Microalgae for CO_2 Fixation

by

Ida Nordiana Binti Abdul Samat

Dissertation submitted in partial fulfilment of
the requirements for the
Bachelor of Engineering (Hons)
(Chemical Engineering)

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CERTIFICATION OF APPROVAL

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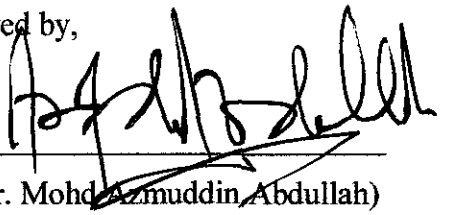
Approved by,

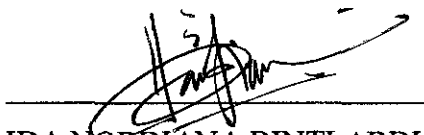


(AP. Dr. Mohd Azmuddin Abdullah)

UNIVERSITI TEKNOLOGI PETRONAS
TRONOH, PERAK
May 2012

CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, that the original work is my own except as specified in the references and acknowledgements, and that the original work contained herein have not been undertaken or done by unspecified sources or persons.



IDA NORDIANA BINTI ABDUL SAMAT

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ABSTRACT

The problem of climate change arising mainly from CO_2 emission is currently a critical environmental issue. Biofixation using microalgae has recently become an attractive approach to CO_2 capture as algal species naturally use CO_2 for photosynthesis. *Nannochloropsis sp., Chaetoceros sp., Pavlova lutheri,* and *Isochrysis galbana* were cultivated in 1 L vessels to determine the best microalgal strain. Cell density of each microalgae culture *Nannochloropsis sp., Isochrysis galbana, Pavlova lutheri* and *Chaetoceros sp.* yield result of $35.0x10^6$, $7.0x10^6$, $2.75x10^6$, and $1.0x10^6$ cells/ml, respectively. Maximum biomass formation rate, X_{max} (mg $L^{-1}d^{-1}$) and and lipid content of selected microalgal strain, *Nannochloropsis sp.* were 22 and 46, respectively. *Nannochloropsis sp.* cells were further cultured in 5 L working volume of bioreactor. Microalgal strains were grown under different temperatures and different air flow rates to find out optimum range. The effects of CO_2 concentration on growth and lipid content were also investigated. *Nannochloropsis sp.* was considered the best species among all other species with maximum biomass formation rate, X_{max} (mg $L^{-1}d^{-1}$) and and lipid content of 22 and 46 mg/L, respectively. Optimum temperature and CO_2 fixation rate (mg/L) was 25°C and 488.8, respectively. CO_2 supply with continuous aeration gave a significant difference in result, culture with CO_2 supply lead to higher biomass productivity of 420 mg/L. Air flow rate of 2 L/min gives better result compared to 1 L/min aeration, with 520 mg/L dry weight and 110 mg/L lipid content; the higher flow provides more CO_2, thus accelerate growth. Maximum specific growth rate of 5 L cultivation was higher than 1 L cultivation of 39×10^6 and 35×10^6 cells d^{-1}, respectively. While doubling time was 8.8 and 10.8 day for 1 L cultivation and 5 L cultivation, respectively.

Keywords: Microalgae; *Nannochloropsis sp., Chaetoceros sp., Pavlova lutheri, Isochrysis galbana,* CO_2, Bioreactor, Biomass, Lipid

CHAPTER 1
INTRODUCTION

1.1 Background of Study

Global warming from greenhouse gases emission such as CO_2 has become a major concern. The growth in CO_2 emission was mainly led by coal, natural gas and oil with 44%, 20%, and 36% respectively. Carbon dioxide emissions in 2011 at 391.76 ppm are the highest, breaking the prior record in 2010 at 389.82 ppm. In February 2012, the CO_2 emissions reported to be 393.65 ppm [1]. According to the latest report by the Intergovernmental Panel on Climate Change (IPCC), if CO_2 concentrations in the atmosphere doubled in relation to preindustrial levels, the temperature of the Earth's surface would rise an average of more than 4°C by 2100. Emissions of CO_2 from fossil fuel combustion, with contributions from cement manufacture, are responsible for more than 75% of the increase in atmospheric CO_2 concentration since pre-industrial times (IPCC, 2011). During the last 2 decades, many attempts have been made to reduce atmospheric CO_2. The need for CO_2 fixation and sequestration has become urgent. Among many attempt to reduce the quantity of CO_2 in the atmosphere, the CO_2 fixation using microalgae has extensively been studied since the beginning of the 1990's. Algal green technology for CO_2 removal can be converted into biodiesel and bioenergy. The CO_2 fixation rate is influenced by factors such as CO_2 concentration, lighting, growth media or cultivation techniques. A lot of methods developed in assessing the potential of microalgae in fixing the CO_2, either by biomass measurement or growth rate as well as direct measurement of CO_2 consumption of microalgae.

In the present study, the effects of temperature, air flow rate and CO_2 supply on growth of *Nannochloropsis sp.* culture was investigated. The lipid content in a batch bioreactor system was also evaluated.

[1] Scripps CO_2 Program UCSD / Scripps Institution of Oceanography, National Oceanic and Atmospheric Administration (NOAA)

1.2 Problem Statement

Removal of CO_2 using membranes or amine can be an expensive option and not green. Strategies for CO_2 removal for example by absorption in aqueous amine e.g. Monoethanolamine (MEA) led to the formation of corrosive compounds on decomposition. The bio-fixation of CO_2 by microalgae is a natural capture because microalgae utilize use CO_2 for photosynthesis. Thus, microalgae consumption rate can be used to reflect its ability to fix CO_2.

1.3 Objectives

The objectives of this research are:

1. To determine the best microalgal strain among *Nannochloropsis sp.*, *Chaetoceros sp.*, *Pavlova lutheri,* and *Isochrysis galbana*, optimum temperature and air flowrate for microalgae growth.
2. To investigate the effects of CO_2 concentration on growth and lipid content of selected microalgae species.
3. To establish the kinetics parameters such as maximum specific growth rate, μ_{max}, and doubling time t_d.

1.4 Scope of Study

The main focus of this work is to develop a system using microalgae for CO_2 fixation. Analysis of cell growth, lipid content, biomass production and CO_2 fixation rate will then be evaluated.

1.5 Relevancy of the Project

CO_2 fixation by microalgae is an environmentally friendly biological based system, therefore is a good option of making the CO_2 removal less harmful. Research on the commercial usage of this microalgae is still ongoing to decrease reliance the on the chemical reaction based CO_2 mitigation, microalgae have been looked at as a potential substitute for reduction of CO_2 in atmosphere.

CHAPTER 2
LITERATURE REVIEW

A number of research and development efforts have been directed at reducing CO_2 emissions. Various CO_2 mitigation strategies have been investigated, and can be generally classified into three strategies: (1) chemical reaction-based approaches; for instance, washing with alkaline solutions, and amine coating activated carbon (2) direct injection to underground or to the ocean (3) biological CO_2 mitigation, with CO_2 being biologically converted to organic matters (Ho et al., 2010). Biological CO_2 fixation is currently achieved through the photosynthesis of all earthly plants and a various number of photosynthethic microorganisms. However, plants are expected to contribute to only 3-6% reduction in global CO_2 emissions (Skjanes et al., 2007). Microalgae and cyanobacteria can grow much faster than terrestrial plants, and their CO_2 fixation efficiency is about 10-50 times better (Costa et al., 2000)

2.1 Microalgae

Microalgae are defined as a diverse group of prokaryotic and eukaryotic photosynthetic microorganisms that grow rapidly due to their simple structure (Li et al., 2008). Others claimed microalgae comprise a vast group of photosynthetic, heterotropic organisms which have an extraordinary potential for cultivation as energy crops (Bajhaiya et al., 2010). Today, more than 40 different species of microalgae, isolated in different parts of the world, are cultured as pure strains in intensive systems. Microalgae are very important in the commercial rearing of various species of marine animals. They need microalgae as foods source as to support the growth. Besides, for rearing marine fish larvae, algae are used directly in the larval tanks, where they are believed to play a role in stabilizing the water quality, nutrition of the larvae, and microbial control. Example of the most frequently used species in commercial mariculture operations are the diatoms *Skeletonema costatum*, *Thalassiosira pseudonana*, *Chaetoceros gracilis*, the flagellates *Isochrysis galbana*, *Tetraselmis suecica*, and the

chlorococcalean *Chlorella* spp. (Coutteau P., 1996). Some types of marine microalgae under microscope shown in Figure 1.

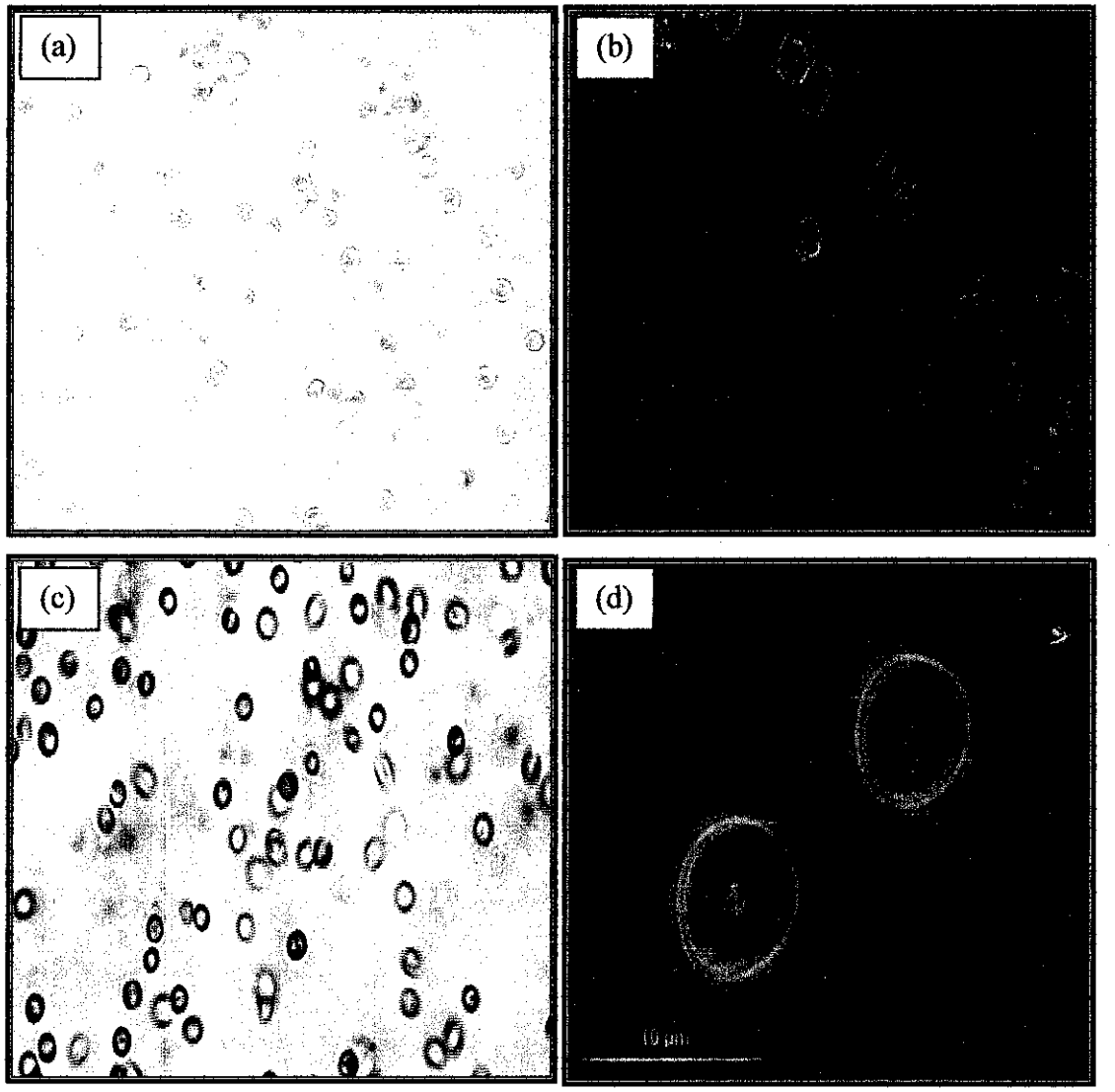


Figure 1: Microalgae species viewed under a light microscope (a) *Nannochloropsis sp.* (b) *Chaetoceros sp.* (c) *Isochrysis galbana* (d) *Pavlova lutheri.* (Source: Provasoli-Guillard National Center for Culture of Marine Phytoplankton)

2.2 Microalgal Photosynthesis

Microscopic algae use photosynthetic process similar to that of higher-developed plants, thus able to be utilized as medium for CO_2 mitigation. Microalgae assimilate inorganic carbon in the photosynthesis with the present of light either sunlight or from other sources. The solar energy will then converted to chemical energy with oxygen (O_2) as a

by-product, and in a second step the chemical energy is used to assimilate carbon dioxide and convert it to sugars (Larsdotter, 2006). Biofixation of carbon dioxide using microalgae has emerged as a potential option as microalgae have the advantages of efficient photosynthesis. According to Iverson (2006), photosynthesis is a process that contains two steps, light reactions that only occurs when the cells are illuminated,

$$2H_2O + 2NADP^+ + 3\ ADP + 3P_i + light \rightarrow 2NADPH + 2H^+ + 3ATP + O_2 \quad (1)$$

and carbon-fixation reactions, also known as dark reactions, that occur both in the presence and absence of light.

$$3CO_2 + 9ATP + 6NADPH + 6H^+ \rightarrow C_3H_6O_3\text{-phosphate} + 9ADP + 8P_i + 6NADP^+ + 3H_2O \quad (2)$$

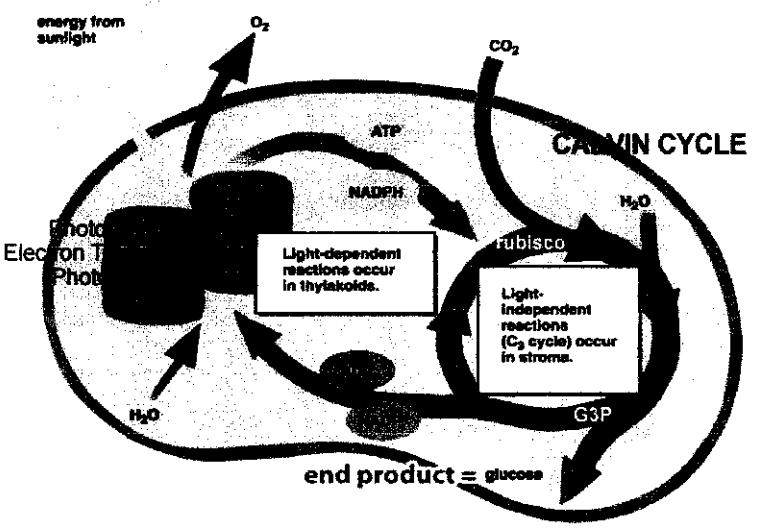


Figure 2: Diagram explains the photosynthesis reaction (Source:http://withfriendship.com/user/kethan123/calvin-cycle.php)

2.3 Factors affecting CO_2 fixation of microalgae

Many factors contribute to carbon dioxide fixation rate by microalgae such as light intensity, CO_2 concentration and bioreactor design.

2.3.1 Light Intensity

Since microalgal-CO_2 fixation involves photoautotrophic growth of cells, the CO_2 fixation capability of specific species should positively correlate with their cell growth rate and light utilization efficiency (Jacob-Lopes et al., 2009). Generally, the amount of light energy received and stored by the cells is directly proportional with the carbon fixation capacity, consequently determining the productivity in the biomass and cell growth rate. Higher light utilization efficiency can be increased by increasing surface area and shortening the light path and layer thickness (Pulz, 2001). Normally, the cell growth is directly proportional to the light intensity or light periods until an extremely high illumination level is reached that can damage photosystem (Melis, 1999). In natural solar energy, the photosynthesis of most microalgae is saturated at about 30% of total solar radiation (Pulz, 2001).

2.3.2 CO_2 Concentration

Much work has been done on the effect of different concentration of carbon dioxide on microalgal growth rates and carbon dioxide fixation including the source from the atmosphere and the flue gases. Cheng et al. (2006) investigated using normal room air (0.04% CO_2), the CO_2 concentration in the discharged gas could be decreased to 0.015%, indicating that the microalgae *Chlorella vulgaris* function well for CO_2 reduction in atmosphere. Experimental work in batch operation strategies of *Chlorella vulgaris* and *Chlorella emersonii* with CO_2 concentration of air resulted in low amount of CO_2 consumption, 75 mg L^{-1} d^{-1}, 77 mg L^{-1} d^{-1} respectively (Scragg et al, 2002). In other investigation using *Anabaena sp* with continuous operation, much higher CO_2

consumption rate is gained 1450 mg L^{-1} d^{-1} (Lopez et. al., 2009). This indicates the continuous operation is better than batch operation (Cheng et al, 2006). A comparison of *Chlorella sp* growth and biofixation of CO_2 in two different cultivation modes was also studied, shows that the closed system microalgae able to rapidly grow and effectively fix CO_2 when using the proper cultivation mode (Zhao et al., 2011). Since the ambient atmospheric concentration of CO_2 is far below optimum for algal growth, supply of extra carbon dioxide may be necessary (Jeong et al, 2003), providing the cultures with air enriched with 1 % (Cheng et al, 2006), 2% (Chiu et al, 2008) to 5% CO_2 (Sydney et al, 2010). In other experimental result showing *C. vulgaris* grew well in different concentrations of biogas slurry. This species had excellent growth (Jeong et al., 2003) and tolerance to high concentrations of CO_2, and the largest biomass productivity was obtained at the aeration of 1.5% CO_2, also results in CO_2 removal at a rate of 60 mL min^{-1} (Wang et al., 2011). Also, the maximum efficiency of CO_2 reduction reached 58% in the culture aerated with 2% CO_2 (Chiu et al., 2008)

Typical power plant flue gases have carbon dioxide levels ranging from 10%-15% (Cheng et.al, 2006; Stepan et al, 2001). At such percentages, microalgae show no signs of significant growth inhibition (Stepan et al, 2001). On the other hand, studies have shown the photosynthetic efficiency of microalgae could well be in the range of 10%-20% or higher (Richmond A., 2000). Lee (2010) however, claimed the growth rate was declined to about half at 30% CO_2 concentration for *C. littorale*, a marine species of microalgae. CO_2 resistance of fresh water microalgae *Chlorella HA-1* on the other hand exhibits the growth inhibition at concentration higher than 10%.

Microalgal-CO_2 fixation is environmentally sustainable when combined with other environmental-protecting processes such as waste water treatment or heavy metal removal. A combination of CO_2 fixation, biofuel production, and waste water treatment may thus provide a very promising alternative to current CO_2 mitigation strategies (Ho et al., 2010). Research on the ability of microalgae to fix CO_2 is summarized as table below:

Table 1: Comparison of Biomass Productivity and CO_2 Fixation Ability of Microalgae Reported in the Literature (Ho et al., 2010)

Microalgal species	CO_2 (%)	Biomass Productivity (mg L^{-1} d^{-1})	CO_2 Consumption Rate (mg L^{-1} d^{-1})	Reference
Nannochloropsis sp.	15	270	508	Negoro et al.(1991)
Chlorella sp.	20	700	1316	Sakai et al.(1995)
Chlorella sp.	10	940	1767	Sung et al.(1999)
Chlorella sp.	2	171	857	Chiu et al.(2008)
Chlorella sp.	10	381.8	717.8	Chiu et al.(2008)
Chlorella sp.	10	610	1147	Chiu et al.(2008)
Chlorella sp.	5	335	700.2	Ryu et al.(2009)
Chlorella vulgaris	Air	40	75	Scragg et al.(2002)
Anabaena sp.	Air	-	1450	Lopez et al.(2009)

2.3.3 Photobioreactor design for CO_2 emissions

The effective photobioreactors is the key towards a successful microalgal-CO_2 sequestration process. The design of photobioreactor is important as most of the microalagae are photoautotrophs, that dependent on light. Photobioreactors can be divided into open and closed systems. It is evident that a closed system has certain advantages in term of amount of CO_2 fixation, biomass productivity, great process control as well as photosynthetic efficiency.

2.3.3.1 Open system

This open system can be classified as one that utilizes natural or artificial water. Most open systems are much simpler than closed photobioreactor thus lower construction and operating cost to build such system. Open system microalgae cultivation using natural environment are widely used in commercial microalgae production, representing 80% of all such projects (Ho et al, 2010). This system however have certain limitations with regard to cell growth due to environmental issues such as vapor losses, risk of contamination and difficulty in temperature and light control that lead to lower productivity (Ho et al., 2010). Due to these drawbacks, the microalgae cultivation is not feasible using open system (Pulz, 2001).

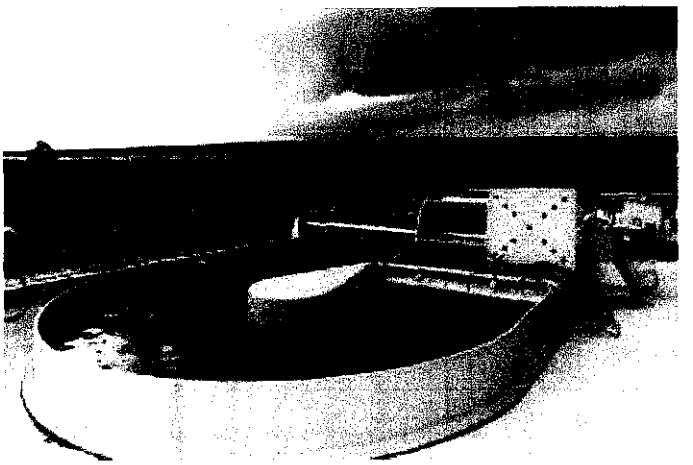


Figure 3: A raceway filled with algae (Source: Wageningen University and Research Centre, 2011)

2.3.3.2 Closed system

Chiu et al (2008) demonstrated that by using photobioreactor in closed system is an effective and promising method. This system is well-controlled with low contamination risk as well as high CO_2 fixation rate compared to open systems (Sierra et al., 2008). Such systems usually have a larger surface area exposed to the light source. However the scaling up of most closed photobioreactors face some limitations to biomass circulation and the growth parameters control. Good photobioreactor system will have a good mixing, gas transfer and light distribution (Chiu et al., 2009).

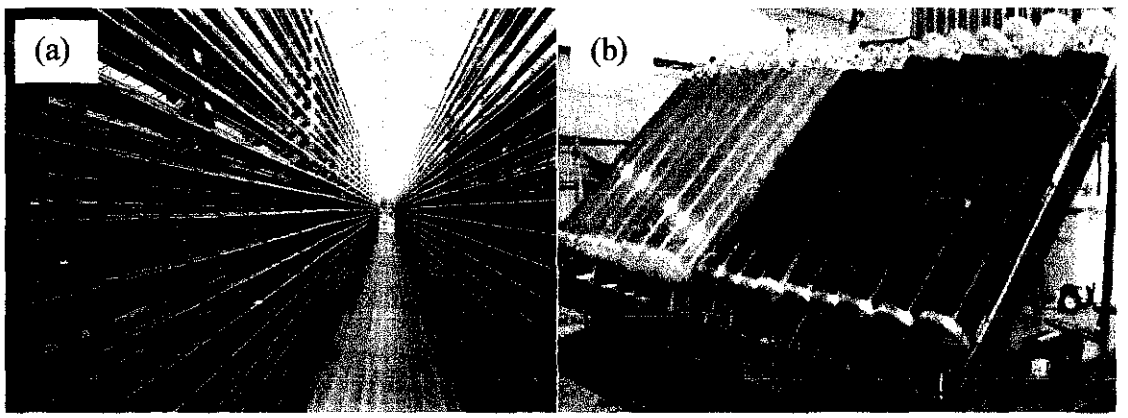


Figure 4: Reactor configurations for microalgal cultivation (a) Bubble column reactor (b) Horizontal tubular reactor. (Source: Green Fuel Technologies)

Few types of this photobioreactor are vertical tubular systems, plate-type system and column system. Basically, column photobioreactor systems are relatively low cost, tubular type systems are the most attractive option for large scale cultivation, while vertical tubular type can achieve the most CO_2 fixation because of their better circulation and mass transfer (Chiu et al., 2009). The comparison of each type of photobioreactors is represented in Table 2.

Table 2: Advantages and disadvantages of different type of photobioreactors (Ho et al., 2010)

Type of closed photobioreactor	Advantages	Disadvantages
Tubular	• Large illumination surface area • Fairly high biomass productivity • Relatively cheap • Suitable for outdoors	• Fouling • Large area of land needed • Poor mass transfer • High O_2 accumulation • Photoinhibition risk • Hard to control temperature
Flat-type	• Huge illumination surface area • Good light path • High biomass productivity • Easy to scale up • Suitable for outdoors • Relatively low O_2 accumulation • High photosynthetic efficiency	• High hydrodynamnic stress • Hard to control temperature
Column	• Low shear stress • Low energy consumption • Relatively cheap • Easy to operate	• Small illumination surface area

CHAPTER 3
METHODOLOGY

Research methodology in the following description covers the procedures for microalgae strain selection, based on temperature, air flow rate and with pure CO_2 supply. Preparation of the Conway media, determination of CO_2 fixation rate and other assay procedures are as follows:

3.1 Research Methodology

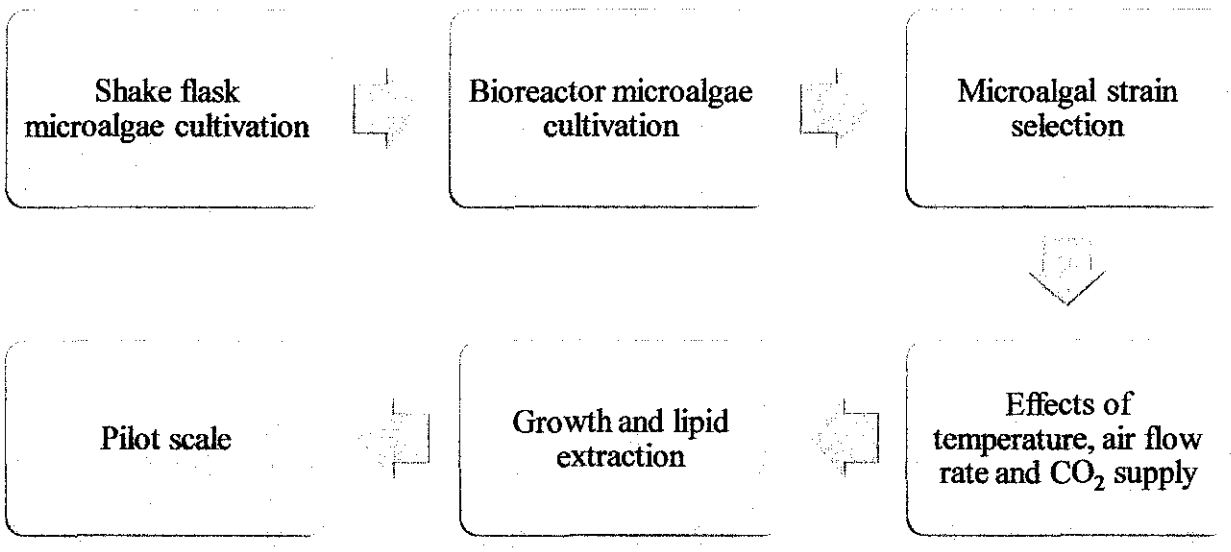


Figure 5: Experimental work methodology

3.2 Materials and Methods

3.2.1 Conway Media Preparation

Erlenmeyer flasks (250 mL), each containing 100 mL of the medium, were inoculated with 10% (v/v) of inoculums and *Nannochloropsis sp., Chaetoceros sp., Pavlova lutheri,* and *Isochrysis galbana* were grown in a medium of the following composition (in g L^{-1}): $NaNO_3$, 100; Disodium EDTA, 45; H_3BO_3, 33.60; $Na_2HPO_4.4H_2O$, 20; $FeCl_3.6H_2O$, 1.30; $MnCl_2.4H_2O$, 0.36; $ZnCl_2$, 2.10; $CoCl_2.6H_2O$, 2.0; $(NH_4)_6MO_7O_4.4H_2O$, 0.90; $CuSO_4.5H_2O$, 2.0; Thyamine chlorohydrate B_1, 0.2; Cyanocobalamin B_{12}, 0.01; KNO3, 100. All constituents were added aseptically after sterilization.

3.2.2 Microalgae Cultivation and Maintenance

1 ml of main mineral solution contained trace metal solution, 1 ml nitrate solution and 0.1 ml vitamin solution was added for each liter of artificial 30 ppt sea water. Culture of microalgae was added in range of 5-10% of media, e.g. for a shake flask 250 ml media would require 12.5-25 ml of stock culture. Sterilization of equipment and sea water using auto clave must was done in 15 minutes at 120°C. The culturing workplace was cleaned by ethanol to ensure no contamination before sub-culturing each species of microalgae takes place. The flasks were sealed and arranged with certain gaps to ensure adequate exposure to light and be put on a shaker (130 rpm) to ensure uniform nutrient uptakes and to avoid accumulation.

Figure 6: 250 ml microalgae cultivations in laboratory

3.2.3 Experimental System and Condition

1 L cultivation of *Isochrysis galbana, Pavlova lutheri, Chaetoceros sp,* and *Nannochloropsis sp* with continuous aeration is shown in Figure 7.

Figure 7: 1 L cultivation set up system

Cultivation of larger volume was done using 5 L bioreactor is shown in Figure 8.

Figure 8: *Nannocholropsis sp.* culture in 5 L bioreactor

Experiment with pure CO_2 supply for microalgae growth is illustrated in Figure 9. Each sample of microalgae in 250 ml shake flasks were sub-cultured into 1 L vessels and 5 L bioreactors. Lighting system was not built-in the bioreactor. External source of light was supplied by white florescence tubes (Philips). The first experiment was done to determine the best microalgae strain: *Isochrysis galbana, Pavlova lutheri, Chaetoceros sp,* and *Nannochloropsis sp.* and second experiment were to determine the optimum temperature and air flow rate of selected microalgae species. To perform the experiment of CO_2 fixation with pure CO_2 supply, microalgae culture were placed in 2 different bioreactors; 1 as controlled culture without CO_2 addition and 1 with CO_2 supply. The bioreactors were continuously aerated at 2 L/min air flow rate. The cultivation period was 15 days. The initial pH of cultivation was 7.5-8.5. Sparging CO_2 took place every 3 days for 24 hours. At the same time, the fluorescent lamp was turned on for the whole cultivation days.

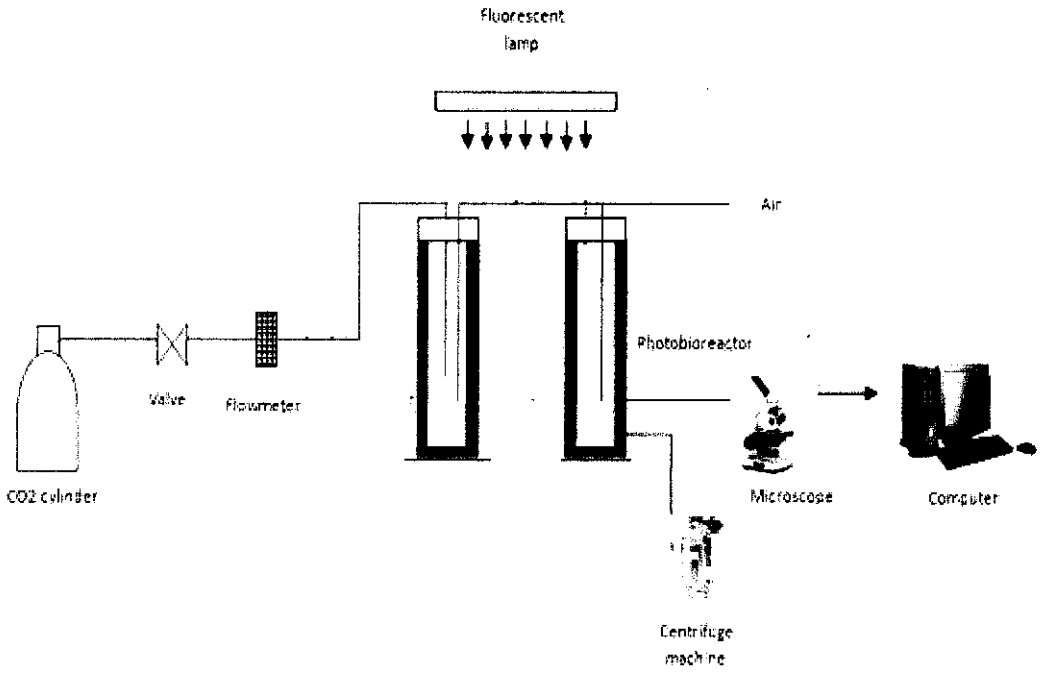


Figure 9: Schematic diagram of CO_2 supply experimental system

3.3 Experimental Analysis

Three experimental analyses conducted: cell growth rate, cell dry weight and lipid content analysis of each species of microalgae.

3.3.1 Cell Growth Rate

The growth of microalgae was measured through counting the number of cells by haemocytometer. Cell density was monitored using a haemocytometer. It contains a quantitative grid and is designed to be used with a microscope to determine bodily cell counts. When the required number of days of algae growth was passed, flasks were removed from the incubation tray and the flasks were swirled to ensure even distribution of algae throughout and then sample was lifted approximately 10μL by using capillary dropper. Sample was then transferred to the filling slide chamber and examined under high power (10 x 40 MAG).

3.3.2 Cell Dry Weight

100 ml of algal suspension was filtered through a pre-dried and pre-weighed glass-fiber filter (Whatman GF/C). The biomass was washed with de-mineralized water and dried at 105 ^{0}C in oven overnight, cooled in a desiccators and dry weight was measured. The formulations are as follows:

Weight of filter paper = a

Weight retained on algal filter = b

Avg. dry weight retained on algal filter = DW_A

Avg. dry weight retained on control filter = DW_c

Volume used = V

$$\text{Fresh weight} = DW_A - a \quad (3)$$

$$\text{Dry weight} = DW_A - DW_c / V \quad (4)$$

3.3.3 Harvesting and Lipid Content analysis

Microalgae harvesting is referred to the process where fairly diluted microalgae have been concentrated until slurry or paste is acquired. In this experiment, cells were harvested after 16 days by centrifugation at 3500 rpm for 10 min. The sample subjected to centrifugal forces and makes the solid particles separated from liquid supernatant. Large scale culture samples were flocculated by using appropriate quantity of alum.

The lipid content analysis was conducted based on Bligh and Dyer (1959) method. 200 ml of sample was taken for centrifugation at 3500 rpm for 10 minutes. The pellet generated from this centrifugation was put into flasks containing distilled water, methanol, and chloroform with ratio 4:10:5. After overnight, 5 ml of distilled water and 5 ml of chloroform was added (final ratio 9:10:10) and the mixture was centrifuged again. The lower layer in the centrifuge tube that contained lipid and chloroform was extracted and put into pre-weighed vials using a dropper. All vials were placed into a water bath at 65°C until 1/3 left and put in an oven at 80°C for 1 hour to evaporate the chloroform and methanol. The % extracted lipids from chloroform finally calculated.

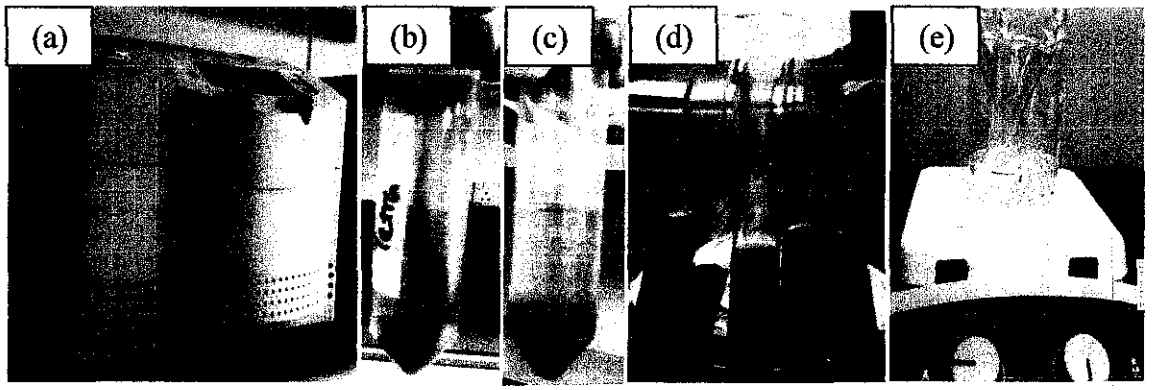


Figure 10: Lipid Extraction Procedures (a) Centrifugation (b) Separation of pellet (c) Media addition (d) Lipid extraction (e) Evaporation

3.4 Kinetics of cell growth in batch culture

For the scale-up of a production process, a number of basic laboratory-scale experiments have to be performed to yield essential data like growth rate (Schlatmann et al, 1996). The growth of cells is autocatalytic. That is mean the rate of increase in cell dry weight is proportional to the concentration of cells initially present (Scragg, 1991b). Cells undergo lag, exponential, stationary and death phase as in Figure 11.

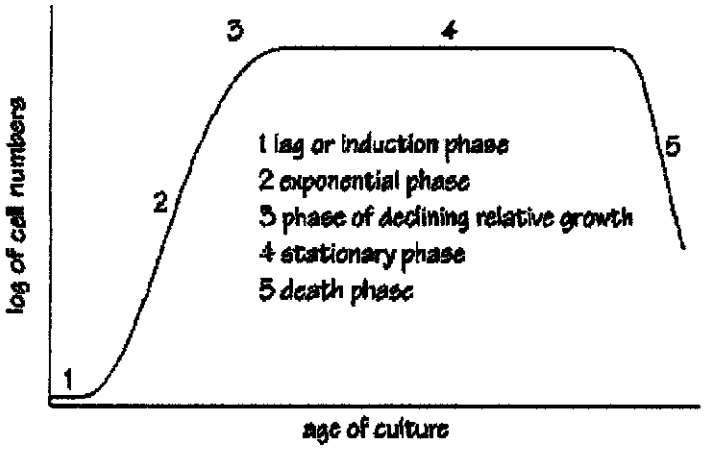


Figure 11: Five growth phases of micro-algae cultures (Coutteau et al., 1996)

Growth rate, r (mg/L.day) refers to the rate of increase in algal cell concentration, X with time as measured in batch culture:

$$r = dX/dt \tag{5}$$

The specific growth rate, μ (day^{-1}) is the growth rate normalized by tha algal biomass concentration, X:

$$\mu = \frac{r}{X} = \frac{dX}{dt}\left(\frac{1}{X}\right) \tag{6}$$

Equation (6) can be integrated to give:

$$X_t = X_o e^{\mu t} \qquad (7)$$

where X_o and X_t are initial and final cell concentrations in mg/L, respectively.

The maximum specific growth rate, μ_{max} is the value of the specific growth rate that is measured during the exponential growth phase.

$$\frac{dX}{dt} = \mu X$$

$$\frac{dX}{dt} = \mu_{max} X$$

$$\frac{dX}{dt} = \int_{x_1}^{x_2} \frac{1}{X} dX = \mu_{max} \int_{t_1}^{t_2} dt$$

$$ln\frac{X_2}{X_1} = \mu_{max}(t_2 - t_1)$$

$$\mu_{max} = \frac{ln\frac{X_2}{X_1}}{(t_2 - t_1)} \qquad (8)$$

During exponential growth also, the cell dry weight doubles from X_o and $2X_o$at regular intervals, t_d known as doubling time.

$$\mu_{max} = \frac{ln2}{t_d}$$

$$t_d = \frac{ln2}{\mu_{max}} \qquad (9)$$

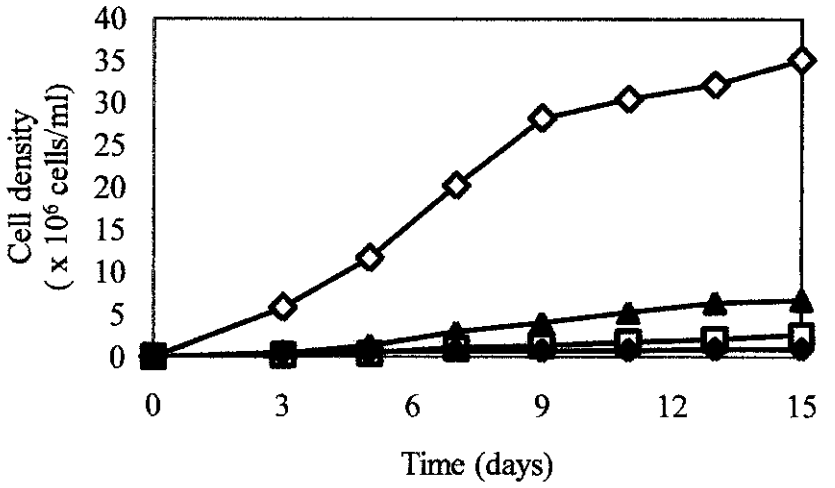
CO_2 fixation rate (Chisti, 2007) calculated from the biomass productivity according to the equation:

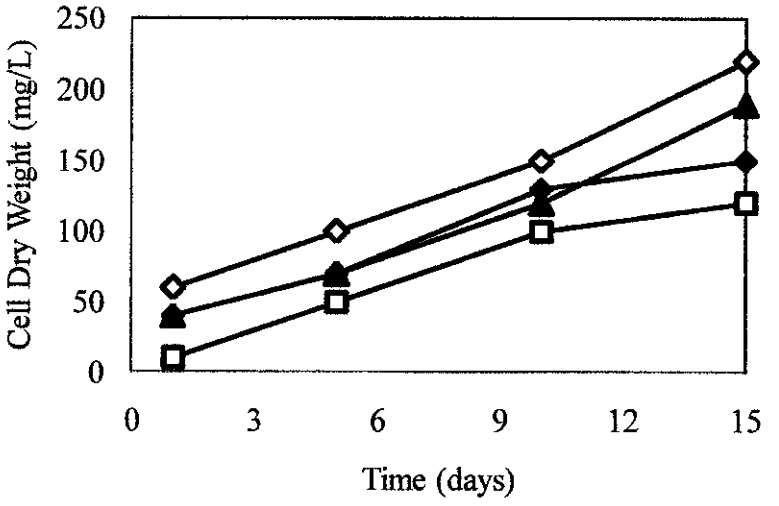
$$CO_2 \text{ fixation rate} = 1.88 \text{ x biomass productivity} \qquad (10)$$

CHAPTER 4
RESULTS AND DISCUSSIONS

Four microalgal species namely *Isochrysis galbana, Pavlova lutheri, Chaetoceros sp., Nannochloropsis sp.* for cell density, cell dry weight and lipid content were evaluated and the results are as follows:

4.1 Selection of microalgal strain in 1 L vessels





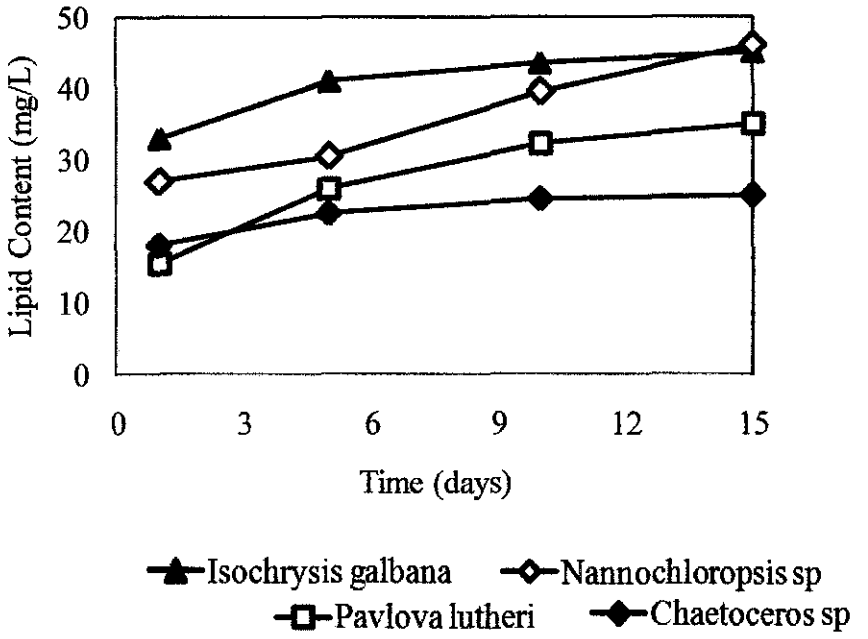


Figure 12: Cell density, cell dry weight and lipid content for different microalgal species in 1L vessels

Growth of *Isochrysis galbana, Pavlova lutheri, Chaetoceros sp.,* and *Nannochloropsis sp.* cell cultures in 1 L vessels were affected by the type of microalgal strains. Among the four species, only *Nannochloropsis sp.* is unicell green microalgae, others are unicell brown microalgae while *Chaetoceros sp.* is a diatom brown microalgae species. For experiment of selection the best microalgae strain, the growth rate up to day 15 was exceptionally high for *Nannochloropsis sp.*, followed by *Isochrysis galbana, Pavlova lutheri* and *Chaetoceros sp.* in decreasing order with maximum cell density values on day 15 of $35.0x10^6$, $7.0x10^6$, $2.75x10^6$, $1.0x10^6$ cells/ml, respectively. Growth of *Nannochloropsis sp* was the best although cells are the smallest (2-4 microns in diameter) among other species. The observation was made on each cultivation day showed the color change from light green to dark green (Figure 13), while the brownish color of other species was hardly observed. This species is very stable in Conway Media with continuous lighting and aeration.

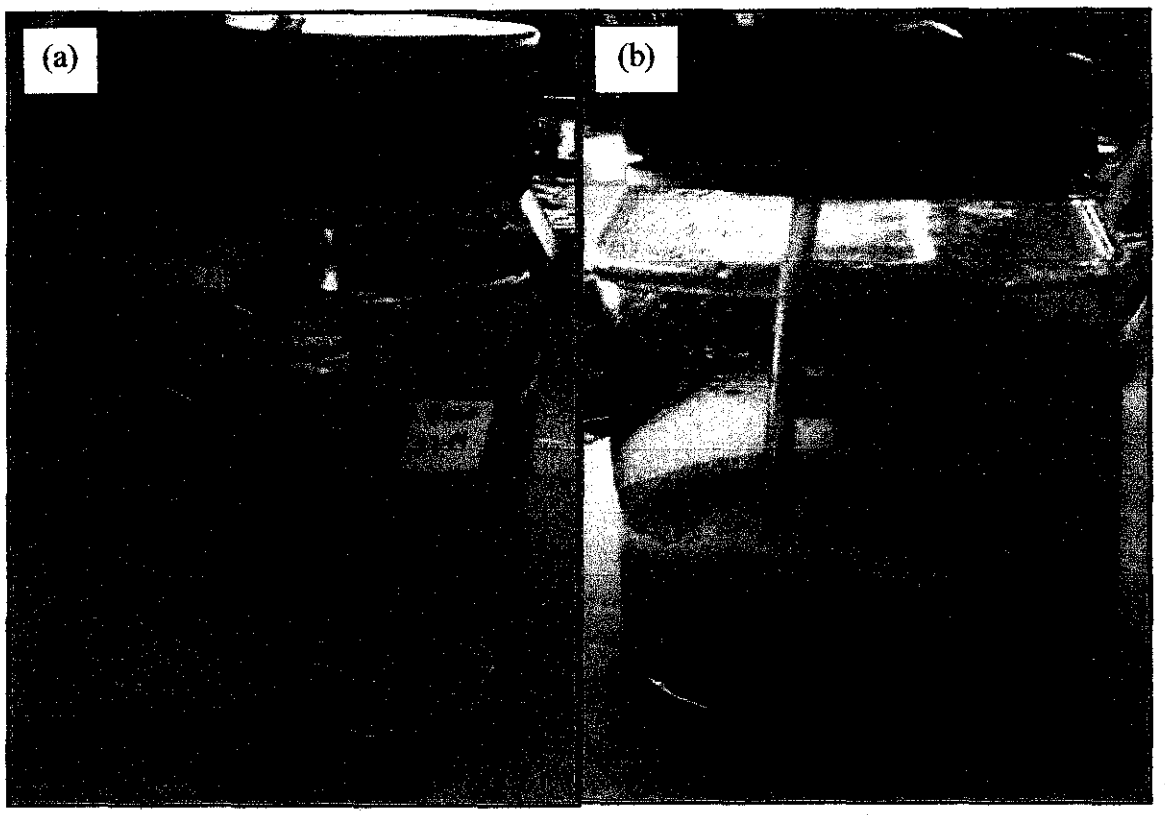


Figure 13: *Nannochloropsis sp.* culture in 1 L vessel. Cultivation on (a) day 1 (b) day 15.

For cell dry weight analysis, *Pavlova lutheri* showed the lowest biomass productivity followed by *Chaetoceros sp.*, *Isochrysis galbana*, and *Nannochloropsis sp.* of 12 mg/L, 15 mg/L, 18 mg/L, 22 mg/L, respectively. Cell density of *Pavlova lutheri* was higher than *Chaetoceros sp*, however the cell dry weight of this species was lower but the difference was quite small. These two brown microalgae species were not grown well during the 15-day cultivation as there were not much color change on the filter paper, and short time required (5-10 minutes) to filter the sample indicated the cells were few. While, 10-15 minutes time required for *Nannochloropsis sp.* cells to be fully filtered shows this species contained more cells thus the highest dry weight obtained.

Values in decreasing order of lipid content in 1 L vessels experiment were 46, 45, 35, and 25 mg/L of *Nannochloropsis sp., Isochrysis galbana, Pavlova lutheri,* and *Chaetoceros sp.,* respectively. Lipid content of two species *Nannochloropsis sp.* and *Isochrysis galbana* were quite similar, and can be further studied on their potential for biodiesel production. Based on the result discussed, *Nannochloropsis sp.* was seen to contain the highest cell density, cell dry weight and lipid content with CO_2 fixation rate 413.6 mg/L thus would become the chosen species in the next experiment aimed to get the optimum cultivation temperature and air flow rate.

Table 3: Biomass and CO_2 fixation rate of microalgal species in 1 L cultivation

Microalgal species	**Biomass productivity (mg/L)**	**CO_2 fixation rate (mg/L)***
Isochrysis galbana	190	357.2
Pavlova lutheri	120	225.6
Chaetoceros sp.	150	282.0
Nannochloropsis sp.	220	413.6

4.2 5 L Bioreactor

Further studies were carried out to see the effects of temperature and air flow rate to cell growth in 5 L bioreactors.

4.2.1 Effect of Temperature

Nannochloropsis sp. in bioreactors with three different temperatures (25°C, 30°C, 35°C) and fixed air flow rate of 2 L/min was studied and the result of cell dry weight and lipid content presented as follows:

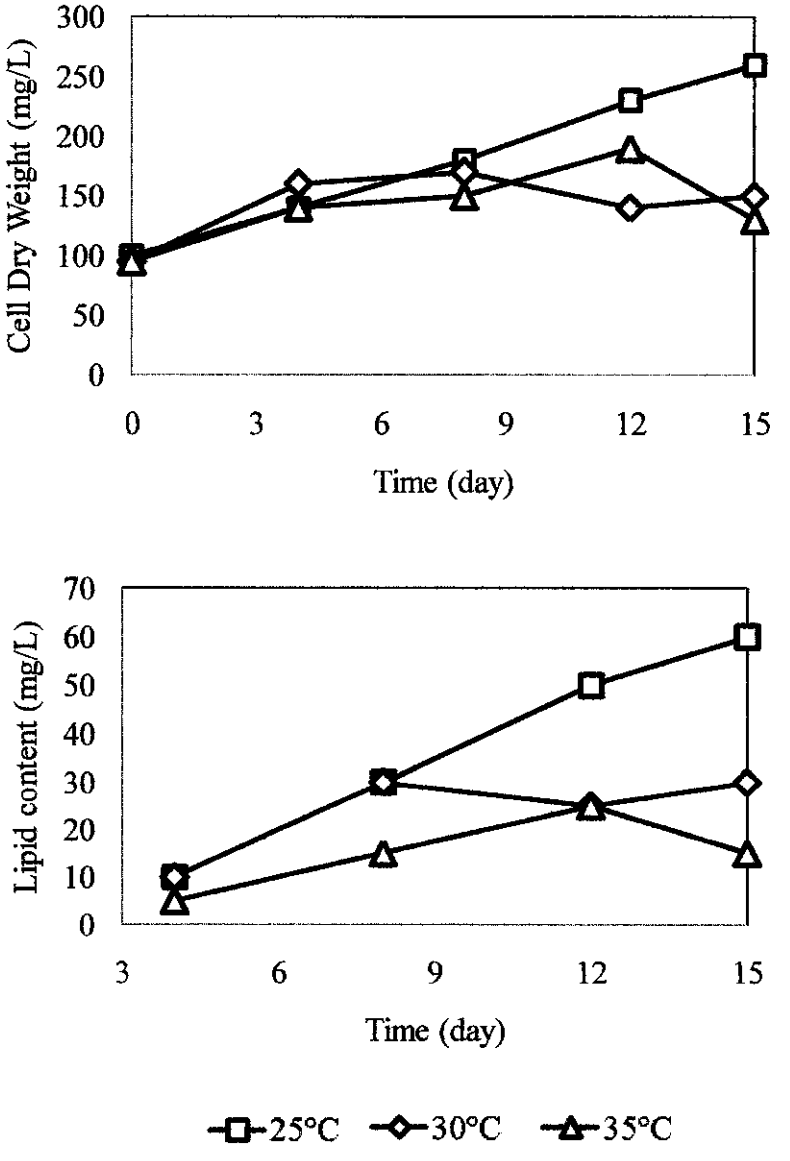


Figure 14: Cell dry weight and lipid content for *Nannochloropsis sp.* in 5 L bioreactor

Cell dry weight and lipid content with respect to time of *Nannochloropsis sp.* was shown in Figure 14. 25°C cultivation (room temperature) showed the highest cell dry weight of 260 mg/l compared to controlled temperature of 30°C and 35°C cultivation. This shows no temperature control is required as the cells grow well in room temperature.

For lipid content analysis, all cultivation at first showed a good rise but for 30°C profile, it dropped at day 10 onwards, 35°C drop at day 13 while the 25°C continues to rise. Result showed the room temperature is the optimum temperature to be used in the next CO_2 experiment.

Table 4: CO_2 fixation rate of 25°C, 30°C and 35°C cultivations

Microalgal species (5L cultivation)	**Biomass productivity (mg/L)**	**CO_2 fixation rate (mg/L)**	**Literature**
Nannochloropsis sp (25°C)	260	488.8	508 mg/L (at 15% CO_2 concentration)
Nannochloropsis sp (30°C)	150	282.0	
Nannochloropsis sp (35°C)	130	244.4	

Table 4 shows the CO_2 fixation rate of each cultivation temperature by mean of natural aeration contains 0.04% CO_2. As reported in literature Negoro et al.(1991), *Nannochloropsis sp* with 15% CO_2 concentration yield a biomass productivity of 270 mg/l and 508 mg/l CO_2 fixation rate. In this experiment with air and room temperature, CO_2 fixation rate at last day cultivation yield 488.8 mg/L which was quite a good result, thus indicated the *Nannochloropsis sp.* can be commercially developed for CO_2 removal in future.

4.2.2 Effect of Air Flow rate

Nannochloropsis sp. in bioreactors with 1 L/min, 2L/min and 3 L/min air flow rate was studied and the result of cell dry weight and lipid content presented as follows:

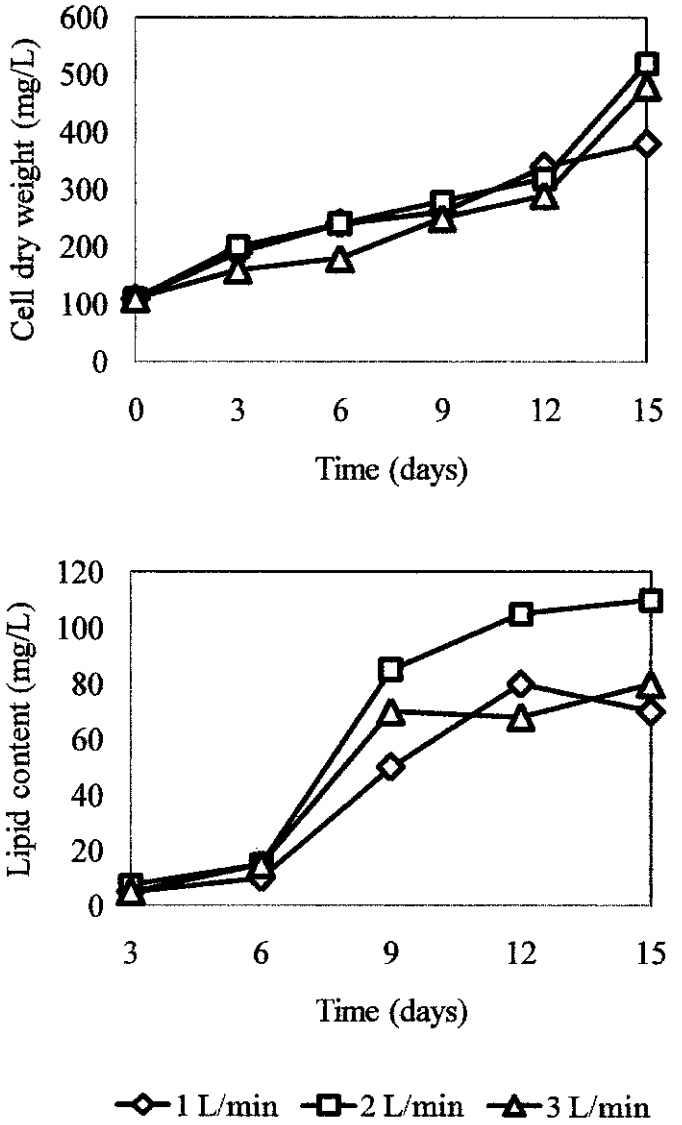
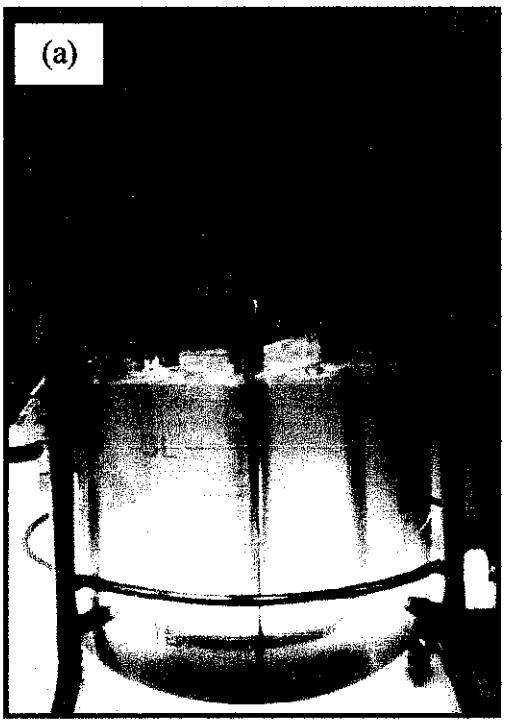


Figure 15: Cell dry weight and lipid content for *Nannochloropsis sp.* in 5 L bioreactor of different air flow rate.

From Figure 15, air flow rate of 1 L/min, 2 L/min and 3 L/min showed result in dry weight analysis of 380, 520 and 480 mg/L, respectively. Few shortcomings occurred during experiment on day 12 and 15 because air flow of 3 L/min bioreactor fluctuated and sometimes stopped. The same goes to 1 L/min bioreactor, the air flow fluctuate on day 15. The lipid content for 1 L/min, 2 L/min and 3 L/min air flow were 70, 110 and 80 mg/L, respectively. This result was proportional to the dry weight analysis. The comparison can only be made to 1 L/min and 2 L/min aeration, concluded that the higher air flow rate gave higher cell dry weight and lipid content as CO_2 supply is more, thus accelerate growth. As the 2 L/min gave a better profile, this amount of flow rate was fixed in later CO_2 experiment.

4.2.3 Effect of CO_2 supply

Nannochloropsis sp. was cultured in two 5 L bioreactors (4 L working volume). One bioreactor supplied with pure CO_2 whiles the other one without CO_2 addition (controlled culture), but both under aeration of 2 L/min. The change in color observed as Figure 16. pH, dry weight and lipid content were analyzed once in every 3 days as shown in Figure 17.



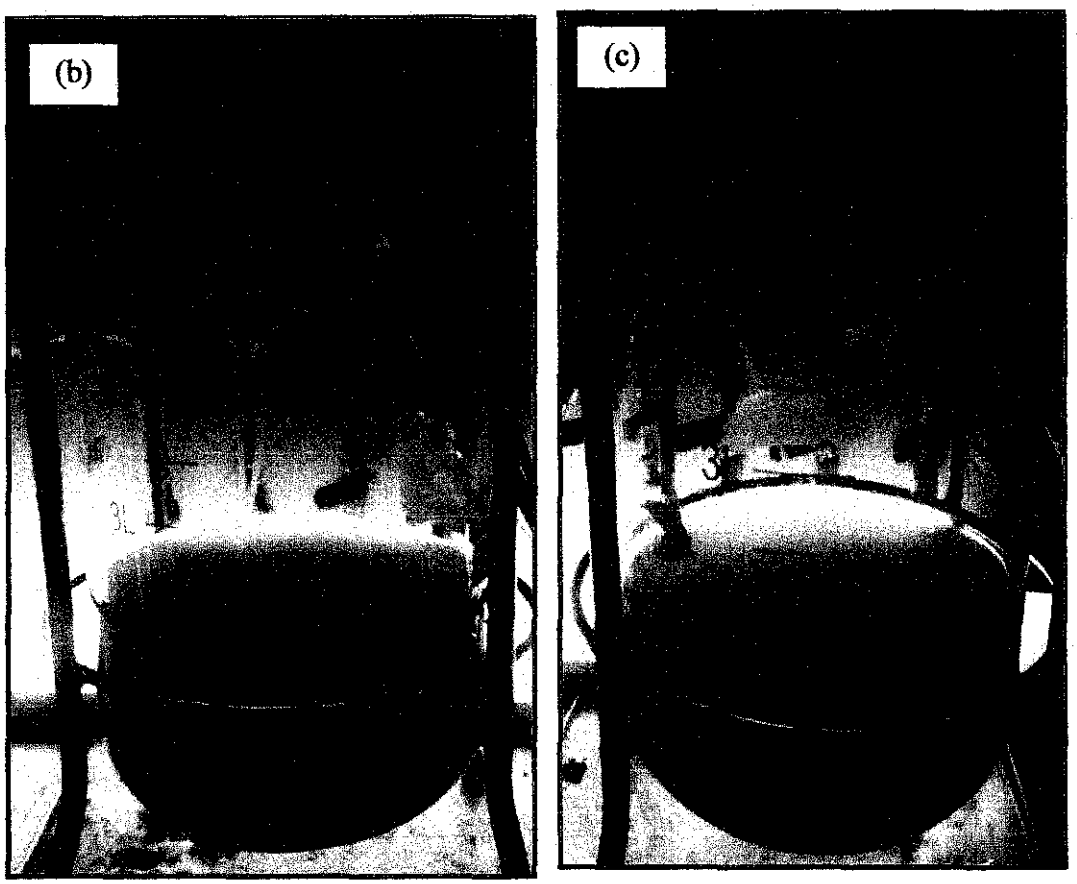
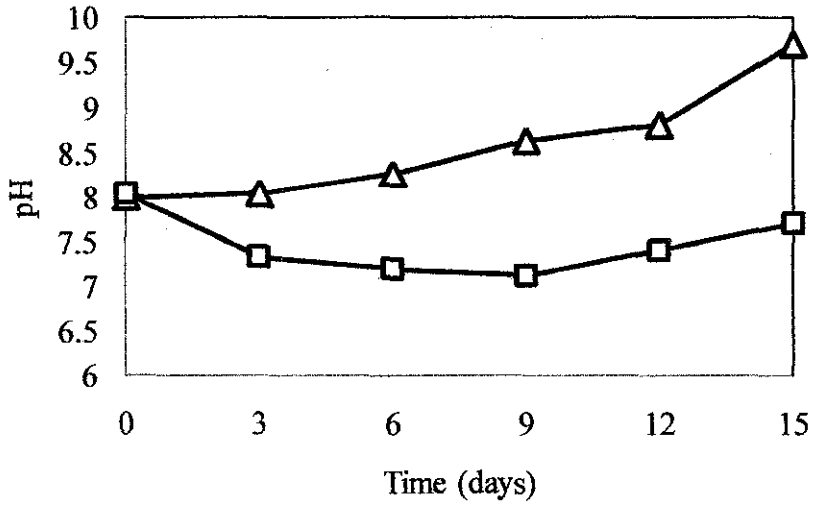


Figure 16: *Nannochloropsis sp.* culture in 5 L vessel. Cultivation on (a) day 1 (b) day 15 controlled culture (c) day 15 culture with CO_2 supply.



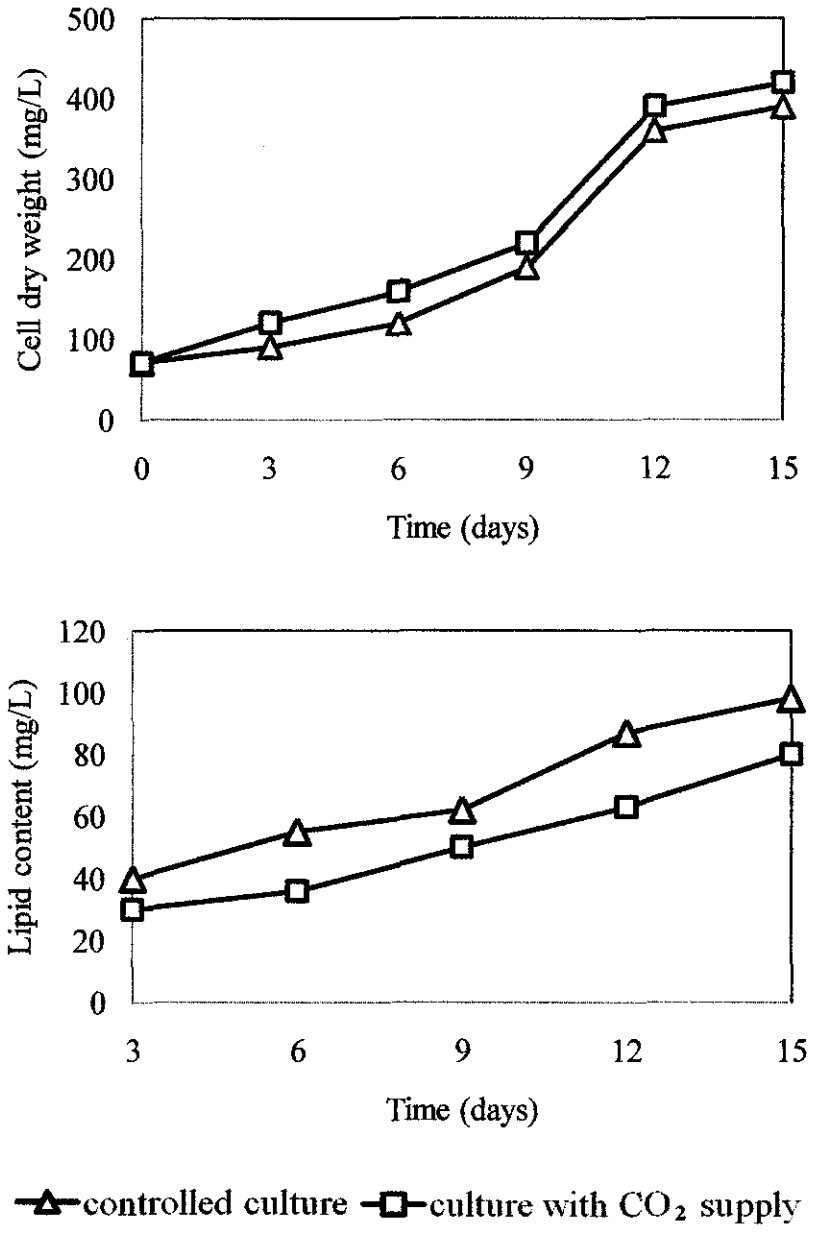


Figure 17: pH, cell dry weight and lipid content with respect to time of *Nannochloropsis sp.* in 5 L bioreactor with and without CO_2 supply.

Initial pHs of both cultures with and without CO_2 addition were quite similar of 8.05 and 8.01, respectively. On days onwards, the pH profile for culture provided with pure CO_2 decreased while increment pH for culture without CO_2 addition clearly observed with value of 7.72 and 9.71, respectively at day 15. There is a complex relationship between CO_2 concentration and pH in microalgal bioreactor systems. Increasing CO_2

concentrations (in this case, increase from CO_2 concentration in air to a higher concentration by external CO_2 supply) can lead to higher biomass productivity, 420 and 390 mg/L, respectively, but will also decrease pH. The decrease in pH can have an adverse effect upon microalgal physiology (Kumar et. al, 2010). This statement was proven as in this experiment, culture with CO_2 supply turned to be a yellowish green color as in Figure 16 although result showed its biomass productivity higher than controlled culture. Lipid content of culture with CO_2 supply also higher compared to controlled culture with 98 and 80 mg/L respectively.

4.3 Kinetics of Cell Growth and Lipid

The growth profile of 15-day cultivation of *Nannochloropsis sp.* is shown in Figure 12 and Figure 14. Comparison of kinetics parameters for cell growth in 1 L culture and 5 L culture in Conway media is shown in Table 5. The t_d and μ_{max} are calculated according to equation (8) and (9) in Chapter 3. The doubling time of 8.8 days was shortest in 1 L cultivation while at 10.8 days in 5 L cultivation. The maximum biomass production in 5 L cultivation was higher than in 1 L cultivation with 0.26 g/L and 0.22 g/L, respectively. Lipid content for 5 L cultivation was also higher than 1 L cultivation with 60 g/L and 46 g/L, respectively.

Table 5: Kinetics parameters of *Nannochloropsis sp.* in different cultivations

Experimental Conditions	Specific Growth Rate, μ_{max} (10^6 cells d^{-1})	Maximum biomass conc., μ_{max} (g $L^{-1}d^{-1}$)	Doubling Time t_d (day)	Lipid Content (%)
1 L Cultivation	35.0	0.22	8.8	1.62
5 L Cultivation	39.0	0.26	10.8	2.12

CHAPTER 5
CONCLUSIONS AND RECOMMENDATIONS

Studies on microalgae species of *Nannochloropsis sp., Chaetoceros sp., Pavlova lutheri,* and *Isochrysis galbana,* indicated that *Nannochloropsis sp.* contained the highest cell density, cell dry weight and lipid content thus considered the best microalgae species for CO_2 fixation. The optimum temperature of cultivation is room temperature 25°C, the higher temperature will inhibit cell growth. Air flow rate of 2 L/min gives better result compared to 1 L/min aeration; the higher flow provides more CO_2, thus accelerate growth. Culture with external CO_2 supply also promotes better growth rather than supply with air alone. Kinetics parameter of maximum specific growth rate and doubling time for 1 L cultivation is less compare to 5 L cultivation.

The lipid content and biomass productivity in this experiment can be used to reflect the possible usage of microalgae in biodiesel production. Investigation on effect of known CO_2 concentrations and determination of CO_2 consumption using sensors must be conducted for better analysis of ability of microalgae species to fix CO_2. Further commercialization of microalgae as method for CO_2 removal with waste water treatment combination is a good merit to be implemented in future.

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APPENDIX

Gantt Chart

Final Year Project I (FYPI)

No	Activities	Week													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Selection of project title	■	■					Mid Semester Break							
2	Extended proposal preparation		■	■	■										
3	Submission of extended proposal					X									
4	Proposal defense								X						
5	Familiarize with laboratory work			■	■	■	■		■	■	■	■	■	■	■
6	System development								■	■	■	■	■	■	■
7	Preparation of interim report					■	■		■	■	■	■	■	■	■
8	Submission of interim report														X

Final Year Project II (FYPII)

No	Activities	Week													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	System development	■	■					Mid Semester Break							
2	Media preparation, microalgae cultivation		■												
3	CO_2 fixation experiment (2% concentration)		■	■	■										
4	CO_2 fixation experiment (5% concentration)					■	■								
5	Cell growth evaluation		■	■	■	■	■								
6	Biogas determination			■	■	■	■								
7	Lipid extraction			■	■	■	■								
8	Kinetic parameters calculation						■		■	■					
9	Pre-EDX											X			
10	Preparation of report	■	■	■	■	■	■		■	■	■	■	■	■	
11	Submission of draft report												X		
12	Submission of dissertation (soft copy)													X	
13	Submission of technical paper													X	
14	Oral presentation														X
15	Submission of project dissertation (hard bound)														X