BIOMASS AND LIPID PRODUCTION FROM GREEN MICROALGA *ANKISTRODESMUS* SP.

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ID: 53920102

A THESIS SUBMITTED AS A PART OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
IN ENVIRONMENTAL TECHNOLOGY

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AT KING MONGKUT'S UNIVERSITY OF TECHNOLOGY THONBURI

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ABSTRACT

Light intensity directly affects the growing and photosynthesis of microalgae, which requires optimal light intensity during the cultivation. Photoinhibition may occurred at high light intensity and low algal densities. In order to in dense algal culture, light penetration may be limited. The effect of light intensity and temperature on the cell growth and biochemical compositions of *Ankistrodesmus* sp. were investigated. The lipid content was strongly influenced by the temperature. Increasing temperature seemed to lower the lipid accumulation in this alga. The maximum biomass concentration occurred at 30 klux.

Carbon dioxide is a greenhouse gases and a main cause of global warming. Microalgae could uptake carbon dioxide as carbon source for growth. *Ankistrodesmus* sp. was cultivated for the production of microalgal lipid. pH in the range from 6 to 8 was not found to give significant effects on growth and lipid production where the culture seemed to grow best at pH 8. Although the algal growth remained unaltered, lipid production could be enhanced when the culture was aerated with additional CO_2. It was found that as much as 30% lipid could be achieved when 5% by vol. of CO_2 was mixed with the air supply, i.e. lipid productivity increased from 97.45 to 109.99 mg L^{-1} d^{-1}. Analysis indicates that CO_2 helped promote the accumulation of palmitic acid which is the dominant lipid species.

This work investigated the effect of the nutrient on lipid accumulation in *Ankistrodesmus* sp. culture. Batch cultures were carried out using fresh BG11 medium, and after the harvest, the medium was reused for the next culture, and this method was repeated two times. The maximum lipid productivity of 29.75 mg L^{-1} d^{-1} was obtained from the culture with the 2^{nd} reuse medium. In continuous cultures, *Ankistrodesmus* sp. was cultured in both fresh and modified BG11 mediums. The modified BG11 medium was adjusted to resemble the content of the 1^{st} reuse medium. As a comparison between batch and continuous cultures, it was proven that the productivity in the continuous culture was better than the batch where the achievable maximum biomass and lipid were 188.30 and 38.32

mg L^{-1} d^{-1}. The maximum lipid content of 34.22% was obtained from the continuous culture at the dilution rate of 0.08 d^{-1}, whereas the maximum saturated and unsaturated fatty acid productivities of 79.96 and 104.54 mg L^{-1} d^{-1} were obtained at the dilution rate of 0.16 d^{-1}.

Keywords: *Ankistrodesmus* sp., Photobioreactor, Lipid production, Carbon dioxide, Continuous culture, Modified nutrients

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NOMENCLATURE

BG11	Blue-green medium
C	Carbon
°C	Celsius
cm	Centimeter
CO_2	Carbon dioxide
d	Day
D	Dilution rate
g	Gram
k	Kilo-
L	Liter
m	Milli-
MUFA	Monounsaturated fatty acid
N	Nitrogen
P	Phosphorous
PUFA	Polyunsaturated fatty acid
s	Second
SFA	Saturated fatty acid
THB	Baht
UFA	Unsaturated fatty acid
Usg	Superficial gas velocity
V	Volume

CHAPTER 1
INTRODUCTION

1.1 Rationale

The energy crisis on the finite energy sources, e.g., petroleum, natural gas, and coal, has its direct impact on the economy worldwide. The limited energy sources might result in instability of energy's security and economy. Additionally, a luxurious use of fossil fuels yields significant benefits the industries, but with costs of environmental concerns, e.g. resource depletion, climate change, waste disposal, etc. which could have their effect worldwide. It is therefore important to pursue material and energy recycling, while minimizing waste, at a global level. In order to reduce the dependency of the finite energy sources, much attention have been focused on renewable energy sources, such as geothermal, wind, solar, hydro-energy, and bioenergy. These alternative energy sources will play an important role in the very near future [1].

Thailand has supported the research and development on alternative fuels, such as algal biodiesel, biogas and ethanol. The ministry of Energy had developed target and roadmap of the Renewable Energy Development Plan (REDP) for 15 years (2008-2022). The renewable energy installation included the five policies, i.e. support the domestic renewable energy in industry, improve renewable technology efficiency, more security for the national energy, promote green local energy and use the national main energy replacement. REDP with a share of renewable energy sources (2.4% power, 7.6% thermal, 6.2% NGV and 4.1% biofuels) was set at 20.3% of the final energy demand in the year 2022 [2].

Biodiesel has better properties than does petroleum diesel fuel, as biodiesel is non toxic, biodegradable, renewable and free from sulfur and aromatics. Biodiesel is usually produced from vegetable oils including rapeseed, soybean, mustard, palm, sunflower, algae and animal fats such as tallow, lard and yellow grease [3].Vegetable oil has been much attention for biodiesel production; however, lesser attention was paid to the aquatic algal biomass despite its high productivity. Many research reports stated that microalgae provided a much greater oil productivity per unit area when compared with other plant sources. Microalgae can be grown rapidly in various types of environments. The use of algae as a biodiesel source is therefore considered a promising alternative to solve the

world energy crisis. This will not only help achieve environmental and economic sustainability, but the algal cultivation is also capable of sequestering atmospheric carbon dioxide (CO_2) and lessens the global warming problem.

Current large scale cultivation microalgae for fuel-oil production is still economically infeasible due primarily to the major costs in the use of energy and nutrient-medium preparation. One of the options to minimize the production costs is to minimize the amount of nutrients through the reuse of a spent medium. This can be achieved via a proper separation technique to remove algal biomass from the nutrient. For most algae, inorganic coagulants and bioflocculant were used for the cell harvest and the flocculated medium was re-used as nutrient in the next culture. The reuse of flocculated medium helps minimize the demand for water and reducing the nutrient cost, however, this might result in a slight decrease in algal growth [4, 5]. The medium composition could also be adjusted to best suit microalgal growth. This may also reduce the nutrient cost and there were reports that, by achieving specific nutrient requirement for certain algal species, the growth of the algae could be significantly enhanced [6]. Selecting proper culture mode could also affect the feasibility of the cultivation, where continuous and batch cultures might offer specific advantages and disadvantages depending on the type of algae, reactor controllability, and the ease of maintenance [7].

Recently, Maeda *et al*. [8] studied the effects of dilution rate on the lipid productivity of *Chlamydomonas* sp. under continuous system by using chemostat and turbidostat method. Furthermore, Lamers *et al*. [9] investigated the effect of light intensity on fatty acid of *Dunaliella salina* under turbidostat method. Tang *et al*. [10] studied the influences of light intensity, CO_2 concentration on the growth, lipid content, and fatty acid composition of *Chlorella minutissima* and *Dunaliella tertiolecta*. They reported that both microalgae presented the high biomass and fatty acid productivity as biodiesel feedstock.

Many species of microalgae that appear to be promising alternative sources of biodiesel production are mentioned in the list with lipid content as a percentage of dry weight of biomass: *Chlorella* sp. (14-22%) [11], *Dunaliella primolecta* (23%) [12], *Isochrysis* sp. (25-33%) [13], *Nannochloropsis* sp. (25-35%) [14] and *Scenedesmus* sp. (8-14%) [15]. In this case, *Ankistrodesmus* sp. is an interesting freshwater microalga capable of producing oil due primarily to its high lipid content, about 28-40% [16]. *Ankistrodesmus* sp. is a species of green photoautotrophic and unicellular microalgae which can be easily cultivated in the laboratory and grow fast under suitable conditions. Literature

demonstrates that *Ankistrodesmus* sp. cultivated under well temperature controlled chamber with proper light:dark cycle, i.e. 12:12 h, could provide a maximum specific growth rate and cell density of 0.85 d^{-1} and 8.5×10^{6} cells mL^{-1}, respectively. This lipid content could be enhanced when cultivated under low nutrients [17, 18, 19, 20]. Apart from that, *Ankistrodesmus* sp. could also accumulate a relatively high protein content of 47.31% [21], while keeping a low carbohydrate content of 14.5% [16].

This study examined the lipid production from *Ankistrodesmus* sp., which was cultivated in an airlift photobioreactor. This started from the investigation on the mode of biomass production, i.e. batch and continuous cultures, followed by the study of the effect of environmental/operating conditions on the stimulation of algal lipid. Aim to investigate the effect of pH and carbon dioxide concentration in batch culture. Furthermore, aim of the present study was to minimize the algal operating cost for the cultivation of green microalgae *Ankistrodesmus* sp. by reusing the medium in both batch and continuous cultures. The culture was optimized to determine proper operating conditions that provide the best lipid yield where the relationship between nutrients consumptions and biodiesel production was examined. For batch cultures, the culture medium was used in three consecutive growth cycles, whereas the continuous cultures were cultivated with a concocted reused medium. The influences of nutrient on growth and the accumulation of fatty acid in *Ankistrodesmus* sp. were also discussed.

1.2 Objectives

1.2.1 To optimize cultural conditions in order to maximize lipid production of *Ankistrodesmus* sp.

1.2.2 To investigate the medium recycling algorithm to minimize operating costs during the culture of such alga.

1.3 Scope of Research Work

The determination of the optimal conditions for *Ankistrodesmus* sp. cultivation was conducted in a steady state, continuously operating airlift photobioreactor located within an evaporation chamber where the temperature was controlled at 30°C by an evaporative system. The system was operated under natural light intensity. The research was carried

out with three experiments, (1) batch culture of *Ankistrodesmus* sp. in Duran bottle and airlift photobioreactor, (2) enhancing *Ankistrodesmus* sp. growth with carbon dioxide supplement and (3) increasing lipid production with reuse and modified BG11 media. The manipulating variables are:

- pH levels at 6, 7 and 8
- CO_2 concentrations at 1, 2, 5 and 10%
- Dilution rates at 0.08, 0.16 and 0.24 d^{-1}
- Nitrogen and phosphate concentrations in the recycling medium

CHAPTER 2
THEORIES AND LITERATURE REVIEW

2.1 Microalgae

Microalgae are prokaryotic or eukaryotic photosynthetic microorganisms. Examples of prokaryotic algae are cyanobacteria (*Cyanophyta*), the traditional name of blue green algae. Eukaryotic microalgae included green algae (*Chlorophyta*) and diatom (*Bacillariophyta*) [22, 23]. Microalgae can live in a wide range of environmental conditions, not only aquatic but also terrestrial. Microalgae are living in saline and fresh water that convert sunlight, water and carbon dioxide to biomass. All algae contain protein, carbohydrate, lipid and nucleic acid while the proportions are vary with the species of algae. Many microalgal species can accumulate substantial amount of lipid [1].

Microalgae are a promising alternative sources for biodiesel production, because they are grow much faster than plants, easily cultivated, low nutrients requirement, could be cultured in some wastewater, reduce carbon dioxide emission. The advantages of microalgae for producing biodiesel is as follows [1, 23, 24]:

- Grow rapidly and contain high content of lipids
- Grow anywhere and live in harsh conditions
- Treat agricultural wastewater that contain excess nitrogen and phosphorus nutrients
- Harvest algal daily
- Produce useful by-products, including long-chain polyunsaturated fatty acids, and carotenoids for foodstuffs
- Not require arable land
- Fix CO_2 and reduce carbon emissions via photosynthesis
- Release to minimize concentrations of sulphur dioxide, nitrous oxide and other contaminants when using algae biofuel

Each fresh water microalgae species constitutes a different level of biochemical composition, as shown in Table 2.1.

Table 2.1 Lipid content of various freshwater microalgae species

Microalgae species	Lipid content (%)	Protein content (%)	Carbohydrate content (%)	Reference
Ankistrodesmus sp.	40	20	20	Singh *et al*. [25]
Botryococcus braunii	25-75	17	32	Ashokkumar and Rengasamy [26]
Chlorella vulgaris	14-22	51-58	12-17	Carioca *et al*. [27]
Dunaliella salina	6	57	32	Chisti [28]
Isochrysis sp.	25-33	25	5-16	Chisti [28] Sánchez *et al*. [29]
Nannochloropsis sp.	31	36	18	Fábregas [30]
Scenedesmus obliquus	12-14	50-60	10-17	Carioca *et al*. [27]

2.2 *Ankistrodesmus* sp.

Ankistrodesmus sp. is a freshwater species of green photoautotrophic and unicellular microalgae under the scientific classification as detailed in Table 2.2. *Ankistrodesmus* sp. grows in freshwater. It can be easily cultivated in a laboratory. The various shapes of cells are long and needle, spindle, curve or slightly crescent. The cells may be found individually, clustered, or twisted around each other. The parietal chloroplasts sometimes have pyrenoids. *Ankistrodesmus* is a form of asexual reproduction, the parent cells divides to give the new cell. The parental cell wall ruptures to release 1-16 spores that develop into new cells. *Ankistrodesmus* biosynthesize essential nutrients like protein, lipid, minerals, fiber, essential amino acids, and polyunsaturated fatty acid. Radakovits *et al*. [31] reported that *Ankistrodesmus densus* secretes polysaccharides when exposed to light during stationary phase. Thomas *et al.* [12] analyzed the fatty acid

compositions of *Ankistrodesmus* sp. are C16:4 (hexadecatetraoneic acid) and C18:4 (octadecatetraenic). Tavares and Pereira [32] reported that *Ankistrodesmus gracilis* is used as food source for fish larvae due to its relatively high protein content. The fertilizer NPK (20-5-20) is used as the medium to improve the biomass production under large scale laboratory culture in 850 L tanks was achieved. *Ankistrodesmus convoiutus* was cultured in agro-industrial effluent, including latex concentrate effluent, standard Malaysian rubber effluent and digested palm oil mill effluent, containing high organic matter. The amount of crude protein and lipid of *Ankistrodesmus convoiutus* cultured in effluent mediums were higher than control. The lipid of *Ankistrodesmus convoiutus* contained mostly polyunsaturated fatty acids of C18 and C20 [33]. National Institute for Environmental Studies, Japan [34] reported that *Ankistrodesmus densus* TISTR 8505 found at the first time at Nakorn Pathom, Thailand. Table 2.3 displays the various conditions for *Ankistrodesmus* sp.

Table 2.2 Scientific classification of *Ankistrodesmus* sp. [35]

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

Table 2.3 Reviews of *Ankistrodesmus* cultivation

Author	Year	Strains	Reactor	Medium	Volume	Temp (°C)	Light intentsity (Lux)	pH	Time (d)	Biomass concentration ($g\ L^{-1}$)	Productivity	μ (d^{-1})	Cell concentration ($\times 10^4$ cells mL^{-1})
Griffith et al. [7]	2009	*A. falcatus*	Airlift	Bold Basal (BBM)	3.2 L	25	18,500		14	1.12-1.48	13-55 $mg\ L^{-1}\ d^{-1}$	0.99	
Weissman [36]	1988	*A. falcatus*	Pond	Bold Basal (BBM)	1.4 m^2			6.5-9.6			18 $g\ m^{-2}\ d^{-1}$		
Nayak et al. [37]	1996	*A. falcatus*	Flask	Liquid sterilized	100 mL	27	5,180	7.0	7				
Chu et al. [38]	1995	*A. convolutus*	Flask	Bold Basal (BBM)	1 L	28	3,108	7.0-7.6		0.453		0.93	
Pinto et al. [39]	2003	*A. braunii* (no. 202.7a)	Flask	Bold Basal (BBM)	50 mL	25	7,400	6.2	5			3.85	
Burrell et al. [40]	1985	*A. braunii* (ATCC12744)	Flask	Bristol Medium	500 mL	24	2,220						
LH and AML [21]	2008	*A. gracilis*	Tank	CHU_{12}	850 L	24	5,782	6.7-7.4	15			0.15	6.72
Salim [41]	2013	*Ankistrodesmus* sp.	Tube	Bold Basal (BBM)	500 mL	26	2,970	6.5	14	1.6			40
Kalita et al. [42]	2011	*A. falcatus*	Flask	BG11 BBM	500 mL 500 mL	25 25	1,500-2,000 1,500-2,000		24 24				90 117

2.3 Photosynthesis in microalgae

Photosynthesis is a process in which green plants and many microbes utilize the energy of sunlight to produce carbohydrate and water. Photosynthesis can be summarized in Fig. 2.1. Photosynthesis occurs in chloroplasts, where the light trapping pigments are located on the thylakoids. Pigments are including three major classes such as chlorophylls *a*, *b*, c and *d*, carotenoids, and phycobilins. All chlorophylls have two major absorption bands at 450-475 nm (blue or blue-green) and 630-675 nm (red). Chlorophyll *a* contains four pyrrole rings. Chlorophyll *b* is the similar structure as chlorophyll a but a keto group links with the second pyrrole ring. And Chlorophyll *c* has only the hydrophilic porphyrin head without the phytol tail. Carotenoids are C_{40} hydrocarbon chains which strongly hydrophobic. An absorption range of between 400 and 550 nm. Phycobilins are linear tetrapyrroles, there are not a magnesium atom. These pigments absorb at 500-650 nm [22, 43].

Algae use water as electrons and proton donors. Oxygen is released as a by-product. Photosynthesis mechanism is divided into 2 stages. The first stage is the light-dependent (light) reaction. The photosynthetic light reactions occur in the thylakoid membranes of chloroplast in algae. Electron transfer through photosystem I (P700) and photosystem II (P680). Chemical energy is converted from light energy with providing 2 moles of $NADPH_2$ and 3 moles of ATP. The second stage is the light-independent (dark) reactions or called Calvin-Benson cycle, which take place in the stroma. The sequence of reaction, these electrons are used along with energy from ATP to reduce CO_2 to carbohydrate. This synthesis of carbohydrate by using carbon atom from CO_2 gas is also called carbon fixation.

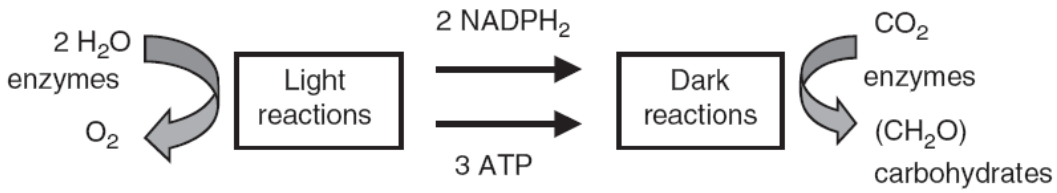

Fig. 2.1 Light and dark reactions of photosynthesis process

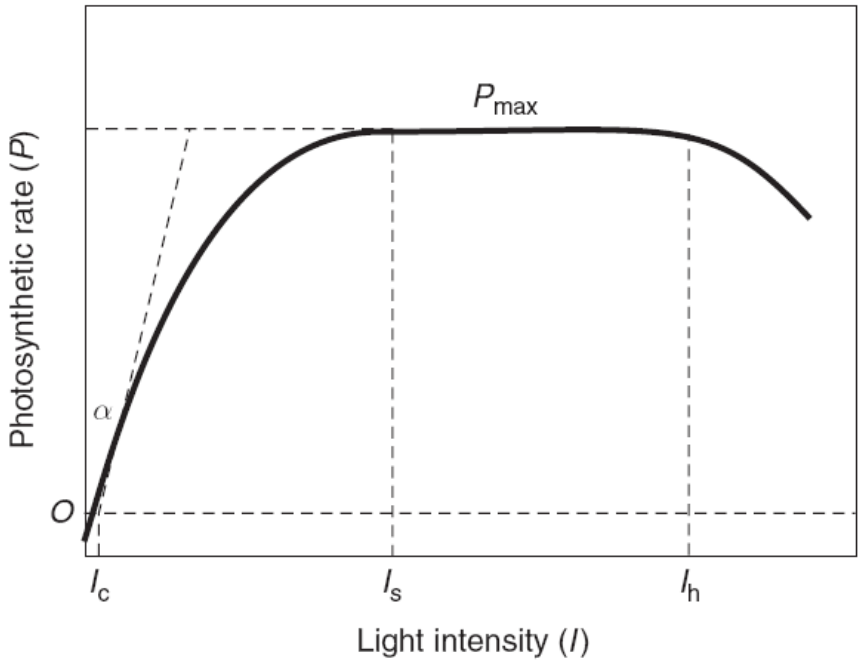

Fig. 2.2 Light response curve of photosynthesis

Proportion of oxygen evolution from light intensity could measure photosynthetic activity. The photosynthetic light respond curve (*P/I* curve) is a relationship between the photosynthetic rate (*P*) and light intensity (*I*), as shown in Fig. 2.2. The *P/I* curve can be divided into three region; a light limited region which photosynthesis increases with light intensity increased, a light saturated region which photosynthesis is independent of irradiance and a photoinhibited region which photosynthesis decreases with increasing light intensity. P_{max} is the maximum rate of photosynthesis.

2.4 Environmental factors

A multicriteria strategy considering factors such as light, pH and temperature has to be adapted for microalgae cultivation

2.4.1 Light

Light intensity plays an important role for algae. Light is the source of energy that drives the photosynthetic reaction. The intensity of light, spectral quality and photoperiod should be considered. Furthermore, Microalgae densities and the depth of photobioreactor also investigated. The light intensity must be increase at higher depth and cell concentration of algae. The range of the light intensity between 100 and 200 $\mu E\ s^{-1}\ m^{-2}$ are

the most operative in the culture, which corresponds to about 5-10% of full daylight (2000 μE s^{-1} m^{-2}). In case where the light intensity is too high, the photoinhibition may occur. At constant light intensity, some species of microalgae do not grow well, therefore a light/dark cycle is applied. The daily light/dark cycle is usually performed at 14:10 or 12:12 h:h (night:day). Jacob-Lopes *et al*. [44] were reported that the photoperiod has an effect on biomass and carbon dioxide fixation rate. The carbon fixation rate can reached up 99.69% with culture under continuous illumination (24:0) and a linear reduction in biomass production and carbon dioxide fixation with reductions in the duration of the light period was evident except in the light cycle of the 12:12. Apart from that, typically this research use sunlight.

2.4.2 Temperature

The most important factors for microalgae cultivation is the temperature, which it has influence on accumulation of biochemical composition of microalgae. The temperature between 16 and 27 °C is the most temperature for algal cultivation, which vary with the species of microalgae and composition of culture medium. The growth of algae will slow down with the temperature lower than 16 °C, whereas some species of microalgae are dead at temperature higher than 35 °C.

2.4.3 pH

The most pH range for microalgae cultivation is between 7 and 9. In the case of high density algal culture, carbon dioxide is usually added to adjust the pH, which may higher pH 9 during algal growth.

The summary of the cultivation systems and their operating conditions along with the growth parameters is illustrated in Table 2.4.

Table 2.4 Literature review

Author	Year	Strains	Reactors	Medium	Volume (L)	T (ºC)	Light intensity (Lux)	pH	Cell concentration ($x10^6$ cells mL^{-1})	Biomass concentration ($g\ L^{-1}$)	Biomass productivity ($g\ L^{-1}d^{-1}$)	Specific growth rate (d^{-1})
Imamoglu et al. [45]	2007	*Haematococcus pluvialis*	Flask	Rudic	0.25	25	2,960	8	0.95			0.195
Solovchenko et al. [46]	2008	*Parietochloris incisa*	Glass column	BG11	1	25	29,600			8		0.47
Choochote et al. [47]	2010	*Chlorella* sp.	Glass column	Modified N-8	0.3		5,000		388	19.82		
Renaud et al. [48]	2002	*Chaetoceros* sp.	Flask	F/2	1.5	30	5,920	8.3	3.28			0.87
		Rhodomonas sp.				25			0.59			0.35
		Cryptomonas sp.				25			1.02			0.33
		Prymnesiophyte sp.				27			4.97			0.56
		Isochrysis sp.				27			4.94			0.97
Sancho et al. [49]	1999	*Scenedesmus obliquus*	Photobioreactor	Modified	1	30	15,067	6.5		0.12	0.047	
Jeon et al. [50]	2006	*Heamatococcus pluvialis*	Flask	Sterilized	0.25	25	5,920				0.104	
				Optimal			7,400				0.130	
				Heamatococcus			9,000				0.144	
Bouterfas et al. [51]	2006	*Selenastrum* sp.		Mineral medium	0.019	30	27,010					1.55
		Coelastrum sp.			0.383		28,860					1.59
		Cosmarium sp.			1.738		26,640					0.88
Bartley et al. [52]	2014	*Nannochloropsis salina*	Open pond	F/2				8	95.6			0.19
								9	92.8			0.19
Taraldsvik and Myklestad [53]	2000	*Skeletonema costatum*	Flask	Guillard's f medium	2.5	13	14,800-18,500	6.5	0.51			2.4
								7.5	0.56			2.5
								8.0	0.49			2.3
								8.5	0.51			2.4
								9.0	0.32			1.7
Converti et al. [54]	2009	*Chlorella vulgaris*	Flask	Bold's Basal	2	30	5,180					0.14
						35						0.12
						38						0.01
		Nannochloropsis oculata		Guillard F/2 one		15						0.06
						20						0.13
						25						0.07

2.5 Nutritional factors

2.5.1 Carbon

Carbon is supplied as an inorganic substrate in form of CO_2 and HCO_3^-, which are most important for high rates of autotrophic production. Algal biomass contains 50% C. In water, CO_2 may occur as H_2CO_3, HCO_3^- and CO_3^{2-}depending on pH as demonstrated in Fig. 2.3. The major buffer in freshwater is CO_2-H_2CO_3-HCO_3^--CO_3^{2-}which is useful for maintaining the pH within the optimal range for most cultivated species. The direct injection of CO_2 into the medium culture is the most convenient method to control the pH levels. The gaseous CO_2 can be produced from the bicarbonate and carbonate buffer system for photosynthesis though the following reactions:

$$2HCO_3^- \Leftrightarrow CO_3^{2-} + H_2O + CO_2 \quad (2.1)$$

$$HCO_3^- \Leftrightarrow CO_2 + OH^- \quad (2.2)$$

$$CO_3^{2-} + H_2O \Leftrightarrow CO_2 + 2OH^- \quad (2.3)$$

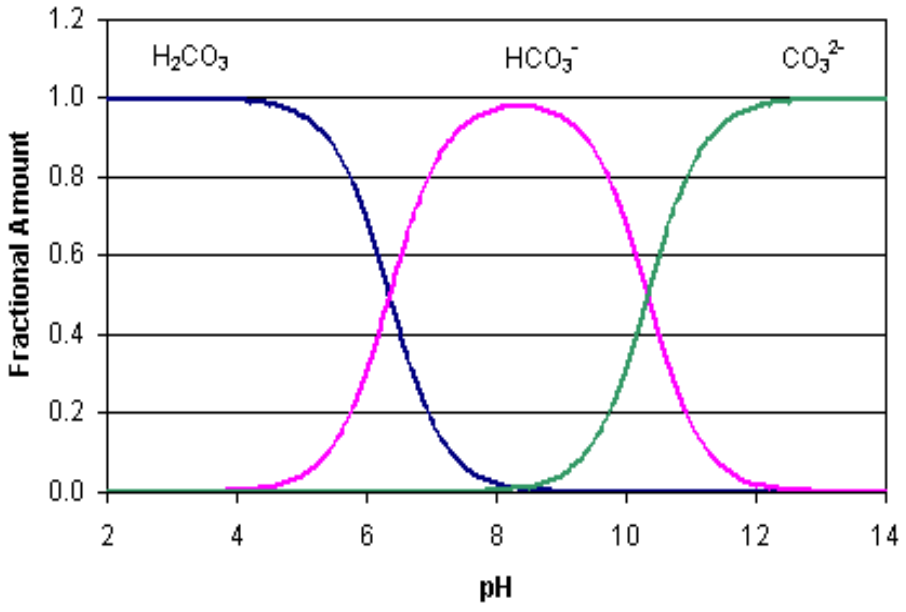

Fig. 2.3 pH-dependency of $H_2CO_3/HCO_3^-/CO_3^{2-}$ equilibrium

2.5.2 Nitrogen

Nitrogen is an important nutrient for the algal biomass. The range of the nitrogen content of the biomass is between 1% and 10%. Nitrate (NO_3^-), ammonium (NH_4^+) and urea (NH_2CONH_2) are commonly used as nitrogen source for algal cultivation, but the ability to use these compounds depends on algal species. An increasing of pH when nitrate is supplied as the only N-source.

2.5.3 Phosphorous

Phosphorus is essential for algae growth as it plays an important role for many cellular processes, such as energy transfer, and the synthesis of nucleic acid. Although algal biomass contains less than 1% P, it is one of the most important growth limiting factors. Most algae acquired phosphorus in inorganic forms, either as $H_2PO_4^-$ or HPO_4^{2-}. The optimal phosphorus concentration in the medium, as well as the phosphorus tolerance, varies with different species, even if all other nutrients are supplied in sufficient concentrations. Phosphorus limitation up to a certain level can potentially increase the production and storage of lipid in microalgae.

2.5.4 Other macronutrients and micronutrients

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

The functions of the minerals in algal cells are summarized in Table 2.5.

Table 2.5 Elements required for algal growth [55]

Element	Examples of function and location in algal cells
N	Amino acid, nucleotides, chlorophyll, phycobilins
P	ATP, DNA, phospholipids
S	Some amino acids, nitrogenase, thylakoid lipids
Na	Nitrate reductase
Ca	Alginates, calcium carbonate, calmodulin
Mg	Chlorophyll
Fe	Ferrodoxin, cytochromes, nitrogenase, nitrate and nitrite reductase, catalase, glutamate synthetase
K	Agar and carrageenan, osmotic regulation, cofactor for many enzymes
Mo	Nitrate reductase, nitrogenase
Mn	Oxygen-evolving complex of photosystem II
Zn	Carbonic anhydrase, Cu/Zn superoxide dismutase, alcohol dehydrogenase
Cu	Plastocyanin, Cu/Zn superoxide dismutase, cytochrome oxidase
Co	Vitamin B12

The literature review of difference nutrients with the growth parameters is illustrated in Table 2.6.

Table 2.6 Literature review

Author	Year	Strains	Reactor	Medium	Volume (L)	CO_2 concentration (% of air)	CO_2 fixation	Specific growth rate (d^{-1})	Biomass concentration ($g\ L^{-1}$)	Cell concentration ($x10^6$ cells mL^{-1})	Productivity ($g\ L^{-1}d^{-1}$)	CO_2 removal efficiency (%)
Morais and Costa [56]	2007	*Spirulina* sp.	Column Photobioreactor	Modified Zarrouk	1.8	0		0.33	0.82			
						6	27.14-37.90 %	0.44	3.40			
						12	6.70-17.06 %	0.33	3.38			
		Scenedesmus obliquus				0		0.15	0.31			
						6	7.40-13.45 %	0.22	1.56			
						12	4.39-8.63 %	0.22	1.80			
Tang et al. [57]	2011	*Scenedesmus* sp.	Flask	Modified BG11	1	0.03	0.150 $g\ L^{-1}d^{-1}$	0.51	1.05		0.083	
						5	0.286 $g\ L^{-1}d^{-1}$	0.94	1.80		0.158	
						10	0.288 $g\ L^{-1}d^{-1}$	0.89	1.84		0.155	
						0.03	0.134 $g\ L^{-1}d^{-1}$	0.69	0.87		0.065	
						5	0.244 $g\ L^{-1}d^{-1}$	0.99	1.44		0.133	
						10	0.260 $g\ L^{-1}d^{-1}$	0.99	1.55		0.144	
Nakanishi et al. [58]	2014	*Chlamydomonas* sp.	Flask	HSM medium		0.04			0.2	0.9		
						2			4.0	7.8		
						4			3.7	7.7		
						8			3.3	5.8		
Cheng et al. [59]	2006	*Chlorella vulgaris*	Cylindrical glass photobioreactor			0.5	65 $mg\ L^{-1}\ h^{-1}$					27
						1	80 $mg\ L^{-1}\ h^{-1}$					13
						2	49 $mg\ L^{-1}\ h^{-1}$					5
						2.5	28 $mg\ L^{-1}\ h^{-1}$					2.5
Zheng et al. [60]	2011	*Tetraselmis subcordiformis*	Rectangular airlift photobioreactor	Modified Walne medium		1.63	39.11 $mg\ L^{-1}\ h^{-1}$	0.0121			0.56	10.18
						5	56.14 $mg\ L^{-1}\ h^{-1}$	0.0341			0.82	3.18
						10	47.27 $mg\ L^{-1}\ h^{-1}$	0.0149			0.70	2.01
						15	46.83 $mg\ L^{-1}\ h^{-1}$	0.0299			0.70	1.93
						18.37	25.97 $mg\ L^{-1}\ h^{-1}$	0.0085			0.38	0.60
Chiu et al. [14]	2009	*Nannochloropsis oculata*	Flask	Modified F/2		0.03		0.194	0.268			
						2		0.571	1.277		0.480	
						5			0.100		0.441	
						10			0.020		0.398	

Table 2.6 Literature review (Cont.)

Author	Year	Strains	Medium	N concentration	P concentration	Other elements concentration	Biomass concentration ($g\ L^{-1}$)	Biomass productivity ($g\ L^{-1}d^{-1}$)	Cell concentration ($x10^6$ cells mL^{-1})	Lipid content (%)	Protein content (%)	Carbohydrate content (%)
Feng et al. [61]	2011	*Isochrysis zhangjiangensis*	F/2	24h with 75 mg L^{-1}			3.1		121	40.9	34.1	23.2
				48h with 75 mg L^{-1}			2.6		65	34.7	23.0	36.0
				72h with 75 mg L^{-1}			2.0		40	29.8	16.0	47.7
				N-depletion					58	26.0	15.0	47.8
Wu et al. [62]	2013	*Monoraphidium* sp.	Artificial medium	1.8 mM NH_4NO_3			0.11			28.0		
				3.6 mM KNO_3			0.65			31.5		
				3.6 mM NH_4Cl			0.17			31.2		
Mata et al. [63]	2013	*Dunaliella tertiolecta*	Artificial seawater medium	10XN				141.79		33.45		
				20XN				83.08		20.45		
						10XFe		124.36		22.55		
						20XFe		100.00		17.50		
Li et al. [64]	2008	*Neochloris oleoabundans*	Modified soil extract	N from nitrate			2.5			38.0		
				N from urea			2.1			17.5		
				N from ammonium			1.1			19.0		
Xin et al. [65]	2010	*Scenedesmus* sp.	Modified BG11	2.5 mg L^{-1}	1.3 mg L^{-1}				0.32	30		
				5.0 mg L^{-1}	1.3 mg L^{-1}				0.48	23		
				10.0 mg L^{-1}	1.3 mg L^{-1}				0.81	21		
				15.0 mg L^{-1}	1.3 mg L^{-1}				1.01	24		
				25.0 mg L^{-1}	1.3 mg L^{-1}				1.34	25		
				10.0 mg L^{-1}	0.1 mg L^{-1}				0.26	55		
				10.0 mg L^{-1}	0.2 mg L^{-1}				0.47	29		
				10.0 mg L^{-1}	0.5 mg L^{-1}				0.60	28		
				10.0 mg L^{-1}	1.0 mg L^{-1}				0.72	24		
				10.0 mg L^{-1}	2.0 mg L^{-1}				0.98	22		
Markou et al. [66]	2012	*Spirulina platensis*	Modified Zarrouk		10 mg L^{-1}		0.86			3.78	21.11	66.60
					20 mg L^{-1}		2.16			7.43	23.38	56.94
					30 mg L^{-1}		2.27			7.87	41.12	36.61
					40 mg L^{-1}		2.19			8.17	37.89	31.36
					50 mg L^{-1}		2.03			8.41	40.98	13.76
					500 mg L^{-1}		1.93			8.20	42.92	10.99
Nigam et al. [67]	2011	*Chlorella pyrenoidosa*	Fogg's medium	0 g L^{-1}			0.076			19		
				0.05 g L^{-1}			0.127			26		
				0.10 g L^{-1}			0.246			18		
				0.20 g L^{-1}			0.296			15		
				0.40 g L^{-1}			0.315			11		

Table 2.6 Literature review (Cont.)

Author	Year	Strains	Medium	N concentration	P concentration	Other elements concentration	Specific growth rate (d^{-1})	Cell concentration ($x10^6$ cells mL^{-1})	Lipid content (%)	Protein content (%)	Carbohydrate content (%)
Cakmak et al. [68]	2012	*Chlamydomonas reinhardtii*	Tri-acetate-phosphate	Control				12.27	16.8		
				N-starved				2.09	39.8		
						S-starved		4.22	37.6		
Liu et al. [69]	2008	*Chlorella vulgaris*	F/2-Si			0		14	7.8		
						Fe 1.2×10^{-8} mol L^{-1}		20	11.8		
						Fe 1.2×10^{-7} mol L^{-1}		20	12.2		
						Fe 1.2×10^{-6} mol L^{-1}		20	16.5		
						Fe 1.2×10^{-5} mol L^{-1}		17	56.6		
Ulloa et al. [70]	2011	*Tetraselmis suecica*	Seawater medium			0		4.2	25	43	23
						Mg 0.01 mM		4.3	26	50	25
						Mg 0.1 mM		4.5	22	40	30
						Mg 1 mM		4.2	20	52	24
						Mg 3 mM		7.0	28	42	26
						Mg 10 mM		6.0	23	40	29
Yeesang and Cheirsilp [71]	2011	*Botryococcus* sp.	Modified Chu 13	N-rich					25.8		
				N-deficient					32.3		
Converti et al. [54]	2009	*Chlorella vulgaris*	Bold's Basal	$NaNO_3$ 0.375 g L^{-1}			0.13		15.31		
				0.75 g L^{-1}			0.14		14.37		
				1.50 g L^{-1}			0.14		5.90		
		Nannochloropsis oculata		0.075 g L^{-1}			0.10		15.86		
				0.15 g L^{-1}			0.10		13.01		
				0.30 g L^{-1}			0.13		7.88		
Rukminasari [72]	2013	*Nannochloropsis* sp.	PhK medium	Control				7.92	0.02 g L^{-1}		
				No-N				2.22	0.14 g L^{-1}		
					No-P			2.21	0.08 g L^{-1}		
Huang et al. [73]	2013	*Tetraselmis subcordiformis*	F/2	0 mmol L^{-1}			0.014		27.50		
				0.88 (control)			0.038		17.50		
				1.76 mmol L^{-1}			0.040		13.40		
		Nannochloropsis oculuta		0 mmol L^{-1}			0.033		34.70		
				0.88 (control)			0.068		24.80		
				1.76 mmol L^{-1}			0.074		22.50		

2.6 Culture methods

There are many types of algal cultivation which used in worldwide. The most commonly used consist of batch, continuous and semi-continuous cultures.

2.6.1 Batch cultures

This is the most common culture system for the cultivation of microalgal cells due to its ease of operation and its simplicity. In batch cultures, a limited amount of culture medium is given at the beginning of the culture and no further input of nutrients. The algal cell density increases constantly until the exhaustion of some limiting factors.

Several different phases may occur in a batch culture, which indicate the changes in biomass quantity. Fig. 2.4 illustrates all the possible six growth phases consisting of 1) adaption (lag phase), 2) accelerating growth phase, 3) exponential growth (log phase), 4) decreasing log growth (linear growth), 5) stationary phase, and 6) accelerated death. Different phases of the growth curve may alter in length and slope according to the condition prevailing in the culture.

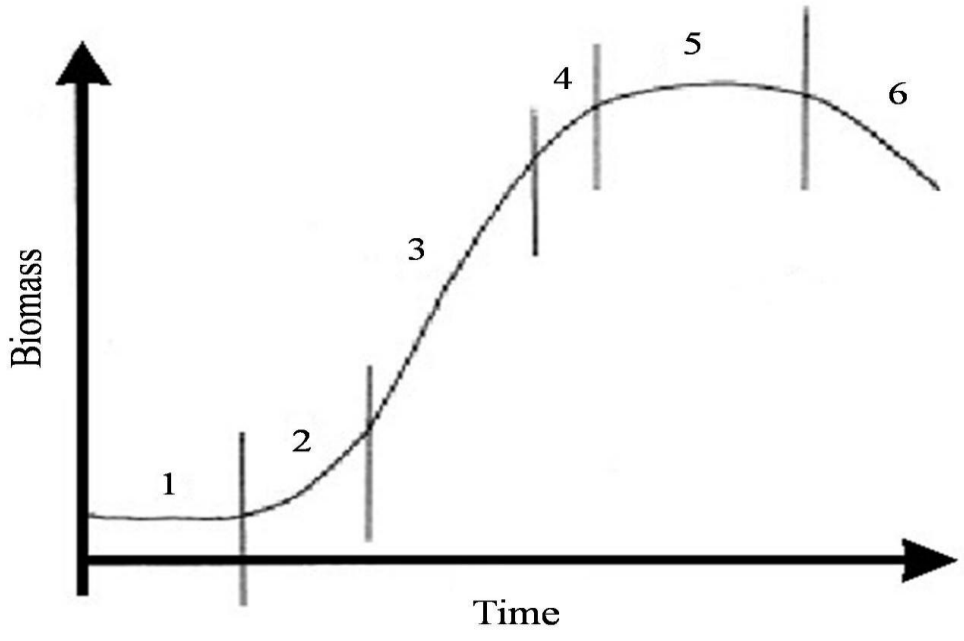

Fig. 2.4 Growth phase of algal under batch culture conditions

Phase 1 (lag phase): the new medium condition is usually different from the previous condition from which microalgae we taken. In general, microalgae are not adapted to the new environment and may even be in an unhealthy condition. At the same time, the algal culture adjusts itself to altered condition. During this phase, the algal growth rate is close to zero.

Phase 2 (accelerating growth phase): after the cell adjusts to the new medium, it starts to grow and multiply.

Phase 3 (log phase): after a short phase of growth acceleration, the cell growth rate increases rapidly at a rate that is controlled by many other growth factors, e.g. nutrient concentrations, light intensity, etc. The cell density increases as a function of time.

Phase 4 (linear growth phase), growth rate reduction is occurred due to either the depletion of nutrients or the accumulation of toxic compounds released during the growth.

Phase 5 (stationary phase), the equilibrium is reached between the cell growth and degradation processes. This phase normally describes the maximum attainable concentration of algal biomass in a closed system.

Phase 6 (accelerated death phase), the algal cell stops growing due to exhaustion of nutrients or high toxic concentration.

2.6.2 Continuous cultures

Nutrients are infinite in continuous cultures. The fresh culture medium is added into the system continuously. The rate constant is controlled by a peristaltic pump. A volume of influent medium is equal to the volume of removed medium. The old medium flows out to harvest tank. Fig. 2.5 shows diagram of a continuous culture.

Continuous culture is a different function from batch culture in that in a continuous culture, the medium is supplied into the culture system with constant flow rate for keep constant working volume. As a result, the cell populations reach a steady state.

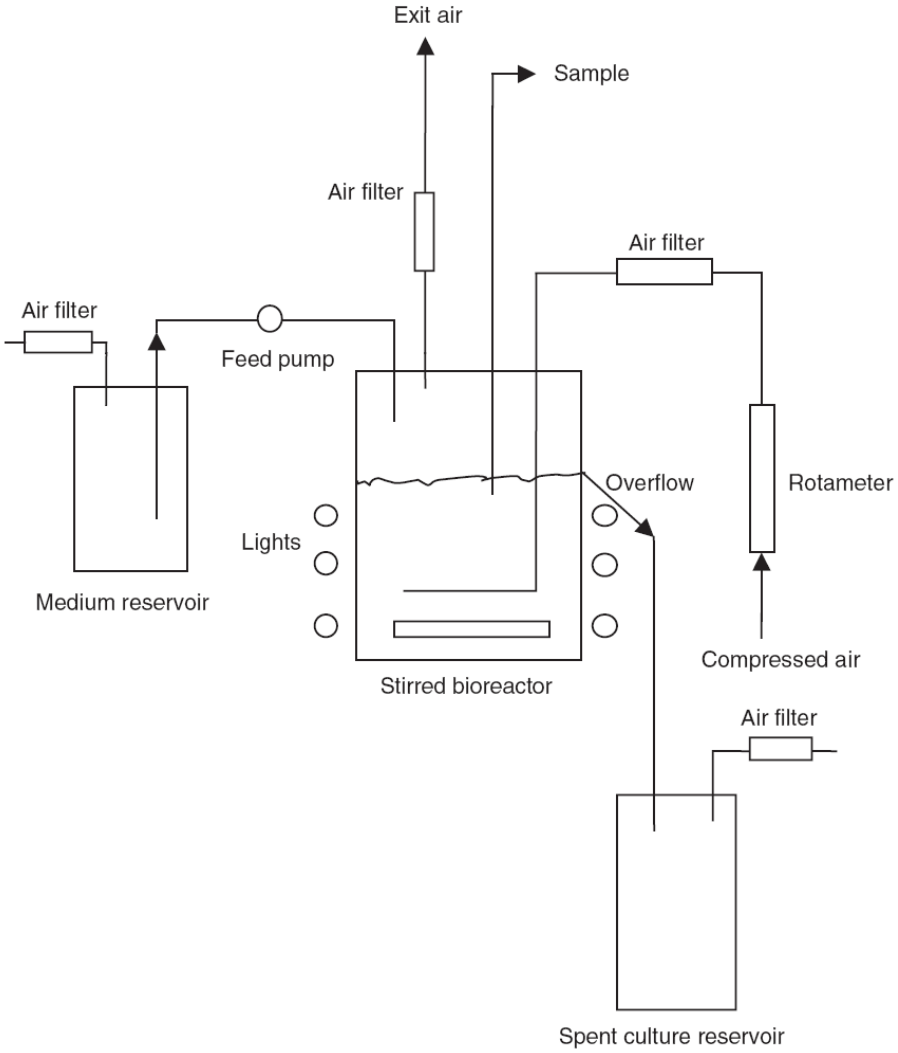

Fig. 2.5 Schematic diagram of a continuous culture setup

The dilution rate of continuous culture (*D*) can be calculated with the rate of inflow medium similarly to the flow rate of the culture (*F*) and the working volume (*V*) as follows:

$$D = F/V \tag{2.4}$$

For an infinitely small time interval, this balance for the culture could be written as:

$$dx/dt = (\mu - D)X \tag{2.5}$$

where,

dx is increase in biomass concentration (g l^{-1})

dt is Infinitely small time interval (h)

μ is specific growth rate (h^{-1})

D is dilution rate (h^{-1})

X is biomass concentration (g l^{-1})

At steady state, an increase in biomass concentration during an infinitely small time interval is zero ($dx/dt = 0$). That is no net increase in the biomass concentration takes place. The specific growth rate equals the dilution rate as follows,

$$\mu X = DX \tag{2.6}$$

$$\mu = D \tag{2.7}$$

Continuous culture can be classified into 2 categories:

- Chemostat culture, the medium flows into the culture system at a steady state and maintaining the specific growth rates at predetermined values. Temperature, pH and substrate concentration as the culture parameters could be adjusted for maintained specific growth rates.
- Turbidostat culture, which is an elaboration of the chemostat. The medium is supplied when the algal density reaches at predetermined point. Turbidity of culture is measured by spectrophotometer. The medium is added into culture system until biomass concentration rises above a chosen level.

The advantages of a continuous culture system are as follows:

- Provide a high degree of control
- Algal densities can be controlled by vary the dilution rate and the culture system extended the cultivation for long time periods.
- Results are easily reproducible at the steady state
- Reduce the need for labor due to its automation

2.6.3 Semi-continuous cultures

In a semi-continuous system, the fresh medium is supplied into the culture system at once and spent culture flow out into a harvest tank. Algae can be grown in the culture medium for 24 hours. Subsequently, the procedure is repeated.

2.7 Photobioreactors for microalgae cultivation

A photobioreactor is a device for microalgae cultivation, the design are varied depending on the environment conditions and available materials [23]. Light is a main energy source for photosynthesis cells therefore light intensity comes in to the photobioreactor should be concerned. High light intensity at photobioreactor surface area may cause of photoinhibitor which resulted in low photosynthetic rate [74]. Microalgae can be grown in open ponds and enclosed photobioreactors, there are limitation and advantages of each type.

2.7.1 Open ponds

Open pond systems often used for commercial algal production, especially raceway ponds and circular ponds [75]. The reasons for this are relatively economical and can utilize natural sunlight. Furthermore, it is cleaning up easily after cultivation. Rawat *et al*. [76] has been reported that this system can be used domestic municipal wastewater as medium for cultivation. In addition, the flue gas may add into the open pond to speed up the photosynthetic rate, if the system is located near power plant. However, this system has many disadvantages which relate to the limiting factors of microalgae production. The major weakness of the open pond system is a little control of culture conditions. The other advantages from the open pond are required large area for cultivation, easily contaminated by other microorganism so it is difficult to maintain a monoculture, expensive cost for harvesting procedure. Furthermore, settling causes low productivity which it is difficult to distribute nutrients and dark zone appearance, therefore the mixing equipment have been provided in the system, such as paddle wheels, rotating arms and pump, which increase the capital and operating costs of these systems (Fig. 2.6).

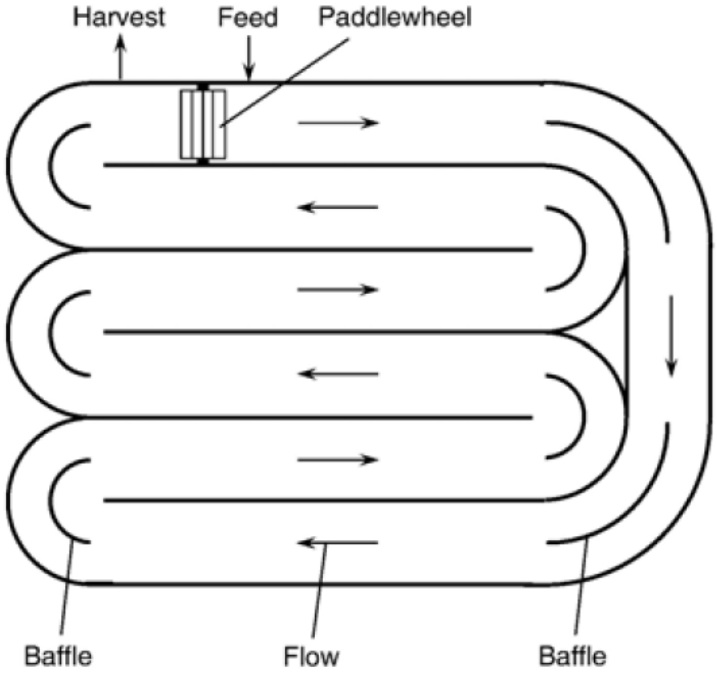

Fig. 2.6 Open pond on top view

2.7.2 Enclosed photobioreactors

Enclosed photobioreactors can be produced high biomass productivity and it is easily control of culture condition. There are various types of photobioreactors designed for different purposes, where the main categories include: (1) tubular; (2) airlift; and (3) flat plate.

- Tubular photobioreactors

Many different designs of photobioreactors have been developed, but tubular is the most successful on a large scale to produce algal biomass for biofuel production [28] due to the tube having solar collectors to capture sunlight for photosynthesis [77]. A tubular photobioreactor consists of one or more relatively transparent tubes that are usually made of glass or plastic, as present in Fig. 2.7 [28]. Although tubular photobioreactor is suitable for commercial large scale culture of microalgae which partly relies on the multiplication of bioreactor unit [78]. However, the limitation of tubular photobioreactor is the length of the tubes. It is limited by oxygen accumulation, carbon dioxide depletion and pH variations [74].

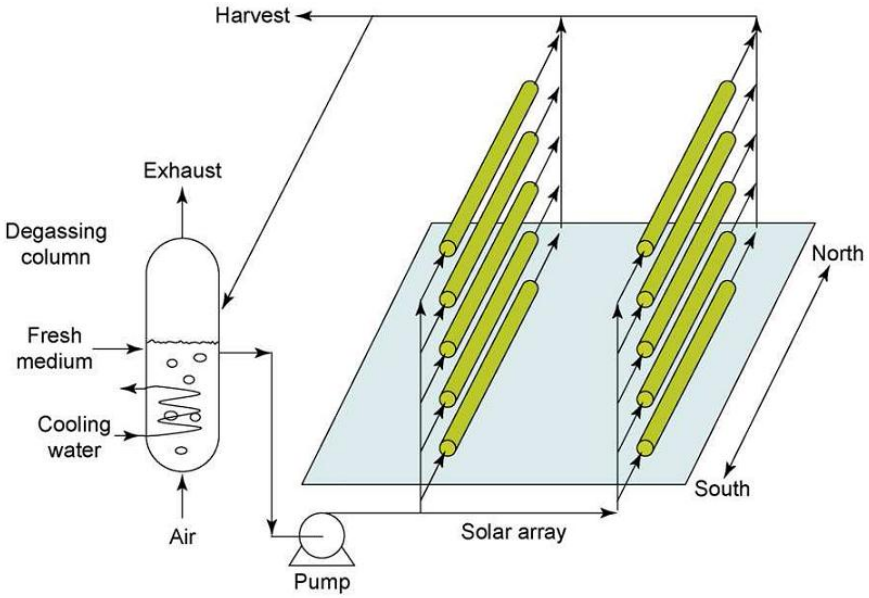

Fig. 2.7 Tubular photobioreactor with fence like solar collectors

- Airlift photobioreactor

An airlift photobioreactor is similar to a bubble column reactor, but differs in that it contains a draft tube. The draft tube is always an inner tube which helps improves circulation and oxygen transfer in the reactor. Fig. 2.8 illustrates the basic structure of an air lift photobioreactor which comprises of four differently sections including (1) riser: the gas is injected at the bottom of this section and the flow of gas and liquid is upward. (2) downcomer: this section is parallel to the riser which the flow of gas and liquid is downward. The difference in mean density between riser and downcomer is causing the pressure gradient for recirculation. (3) base: the bottom connection zone between the riser and downcomer is very simple. It is usually believed that the design of this section can influence gas holdup, liquid velocity and solid phase flow. (4) gas separator: this section at the top of reactor connects the riser to the downcomer which provided facilitates liquid recirculation and gas disengagement. Air lift photobioreactor can increase radial movement of fluid that is necessary for improved light-dark cycling [23]. Airlift photobioreactor has several advantages such as gas is well mixing which effect on homogeneous distribution of nutrient and algae, needed low energy consumption that no stirrer or agitator, high light utilization efficiency lead to high productivities as high biomass and less parts which it is easy to sterilize. Monkonsit *et al*. [79] studied the system performance of airlift photobioreactor compared with bubble column to cultivated *Skeletonema costatum*. The result found that the airlift photobioreactor provided better performance than the bubble

column which the maximum cell concentration, specific growth rate and productivity were considered. This was because the airlift photobioreactor allowed circulatory flow in the system which helps prevent cell precipitation. Krichnavaruk *et al*. [6] showed that the results were the same. The cultivation of *Chaetoceros calcitrans* in the airlift photobioreactor was found to be superior to that in the bubble column. Kaewpintong *et al*., [80] was reported that the most appropriate aeration velocity at 0.4 cm s^{-1} and small riser was shown to have positive influence of on the cell growth. The growth rate of *Haematococcus pluvialis* could be enhanced at 1% CO_2 supplement to the air supply. On the other hand, an airlift photobioreactor has limitations include their small illumination surface area due to diameter and height cannot be much increased and their construction requires sophisticated materials.

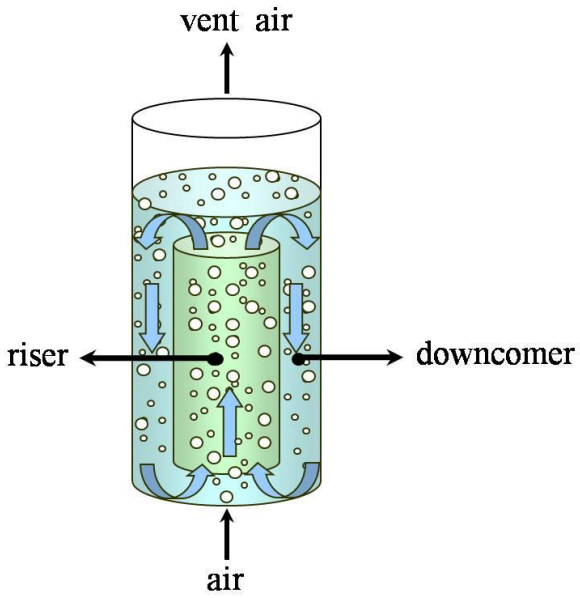

Fig. 2.8 Airlift photobioreactor

- Flat plate photobioreactor

Flat plate photobioreactor or a flat panel photobioreactor support the highest densities of photoautotrophic cells due to their large illumination surface areas, which are suitable for outdoor mass cultures for microalgae [81]. Generally, flat plate photobioreactor is made of transparent materials for maximum utilization of solar light energy. Dissolved oxygen concentration accumulation in flat plate photobioreactor is relatively low compared to horizontal tubular. Richmond [82] has been reported that in this reactor has achieved high photosynthetic efficiency. However, it also has some limitations such as difficult to control culture temperature, and scale up require many compartments and support materials. Issarapayup *et al*. [83] was considered efficiency of the system while the ratio between the downcomer and riser cross sectional area and size of the system were changed. The result showed that the cell density and specific growth rate in 17 L flat plate were higher when compare as 90 L flat plate. However, the large scale 90 L flat plate was the most cost effective system.

CHAPTER 3
METHODOLOGY

3.1 Batch culture of *Ankistrodesmus* sp. in Duran bottle and airlift photobioreactor

The strain of green microalgae used for this study, *Ankistrodesmus* sp. (Fig. 3.1) was obtained from the Microbiological Resources Centre (MIRCEN) of the Thailand Institute of Science and Technology Research (TISTR). *Ankistrodesmus* sp. was cultured with a BG11 medium, which contained the components as shown in Table 3.1. The incubation was cultured in 250 mL flask for a week.

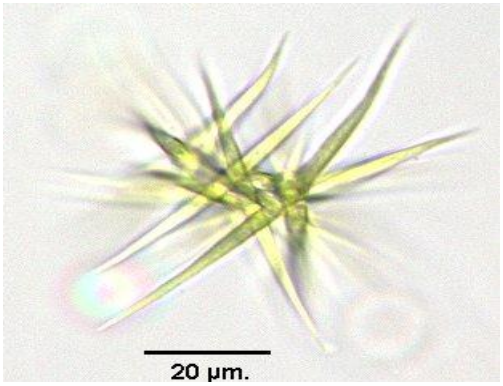

Fig. 3.1 *Ankistrodesmus* sp. under microscope

Ankistrodesmus sp. was cultured in 2 L Duran bottle size containing BG11 medium. The medium was prepared and autoclaved at 121°C for 15 minutes, see in Fig. 3.2. Ten percent of inocumlum (v/v) was used. Three sets of batch experiment were conducted in controlled environment. The batch starter of *Ankistrodesmus* sp. which was cultivated under various light intensities of 10, 20 and 30 klux (light supplied with white fluorescent light), and aerated with sterile air (filtered through 0.2 micron filter, Gelman Acrodisc 50). The batch cultivation was cultured until the stationary growth was reached.

3.1.1 Effect of temperature in Duran bottle

1. Fill inoculum into culture bottle containing BG11 medium
2. Start the culture with initial biomass concentration of about 0.03 g L^{-1}

3. Place the culture bottle in the controlled chamber where temperature is set at 30 °C
4. Aerate the mixture with the air flow rate of 1 cm s^{-1}
5. Take samples for cell dry weight analysis once a day till stationary growth phase is reached
6. Repeat Steps 1-5 and adjust temperature to 35 and 40 °C
7. Harvest cells for biochemical composition analysis

3.1.2 Effect of light intensity in Duran bottle

1. Fill inoculum into culture bottle containing BG11 medium
2. Start the culture with initial biomass concentration of about 0.03 g L^{-1}
3. Put the culture bottle in the controlled chamber where temperature is set at 30 °C and light intensity at 10, 20 and 30 klux
4. Aerate the mixture with the air flow rate of 1 cm s^{-1}
5. Take sample for cell dry weight analysis once a day till stationary growth phase is reached
6. Harvest cells for biochemical composition analysis

Fig. 3.2 Batch cultivation of *Ankistrodesmus* sp. in 2 L Duran bottle to determine growth curve within controlled chamber

Table 3.1 Compositions of BG11 medium (per liter) [84]

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

3.1.3 Batch cultivation in airlift photobioreactor

The airlift photobioreactor used in this study was made of clear acrylic plastic with the dimensions, as shown in Fig. 3.3, i.e. wall thickness of outer column is 3 mm, wall thickness of the inner tube is 2 mm, inner height is 40 cm, outer height is 60 cm, inner column with 5 cm i.d., and outer column of 10 cm i.d. The working volume in the photobioreactor was 3 L. The air was provided from the bottom of the photobioreactor. The internal loop airlift was divided into 2 parts including riser and downcomer. In the riser, the air flows up and induces the liquid up-flow, most of the air bubbles leave the system at the gas-liquid separator part where the liquid flows downwards in the downcomer section.

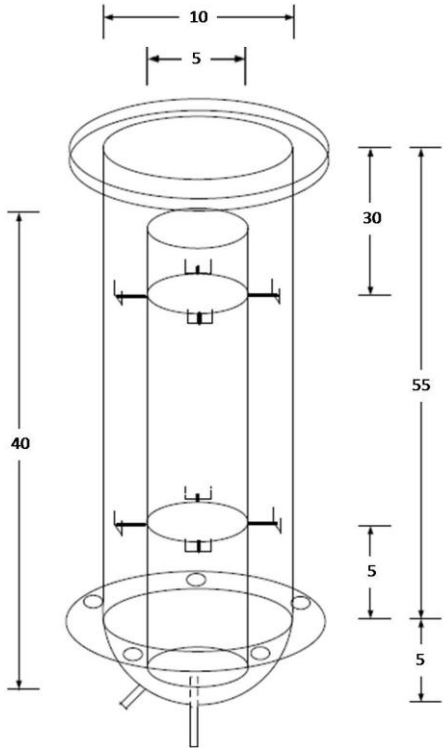

Fig. 3.3 Schematic of airlift photobioreactor

The experimental set up was performed as follows:

1. Prepare BG11 medium with freshwater
2. Fill 2.5 L of medium into airlift photobioreactor
3. Overnight sterilize medium with troclosene sodium (3.0 g/ tablet), which has free available chlorine of 1.97 g
4. Supply air through the porous sparger at the bottom of the reactor for 24 hours

5. Check for residual chlorine with potassium iodide, if the chlorine is exhausted, the sample is clear, otherwise a yellow solution appears. Remove chlorine with sodium thiosulfate if this is the case
6. Add 0.5 L of initial cell from inoculum into airlift photobioreactor
7. Supply air and record light intensity and temperature
8. Take sample for analysis, i.e. cell dry weight, nitrate and phosphorous concentration at once a day frequency until the stationary growth is reached (see Section 3.6 for analysis procedures)
9. Calculate the specific growth rate and doubling time (see Section 3.5.1 and 3.5.2 for procedures)
10. Harvest cells for biochemical compositions analysis

3.2 Enhancing *Ankistrodesmus* sp. growth with carbon dioxide supplements

Fig. 3.4 shows a schematic drawing of the photobioreactor with pH control. It consists of the airlift photobioreactor connected to a carbon dioxide tank. A pH electrode in the culture airlift photobioreactor was coupled to a pH controller. pH was measured continually, and kept within an interval of 6 ± 0.5, 7 ± 0.5 and 8 ± 0.5 by automatic CO_2 feeding.

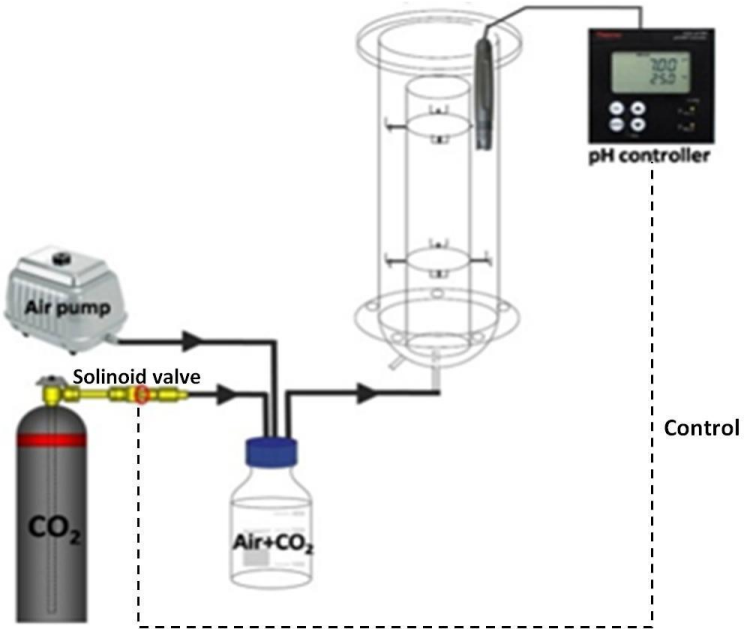

Fig. 3.4 Schematic of experiment set up of the pH control photobioreactor

3.2.1 Effect of pH in medium

1. Repeat steps 1-6 in Section 3.1.3
2. Sparge a mixture of air and CO_2 where pH is controlled at 6
3. Take sample for analysis (see step 8 in Section 3.1.3)
4. Harvest cells for biochemical compositions analysis after 8 days of cultivation
5. Repeat Steps 1-4 and adjust pH at 7 and 8

3.2.2 Cultivation with air and carbon dioxide mixture

1. Set up the experiment as Fig. 3.5 within evaporation chamber
2. Repeat Steps 1-6 in Section 3.1.3
3. Sparge a mixture of air and CO_2 concentration at 1% with aeration of 1.5 L min^{-1}
4. Take sample for analysis (see step 8 in Section 3.1.3)
5. Harvest cells for biochemical compositions analysis after 8 days of cultivation
6. Repeat Steps 1-4 and adjust to 2, 5 and 10%

Fig. 3.5 Setup of the cultivation of carbon dioxide supplement

3.3 Increasing lipid production with reuse and modified BG11 media

3.3.1 Batch cultivation with reused medium

Ankistrodesmus sp. was cultivated in the airlift photobioreactor (at least two replications). The experiment was conducted with 3 different media, i.e. fresh medium, 1st reused medium and 2nd reused medium. The fresh medium was prepared following as Table 3.1. The culture cycle was 14 days, and after the harvest, the medium and microalga were separated by centrifugation at 2150×g for 5 minutes and the supernatant was reused in the next cultivation (referred to as 1st reused medium). Similar procedure was applied for the 2nd reused medium. Note that our preliminary results suggested that the 3rd reused medium was no longer suitable for the cultivation of such alga.

3.3.2 Continuous cultivation

A continuous culture of *Ankistrodesmus* sp. was also conducted in the airlift photobioreactor (at least three replications), with superficial gas velocity (Usg) of 1.33 cm s^{-1} under outdoor conditions, as described in Fig. 3.6. The medium was continuously added into the system with a peristaltic pump (Cole-Parmer Masterflex L/S). The spent medium and microalgae overflowed to the product tank. The dilution rates were adjusted by varying the flow rates. The fresh medium was the BG11 standard medium, whereas the modified BG11 medium was concocted following the content of the 1st reuse medium from the batch experiment (per liter), i.e. 0.95 g $NaNO_3$, 0.0015 g $K_2HPO_4 \cdot 3H_2O$, 0.047 g $MgSO_4 \cdot 7H_2O$, 0.013 g $CaCl_2 \cdot 2H_2O$, 0.0036 g Ammonium ferric citrate. Other elements were maintained according to the level specified in the BG11.

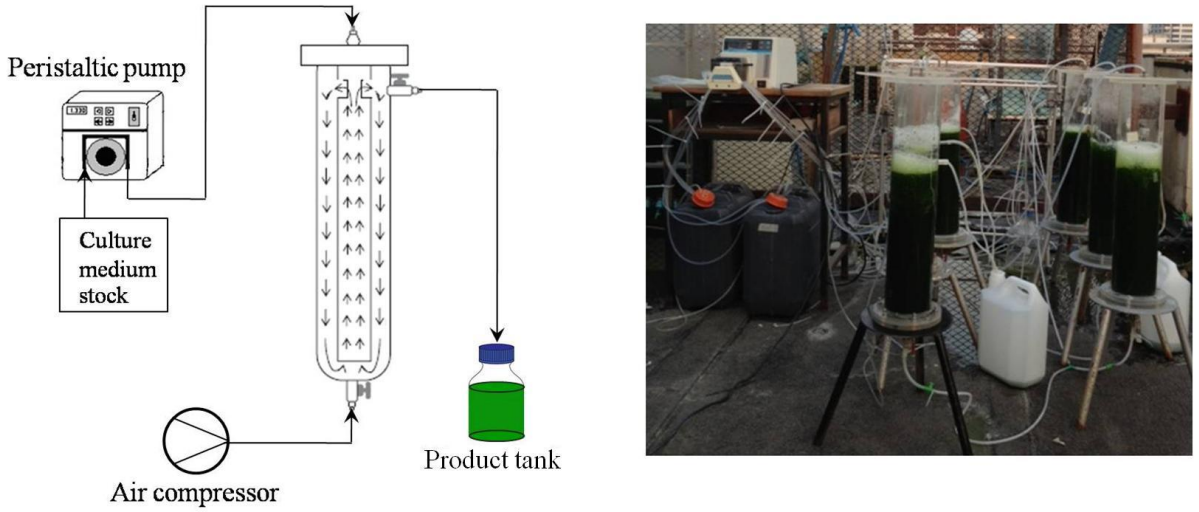

Fig. 3.6. Setup of the bench scale culture (schematic: left, actual setup: right)

3.4 Measurement of microalgal growth

The estimation of cell dry weight is one of the most direct ways to determine biomass production and involves the following steps.

1. Collect 25 mL samples
2. Dry the Whatman GF/C filter paper with 1.6 μm pore size membrane and 50 mm of diameter in an oven at 80 °C for 24 hours or until weight is constant
3. Weigh the filter paper
4. Filter 25 mL of the microalgal through the Whatman GF/C filter paper
5. Wash the microalgal on the filter paper with 20 mL of DI water
6. Put the paper filter in an oven at 80 °C for 24 hours or until weight is constant
7. Cool down the paper filter at room temperature
8. Put the filter paper in desiccators
9. Weight the filter paper
10. Calculate cell dry weight using Equation 3.1

$$Algal\ dry\ weight\ (g\ L^{-1}) = \frac{Wt_A - Wt_B}{V} \times 1000 \qquad (3.1)$$

where

Wt_A is weight of filter paper and algae (g)

Wt_B is weight of filter paper (g)

V is volume of culture (mL)

3.5 Calculations

3.5.1 Determination of specific growth rate

Growth curve is plotted and the specific growth rate can be calculated from Equation 3.2 as follows:

$$\mu = \frac{\ln x_2 - \ln x_1}{t_2 - t_1} \quad (3.2)$$

where

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

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

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

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

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

3.5.2 Determination of doubling time

Doubling time (t_d) is the period of time required for double the amount of cells in the exponential growth phase. Doubling time for biomass in batch culture can be calculated by specific growth rate (μ) as follows (Equation 3.3):

$$t_d = \frac{ln2}{\mu} = \frac{0.693}{\mu} \quad (3.3)$$

3.5.3 Determination of dilution rate

The dilution rate in continuous cultivation can be calculated from Equation 3.4 as follows:

$$D = \frac{F}{V} \quad (3.4)$$

where

D is the dilution rate (d^{-1})

F is the flow rate (L d^{-1})

V is the working volume of bioreactor (L)

3.5.4 Determination of productivity

The productivity of microalgal can be calculated from Equation 3.5 as follows:

$$P = \frac{N_2 - N_1}{t_2 - t_1} \times V \quad (3.5)$$

where

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

N_1 is biomass concentration at t_1 (g L^{-1})

N_2 is biomass concentration at t_2 (g L^{-1})

t_1 is first sampling time (d)

t_2 is second sampling time (d)

V is harvest volume (L)

3.5.5 Determination of lipid productivity

$$\textit{Lipid productivity (mg } L^{-1} d^{-1}) = \textit{Biomass productivity (mg } L^{-1} d^{-1}) \times \frac{Lipid\ content\ (g)}{100\ (g)} \quad (3.6)$$

3.5.6 Determination of specific productivity

The specific productivity for the cultivation of alga can be calculated from Equation 3.7:

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

where

SP is specific productivity (g L^{-1} d^{-1})

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

V is harvest volume (L)

3.5.7 Determination of carbon dioxide fixation

Carbon dioxide fixation is calculated from the relationship between the carbon content of the cells and the specific productivity as follows (Equation 3.8):

$$R_{CO_2} = C_C P_X \left(\frac{MW_{CO_2}}{MW_C}\right) \quad (3.8)$$

where

R_{CO_2} is carbon dioxide fixation (g L^{-1} d^{-1})

C_C is percent of carbon in biomass (%)

P_X is specific productivity (g L^{-1} d^{-1})

MW_{CO_2} is molecular weight of CO_2 (g mol^{-1})

MW_C is molecular weight of carbon (g mol^{-1})

3.6 Determination of medium composition

3.6.1 Nitrate analysis

Nitrate concentration can be measured by UV-Visible spectrophotometer at wavelengths of 220 and 275 nm.

1. The volume of sample is collected at 5 mL
2. Filter the microalgal through the Whatman GF/C filter paper which has 1.6 μm pore size membrane and 25 mm of diameter
3. Dilute the sample if the optical density is greater than 1.0
4. Measure solution by UV-Visible spectrophotometer at wavelengths of 220 and 275 nm as follows (Equation 3.9):

$$\text{Nitrate (ppm)} = \frac{(\text{Abs}_{220\text{nm}} - \text{Abs}_{275\text{nm}}) \times \text{A}}{\text{B}} \quad (3.9)$$

where

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

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

3.6.2 Phosphate analysis

Phosphate concentration can be measured by UV-Visible spectrophotometer at a wavelength of 885 nm.

1. The volume of sample is collected at 5 mL
2. Filter the microalgal through the Whatman GF/C filter paper which has 1.6 μm pore size membrane and 25 mm of diameter
3. Dilute the sample if the optical density is greater than 1.0

4. Prepare the reagents as follows:
    - Stock Ammonium molybdate solution: 15 g of $(NH_4)_6Mo_7O_{24}.4H_2O$ is dissolved in 500 mL of distilled water. The solution is stored in a plastic bottle.
    - Sulfuric acid solution: 140 mL of concentrate sulfuric acid is dissolved in 900 mL of distilled water. The solution is stored in a glass bottle and kept in refrigerator.
    - Ascorbic acid solution: 27 g of ascorbic acid is dissolved in 900 mL of distilled water. The solution is stored in glass or plastic bottle and kept in refrigerator.
    - Potassium antimonyle tartrate solution: 0.34 g of $KNaC_4H_4C_6.4H_2O$ is dissolved 250 mL of distilled water. The solution is stored in glass or plastic bottle and kept in refrigerator.
    - Mixed reagent: 2 mL of Ammonium molybdate solution, 5 mL of Sulfuric acid solution, 2 mL of Ascorbic acid solution, and 1 mL of Potassium antimonyle tartrate solution are mixed.
5. Add 100 μL of mixed reagent into 1 mL of sample
6. Leave to reaction for 30 minutes
7. The solution is measured by UV-Visible spectrophotometer at wavelength of 885 nm

3.6.3 Other elements analysis

The amount of other elements included in BG-11, such as B, Ca, Co, Cu, Fe, K, Mg, Mn, Mo, Na and Zn, were analyzed with ICP-OES (700 series Inductively Couple Plasma-Optical Emission Spectrometer, Agilent technologies).

1. Collect 5 mL samples
2. Filter the microalgal through the Whatman GF/C filter paper with 1.6 μm pore size membrane and 25 mm of diameter
3. Prepare the standard solutions from the standard mixture
4. Measure the concentration of elements by using ICP-OES

3.7 Determination of cell composition

3.7.1 Determination of total lipids

1. Weight the dried algae 1 gram into the thimble and record it. Put it into the soxhlet extractor
2. Weigh the round bottom flask and record them, fill chloroform 120 mL and methanol 60 mL as mixed solvent, after that heat over until colorless
3. Take the round bottom flask to the evaporator until all solvent is removed. Leave it to cool in desiccator for 2 hours and then record the weight of the sample with the flask

3.7.2 Determination of protein

The crude protein content was obtained by multiplying the amount of nitrogen content by the factor of 4.44 [85]. The nitrogen content was analyzed by CHNS/O Analyzer (Perkin Elmer, PE2400 Series II) from Scientific and Technological Research Equipment Centre, Chulalongkorn University. The crude protein content, W_p, was calculated as a percentage by mass, using the following equation:

$$W_p = W_N \times F \tag{3.10}$$

where

W_N is the nitrogen content of the sample, expressed as percentage by mass

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

3.7.3. Determination of carbohydrate

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

$$\text{Total carbohydrate (\%)} = [100 - (\text{lipid} + \text{protein} + \text{ash} + \text{moisture}) (\%)] \tag{3.11}$$

3.7.4 Determination of moisture

1. Keep crucible dried in an oven at 100 °C for 2 hours and then put it in a desiccator for 2 hours. After that record them
2. Weigh the dried algae 1 gram into the crucible and record, calcined at 100 °C for 2 hours and then put it in desiccators for 2 hours. After that record them

3.7.5 Determination of ash

1. Keep crucible dried in an oven at 800 °C for 2 hours and then put it in a desiccator for 2 hours. After that record them
2. Weight the dried algae 1 gram into the crucible and record, calcined at 750 °C for 2 hours and then put it in a desiccator for 2 hours. After that record them

3.7.6 Transmethylation

After identification, the lipid bands on TCL aluminium sheet were scraped off for fatty acid analysis. The following steps are recommended for transmethylation.

1. Weigh 100 mg of dry cell into vial
2. Add 2 mL of methanol: hydrochloric (95:5)
3. Add 100 μL of standard heptadecanoic acid (C17:0) solution
4. Mix and heat at 80 °C for 1.5 hours
5. After cooling, dilute with 1 mL of water and extract with 1 mL of hexane (dissolve 0.01% butylatedhydroxytoluene to hexane)
6. Mix well and leave for separation by centrifuge for 5 minutes
7. Separate and past the upper liquid to sodium sulphate anhydrous
8. Dry up the fatty acid methyl ester by nitrogen gas
9. Analyze by GC-17A gas chromatograph (Shimadzu) equipped with a flame ionization detector (FID)

CHAPTER 4
BATCH CULTURE OF *ANKISTRODESMUS* SP. IN DURAN BOTTLE AND AIRLIFT PHOTOBIOREACTOR

4.1 Batch culture of *Ankistrodesmus* sp. in Duran bottle

This experiment investigated the effect of temperature and light intensity on microalgae growth. *Ankistrodesmus* sp. was cultivated in a 2L Duran bottle as a batch culture within the controlled chamber where temperature and light intensity could be well controlled (Fig. 4.1). The light intensity provided by fluorescent lamps was adjusted at 10, 20 and 30 klux at the surface of a Duran bottle whereas the temperature was at 30, 35 and 40°C. The culture was aerated at the superficial velocity of 1 cm s^{-1}. Each experiment was triplicated.

Fig. 4.1 Controlled chamber for small scale algal cultivation

To see the effect of temperature, the light intensity was fixed at either 10, 20 or 30 klux. Fig. 4.2 (a) shows the growth curves of *Ankistrodesmus* sp. with light intensity of 10 klux, which suggested that the highest biomass density and specific growth rate of 1.22 g L^{-1} (20.9×10^{6} cells mL^{-1}) and 1.38 d^{-1}, respectively, could be achieved at 30°C. A significant drop in biomass density (1.22 to 0.44 g L^{-1}) could be observed when the temperature changed from 30 to 35°C. At 40°C, the maximum biomass concentration shifted a little higher to 0.58 g L^{-1}.

Fig. 4.2 (b) and (c) are the results from similar experiments but with higher light intensity (to 20 and 30 kLux, respectively). Similar results were obtained from these experiments where the maximum biomass concentration occurred at 30 °C for both cases, i.e. 1.24 g L^{-1} at 20 klux and 1.48 g L^{-1} at 30 klux. For instance, the cell density of *Chlorococcum littorale* increased with increasing irradiance using a flat plate photobioreactor [86], and similar results were reported with *Chlorella* sp. [47]. It was reported that light intensity was important for the conversion of incident light energy to algal biomass [87], whereby cells grown under saturated light conditions could potentially accumulate carbohydrate as storage materials [88].

After harvesting at 7 days of cultivation, the medium and microalgae were separated by centrifugation (3,500 rpm) at 850×g for 5 minutes. The supernatant was removed and the microalgal sediment was dried in a freeze drier. Chloroform/methanol mixture and hexane were used as a solvent for the extraction. The highest lipid content was obtained from the growth at 30°C for all light intensity levels, i.e. lipid contents from the cultivation at 10, 20 and 30 klux were 26.04, 24.58 and 25.54 % by wt, respectively (Table 4.1). Increasing temperature seemed to lower the lipid accumulation in this alga, which corresponded to its growth where it was best at 30^{o}C. It was also observed that most lipids were of polar type as they could not be well dissolved in hexane (approximately 2-4 %wt) and polar lipid accumulated better at low light intensity. Temperature only slightly affected carbohydrate and protein accumulations, however, carbohydrate seemed to be a little more accumulated at low light intensity. Protein was observed to be slightly more accumulated at 20 klux, but such effect was not so significant.

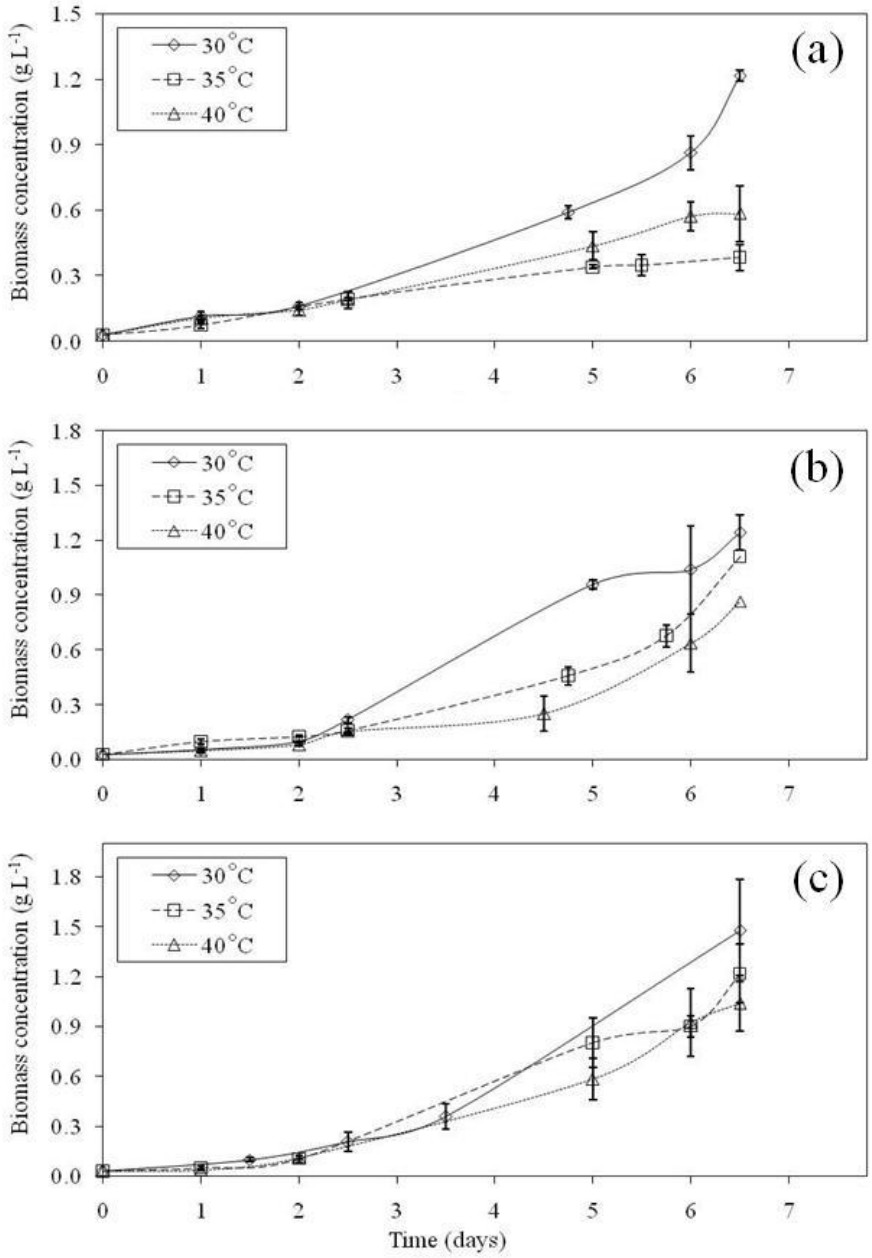

Fig. 4.2 Effect of temperature on growth of *Ankistrodesmus* sp. cultivated within a controlled chamber at 30, 35 and 40 °C under continuous light of (a) 10, (b) 20 and (c) 30 klux

Table 4.1 Biochemical compositions for *Ankistrodesmus* sp. cultured under various temperatures and light intensities

Light intensity (klux)	Temperature (°C)	Lipid (%)		Protein (%)	Carbohydrate (%)
		Chloroform: methanol	Hexane		
10	30	26.04	3.36	24.91	37.55
	35	25.95	3.03	25.57	36.02
	40	22.14	4.02	22.20	41.28
20	30	24.58	3.05	26.18	37.93
	35	24.08	2.97	27.31	31.14
	40	23.92	2.17	32.32	33.55
30	30	25.54	3.21	26.73	35.89
	35	23.84	3.24	25.53	38.70
	40	23.99	2.76	24.29	33.19

4.2 Batch cultivation in airlift photobioreactor

This experiment investigated the growth profile and specific growth rate of *Ankistrodesmus* sp. cultivated in a 3 L batch airlift photobioreactor within an evaporation chamber (Fig. 4.4), where the temperature was controlled at 30 °C. The culture system was aerated with normal air with superficial gas velocity (Usg) of 1.13 cm s^{-1} (aeration flow rate of 1.5 L min^{-1}). Fig. 4.5 illustrates the biomass concentration time profile of *Ankistrodesmus* sp. in this airlift photobioreactor system. The maximum biomass of about 0.34 g L^{-1} occurred at Day 6 with the growth rate of 0.02 g L^{-1} d^{-1}. The specific growth rate and doubling time was determined from the growth results at Days 1-2 which were approximately 1.30 d^{-1} and 0.53 d, respectively.

Although an evaporation chamber was designed to use the latent heat of water (from evaporation) to control the temperature at 30°C, the evaporative power of this chamber did not seem to be adequate to remove the solar energy as the temperature went up to as high as 38°C during hot summer days. The time profiles of temperature and light intensity are shown in Fig. 4.6 (a) and (b), respectively. These measurements reveal that there was a large variation in outdoor conditions especially on light intensity which could vary from as high as 110,000 lux (during Day 5) to as low as 33,000 lux (during Day 8). Therefore this significantly affected the growth of such alga as photosynthetic activities depend markedly on the level of the exposed light intensity. Nevertheless, *Ankistrodesmus* sp. exhibited a relatively good tolerance to these natural disturbances and continued to grow, but with a sacrifice in the growth rate when compared with the well controlled chamber as reported in the previous section.

Fig. 4.3 Experimental set up in airlift photobioreactor within evaporation chamber

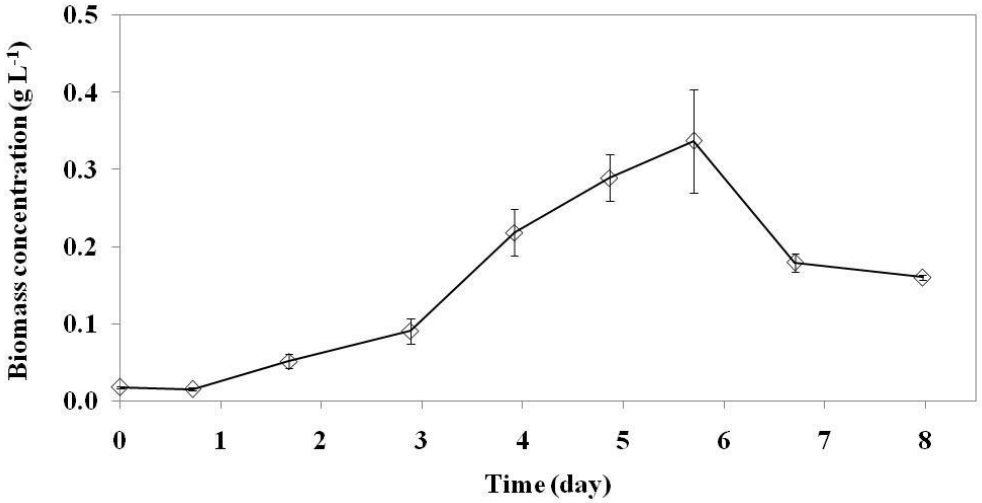

Fig. 4.4 Growth curve of *Ankistrodesmus* sp. in airlift photobioreactor

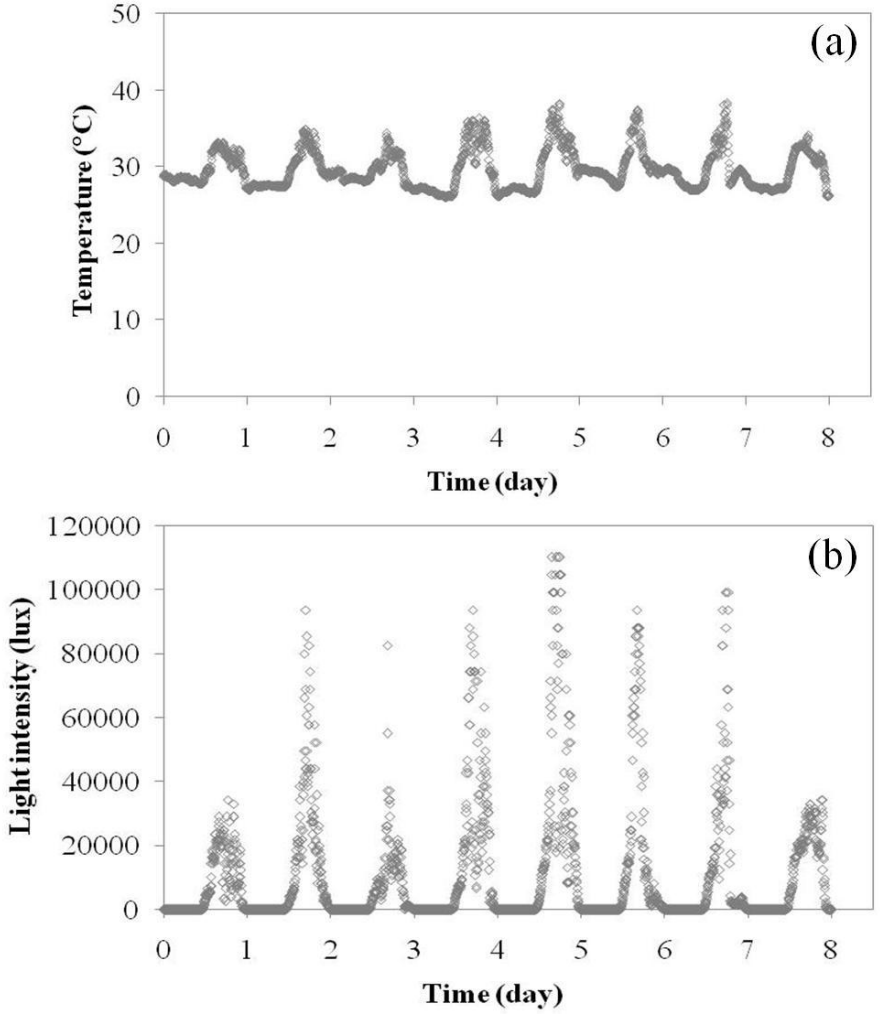

Fig. 4.5 (a) Temperature and (b) light intensity on each day of cultivation

The concentrations of nitrogen and phosphorous remaining in the different mediums are shown in Fig. 4.7 (a) and (b), respectively. A large quantity of nitrogen still remained until the harvest day. The amount of nitrogen being consumed in mediums ($\Delta N/\Delta X$) was 337.05 mg-N g^{-1} (calculated at Day 6 of cultivation). On the other hand, phosphorous was uptaken significantly into microalgae where $\Delta P/\Delta X$ was approximately 26.16 mg-P g^{-1}. Phosphorous in the medium decreased rapidly until the 5^{th} day of cultivation after which it was constant. These were in a similar range as reported in literature, e.g. Bellou and Aggelis [89] reported that the freshwater algae *Chlorella vulgaris* consumed nitrogen and phosphorous at 360 mg-N g^{-1} and 47 mg-P g^{-1}, respectively.

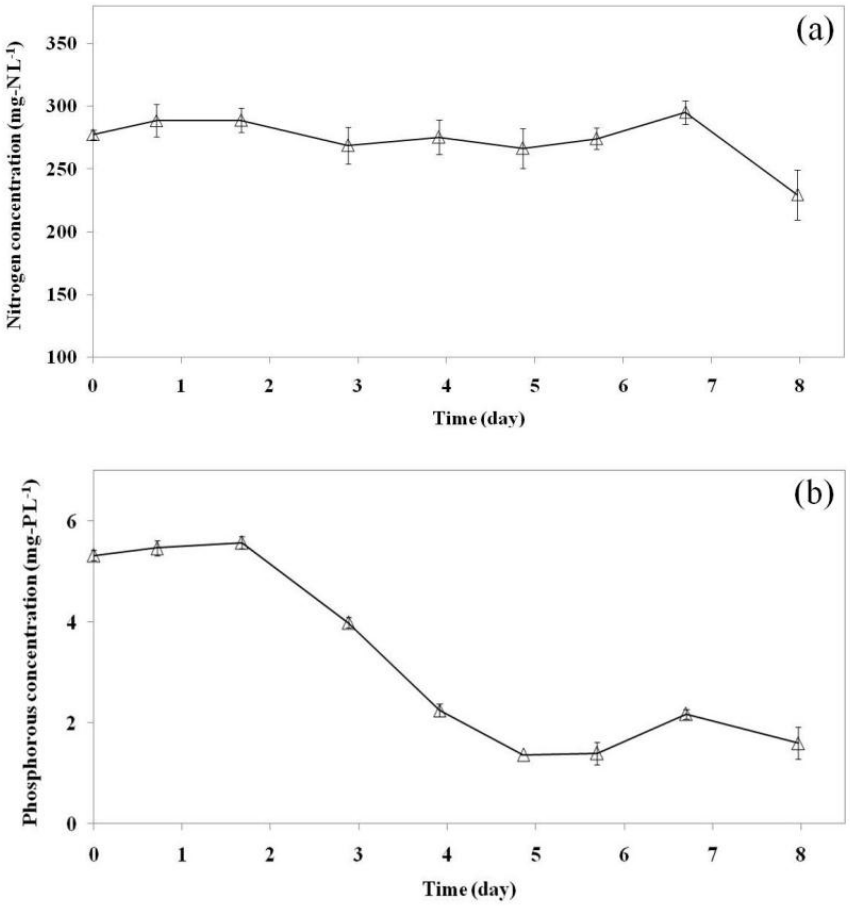

Fig. 4.6 Time profiles of (a) nitrogen and (b) phosphorous remaining in the medium

4.3 Concluding remarks

This chapter reported findings regarding the growth of *Ankistrodesmus* sp. both in a well controlled chamber and an outdoor airlift photobioreactor operated under nonsterile normal conditions, i.e. pure air and standard BG11 medium. It was proven that *Ankistrodesmus* sp. could withstand the outdoor condition adequately well, despite of its low growth profile. The next chapter describes how to enhance the growth of such alga by supplementing carbon dioxide as an additional carbon source.

CHAPTER 5
ENHANCING *ANKISTRODESMUS* SP. GROWTH WITH CARBON DIOXIDE SUPPLEMENTS

This experiment was carried out with two different methods of CO_2 supplements, i.e. (1) continuous carbon dioxide addition at various concentrations; and (2) adding carbon dioxide for pH control.

5.1 Growth profile of *Ankistrodesmus* sp.

For the first case with continuous supply of CO_2, *Ankistrodesmus* sp. was cultured with air at the flow rate of 1.5 L min^{-1} mixed with CO_2 gas at various volume compositions, i.e. 1% (15 mL min^{-1}), 2% (30 mL min^{-1}), 5% (75 mL min^{-1}) and 10% (150 mL min^{-1}), and the resulting growth curves are presented in Fig. 5.1(a). In all experiments, the culture started with the initial biomass concentration of 0.2 g L^{-1} (approximately $1.2x10^6$ cells mL^{-1}). Cell growth was found to vary steadily with CO_2 addition range of 1 to 5%, after which no further positive effect of CO_2 was observed. The maximum biomass yield was obtained at the end of the experiment with 5% CO_2 at 2.91 g L^{-1} whereas the cell growths with 1%, 2% and 10% were 1.79, 1.97 and 2.15 g L^{-1}, respectively. This result could be explained as follows.

The photosynthesis involved in the growth of microalgae was affected significantly by the level of CO_2 supply [90]. In the experiments with CO_2 supplement, cell biomass obtained when 5% CO_2 was supplied to the system was 1.26-fold greater than that obtained from the control experiment (0.04% CO_2). Similar results were reported by several other works, for instance, Baba *et al*. [91] reported that the growth rate and the amount of total protein content of *Chlamydomonas reinhardtii* increased about 1.5-fold when the CO_2 concentration was increased from atmospheric level to 3%.

For the second case with feeding CO_2 for control pH, the cultivation of *Ankistrodesmus* sp. was subject to the pH adjustment environment where the pH controller was connected to the CO_2 feeding pump to control the pH of the nutrient at the set point. The results are displayed in Fig. 5.1(b) where the maximum biomass at pH 6, 7, 8 and control (no pH control) were 1.34, 1.35, 1.50 and 1.23 g L^{-1}. The biomass yield showed a slightly increasing trend with an increase in pH. Within the range of pH examined in this

work, the operation under pH 8 seemed to give the best performance; however, as an overall observation, the effect of pH did not seem to be significant.

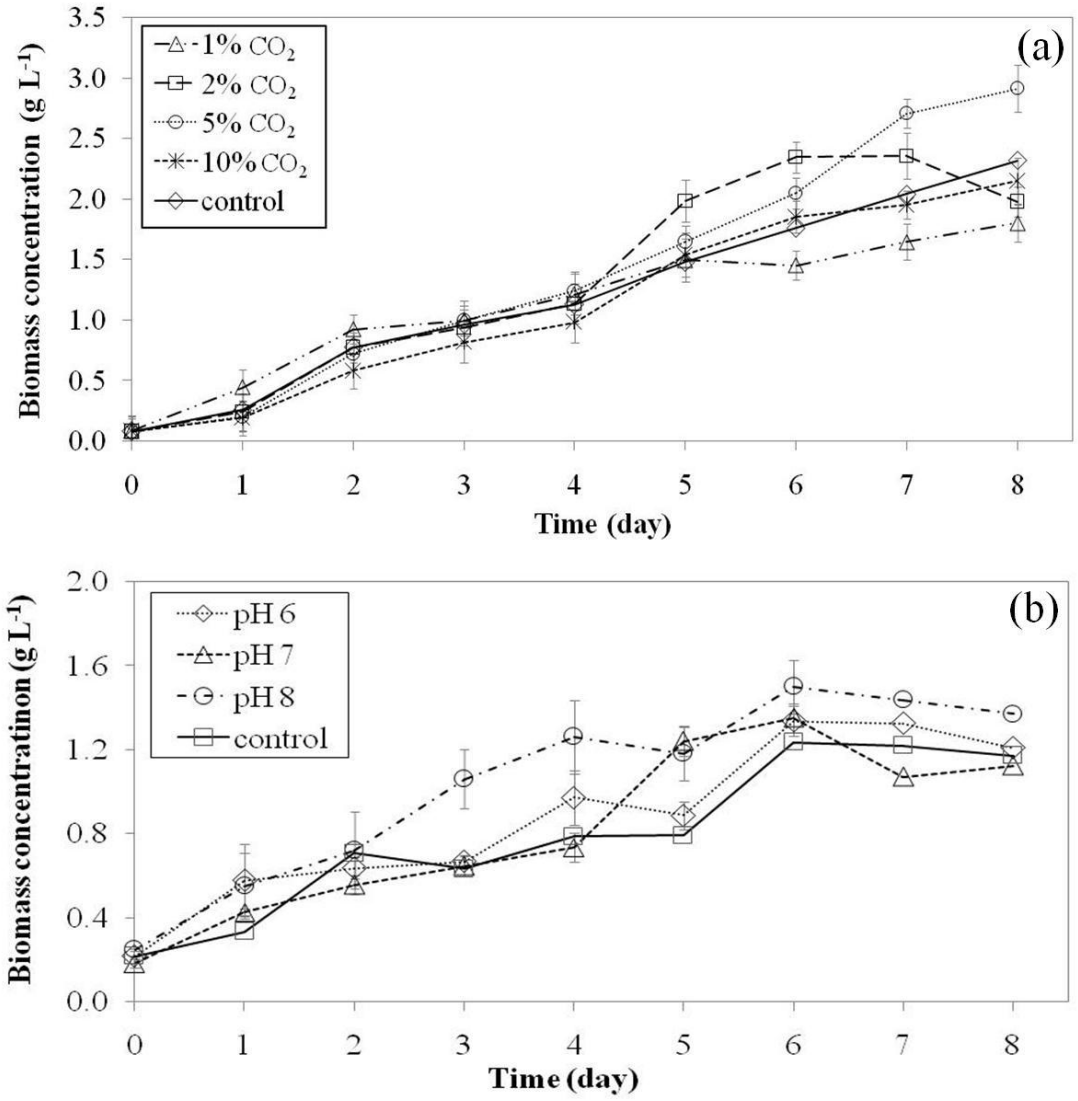

Fig. 5.1 Growth profile of *Ankistrodesmus* sp. under (a) CO_2 supplement and (b) pH control

It is interesting to note that there was not a strict relationship between the algal growth and major nutrient consumptions, as illustrated in Fig. 5.2. Although there might seem to exist some dependency between growth and nutrient consumption in the experiments with CO_2 supplement as suggested in Fig. 5.2(a) and (c), Fig. 5.2 (b) and (d), which is the result from the experiments with pH control, clearly indicates that growth remained constant regardless of the nutrient uptake. Our observation also shows that the

ranges of nitrogen and phosphorus concentrations did not vary much in each experiment. Hence, it could be concluded from this experiment that there was no direct relationship between growth and nutrient consumption, at least within the range of nutrient concentration as employed in this work. At other conditions, the results may change, for instance, Cakmak *et al*. [68] reported that the biomass of *Chlamydomonas reinhardtii* decreased under nitrogen deprivation, but this was observed at very low nitrogen supply. There was also evidence a much too high supply of phosphorus could lead to a luxury uptake of the algae, which did not result in a better growth [92].

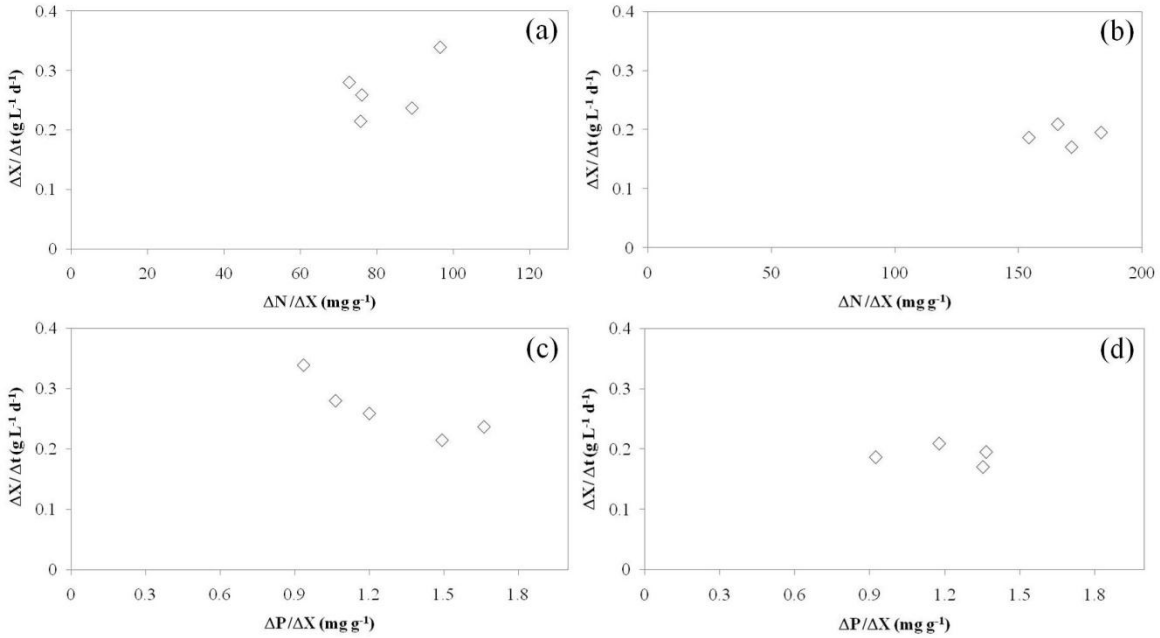

Fig. 5.2 Nutrient consumption and growth rate of *Ankistrodesmus* sp. under (a and c) the continuous CO_2 supplement and (b and d) the pH control

5.2 Accumulation of biochemical compositions

In this section, the effect of CO_2 on the accumulation of various biochemical compositions, i.e. lipids, proteins and carbohydrates, was investigated.

Considering the lipid composition in the biomass, Table 5.1 demonstrates that the lipid contents of 34.83, 28.71, 33.72, 31.00 and 29.17% were observed for the culture with CO_2 concentrations of 0.04% (control), 1%, 2%, 5% and 10%, respectively. Lipid accumulation in the 2% CO_2 and control (0.04% CO_2) reached similar levels. For the second case with pH control, no significant differences in lipid contents were observed

regardless of the pH level, and the lipid contents were in the range of approximately 33-36%.

High carbohydrate contents were obtained when the culture was fed with 5 and 10 % CO_2. Carbohydrate was observed also to be high (31.12%) at pH 6, and this dropped to 23.87 and 26.97% at pH 7 and 8, respectively. Furthermore, experiments with continuous CO_2 supplement saw a higher carbohydrate accumulation as compared to the experiment with pH control. *Ankistrodesmus* sp. cultured with carbon dioxide could accumulate high carbohydrate as more CO_2 could enter the Calvin cycle leading to a greater production of carbohydrate in such cells.

Experiments at pH 7 and 8 led to an alga with a large amount of protein at approximately 38% (Table 5.1). In the case of the continuous CO_2 supplement, the control (0.04%), 1% and 2% CO_2 could see a high protein accumulation (33-35%) when compared to the culture with 10% CO_2. *Ankistrodesmus* sp. accumulated high protein at low CO_2 as cells produce high carbonic anhydrase (CA), see in Fig. 5.3(a), which is an enzyme that catalyzes the reversible reaction between bicarbonate dehydration and carbon dioxide hydration to facilitate the transport of carbon into the cell. This enzyme is a part of the protein that algal cells produced and this is the reason why protein accumulation became quite high at low CO_2 condition. Note that at other conditions where CO_2 level was high, the cells would no longer need CA enzyme (see Fig. 5.3(b)), and therefore, resulted in low protein storage.

Table 5.1 Biochemical compositions in *Ankistrodesmus* sp.

Condition		Biochemical composition (%)		
		Lipid	Protein	Carbohydrate
CO_2 supplement	1%	28.71 ± 0.94	35.18 ± 2.34	36.11 ± 0.81
	2%	33.72 ± 1.43	33.72 ± 2.35	29.21 ± 1.16
	5%	31.00 ± 1.00	31.00 ± 1.97	37.78 ± 1.18
	10%	29.17 ± 1.12	29.17 ± 1.76	36.46 ± 1.24
	Control	34.83 ± 1.14	34.83 ± 1.56	31.30 ± 0.93
Controlled pH	pH 6	33.04 ± 1.32	35.84 ± 2.10	31.12 ± 1.83
	pH 7	36.99 ± 1.45	39.15 ± 2.04	23.87 ± 1.67
	pH 8	34.83 ± 1.67	38.20 ± 1.93	26.97 ± 1.12
	Control	35.49 ± 1.95	32.51 ± 1.75	32.00 ± 0.99

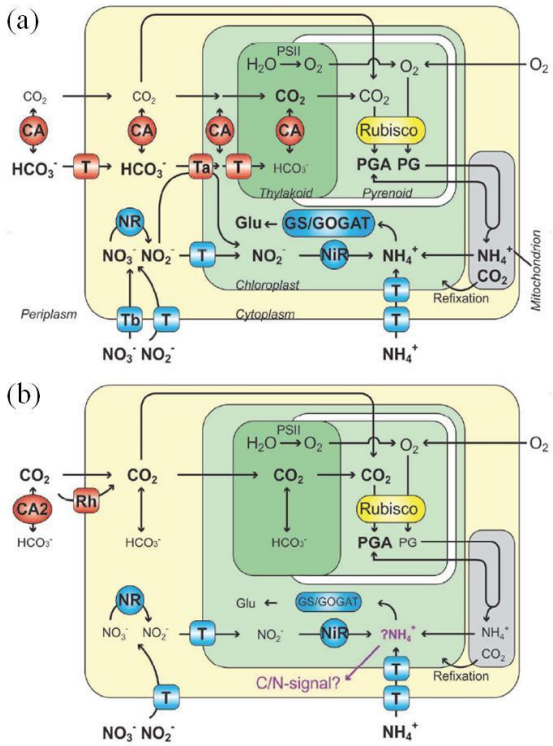

Fig. 5.3 Schematic illustration of a C/N-status model in (a) low and (b) high CO_2-acclimated cells [93]

Fig. 5.4(a) and (b) present the relationship between biochemical compositions and nitrogen consumption at various carbon dioxide concentrations and at various pH levels, respectively, which illustrates that lipid content stayed constant regardless of the nitrogen uptake for each cultivating condition (Fig. 5.4(a)). Similarly, phosphorus uptake did not show any impact on the biochemical composition within the cells (Fig. 5.4(c)). In cases with pH control, lipid and protein contents slightly increased with increasing both N and P consumptions, while carbohydrate decreased, see in Fig. 5.4 (b) and (d).

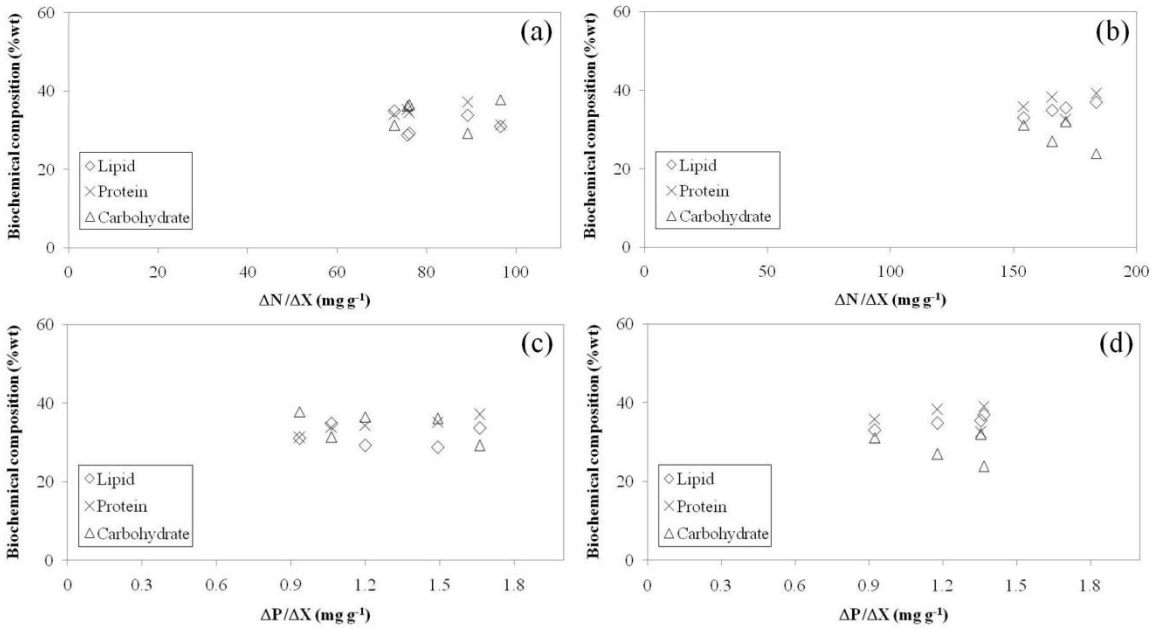

Fig. 5.4 Nutrient consumption and biochemical accumulation in *Ankistrodesmus* sp. under (a and c) the continuous CO_2 supplement and (b and d) the pH control

Fig. 5.5, shows the productivities of the biomass, lipids, proteins and carbohydrates where the maximum of 355, 110, 111 and 134 mg L d^{-1}, respectively, were observed at 5% CO_2. It can be observed that the highest productivity did not necessarily take place at the condition which gave the highest content. This was due to the biomass productivity which occurred at different conditions as discussed in the previous section. In other words, the biochemical productivities would depended significantly on the compromise between the biochemical composition and the biomass productivity.

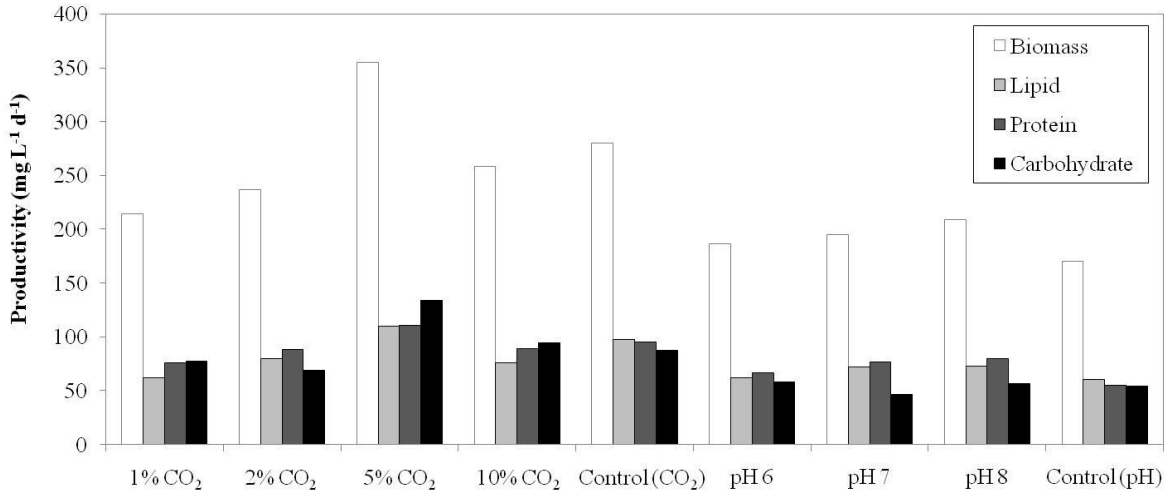

Fig. 5.5 Biomass and biochemical productivity for the culture with CO_2 supplements and pH control

5.3 Fatty acid classification

The fatty acid productivity profiles obtained from the cultivation of *Ankistrodesmus* sp. under various operating conditions as examined in this work are presented in Fig. 5.6. This demonstrates that the main fatty acids found from this specific culture were lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), gamma linolenic acid (C18:3 (ω6)), alpha linolenic acid (C18:3 (ω3)), stearidonic acid (C18:4 (ω3)), and lignoceric (C24:0) were analyzed among all the conditions. Among all, Palmitic acid (C16:0) was the major fatty acid. Palmitic acid productivity increased with increase in carbon dioxide concentration up to 5% CO_2 where the highest palmitic acid productivity was obtained (Fig. 5.6(a)). The fatty acid productivity from the pH controlled experiments (Fig. 5.6(b)) seemed to be lower than those obtained from the continuous CO_2 supplementing experiments. However, palmitic acid productivity was also presented the dominant saturated fatty acids, followed by oleic acid and linoleic acid productivities. Furthermore, margaric acid productivity (C17:0) was found in the pH control (0.63, 0.16 and 0.15 mg L^{-1} d^{-1} at pH 6, 7 and 8, respectively), while the continuous CO_2 supplements disappear.

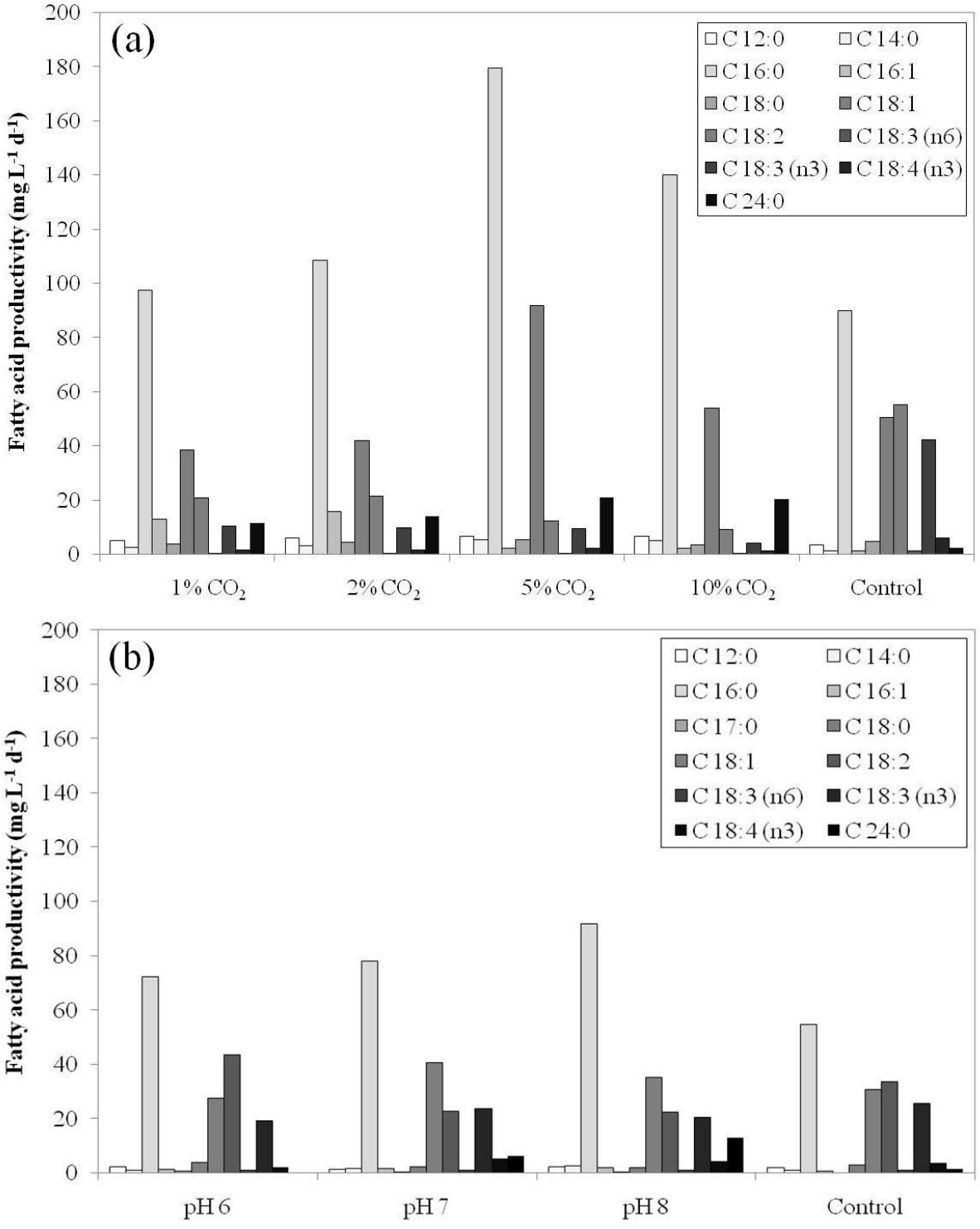

Fig. 5.6 Fatty acid accumulation in *Ankistrodesmus* sp. under (a) the continuous CO_2 supplement and (b) the pH control

5.4 Carbon dioxide fixation

Carbon dioxide concentration in the flue gas emitted from the power plant is about 30%. The CO_2 concentration in the atmosphere is around 380 ppm [94]. Carbon dioxide is the main component of greenhouse gases, one of the ways to reduce greenhouse gas emission is to generate energy from reduce carbon emission source. Photosynthetic microalgae use inorganic carbon for growth, hence, can be used for the biofixation of carbon dioxide.

This section illustrates how carbon could be fixed into the biomass of the green alga *Ankistrodesmus* sp. and the accumulation of fixed carbon dioxide under various CO_2 concentrations and the pH control are displayed in Table 5.2. The control (0.04%) and the 1% CO_2 supplement could not induce a better carbon uptake and only a low carbon content biomass (44.33 and 45.32%, respectively) was obtained. A higher carbon content could be achieved at elevated CO_2 feeding conditions and this carbon content became as high as 47-50%. The carbon content could be further converted to CO_2 fixation and fixation rate as also summarized in Table 4. It is clear that, for the CO_2 feeding condition, the CO_2 fixation could be managed at higher than 8 g or 356 mg $L^{-1}d^{-1}$. Unfortunately, a much lower carbon fixation of 6 g and 250 mg L^{-1} d^{-1} was obtained from the system with pH control. The maximum CO_2 fixation of *Ankistrodesmus* sp. was 15.51 g with the 5% CO_2 concentration (Table 5.2).

Table 5.2 Carbon content and average carbon dioxide fixation of *Ankistrodesmus* sp. under various CO_2 concentrations and the pH control

Condition	Carbon content (% wt.)	CO_2 fixation (g)	CO_2 fixation rate (mg L^{-1} d^{-1})
CO_2 concentration			
1% CO_2	45.32 ± 0.31	8.56 ± 0.06	356.70 ± 2.46
2% CO_2	47.16 ± 0.14	9.84 ± 0.03	409.91 ± 1.23
5% CO_2	49.67 ± 0.32	15.51 ± 0.10	646.17 ± 4.13
10% CO_2	50.66 ± 0.25	11.53 ± 0.06	480.42 ± 2.41
Control (0.04% CO_2)	44.33 ± 0.31	10.91 ± 0.08	454.76 ± 3.14
pH control			
pH 6	45.36 ± 0.37	4.96 ± 0.04	206.50 ± 1.66
pH 7	49.50 ± 0.40	5.14 ± 0.04	214.06 ± 1.71
pH 8	48.41 ± 0.36	6.00 ± 0.04	249.81 ± 1.86
Control	45.91 ± 0.35	4.83 ± 0.04	201.18 ± 1.54

5.5 Concluding remarks

The biomass productivities of *Ankistrodesmus* sp. could be enhanced with carbon dioxide addition. Although the various biochemical compositions could be adjusted within a small range of productivity, this allows a slight improvement of the system to manage the properties of the final biomass. In the next chapter, lipid enhancement was selected as the model study where the operation of the algal cultivation system was adjusted to maximize the production of lipid content in the biomass.

CHAPTER 6
INCREASING LIPID PRODUCTION WITH REUSE AND MODIFIED BG11 MEDIA

6.1 Effect of medium and dilution rate on cell growth

Ankistrodesmus sp. was cultured in both fresh and reuse mediums under batch mode. Fig. 6.1 demonstrates the growth with an initial cell density of 0.14 g L^{-1} under three different medium conditions, i.e. fresh, 1st reused, and 2nd reused. The 1st reused medium is defined as the medium used after the first cultivation where the biomass was removed by centrifugation whereas the 2nd reuse medium is, in a similar manner, the medium after the second cultivation. At the last day of cultivation, the biomass density in the 2nd reuse medium reached the maximum value of about 1.04 g L^{-1}. In the fresh and 1st reuse mediums, the final biomass densities were 0.69 and 0.73 g L^{-1} which were lower than that obtained from the 2nd reuse medium at about 34% and 30%, respectively. The culture with the 1st reuse medium grew better than that with the fresh medium suggesting that the initial nutrient concentration might be slightly too high for an effective growth of such culture. From Day 6 up to Day 8, the biomass density decreased rapidly which might be due to the contamination with bacteria and protozoa in the last period of the 2nd reuse medium culture. Note that the system was operated with the top surface exposed to open air to resemble the actual outdoor culture which could not totally prevent contamination. Our indoor culture provided similar results where cell density in the reused medium was higher than the fresh medium. The final cell dry weight of *Ankistrodesmus* sp. in the 1st reuse medium was 0.76 g L^{-1} followed by that in the 2nd reuse medium and fresh medium with the cell dry weight of 0.60 and 0.55 g L^{-1}, respectively. However depending on the medium, this might not always be the case for other algal species, e.g. an opposite result was reported by Rodolfi *et al*. [95] who described that the biomass concentration of *Nannochloropsis* sp. in the fresh media (control) was higher than the recycled media.

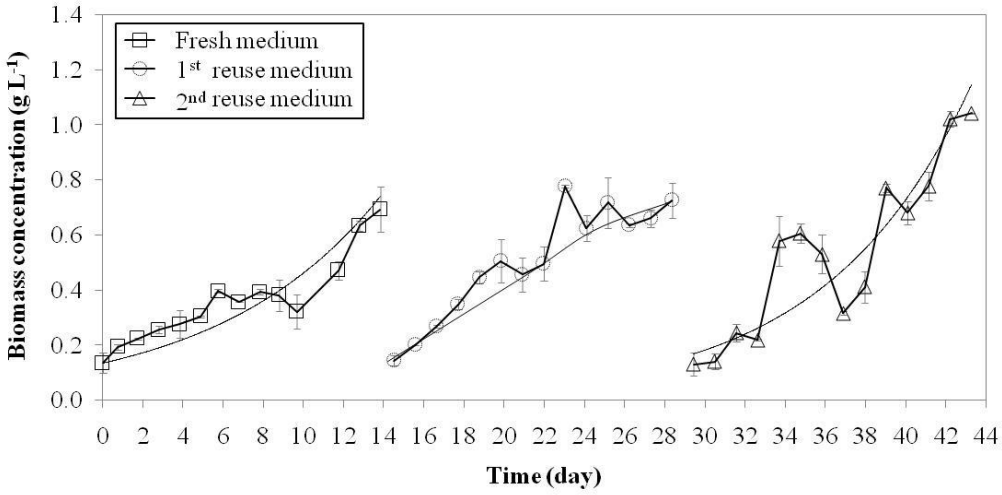

Fig. 6.1 Growth profile of *Ankistrodesmus* sp. from batch culture (average data from two batches)

Continuous cultures of *Ankistrodesmus* sp. at various dilution rates were operated for 28 days and the resulting biomass concentrations are displayed in Fig. 6.2. The two experiments with two different mediums, i.e. fresh and modified BG11, were conducted simultaneously to minimize the effect of environmental conditions that might vary from batches to batches. It is emphasized here that the modified BG11 medium contains 36.67% lower nitrogen content and 96.25% lower phosphorus content than those in the fresh BG 11 medium. These levels of nitrogen and phosphorus were similar to those in the 1st reuse medium as described above. At steady state, microalgal densities in the fresh medium at the dilution rates of 0.08, 0.16 and 0.24 d^{-1} were 1.01, 0.95 and 0.52 g L^{-1}, respectively. *Ankistrodesmus* sp. grew better in the modified BG11 medium than in the fresh BG11 medium and biomass concentrations obtained from the modified BG11 medium were 1.56, 1.36 and 0.64 g L^{-1} at the same range of dilution rates, respectively.

At Day 12 of cultivation, the cell density was re-adjusted to approximately 1 g L^{-1} both in fresh and modified BG11 mediums before adjusting the dilution rate from 0.08 to 0.16 d^{-1} and this new dilution rate was maintained during Days 12-20 of cultivation. Changes in the dilution rate seemed to have a significant impact on cell density; however, the biomass concentration obtained from the modified BG11 medium was still higher than that from the fresh medium in a similar fashion with the results at 0.08 d^{-1}. The maximum biomass concentrations in the fresh and modified BG11 mediums were 0.99 and 1.37 g L^{-1}, respectively. When considered the nutrients cost, as the modified nutrient contained lesser

quantity of some specific nutrients, it was cheaper than the standard BG11, and the analysis demonstrated that using the modified BG11 could significantly reduce the nutrient cost down from 0.11 to 0.08 THB/L.

At Days 21-28 of cultivation, the dilution rate was adjusted to 0.24 d^{-1}. *Ankistrodesmus* sp. in the modified BG11 medium declined rapidly at Day 25 and reached its new steady state biomass concentration at 0.64 g L^{-1}. At the same time, *Ankistrodesmus* sp. in the fresh medium could only reach the steady state concentration of about 0.52 g L^{-1}.

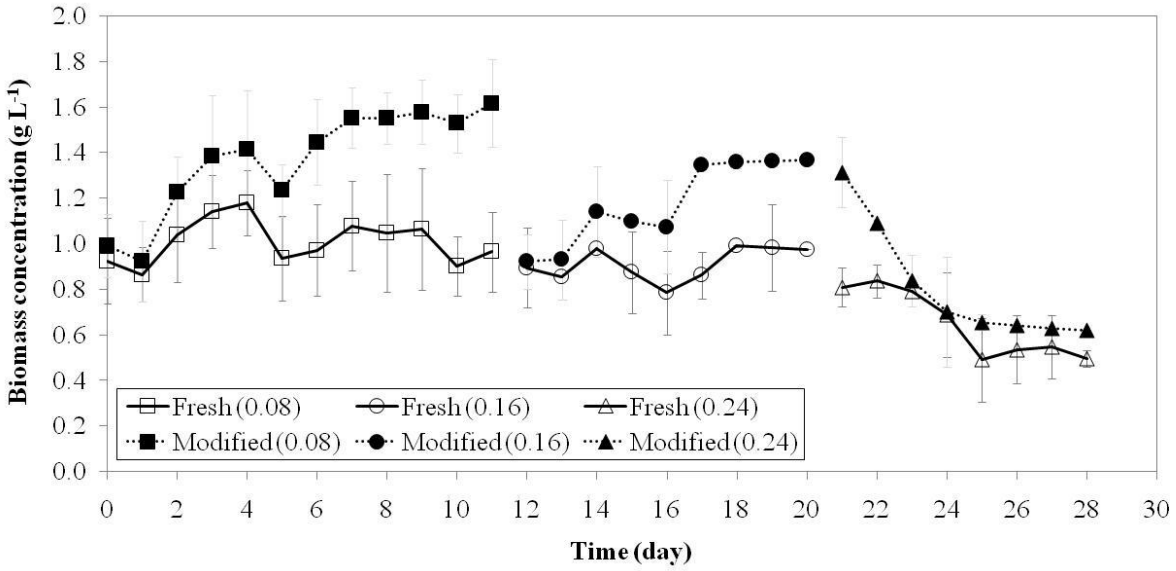

Fig. 6.2 Growth profile of *Ankistrodesmus* sp. from continuous culture

6.2 Effect of nutrient consumption on biochemical composition

Table 6.1 reports the lipid content and productivity from the cultivations of *Ankistrodesmus* sp. with various mediums both in batch and continuous modes. Note that batch cultures harvested on Days 4.16, 6.25 and 12.5 were equivalent to continuous cultures at the dilution rate of 0.24, 0.16 and 0.08 d^{-1}, respectively. In batch cultivation, the highest lipid content (57.70%) was obtained from the culture with the 1st reuse medium, followed by the 2nd reuse and fresh mediums with lipid contents of 44.80 and 32.40%, respectively. It is obvious that both reused medium conditions could provide higher lipid content than the fresh medium. Although lipid content from batch cultivations was higher than that from continuous cultivation, the lipid productivity from the continuous culture was greater than that in the batch culture. This was because the biomass productivity from

the continuous cultivation was significantly greater (almost 2.8 times) than that from the batch. The maximum lipid productivity of 38.32 mg L^{-1} d^{-1} was obtained from the culture with modified BG11 medium at the dilution rate of 0.16 d^{-1}. Statistically, there was no a significant difference of biomass and lipid productivity at continuous culture between dilution rate of 0.16 and 0.24 d^{-1} ($p<0.05$).

Table 6.1 Comparison productivity between batch and continuous cultures

Cultivation mode	Medium	Residence time (d)	Dilution rate (d^{-1})	Lipid content (%)	Biomass ($g\ L^{-1}$)	Biomass productivity (mg L^{-1} d-1)	Lipid productivity (mg L^{-1} d^{-1})
Batch†	Fresh	4.16		32.40	0.21	18.03	5.84
	1st Reuse			57.70	0.34	47.48	27.39
	2nd Reuse			44.80	0.30	40.91	18.33
	Fresh	6.25		32.40	0.28	23.20	7.52
	1st Reuse			57.70	0.44	47.60	27.47
	2nd Reuse			44.80	0.41	44.83	20.08
	Fresh	12.5		32.40	0.60	37.20	12.05
	1st Reuse			57.70	0.69	43.80	25.27
	2nd Reuse			44.80	0.96	66.42	29.75
Continuous††	Fresh		0.08	31.03	1.01±0.09	80.62 ± 7.77	25.81 ± 2.67
	Modified			34.22	1.37±0.13	109.53 ± 18.49	37.48 ± 6.33
	Fresh		0.16	18.21	0.91±0.07	145.45 ± 11.78	26.49 ± 2.15
	Modified			20.35	1.18±0.19	188.30 ± 22.69	38.32 ± 4.62
	Fresh		0.24	16.23	0.65±0.14	132.17 ± 19.28	25.24 ± 3.13
	Modified			20.48	0.81±0.05	177.02 ± 19.53	36.25 ± 4.00

Note: † average from two cultivation batches

†† data from three experimental replications

This implies that the cells had accumulated a larger quantity of lipids when they were subject to unfavorable culture conditions, and in this case, lower nitrogen supply, as illustrated in Fig. 6.3(a) (in reused medium). Similar results were observed from continuous cultures where high lipid level was obtained when cultivated in the modified BG11 medium. Nitrogen deficiency may have a significant effect on this lipid accumulation as also reported elsewhere [67, 96, 97]. On the other hand, opposite results were observed for the case of phosphorus, i.e. lipid seemed to accumulate in a greater extent when cells were exposed to a higher phosphorous concentration in the medium (Fig. 6.3(b)).

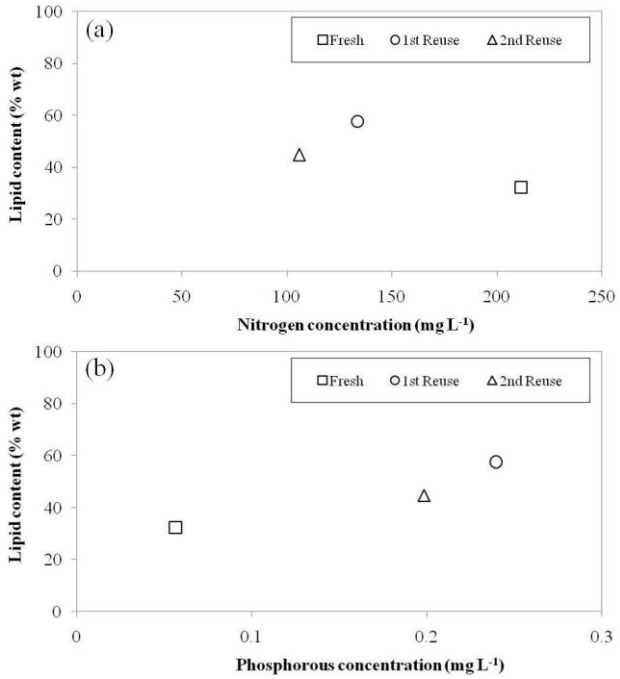

Fig. 6.3 Nutrient concentrations and lipid content in *Ankistrodesmus* sp. under batch cultivation: (a) nitrogen and (b) phosphorous

Nitrogen in the mediums seemed to have notable influence on protein accumulation. A large amount of nitrogen consumption (113.29 mg L^{-1}) gave a relatively high protein content (31.20%). This occurred with the culture grown in the fresh medium. The 2nd and 1st reuse mediums saw proteins decreasing to 20.87 and 17.42%, respectively (Table 6.2).

Table 6.2 Amount of nitrogen in different mediums and microalgae cells

Medium	Protein content (%)	Growth rate (d^{-1})	Initial nitrogen in medium (N_i) (mg N L^{-1})	Final nitrate nitrogen in medium (N_f) (mg N L^{-1})	Nitrate Nitrogen consumption ($\Delta N = N_f - N_i$) (mg N L^{-1})	Total Nitrogen in cell (N_{cell}) (mg N L^{-1})	NH^+_4 Nitrogen Released from cell ($N_{NH4} = \Delta N - N_{cell}$) (mg N L^{-1})
Fresh	31.20	0.47	324.77	211.48	113.29	48.66	64.63
1st Reuse	17.42	0.32	205.66	133.58	72.07	28.44	43.63
2nd Reuse	20.87	0.52	160.71	105.87	54.84	48.93	5.91

Table 6.2 illustrates that although the 2nd reuse medium contained a lower level of nitrogen (160.71 mg N L^{-1}) than that in the standard BG11 medium, the highest growth rate was still observed. This suggested that the amount of nitrogen in the reuse medium was still enough for microalgal growth, and the excess nitrogen in the fresh medium was only used extravagantly and was released from the cell in the form of NH^+_4. It is interesting to see that only a minor quantity of nitrogen was released when the 2nd reuse medium was applied. The protein productivity in such cell would therefore depend on both protein content and biomass growth character. It is noted that in Table 7, most initial nitrogen was supplied in the form of nitrate. Alga consumed nitrate nitrogen for their metabolism, and in return, excreted nitrogen in the form of NH^+_4. The simple form of nitrogen uptake and release mechanisms are given in Fig. 6.4.

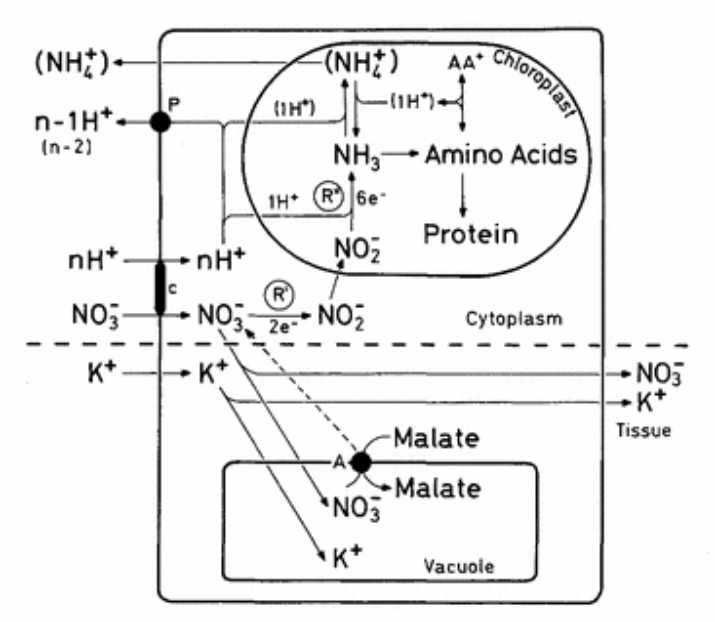

Fig. 6.4 Scheme of nitrate assimilation and related alkalinization under steady state conditions [98]

The result of the highest nitrogen concentration in the fresh medium (211.48 mg L^{-1})) presented the highest amount of protein, as seen in Fig. 6.5(a). The amount of protein in algal cells was variable, and protein might have lost as a result of nitrogen starvation [99]. Protein accumulation of microalgae directly depended on nitrogen concentration, where nitrate entered the cells and converted to ammonium and entered the GS/GOGAT cycle to produce two glutamate molecules, one molecule for the recycled the GS/GOGAT pathway and the other can be used to form complex amino acids (Fig. 6.6). Relationships between nitrogen consumptions and biochemical compositions exhibited similar trends with nitrogen concentrations (data not shown). Protein content slightly decreased with increasing phosphorous concentration Fig. 6.5(b). Similar trends for carbohydrate accumulation are displayed in Fig. 6.7(a) and (b).

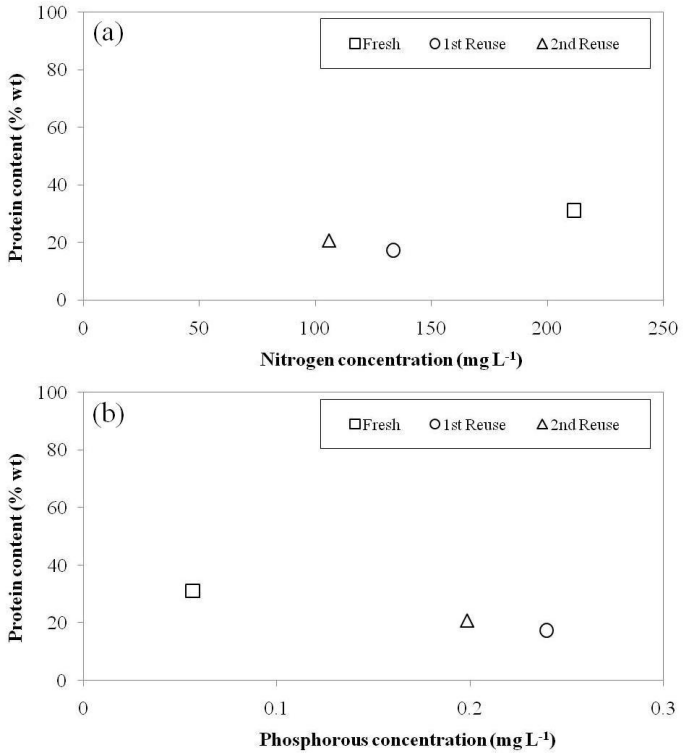

Fig. 6.5 Nutrient concentrations and protein content in *Ankistrodesmus* sp. under batch cultivation: (a) nitrogen and (b) phosphorous

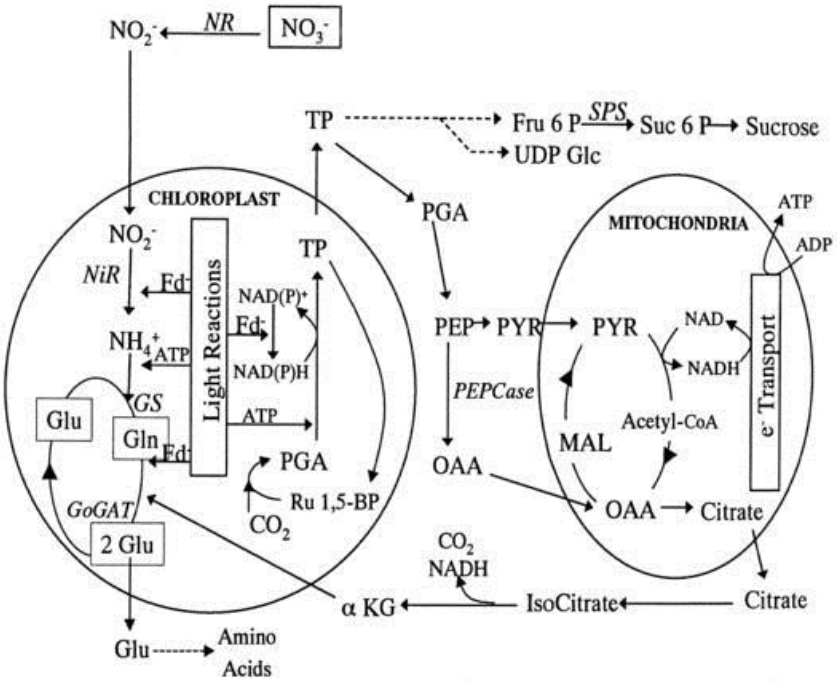

Fig. 6.6 Reactions involved in nitrate uptake and assimilation in plant cells [100]

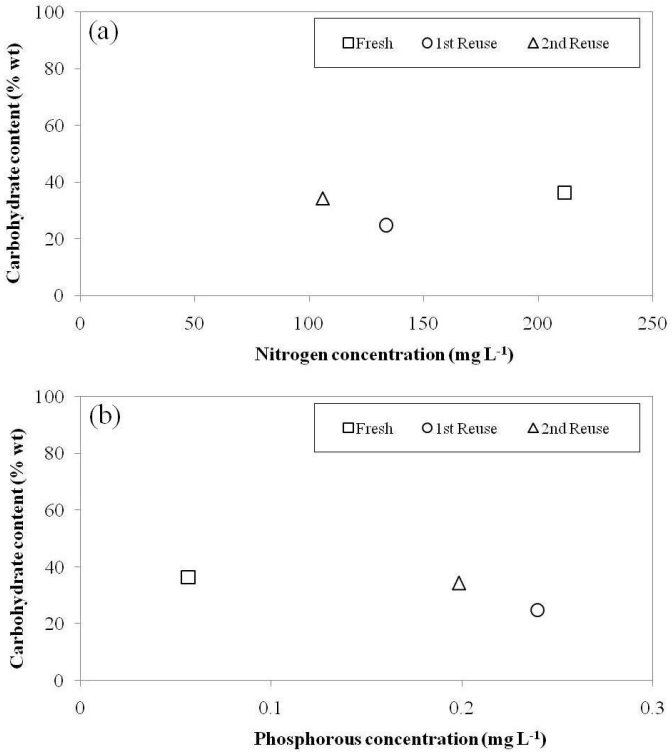

Fig. 6.7 Nutrient concentrations and carbohydrate content in *Ankistrodesmus* sp. under batch cultivation: (a) nitrogen and (b) phosphorous

6.3 Effect of dilution rate on biochemical composition

The reduction of lipid content may be influenced by the increasing dilution rate both in the fresh medium and modified BG11 medium, whereas lipid content was higher in the modified BG11 medium than in the fresh medium (see Fig. 6.8(a)). The highest lipid content of 34.22% was obtained at dilution rate 0.08 d^{-1} in the modified BG 11 medium while the dilution rate 0.24 d^{-1} in fresh medium gave the lowest lipid content of 16.23%. The nitrogen concentrations increased with increasing dilution rate, resulting in a large accumulation of protein (Fig. 6.8(b)), which was discussed in the previous section. It is interesting to note that although dilution rate seemed to have an influence on the accumulation of lipids and proteins, but did not have a comprehensible relationship with carbohydrate content (Fig. 6.8(c)).

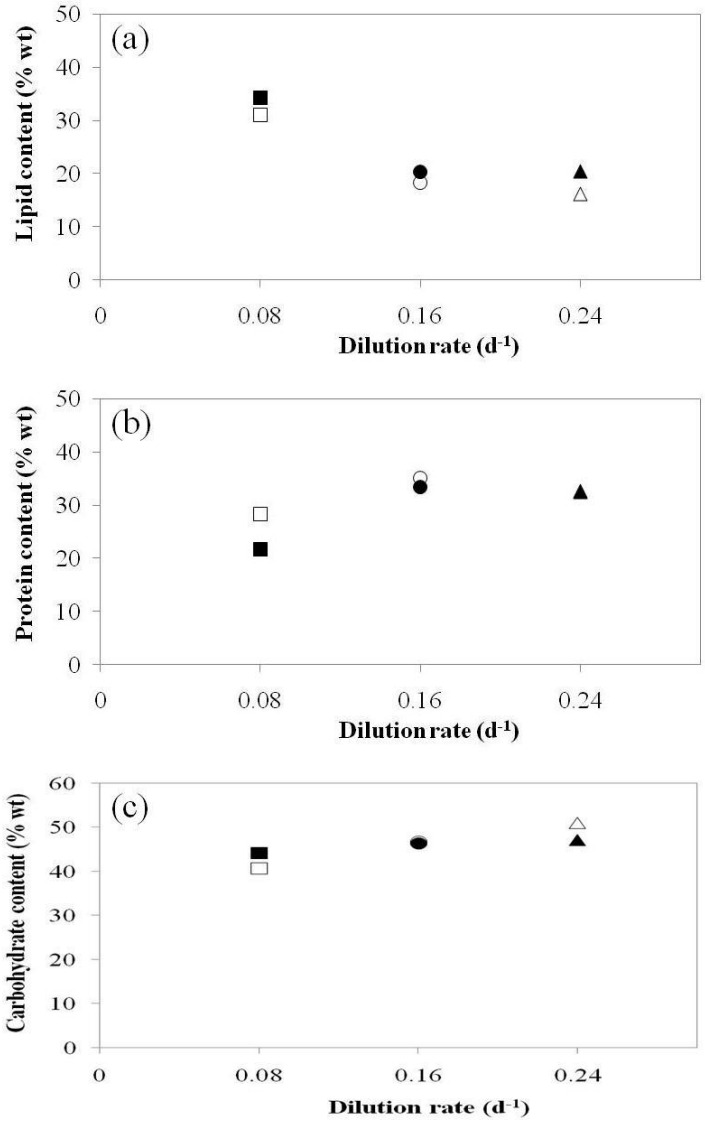

Fig. 6.8 Biochemical compositions accumulation in *Ankistrodesmus* sp. with various dilutions (□,○ and △denote fresh medium at dilution rates of 0.08, 0.16 and 0.24 d^{-1}, respectively, whereas ■, ● and ▲ are for modified BG11 at the same order of dilution rates). Lipid, protein and carbohydrate content with various dilution rates are present in (a), (b) and (c), respectively

6.4 Comparison of fatty acids between batch and continuous cultivations

This analysis was conducted to see if the lipid accumulated in *Ankistrodesmus* sp. was suitable as a raw material for biodiesel production. Table 6.3 demonstrates the mean values of fatty acids obtained from the alga. The majority of fatty acids was palmitic acid (C16:0) belonging to saturated fatty acid, and oleic acid (C18:1) belonging to monounsaturated fatty acid. In most cases, more unsaturated fatty acids than saturated fatty acids were found except the modified BG11 medium in continuous cultivation at the dilution rate of 0.24 d^{-1}. The highest sums of saturated fatty acid and unsaturated fatty acid were approximately 45% and 58%, respectively. The saturated fatty acid content increased with increasing dilution rate, whereas unsaturated fatty acids decreased.

Palmitic acids (C16:0) from the batch culture with fresh and 1st reused mediums were in a similar range whereas that from the culture with the 2nd reused medium was relatively low (data not shown). Palmitic acid (C16:0) became the most abundant fatty acid when cells were cultivated in the fresh medium continuous culture, and it was shown the highest value approximately 40%. Both fresh and modified BG11 mediums when used in continuous culture led to the formation of high amounts of fatty acid in forms of palmitic acid (C16:0), i.e. at 40.66% and 37.81%, respectively. This was in a similar range with other algae, e.g. the palmitic acid contents of *Ankistrodesmus falcatus*, *Tetraselmis suecica*, *Isochrysis galbana* and *Chlorella* sp. were 28.6%, 53.49%, 48.42% and 30.99%, respectively [16, 101]. The continuous culture with modified BG11 medium saw an accumulation of stearic acid (C18:0) at approximately 4-6%; the component not found in all other conditions.

The highest polyunsaturated fatty acid (PUFA) was obtained from the culture in the fresh medium under continuous cultivation at a dilution rate of 0.24 d^{-1} (average values of 36.08%). Linoleic acid (C18:2) and alpha linolenic acid (C18:3 (ω3)) were over 8% while other polyunsaturated fatty acid was quite low. The four fatty acids, i.e. C12:0, C14:0, C16:1 and C18:3(ω6) were always under 2%.

Fig. 6.9 illustrates the distribution of the productivity of the various types of fatty acids from the various cultures of *Ankistrodesmus* sp. The percentage of palmitic acid in the fresh medium continuous culture at the dilution rate of 0.16 d^{-1} was relatively high leading to a high productivity of saturated fatty acid. However, the highest saturated fatty acid content was obtained with the modified BG11 medium with continuous cultivation at

dilution rates of 0.16 and 0.24 d^{-1}. As an overall comparison, the highest polyunsaturated and unsaturated fatty acid productivity was observed in the continuous culture with the modified BG11 medium at the dilution rate of 0.16 d^{-1} (59.13 and 104.54 mg L^{-1} d^{-1}, respectively). This condition, therefore, could be treated as the most suitable if the culture of *Ankistrodesmus* sp. was to be used for the biodiesel production.

Table 6.3 Fatty acid profile from batch and continuous cultures of *Ankistrodesmus* sp.

Type of fatty acids	Total fatty acid (%)							
	Batch		Continuous					
			Fresh			Modified BG11		
	Fresh	1st Reuse	Dilution rate (d^{-1})			Dilution rate (d^{-1})		
			0.08	0.16	0.24	0.08	0.16	0.24
Lauric acid (C12:0)	1.96	0.88	1.36	1.30	1.33	0.27	0.64	0.15
Myristic acid (C14:0)	0.93	0.59	0.72	0.69	0.64	0.36	0.55	0.83
Palmitic acid (C16:0)	37.44	36.16	28.87	40.66	32.26	31.35	32.56	37.81
Palmitoleic acid (C16:1)	0.60	0.31	0.91	1.28	0.93	0.25	0.46	0.35
Stearic acid (C 18:0)	-	-	-	-	-	4.59	3.93	6.38
Oleic acid (C18:1)	23.15	33.48	23.91	24.75	20.84	31.43	23.66	28.25
Linoleic acid (C18:2)	10.19	9.16	13.54	8.01	12.06	14.64	18.90	9.17
Gamma linolenic acid (C18:3 (ω6))	0.27	0.19	0.75	0.97	0.46	0.50	0.56	0.35
Alpha linolenic acid (C18:3 (ω3))	13.07	8.61	14.22	11.76	18.70	8.81	10.33	3.30
Stearidonic acid (C18:4 (ω3)	2.33	3.12	4.91	2.92	4.86	2.34	1.61	1.14
Lignoceric acid (C24:0)	3.50	2.51	3.50	2.84	3.16	0.06	-	-
Unknown	6.51	4.93	7.31	4.82	4.76	5.40	6.80	12.27
SFA[a]	43.88	40.20	34.45	45.49	37.39	36.63	37.68	45.17
MUFA[b]	23.75	33.79	24.82	26.03	21.77	31.68	24.12	28.6
PUFA[c]	25.86	21.08	33.42	23.66	36.08	26.29	31.40	13.96
UFA[d]	49.61	54.87	58.24	49.69	57.85	57.97	55.52	42.56

Note: a saturated fatty acid
b monounsaturated fatty acid
c polyunsaturated fatty acid
d unsaturated fatty acid

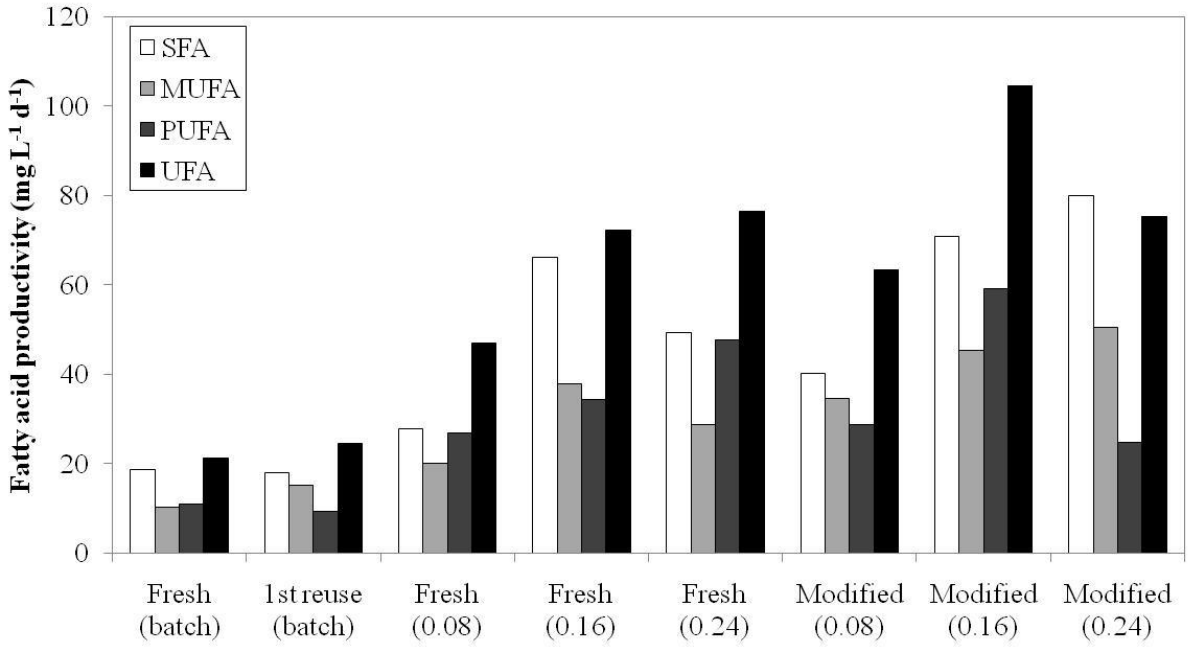

Fig. 6.9 Fatty acid productivities of *Ankistrodesmus* sp. in batch and continuous cultures both with fresh and modified BG11 media

6.5 Concluding remarks

This work reveals that *Ankistrodesmus* sp. was best cultivated in a continuous culture at the dilution rate of 0.16 d^{-1}. The cost of the nutrients was reduced by 27% by adjusting the levels of major nutrients especially nitrogen and phosphorus. The reducing elements of this modified BG11 standard medium were proven to raise the lipid accumulation, where the maximum lipid yield was obtained at the point of maximum growth. Analysis indicated that palmitic acid (C16:0) was the major fatty acid component in the lipid extract.

CHAPTER 7
CONCLUSIONS

The culture of *Ankistrodesmus* sp. in the airlift photobioreactor was shown to be possible. The main findings obtained from this work can be summarized as follows (Fig. 7.1):

7.1 Batch culture of *Ankistrodesmus* sp. in Duran bottle

Light intensity affects directly the growing and photosynthesis of microalgae, which require optimal light intensity during the growth cycle. The cultures of *Ankistrodesmus* sp. grew best with the light intensity of 30,000 lux and temperature at 30 °C. The result achieved the maximum biomass concentration of 1.48 g L^{-1}. The highest biomass and lipid productivity of 222.56 and 56.84 mg L^{-1} d^{-1} were obtained in such conditions.

7.2 Enhancing *Ankistrodesmus* sp. growth with carbon dioxide supplement

Gaseous carbon dioxide is the main carbon source, and the adding of CO_2 was expected to increase the growth of microalgae. pH may be regulated and kept at a fixed level by automatic CO_2 feeding when pH was exceeded the set point. At pH 8, the culture grown best compared to pH 6 and 7. The maximum lipid productivity of 72.85 mg L^{-1} d^{-1} was obtained in such culture.

Carbon dioxide was added into the airlift photobioreactor continuously. At 5% of carbon dioxide, the culture seemed to grow best but this extra CO_2 did not have significant effects on the lipid content in the alga. The attainable maximum lipid productivity of 109.99 mg L^{-1} d^{-1} were relatively high when compared to the result from the cultivation without CO_2 where the maximum lipid productivity of 97.45 mg L^{-1} d^{-1} could be obtained.

7.3 Increasing lipid production with reuse medium and modified BG11 medium

To minimize operating costs, the medium was reused to culture microalgae. The reuse of nutrient does not seem to have negative effect to the lipid productivity. The highest lipid content of 57.70% was obtained in the 1st reused medium operating in a batch mode, the depletion of nitrogen source in reused mediums could be an important factor for the accumulation of lipid.

The control of the flow rate of the fresh medium is an important issue in the continuous culture, and the flow rate should not be higher than the specific growth rate if wash out is to be avoided. The cell growth decreased with increasing dilution rate. Lipid synthesis was not significantly affected by the dilution rate. The maximum biomass and lipid productivity in the modified BG11 medium with continuous system at 0.16 d^{-1} were the highest at 188.30 and 38.32 mg L^{-1} d^{-1}, respectively. Furthermore, the modified BG11 could significantly reduce the nutrient cost down from 0.11 to 0.08 THB/L.

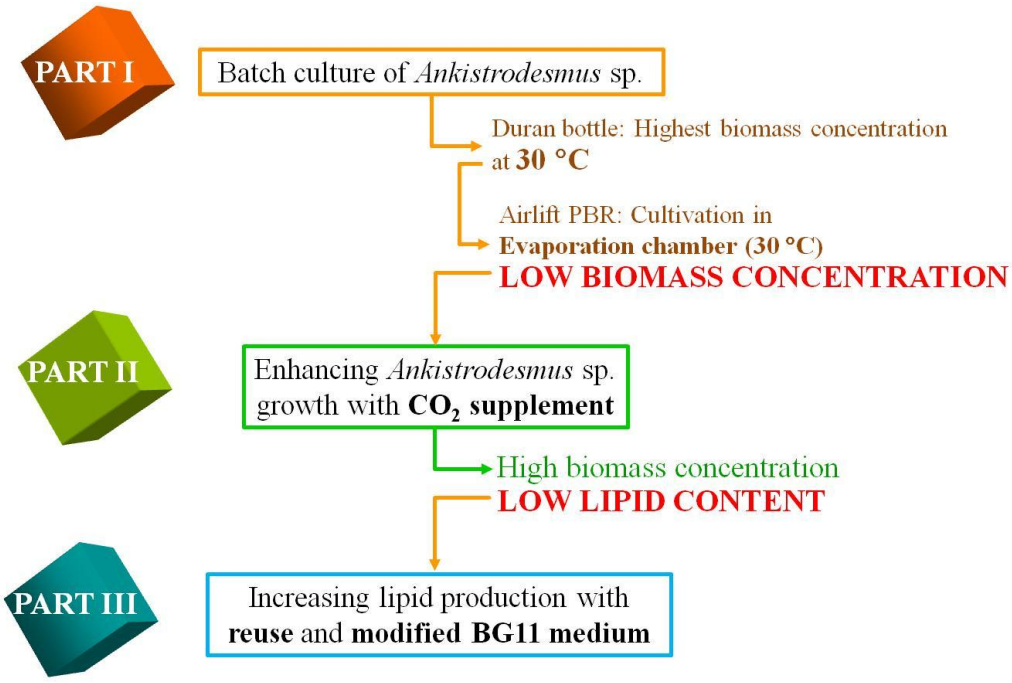

Fig 7.1 Summary of all of the experiments

7.4 Contributions

This research has achieved a steady and successful culture of *Ankistrodesmus* sp. in 3L airlift photobioreactor under batch and continuous systems. The results suggest that the nutrients depletion condition might produced high lipid content, which could used as biofuel feedstock, whereas the nutrients rich condition could induced high protein content, which could used as foodstuff for fish. In addition, carbon dioxide supplement might affect the biomass productivity in such alga.

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APPENDIX A

MEASUREMENTS OF NUTRIENT CONCENTRATIONS

A. 1 Measurement of nitrogen concentration by spectrophotometer [102]

Blank

1 mL of distilled water is measured by a spectrophotometer at wavelengths of 220 and 275 nm, and the blank is set to zero.

Calibration

1. KNO_3 stock solution 100 mg NO_3-N/ L is prepared by dissolved 0.7128 g of KNO_3 in 1000 mL of distilled water and kept with 1 mL of chloroform in dark glass.
2. KNO_3 stock is diluted using distilled water at 0.5, 1.0, 3.0, 4.0, 5.0 and 8.0 mg NO_3-N/ L
3. The solution is measured by a spectrophotometer at wavelengths of 220 and 275 nm

Procedure

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

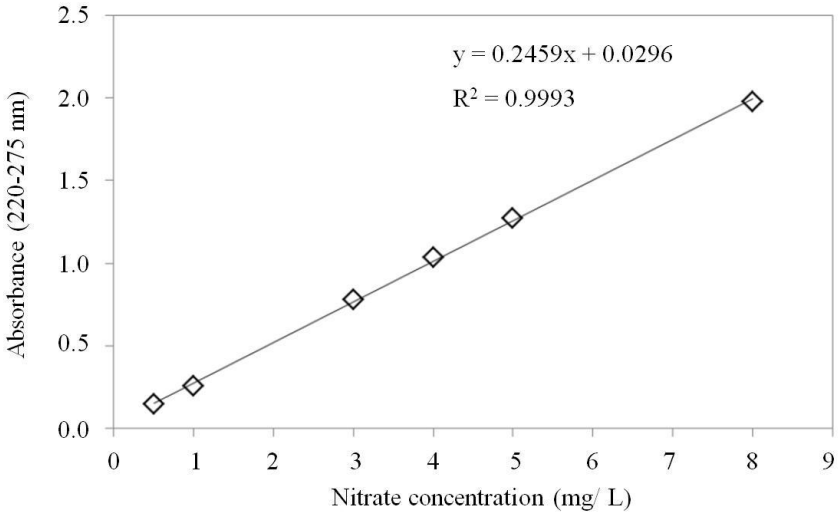


Fig. A.1 Standards of nitrogen concentrations by spectrophotometer

A. 2 The conditions of using measurement phosphorous by ICP

Table A.1 Conditions used in ICP

Power	kW	1
Plasma flow	L min^{-1}	15
Auxiliary flow	L min^{-1}	1.5
Nebulizer flow	L min^{-1}	0.75
Replicate read time	s	5
Instr. stabilization delay	s	15

Table A.2 Sample introduction settings

Sample uptake delay	s	30
Pump rate	rpm	15
Rinse time	s	10

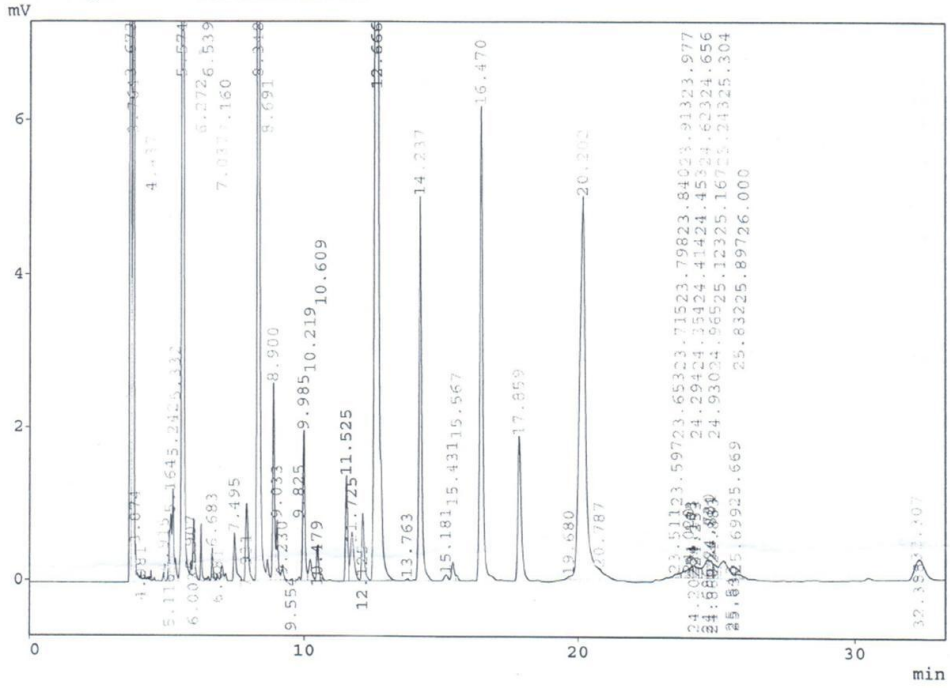
APPENDIX B

CHROMATOGRAM OF FATTY ACIDS

CLASS-GC10 Ver.=2.00 SYS=1 Ch=1 REPORT.NO=10 DATA=L5.D01 00/01/01 04:03:08

Sample : L5
ID : 1
Dilution Factor: 1
Type : Unknown
Detector : FID
Operator : keng

*** Chromatogram *** Filename:L5.C01



*** Peak Report ***

PKNO	TIME	AREA	HEIGHT	MK	IDNO	CONC	NAME
1	3.673	2694822	1002428	E		50.0715	
2	3.764	1935977	1000602	SVE		35.9716	
3	3.874	216	201	T		0.0040	
4	4.081	183	95	T		0.0034	
5	4.437	254	132	TV		0.0047	
6	4.915	455	119			0.0084	
7	5.116	2101	669			0.0390	
8	5.164	3154	885	V		0.0586	
9	5.242	4740	1201	V		0.0881	
10	5.332	1231	361	V		0.0229	
11	5.574	200550	68459	SV		3.7263	
12	5.907	450	149	T		0.0084	
13	6.003	3357	759	TV		0.0624	
14	6.272	2402	740	T		0.0446	
15	6.539	166	55	TV		0.0031	
16	6.683	1699	431	T		0.0316	
17	6.881	287	90	TV		0.0053	
18	7.037	1107	213	TV		0.0206	
19	7.160	379	38	TV		0.0070	
20	7.495	4013	613	S		0.0746	
21	7.931	6603	1013	V		0.1227	
22	8.348	93471	19116	SV		1.7367	
23	8.691	808	197	T		0.0150	
24	8.900	12120	2573	V		0.2252	
25	9.033	4024	713	V		0.0748	
26	9.230	1074	213	V		0.0200	

10 - 1/2 00/01/01 04:37:08

Fig. B.1 Chromatogram of fatty acid under continuous cultivation

APPENDIX C

CALCULATIONS OF NUTRIENT COSTS

C. 1 Calculations of nutrient costs

Table C.1 Prices of nutrients for standard BG11 medium

Chemicals for BG11	Quantity	Unit Price	Amount	Amount/ 1L medium	Price/ 1L medium
$NaNO_3$	25 kg	715	15.0 g	1.5 g	0.0429
K_2HPO_4	25 kg	3350	2.0 g	0.04 g	0.00536
$MgSO_4$	25 kg	275	3.75 g	0.075 g	0.000825
$CaCl_2$	25 kg	325	1.80 g	0.036 g	0.000468
Citric acid	25 kg	1690	0.30 g	0.006 g	0.0004056
Ammonium ferric citrate green	500 g	600	0.30 g	0.006 g	0.0072
$EDTANa_2$	100 g	4250	0.05 g	0.001 g	0.0425
Na_2CO_3	40 kg	880	1.00 g	0.02 g	0.00044
H_3BO_3	25 kg	1400	2.86 g	0.00286 g	0.00016016
$MnCl_2$	500 g	900	1.81 g	0.00181 g	0.003258
$ZnSO_4$	25 kg	1150	0.22 g	0.00022 g	0.00001012
Na_2MoO_4	250 g	2160	0.39 g	0.00039 g	0.0033696
$CuSO_4$	25 kg	2450	0.08 g	0.00008 g	0.00000784
$Co(NO_3)_2$	100 g	840	0.05 g	0.00005 g	0.00042
				Total	**0.11**

APPENDIX D

pH OF MEDIUM IN CARBON DIOXIDE SUPPLEMENTS

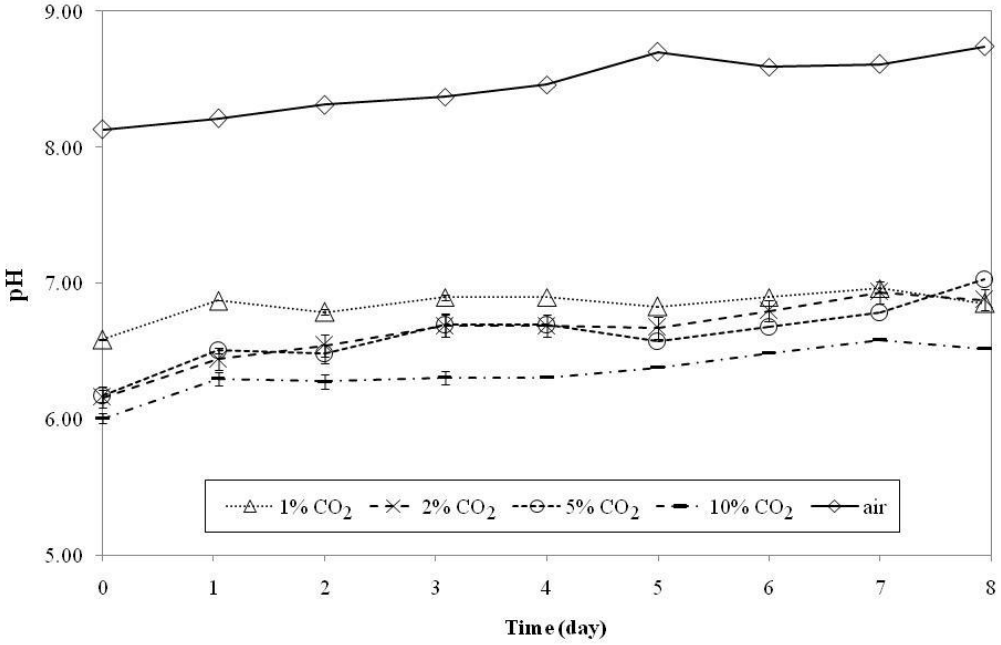


Fig. D.1 pH measure under different CO_2 concentrations