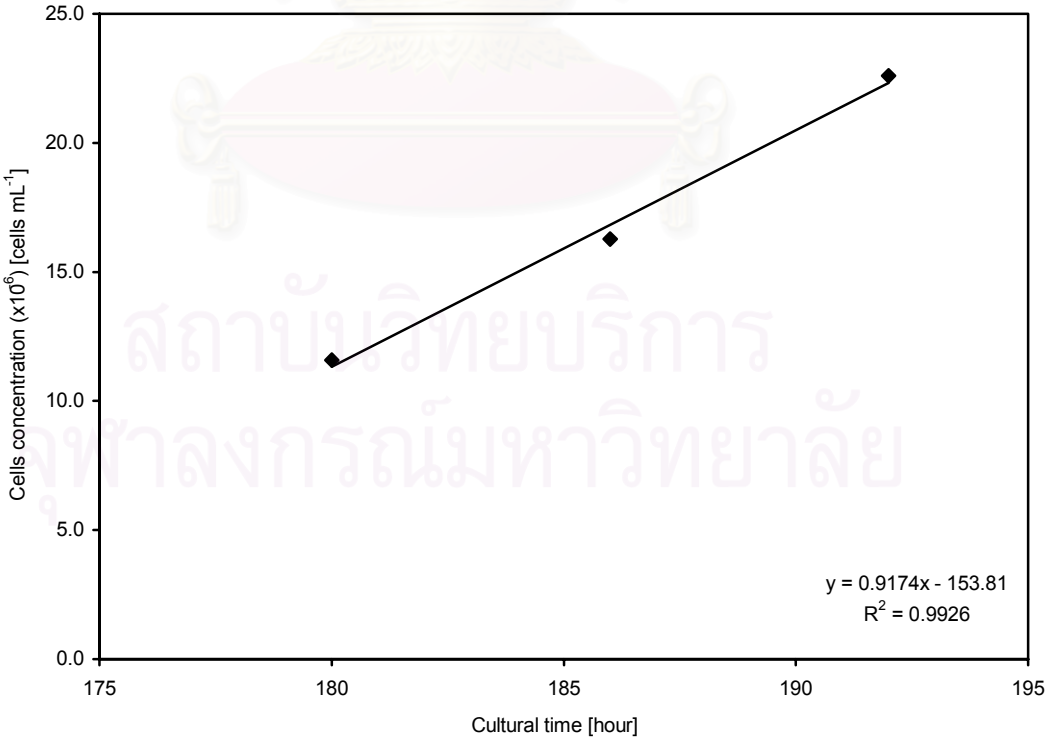

Figure F-12 : Biomass production versus time around 180-192 h.

BIOGRAPHY

Miss Worapannee Loataweesup was born on 5th March, 1977 in Bangkok. She finished her higher secondary course from Triam-udom suksa School in March, 1993. After that, she studied in the major of Biotechnology in Faculty of Agro-Industry at Kasetsart University. Then, she further studied for Master's degree in the major of Chemical Engineering in Faculty of Engineering at Chulalongkorn University. She participated in the Environmental research group in the major of Chemical Engineering and achieved her Master's degree in April, 2003.