The effects of air supply and nitrogen sources on *Scenedesmus Quadricauda*

by

Mohd Fadhli Bin Sayutti

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CERTIFICATION OF APPROVAL

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Approved by,

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(Dr. Lukman Bin Ismail)

UNIVERSITI TEKNOLOGI PETRONAS

TRONOH, PERAK

September 2012

CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, that the original work in 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.

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MOHD FADHLI BIN SAYUTTI

ABSTRACT

Microalgae have been investigated for the production of different biofuels and the feedstock are gaining interest in the present day due to their fast growth potential added with relatively high lipid, carbohydrate and nutrients contents. The purpose of this project is to perform a research to grow the commercial microalgae and to produce significant amount of lipid content from microalgae harvested as a viable alternative renewable energy. The commercial microalga chosen is *Scenedesmus Quadricauda*. The project also intended to study on the effects of air flowrate and nitrogen limitation on cell growth and lipid production. The microalga was cultivated in Afnor Medium with working volume of 5L in cylindrical photobioreactors. The temperature and pH value were in range of 22-25^{o}C and 7-8 respectively for each photobioreactor. The first part of this project was conducted using different flowrates of air supply of range 1.0 to 6.0 L/min. The results show that, as the flowrate of air supply increased from 1.0 to 4.0 L/min, the biomass collected was also increased. However, as the flowrate of air supply was further increased to 5.0 and 6.0 L/min, the biomass collected was decreased. The second part of this project was conducted by controlling the total amount of nitrogen supply to the culture medium in decreasing range of 100% to 20% of nitrogen. The results show that the lipid yield was increased with decreasing amount of nitrogen whereas the biomass obtained was decreased. Besides, the lipid content based on the dry mass percentage was also increased with decreasing amount of nitrogen supplied.

ACKNOWLEDGEMENT

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CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF STUDY

The world has come to a new era where the development of new technologies has been the main focused in the community. This development has put a high demand in energy generation where many vigorous research initiatives aimed at developing alternative renewable and potentially carbon neutral solid, liquid and gaseous biofuels as alternative resources has been made throughout the world (Brennan and Owende, 2009). Nowadays, almost 80% of global energy demand is produced from fossil fuels. Nevertheless, extensive utilization of fossil fuels has led to global climate change, environmental pollution, and health problems (Chen et al., 2010).

Thus, it is a need to develop new sustainable technologies that adopt the policies to reduce impacts of global warming. The Kyoto Protocol of 1997 emphasize for a 5.2% reduction of greenhouse gas emissions worldwide from 1990 values (Wang et al., 2008). One alternative resource that catches the attention of the world is biofuel. The first generation of biofuels derived from terrestrial crops such as sugarcane, sugar beet, maize and rapeseed. This however places an enormous strain on world food markets, which leads to the destruction of the world's forest as well as causing water shortages (IEA, 2007). The second generation biofuels derived from forest residue, lignocellulose agriculture and from non-crop feedstock. The challenge for this generation is over competing land use changes together with the above circumstances (Moore, 2008).

Today, the third generation of biofuels which specifically derived from microalgae; able to make a breakthrough of the major drawbacks related with the first and second generation of biofuels. Microalgae are photosynthetic microorganisms with simple growth requirements which consist of light, nutrients and carbon dioxide. It can produce lipids, carbohydrates and proteins in large amounts over short periods of time which can be further processed into biofuels and valuable co-products (Brennan and Owende, 2009).

1.2 PROBLEM STATEMENT

Despite its inherent potential as a biofuel resource, many factors including low lipid productivity, highly energy-consuming harvesting techniques and expensive oil extraction result in its high costs, such that microalgae has not yet been widely commercialized (Zhang et al., 2010). Thus, extensive researches and developments have recently focused on the improvement of lipid productivity (Li et al., 2008).

Two factors governed the increase in lipid productivity which are; improvements in lipid content and biomass productivity. In photoautotrophic cultures, there are many culture conditions that can increase in lipid content, such as high light intensity, high iron concentration and high carbon dioxide concentration (Feng et al., 2011). But the most effective culture condition is the limitation of the major nutrient nitrogen where the algae cells is put under unfavorable conditions, which normally causes carbon from carbohydrate and protein to be converted into lipid (Harwood and Jones, 1989).

However, before increasing the lipid production from the microalgae by varying the amount of nitrogen supplied to it, the most optimum growing curve of the microalgae must be determined first. The growing curve of microalgae is affected by air supply (amount of carbon dioxide supply), light intensity and nutrients (Brennan and Owende, 2009). For this project, we decided to study on the effects of the air supply and nitrogen content in the nutrients on the growth and lipid accumulation of microalgae.

Based on the findings above, this project will focus on two main objectives which are; determining the optimum flow rate of air supply to the microalgae and increasing lipid accumulation of microalgae in nitrogen limitation environment. The microalgae species used in this project is *Scenedesmus quadricauda.*

1.3 SIGNIFICANCE AND FEASIBILITY OF THE PROJECT

There are several factors why microalgae are chosen for biofuels production; such as high biofuel and biomass productivity. These are the main reasons that make microalgae as the main source for alternative renewable energy resource. Besides, it also has extremely high cell growth rate and seasonal tolerance. In fact, the life cycle of microalgae itself; do contribute in reducing the concentration of carbon dioxide gas in the atmosphere as it has relatively higher photon conversion efficiency which also known as photosynthesis efficiency in fixing the carbon dioxide gas. This will surely decrease the effect of greenhouse gases. Furthermore, it also can effectively accumulate large quantities of lipids and carbohydrates which will be used for oil extraction as well as other valuable co-products (Ho, et al., 2011).

In order to enhance the economic feasibility of microalgae-based biofuel production, it is necessary to increase the microalgae biomass productivity, lipid/carbohydrate content and overall lipid/carbohydrate productivity. Many researches have been done for many species of microalgae targeting for an increase in the overall lipid/carbohydrate productivity (Table 1); as well as for species of *S. quadricauda* microalga. But the gap between the previous researches is almost about two years and not more information we can obtain from it especially on the growth part thus making this project significant to collect more findings about this species of microalga.

Based on Table 1, the lipid productivity for *S. quadricauda* microalga is among the lowest amount of production compared to other species of microalgae. Nevertheless, the *S. quadricauda* microalga is easily available in Malaysia and already stored in university's lab. Besides, this project is also just being started by the university as a new research project which being governed under the Center of Biofuel and Biochemical Research (CBBR). Thus making this project within the scope of Final Year Project 1 which is to study on the available project and come out with the improvement. Furthermore, the growth of microalgae only takes 2 weeks before it can be extracted to find the lipid content from the microalgae. The equipment in UTP lab is also enough to carry out this project.

Table 1.1: Lipid productivity for microalgae species (Brennan and Owende, 2009)

Microalgae species	Lipid productivity (mg $L^{-1}day^{-1}$)	Reference
Chlorella vulgaris	40	Lv et al. (2010)
Neochloris oleoabundans	133	Li et al. (2008)
Scenedesmus sp	39	Yoo et al. (2010)
Botryococcus braunii	21	Yoo et al. (2010)
Nannochloropsis sp	204	Rodolfi et al. (2008)
Isochrysis zhangjiangensis	136	Feng et al. (2011)

There are several conditions that needed by the microalgae for increasing the lipid/carbohydrate productivity which also referred as growth conditions. The main condition that plays this role is the nutrient supply to the microalgae which are nitrogen, phosphorus and potassium. Phosphorus and potassium required by the microalgae in small amount during the growth cycle rather than the nitrogen which mainly contribute to the production of lipids, proteins and carbohydrates depending on the amount of nitrogen supplied to the microalgae (Brennan and Owende, 2009).

As a conclusion, this project is significant as it will help to understand the effects of the nitrogen supply to the microalgae which resulting in the increase of lipid production by the microalgae and then suggest the optimum amount of nitrogen to be used for the growth of *S. quadricauda* microalga.

1.4 AIM, OBJECTIVES AND SCOPE OF STUDY

The aim of this project is to find out the best way to increase lipid production of *S. quadricauda* microalga by the effect of air flowrate and nitrogen supply to the microalga. By increasing the lipid production, will eventually increase in the production of biofuel which can be used as the alternative renewable energy resource in the future.

The objectives of the project are;

1) To determine the optimum flow rate of air supply to microalgae.
2) To increase lipid accumulation of microalgae in nitrogen limitation environment.

The scopes of the study are;

1. The effect of flow rate of air supply to microalgae. Different flow rate of air are supplied to five aquariums (also known as photobioreactors) using air distributor located inside the aquariums. The optimum flow rate of air must be determined in order to find the optimum growth of microalgae. The lower limit for this air flow rate is 1.0 L/min and the upper limit is 6.0 L/min. The optical density for each aquarium is taken every day until the day of harvest which is about 2 weeks. Then the optimum flow rate of air is determined by plotting the growth curve of the microalgae.
2. The effect of nitrogen limitation environment to the lipid accumulation of microalgae. By limiting the nitrogen supply to the microalgae, it will cause the microalgae to be in unfavorable condition which promote the microalgae to convert the protein and carbon stored in the cell into lipids thus will increase the amount of lipid accumulates by the microalgae. The nitrogen is being limited by altering the percentage of nitrogen supply in the Afnor Medium. The percentage of nitrogen supply is in the range of 20-80% decrement of the total nitrogen supply in the standard Afnor Medium. The amount of lipid accumulation by the microalgae is measured at the harvest day.

CHAPTER 2

LITERATURE REVIEW

2.1 ALGAE

Algae represent a large and diverse group of eukaryotic organisms that contain chlorophyll and carry out photosynthesis. These algae do not have a well-organized vascular conducting system and having very simple reproductive structures which differentiate them from other photosynthetic eukaryotes. Being oxygenic phototrophs, algae act as the main sources of oxygen in natural water bodies. This advantage of algae has made the engineers to fully utilize the algae in stabilization lagoons for wastewater treatment.

Most algae are microscopic in size but there are few algae that can grow to several hundred feet in length such as marine *Macrocystic* or giant kelp. This large form of algae is hard to distinguish with other nonalgae organisms such as plants except that the large algae forms produce spores within unicellular structures, but the spores of plants are all produced within multicellular walls. But the microscopic forms of algae are distinctly different from higher plants, and many are unicellular and very small (Prescott et al., 2002).

The algae mostly occur in water (fresh, marine, or brackish) in which they may be suspended (planktonic) or attached and living on the bottom (benthic). There are also algae that live at the water-atmosphere interface. Some algae grow on moist rocks, wood, trees, and on the surface of moist soil. Several algae also grow as endosymbionts within plants, some are attached to the surface of various structures, and a few lead a parasitic existence. There are also algae grow together with fungi to produce linchens (Shuler and Kargi, 2002).

2.1.1 Classification of Algae

Algae contain different kinds of chlorophyll, which has its own abilities to absorb the efficient range of light spectrum. But all algae contain chlorophyll *a*. Some algae

contain other chlorophylls in addition, and it is on this basis that the algae are being distinguished and group from one another. There are seven different taxonomic groups of algae, based largely upon the different chlorophylls and photosynthetic pigments they contain. Their common names are often related to their characteristic color, which is a function of the photosynthetic pigments they contain (Prescott et al., 2002).

Table 2.1: Characteristic of the different groups of algae (Prescott et al., 2002)

Alga Group	Common Name	Chlorophylls	Storage Products	Structural Details
Cyanophyta	Blue-Green	*a*	Starch	Procaryotic, no flagella
Chlorophyta	Green	*a, b*	Starch	0 to several flagella
Chrysophyta	Golden, brown diatoms	*a, c, e*	Lipids	0 to 2 flagella, silica covering
Euglenophyta	Motile green	*a, b*	Polysaccharide	1,2,3 flagella, gullet
Phaeophyta	Brown	*a, c*	Starch	2 flagella, angular plates with furrows
Rhodophyta	Red	*a, d*	Starch, oils	No flagella

2.1.2 Ultrastructure of the Algal Cell

The eukaryotic algal cell is surrounded by a rigid, thin cell wall. Some algae have an outer matrix lying outside the cell wall. This usually is flexible and gelatinous, similar to bacterial capsules. When present, the flagella will act as the locomotor organelles for the cell. Flagella are hair like structure that acts primarily as an organelle of locomotion in the cells of many living organisms. The movement of eukaryotic flagella depends on adenosine triphosphate (ATP) for energy.

Eukaryotes have one to many flagella, which move in a characteristic whip like manner. The flagella closely resemble the cilium in structure. The core is a bundle of nine pairs of microtubules surrounding two central pairs of microtubules (the so-called nine-plus-two arrangement); each microtubule is composed of the protein tubulin. The coordinated

sliding of these microtubules confers movement. The base of the flagellum is anchored to the cell by a basal body (Chelsey, 2007).

The nucleus has a typical nuclear envelope with pores; which consist of nucleolus, chromatin, and karyolymph. The chloroplasts have membrane-bound sacs called thylakoids that carry out the light reactions of photosynthesis. These organelles are embedded in the stroma where the dark reactions of carbon dioxide fixation take place. A dense proteinaceous area called the pyrenoid is associated with synthesis and storage of starch may be present in the chloroplasts (Shuler and Kargi, 2002).

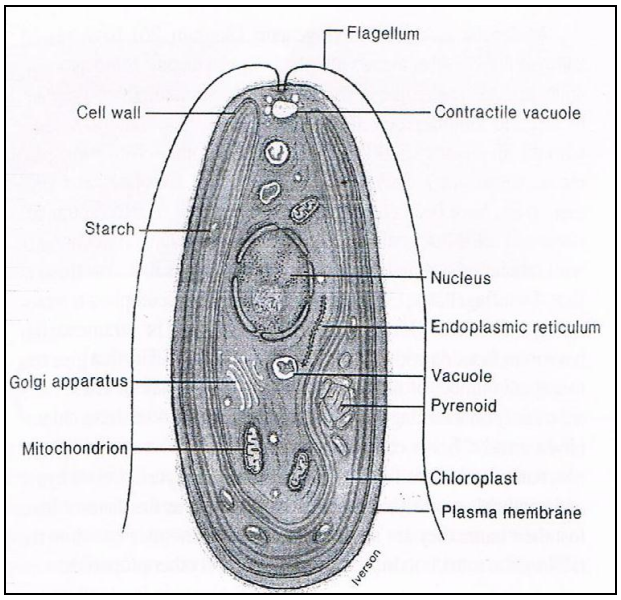

Figure 2.1: Schematic diagram of a typical eukaryotic algal cell showing some of its organelles and other structures. (Prescott et al., 2002)

2.1.3 *Scenedesmus Quadricauda*

S. quadricauda is a genus of algae, specifically of the *Chlorophyta*. *Chlorophyta* or also known as green algae are common in many freshwaters and are among the most important algae in stabilization lagoons for wastewater treatment. Many are single celled, and some are motile by means of flagella. The green algae commonly have one chloroplast per cell (Prescott et al., 2002).

S. quadricauda microalga usually in colonies of 4 (or 2, 8, 16) cells attached side by side, arranged linearly or zigzag; cell body elliptical or spindle or crescent in shape; terminal cells with spiny projections in many species; cell wall usually smooth, but in some species granulated or dented or ridged. Studies had been made and show that this microalga is a promising microorganism for selenium-enriched algal biomass production.

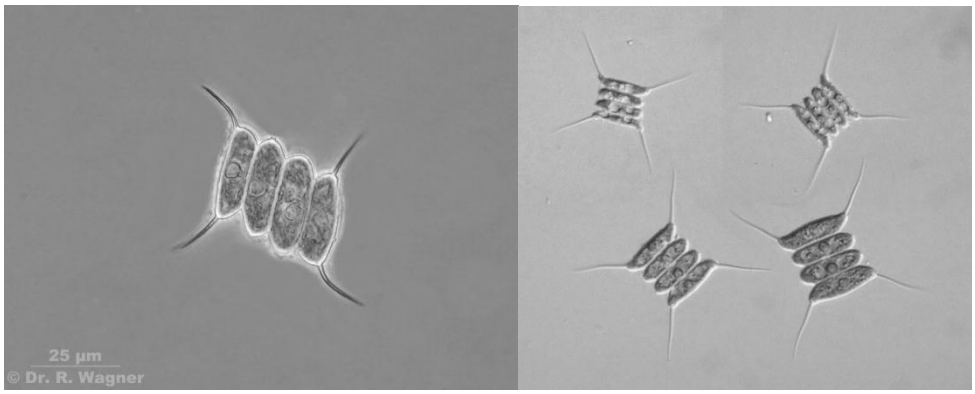

Figure 2.2: S. quadricauda microalga (Ralf Wagner, 2008)

2.2 CELL CONSTRUCTION

Living cells are composed of high-molecular-weight polymeric compounds such as proteins, nucleic acids, polysaccharides, lipids, and other storage materials (fats, polyhydroxybutyrate, glycogen) which also known as biopolymers. These biopolymers constitute the major structural elements of living cells. Besides, cells also contain other metabolites in the form of inorganic salts, metabolic intermediates and vitamins (Shuler and Kargi, 2002).

2.2.1 Lipid Production in Microalgae

Lipids are hydrophobic biological compounds that are soluble in nonpolar solvents but insoluble in water. Few nonpolar solvents that soluble to lipids are benzene, ether and chloroform. These lipids are usually present in the nonaqueous biological phases, such as plasma membrane.

There are many types of lipids present in the cells. Fats are one of the common types of lipids which can serve as biological fuel-storage molecules. The other types of lipids are lipoproteins and lipopolysaccharides which appear in the biological membranes of the cells. Most of the cells can alter the mix of lipids in their membrane in order to adequate with the changes in temperatures or to increase the cells' tolerance to the presence of chemical agents such as ethanol (Kargi, 2002).

The major component for the lipids is fatty acids, which made of straight hydrocarbon chain groups, with a carboxyl group at the end. The hydrocarbon chain of a fatty acid is hydrophobic (water insoluble), but the carboxyl group is hydrophilic (water soluble). A typical fatty acid can be represented as:

$$CH_3 – (CH_2)_n – COOH$$

where the value of n is typically between 12 and 20. Unsaturated fatty acids contain double – C=C – bonds, such as oleic acid (Shuler and Kargi, 2002).

2.2.2 Increase Lipid Production by Nitrogen Limitation

There are three basic growth requirements for microalgae which are light intensity, nutrients supplement and aeration. These three culture conditions have their own effects in increasing the lipid content of the microalgae. However, a study made by Harwood and Jones (1989) revealed that the most effective culture method is the limitation of the major nutrient content which also known as "fattening" strategy. Nitrogen limitation or nitrogen-starvation put the algae cells under unfavorable environmental or stress conditions, which normally causes carbon partitioning from carbohydrate or protein into lipid.

To prove the above hypothesis, based on report of Aquatic Strain Program in the USA (Sheehan et al., 1998), the nitrogen depletion environment has made the cellular lipid content in various classes of microalgae and cyanobacteria increased about an average of 20.2% and 15.1% in total lipids. Furthermore, cyanobacteria also showed an average increase in lipid content of 9.8% under nitrogen-depletion conditions (Hu et al., 2008). The various concentrations of nitrate also increase the lipid content of Nannochloris sp.

UTEX LB1999 from 26.0% to 47.6% (Takagi et al., 2000). An increase from 5.9% to 16.41% in the lipid content of Chlorella vulgaris also being observed for a 75% decrease in the nitrogen concentration (Converti et al., 2009).

Besides, Feng et al., (2011) also revealed that marine microalgae *Isochrysis zhangjiangensis* had high lipid content during sustained nitrate addition and showed high carbohydrate content under nitrate-depletion conditions. This can be concluded that this algal strain can accumulate lipids under nitrogen-repletion conditions and accumulate carbohydrate under nitrogen-depletions conditions.

In conjunction with previous study, Pruvost et al., (2010) have conducted analogous experiments in lab-scale photobioreactor to investigate lipid production by Neochloris oleoabundans, a species known for its ability to accumulate lipids, and especially triacylglycerols (Tornabene et al., 1983). Lipid productivities were quantified in conditions maximizing biomass productivity, and also under nitrogen starvation, which triggers lipid accumulation. Similar productivities were observed for triacylglycerols irrespective of the protocol tested. Although nitrate starvation was necessary to induce triacylglycerols accumulation (18% of dry weight), triacylglycerols productivity was sustained when operating at maximum biomass productivity, because of natural triacylglycerols content in N. oleoabundans has obtained in continuous mode in light-limited condition (3% of dry weight). These results emphasize the difficulty quantifying the utility of a strain for lipid production because of the close dependence on culture conditions and the production strategies applied.

The graph in Figure 3 shows that lipid production is increased when the amount of nitrogen supply to microalgae is limit. This graph is obtained by Goswani and Kalita, (2011) in their paper to investigate the growth and lipid productivity of *Scenedesmus* spp under different concentrations of urea. This can be concluded that microalgae do have an increase in lipid production when they are being put under limitation of the major nutrient nitrogen. The other thing that put in the attention is the variety of organic and inorganic compound that can be used to increase the lipid production.

Luz et al., (2005) stated that an increased abundance of microalgae population cultures does not correspond to an increased uptake of nitrogen per cell in immobilized or co-immobilized cultures. This justification is made based on two possible explanations. First, the total nitrogen content of the cells was related to the size of the population which; the higher population sizes resulted in higher N contents. Second, the higher the number of cells, the older will be the culture physiologically which results due to the cells are less metabolically active and therefore uptake of nitrogen is reduced.

Based on all the above reviews, the work for this project aims to determine the flow rate of air supply which mainly for the carbon dioxide supply to the microalgae for the optimum growth of *S. quadricauda.* This project also aims to know whether *S. quadricauda* can improve in lipid production if it is being put under nitrogen limitation culture. Though, together with this project also, is to find out the effect of extremely high concentration of nitrogen to the lipid production.

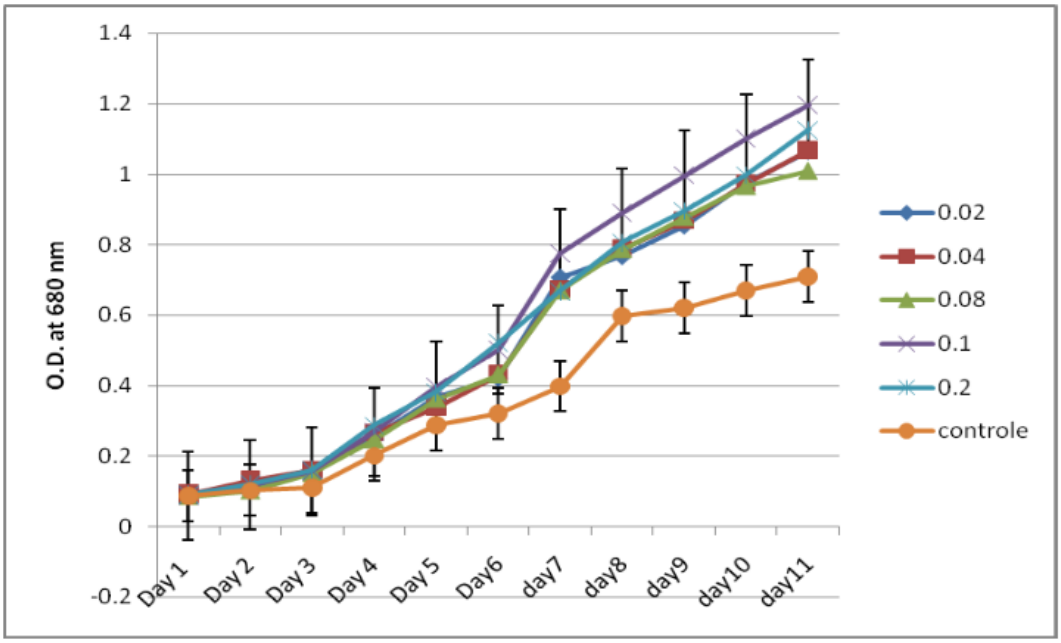

Figure 2.3: Graph of Optical Density versus Time (day)
(Goswani and Kalita, 2011)

CHAPTER 3

METHODOLOGY

3.1 Preparing algal strain and cultivation conditions

The culture medium used is defined medium called Afnor Medium. The medium consist of all nutrients needed by the microalga for growth such as calcium, nitrogen, potassium, magnesium and phosphate. The main composition of Afnor Medium are 40mg/l $Ca(NO_3).4H_2O$, 100mg/l KNO_3, 30mg/l $MgSo_4.7H_2O$, 40mg/l K_2HPO_4, and 0.8125mg/l $FeC_6H_5O_7.5H_2O$ which being stored in different containers as shown in Figure 3.1. The medium was prepared by using certain combination of various nutrients into solution of filtered water. The amount of culture medium inserted was depending on the ratio of working volume used to cultivate the microalgae. 6L of 7 cylindrical photobioreactors were used to cultivate the microalgae with 5L working volume consist of 0.8L inoculation of *S. quadricauda* and 4.2L of filtered water. The temperature of each photobioreactor was measured and the pH was adjusted to the range of 7-8 by adding Na_2CO_3 to the medium.

Figure 3.1: Preparing the medium for culturing S. quadricauda

3.2 Determining the optimum flow rate of air supply

The air was supplied to the photobioreactors by air distributor which consists of tubes connected to the air supply in the lab. There will be one tube for each photobioreactors. The flow rate of the air was measure using flowmeter located at each tube as shown in Figure 3.2 and the optimum air supply was determined based on the growth curve of *S. quadricauda.* The flowrate of air used were 1.0 L/min, 2.0 L/min, 3.0 L/min, 4.0 L/min, 5.0 L/min and 6.0 L/min for each 7 photobioreactors respectively.

Figure 3.2: Flowmeter used to supply the air to photobioreactors

3.3 Manipulating the nitrogen content in the culture medium

The nitrogen content was manipulated based on the total amount of nitrogen supply by the culture medium. The amount of nitrogen supplied by the standard Afnor Medium is assumed to be the optimum amount of nitrogen needed by the microalgae to grow. By limiting the amount of nitrogen supply to the microalgae, the amount of lipid accumulation can be increased due to the unfavorable condition given to the microalgae. The amount of nitrogen decrement is 20%, 40%, 60%, 80% and 100% from the total nitrogen of the standard Afnor Medium and inserted to 5 different photobioreactors as shown in Figure 3.3. The 100% is used as the control for the result to compare with previous method (determining optimum flowrate).

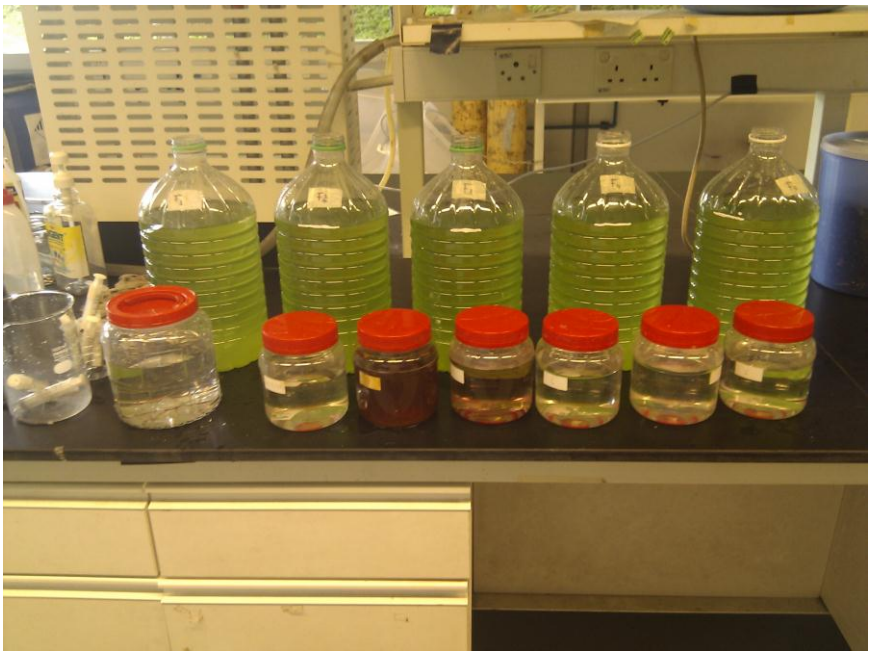

Figure 3.3: Limiting the nitrogen supplement to each photobioreactor

3.4 Varification of lipid accumulation

The *S. quadricauda* was harvested when there was no very large increase in the growth observed inside the tanks. This was done by taking the sample from each tank and tested using the UV-VIS to measure the optical density (OD) of the sample. The expected day for harvesting *S.quadricauda* was about 9 days. The harvested *S. quadricauda* was then dried and the weight of the crude *S. quadricauda* was being reported. Then the lipid from *S. quadricauda* was extracted using Bligh & Dyer method (1959).

The sample was collected from each tank and put into the test tubes with volume of 50mL. The initial weight of empty test tubes was first measured using the electronic balance as shown in Figure 3.4. The sample was then centrifuged at 7800 RPM for 15 minutes and the upper layer of the sample was removed using centrifuge machine as shown in Figure 3.5. Then the sample was put inside the microwave for 5 minutes for cell disruption. Mixture of methanol and chloroform with a volume of 18.75mL with ratio of 2:1 was poured inside each tube and the tubes were vortex well. Then the sample was added with 6.25mL methanol and the tubes were vortex well. Then the sample was added with 6.25mL distilled water and the tubes were vortex well. The

sample was then centrifuge again at 7800 RPM for 15 minutes and the lower layer was collected and put into another test tubes. The lower layer was then dried in the oven as shown in Figure 3.6 at temperature range of 70-80^{o}C for 1 day to dry the solvent. The lipid content left in the tubes was then measured using the electronic balance and the lipid productivity as well as the lipid content for *S. quadricauda* was calculated.

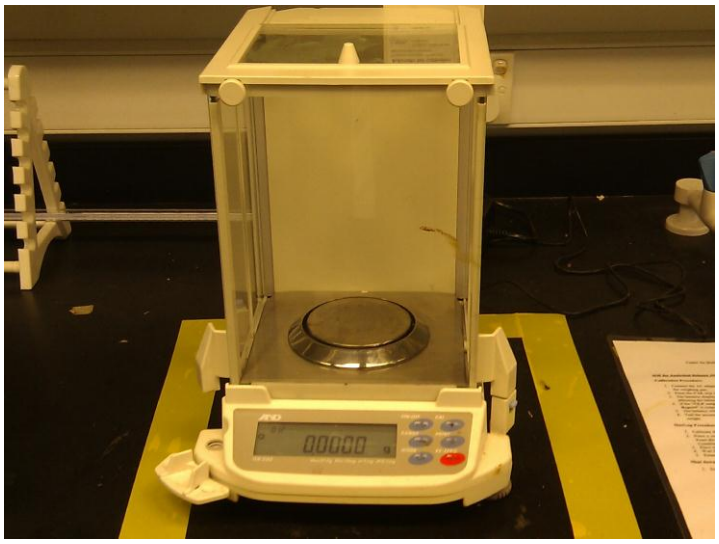

Figure 3.4: Electronic balance for weighing the mass of test tubes and sample

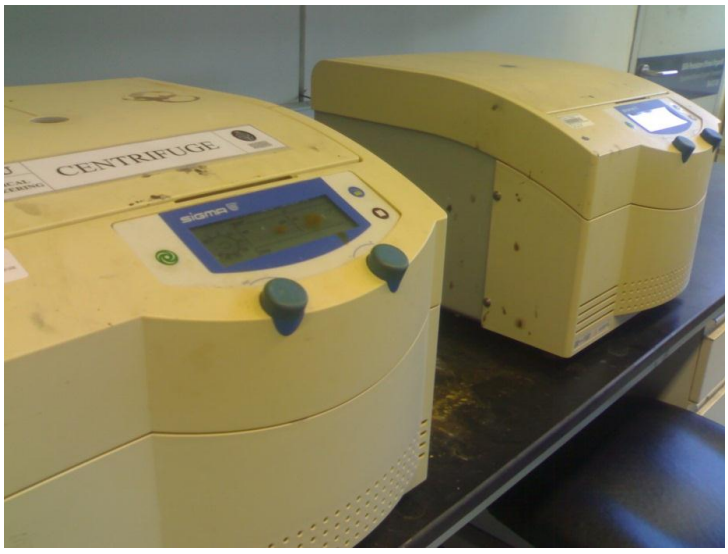

Figure 3.5: Centrifuge machine used for the centrifugation of the sample

Figure 3.6: Oven used for drying process

3.5 Optical Density (OD) measurement

The optical density (OD) of each photobioreactors is important in determining the day for the *S. quadricauda* to be harvested. The OD basically measured the concentration of the microalgae inside the sample. The OD for each photobioreactors was taken daily by taking the samples using the syringe and putting it inside the test tubes as shown in Figure 3.7. The samples were then tested using the UV-Vis Spectrophotometer as shown in Figure 3.8 to measure the OD. The peak graph of the OD obtained was used as the measurement to determine the harvest day of the microalgae. The highest peak of the graph versus time was plotted and the growth curve of the *S. quadricauda* was observed.

Figure 3.7: Taking the samples from each aquarium into test tubes for measuring the optical density

Figure 3.8: Apparatus and UV-VIS equipment to measure the optical density

3.6 Cell counting

To check for cell counting, small amount of sample from each photobioreactor was taken daily and the numbers of cells are counted using microscope. The sample was put into the counting chamber before the cell is being counted under the microscope with X400 magnifation as shown in Figure 3.9. The counting chamber has very numerous small square partitions which designed to help calculating the cell. The counting must be done manually and hence it must be taken slowly and patiently. The counted cell was then used to determine the amount of cell produced at the end of the harvest day.

Figure 3.9: Microscope and counting chamber used to count the cell using Hemocytometer Method

3.7 Chemicals and Equipment/Tools Involved

The chemicals involved during this project are; $Ca(NO_3).4H_2O$, KNO_3, $MgSo_4.7H_2O$, K_2HPO_4, and $FeC_6H_5O_7.5H_2O$, Chloroform, Ethanol and Methanol. Besides the chemicals, these are equipment needed to complete the project; Photobioreactor, Air distributor, Fluorescent Lamp, Flowmeter, UV-Vis Spectrometer, Microscope, Centrifuge, Analytic balance, Oven, Vortex mixer, and Fridge. Besides the chemicals and equipment, glassware also play important role to ensure the project completion which are; Volume flask, Glass test tube, Beaker, Petri dish, Conical Flask, Lamp and Socket.

3.8 Project Flow

In summary, the project flow for this project consists of 4 main steps which can be shown in the Figure 3.10. This flow needs to be done step by step such that the objectives for this project can be achieved.

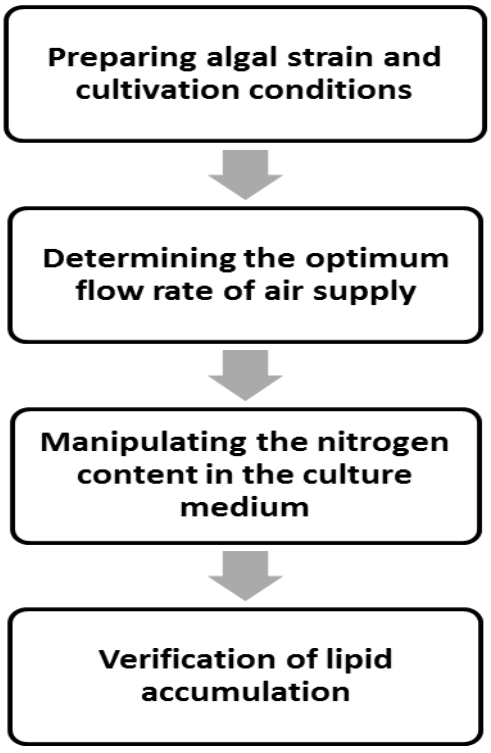

Figure 3.10: Project flow

Table 3.1: Ghantt chart/Key milestones

Work/Activity		Semester 1														Semester 2													
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Selection of Project Topic																												
2	Basic Data Gathering																												
4	Determine the Equipment and Chemicals needed																												
5	Finding the Equipment needed																												
6	Taking the samples																												
7	Lab Familiarisation																												
8	Buying the Equipment																												
9	Studies on Equipments/Software Required																												
10	Preliminary Report Submission																												
11	Result gathering																												
12	Draft Interim Report Submission																												
13	Submission of Interim Report																												
14	Conducting Experiment Part 1																												
15	Conducting Experiment Part 2																												
16	Preparing Analysis and Report																												
17	Analyzing data and performance																												

18	Submission of Progress Report																													
19	Pre-EDX																													
20	Submission of Draft Report																													
21	Submission of Dissertion (Soft Bound)																													
22	Submission of Technical Paper																													
23	Oral Presentation																													
24	Submission of Project Dissertation (Hard Bound)																													

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Optical Density Measurement

The optical density (OD) measurements of the samples are done using the UV-Vis Spectrophotometer. The OD measurement for each sample was done three times and the average was taken to increase the accuracy of the result.

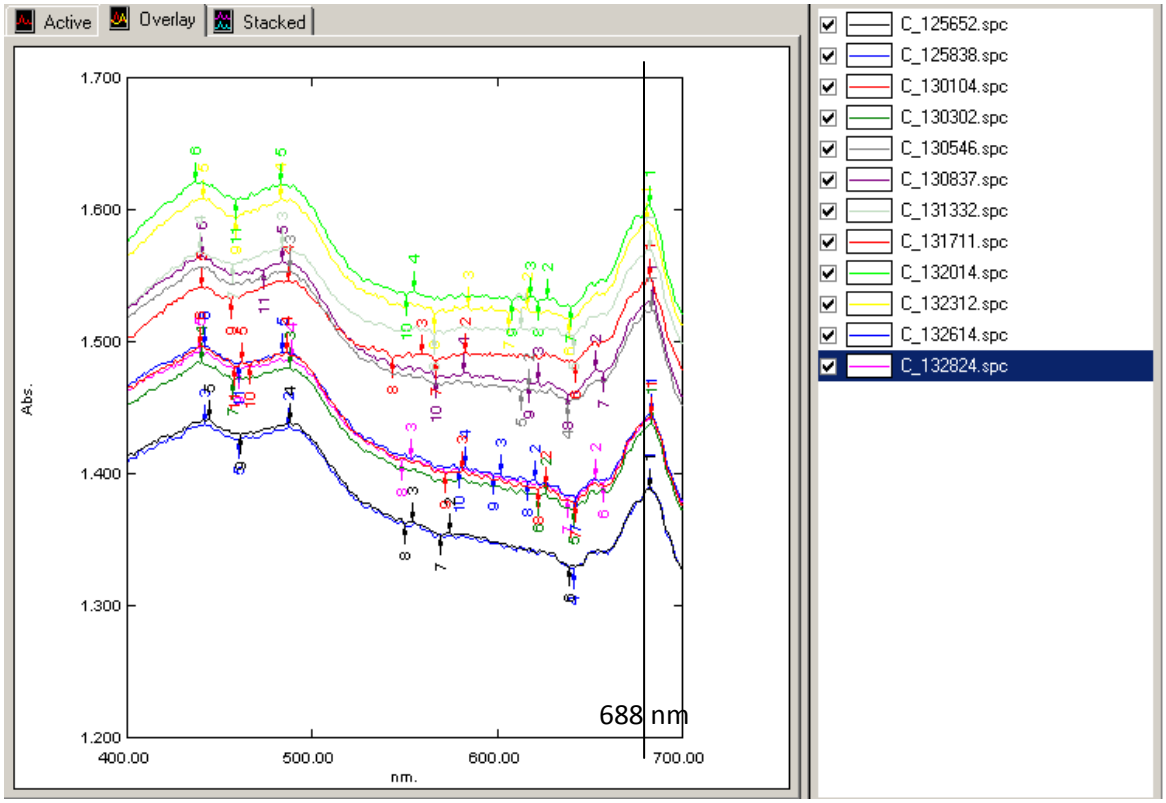

Figure 4.1: The OD for different sample in the range of 400-700nm wavelength

Based on the Figure 4.1 above, we can observe that there were three peaks which can be used for the measurement of the OD of *S. quadricauda.* The peak at the wavelength of 688nm has been chosen as the measurement of OD and was used to plot the graph against time in order to determine the growth curve of *S. quadricauda.*

4.2 Growth Curve of *S. Quadricauda*

The growth curve of *S. quadricauda* was observed by plotting the OD at 688nm versus time until the day of harvest. The growth curve obtained is shown in Figure 4.2 below.

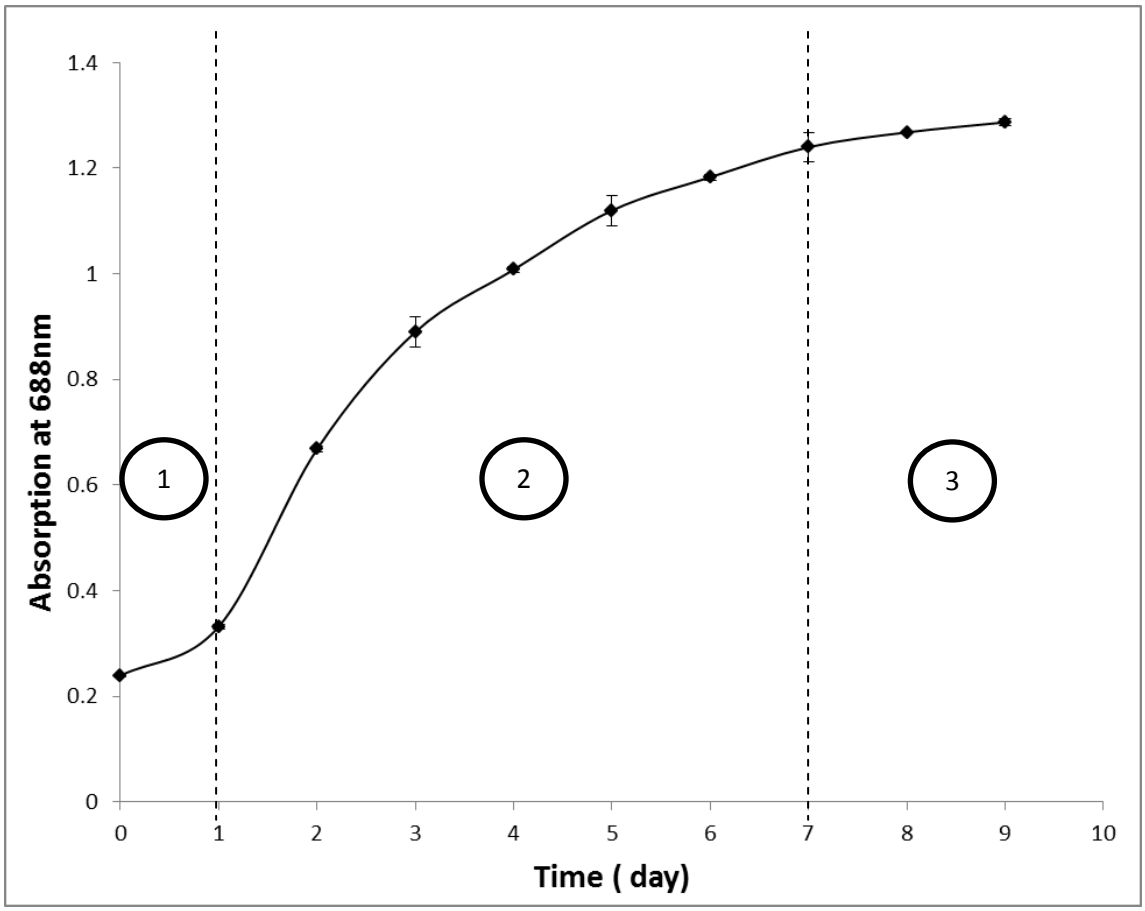

Figure 4.2: Growth curve of S. quadricauda

From the growth curve obtained, the growth curve of *S. quadricauda* can be divided into three phases which are:

1. **Lag Phase**

   This phase occurs from the day when *S. quadricauda* was introduced into fresh culture medium until the first day. During this phase, we can observed that there was not much increased in the OD of the *S. quadricauda* which can also be said that there was not much increase in the amount of *S. quadricauda* cells inside the photobioreactors. This is because no net increase in mass due to cell division does

not take place right away after the *S. quadricauda* was introduced to the fresh medium. However, the cell is actually synthesizing new components in order to adapt with the new environment.

2. Exponential Phase

This phase was when the *S. quadricauda* was growing and dividing at the maximal rate possible which can be observed the rapid increment of OD from day 1 until day 7. At this phase, the growth rate of *S. quadricauda* was constant where the cells were dividing and doubling in number at regular intervals.

3. Stationary Phase

This phase was when the population growth of *S. quadricauda* ceases and the growth curve becomes horizontal which can be observed on the day 7 until day 9. During this phase, the total number of viable microalgae remains constant which can also result from the balance between cell division and cell death.

4.3 Effects of Different Flowrate of Air Supply to *S.Quadricauda*

4.3.1 Growth Curve of *S. Quadricauda*

The experiment was conducted using different flowrate of air supply to the photobioreactors in order to determine the optimum flowrate of air supply to the growth of microalgae. Flowrate of 1.0 L/min, 2.0 L/min, 3.0 L/min, and 4.0 L/min has been chosen based on literature review. The result obtained is showed in the Figure 4.3.

Based on the results obtained in the Figure 4.3, we can see that at flowrate of 4.0 L/min gives the highest growth curve and at flowrate of 1.0 L/min gives the lowest growth curve. This is due to that microalgae need to have enough carbon dioxide in order to growth. Hence, as the higher the flowrate of air supply the higher the growth curve of *S. quadricauda*. Determination of the highest growth curve for the microalgae is important so that we can enhance the growth of *S. quadricauda* at a shorter period of time.

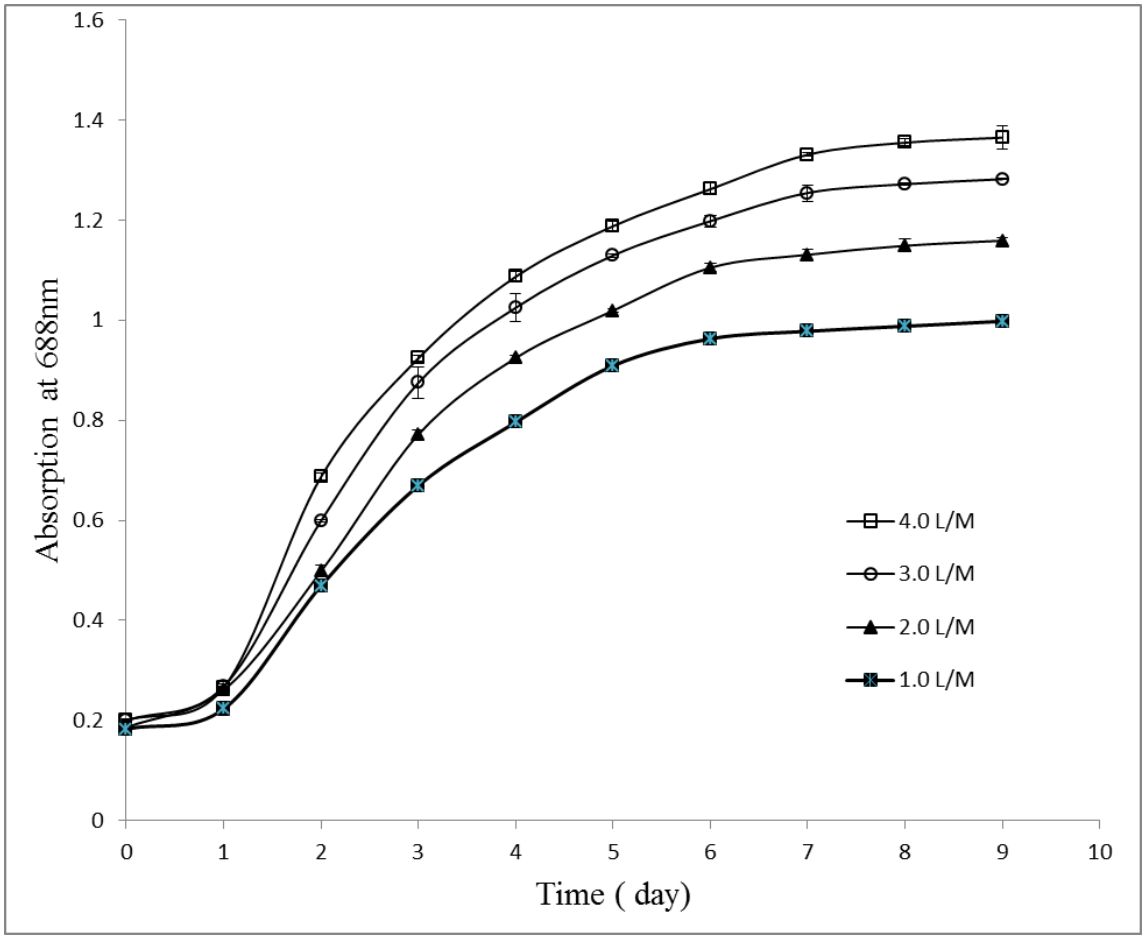

Figure 4.3: Effect of different flowrate to the growth curve of S. quadricauda

However, we still cannot come into conclusion that the flowrate of 4.0 L/min gives the highest growth rate of *S. quadricauda* due to the graph obtained is increasing as the amount of air flowrate supply is increasing. Thus, another experiment is done to determine the most optimum growth rate of *S. quadricauda* with flowrate of 5.0 L/min and 6.0 L/min and the result as shown in the Figure 4.4.

Based on the result obtained in the Figure 4.4, we can observe that the growth curve for the flowrate of 5.0 L/min and 6.0 L/min is almost the same with the growth curve for flowrate of 1.0 L/min. This growth curve is also lower than the growth curve for flowrate of 4.0 L/min. Previously we stated that as the higher the flowrate, the growth curve will also be higher. However this hypothesis has been proven wrong from Figure 4.4 because the optimum flowrate for the growth curve of *S. quadricauda* is at 4.0

L/min. One of the factors that may cause the growth curve become lower although that the flowrate is increased is due to the bad mixing inside the photobioreactor which comes from the air bubbles released in order to supply the air. When the flowrate is too high, the air bubbles released are huge and rapid which makes the microalgae hard to continue growth.

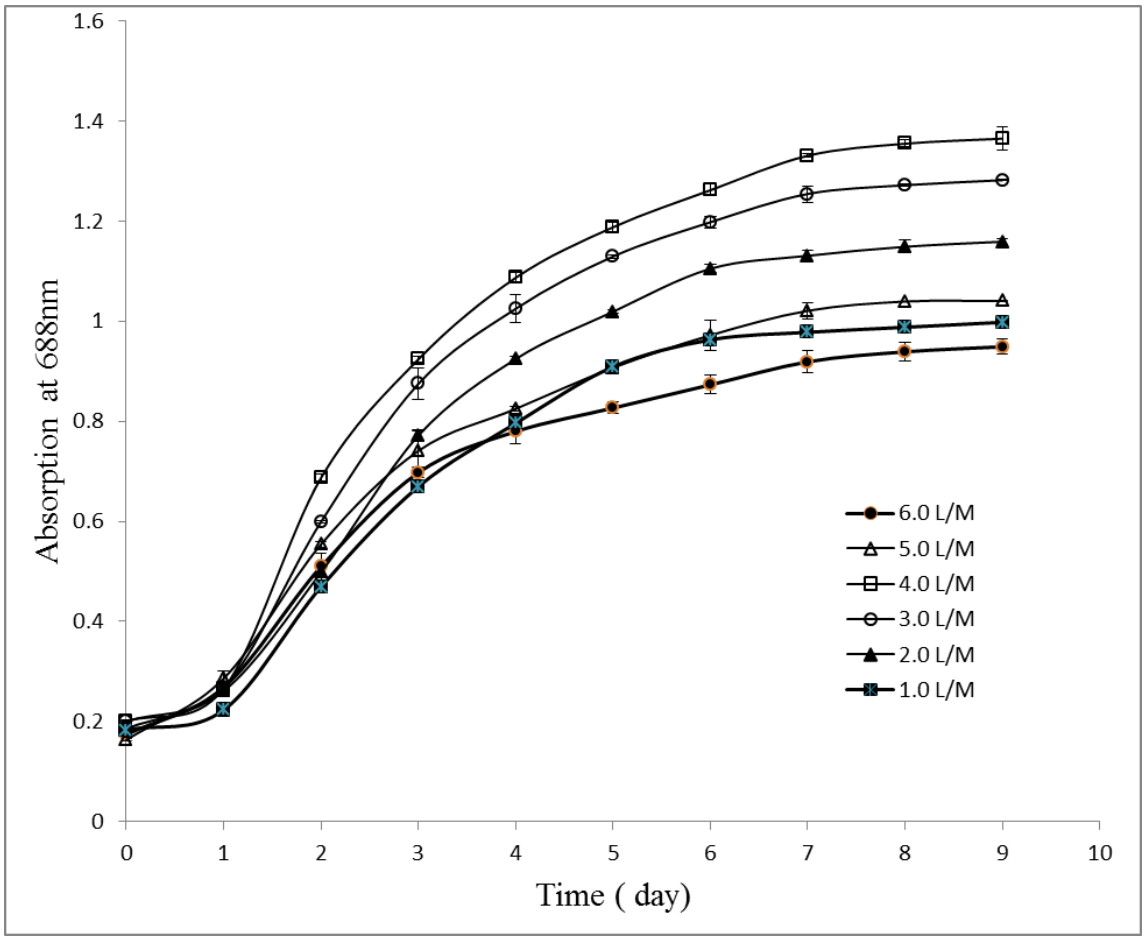

Figure 4.4: Effect of different flowrate to the growth curve of S. quadricauda at high flowrate

4.3.2 Growth Kinetics of *S. Quadricauda*

During the exponential phase, the cells were dividing at constant intervals thus the population will double in number during a specific length of time. This can be used to calculate the Specific Growth Rate (μ) or also known as number of generations of the cells using the formula below (Guillard and Ryther, 1962):

$$\mu = \frac{\ln \frac{N_t}{N_0}}{T_t - T_0}$$

Where,

μ = specific growth rate

Nt= no of cells at the end of log phase.

No= no of cells at the start of log phase

Tt= final day of log phase

To= starting day of log phase

The doublings per day (K) can be calculated by converting T in days from the growth rate (μ) and divide by the natural log of 2 (0.6931).

$$K = \frac{\mu}{0.6931}$$

The time required to achieve a doubling of the no of viable cells is termed as doubling time (Tt) which is calculated by the following formula:

$$T_t = \frac{0.6931}{\mu}$$

The calculated results for the μ, K and T_t are show in the Table 4.1 below.

Table 4.1: Growth Kinetics of S. quadricauda at different flowrate

Air flowrate (L/min)	Specific Growth Rate, μ	Doublings per Day, K	Doubling Time, Tt
1.0	0.243	0.351	2.85
2.0	0.262	0.378	2.65
3.0	0.266	0.384	2.60
4.0	0.275	0.397	2.52
5.0	0.265	0.382	2.62
6.0	0.243	0.351	2.85

Thus, from this kinetics growth of *S. quadricauda*, we can observe that at 4.0 L/min, the specific growth rate is the highest which is 0.275, 0.397 of doublings cells per day which also highest and lowest doubling time taken for the cells which is 2.52. Thus we can conclude that the optimum growth for *S. quadricauda* is at flowrate of 4.0 L/min.

4.3.3 Biomass Concentration of *S. Quadricauda*

The biomass concentration of *S. quadricauda* was calculated by weighing the dry weight of the *S. quadricauda* using the test tube. The result obtained from the biomass concentration shows that the highest biomass was collected at the highest OD which is 0.32 g/L. The results of the biomass concentration are shown in the Table 4.2 below.

Table 4.2: Biomass Concentration and Number of Cells of S. quadricauda

No	A688	Number of Cells, 10^9	Biomass Concentration, g/L
1	1.31	396	0.32
2			
3			
4	1.106	320	0.25
5			
6			
7	0.783	237	0.17
8			
9			
10	0.519	160	0.04
11			
12			
13	0.204	89	0.02
14			
15			

The relationship between the Biomass Concentration in g/L with the OD at 688nm of wavelength is also plotted and shown in the Figure 4.5.

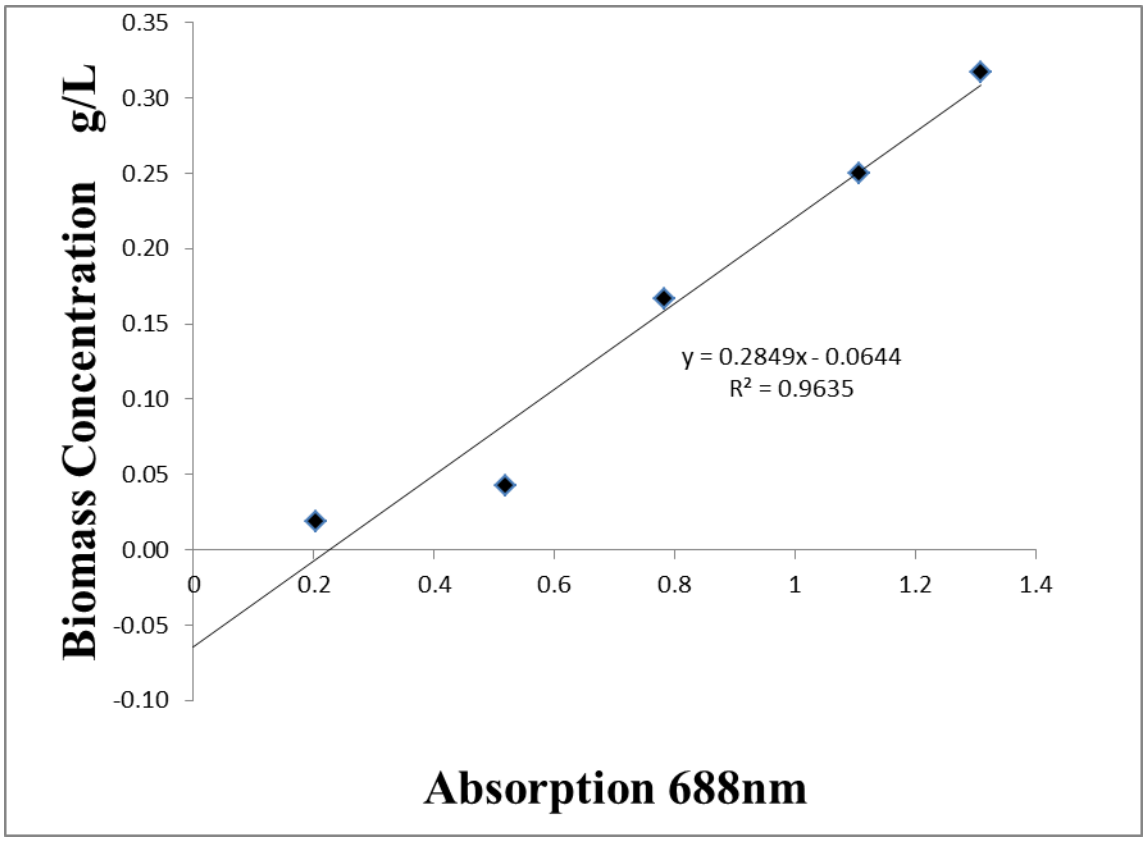

Figure 4.5: Relationship between the Biomass Concentration and OD

Based on the relationship obtained from Figure 4.5, we can conclude that as the OD increase, the biomass concentration is also increase. This relationship is very important for the use in the future in order to determine the amount of biomass concentration had been produced by *S. quadricauda* at certain OD.

4.4 Effects of Nitrogen Limitation Environment to *S. Quadricauda*

4.4.1 Growth Curve of *S. Quadricauda*

The experiment was conducted using different amount of nitrogen supply to the microalgae. The decrement of 20%, 40%, 60%, 80% and 100% (as control) from the total amount of nitrogen supplied by the Afnor Medium had been chosen and applied to five different photobioreactors. The result obtained is shown in Figure 4.6.

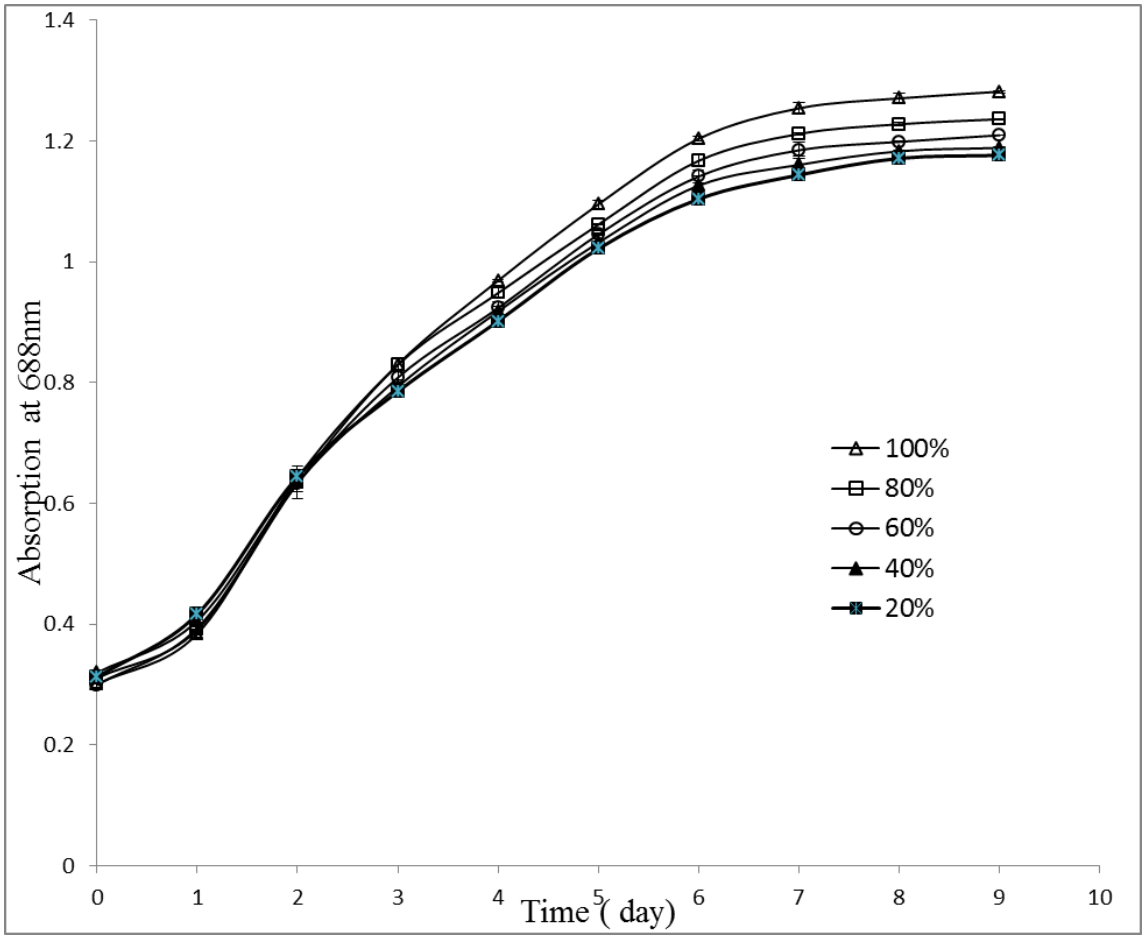

Figure 4.6: Effect of nitrogen limitation to the growth curve of S. quadricauda

Based on the result obtained in Figure 4.6 above, we can observe that by limiting the nitrogen, the growth curve is different for each sample. The highest growth curve is where there is no decrement (100% supply-control) of nitrogen supply to the microalgae and the lowest growth curve is at 20% decrement of total nitrogen supply to the microalgae. This shows that when microalgae is put under unfavorable condition (lack of nitrogen supply), the cell tends to increase in the biomass concentration which can be observe by the OD at the day of 1-2 and then decrease in the OD because there is not enough nitrogen for cell growth. The highest OD is 1.2815 which is for the 100% of nitrogen supply and the lowest OD is 1.176 which is at 20% total nitrogen decrement.

4.4.2 Growth Kinetics of *S. Quadricauda*

The growth kinetics of *S. quadricauda* was also calculated using the same procedure as used in previous experiment. The calculated results for the μ, K and T_t are show in the Table 4.3 below.

Table 4.3: Growth Kinetics of S. quadricauda at nitrogen limitation

Total Nitrogen Decrement (%)	Specific Growth Rate, μ	Doublings per Day, K	Doubling Time, Tt
20	0.189	0.273	3.66
40	0.187	0.270	3.70
60	0.199	0.287	3.48
80	0.197	0.284	3.52
100	0.207	0.299	3.34

Hence, from the growth kinetics of *S. quadricauda* at nitrogen limitation, we can observe that the specific growth rate and doublings of cells per day is the highest at nitrogen 100% supply of nitrogen which is 0.207 and 0.299 respectively. Besides, the doubling time for the cells is also the fastest which is at 3.34 for the 100% supply of nitrogen which support the statement that *S. quadricauda* can increase in the growth of cells if enough nitrogen is supply to the cells.

The kinetics growth of *S. quadricauda* for nitrogen concentration can also be expressed in a kinetic growth model known as Monod Model (Mehregan Jalalizadeh, 2012). The equation used in this model is as follow:

$$\mu = \mu_{\max} \frac{S_N}{(K_{S,N} + S_N)}$$

Where,

μ = growth rate

μ_{max} = maximum specific growth rate

S_N = nitrogen concentration

$K_{S,N}$ = half-saturation constant

For half-saturation constant, the value as per stated in paper research by Mehregan Jalalizadeh, (2012) is 0.035kgm^{-3}. The growth rate obtained from this model is calculated and compared with the growth rate calculated by previous equation for finding the specific growth rate of the algae using experimental data. The result of the growth rate is shown in Table 4.4 below.

Table 4.4: Comparison of Growth Rate for S. quadricauda

Nitrogen Content (%)	Experimental Data	Monod Model
20	0.189	0.1973
40	0.187	0.2298
60	0.199	0.2431
80	0.197	0.2504
100	0.207	0.2549

The Growth Rate obtained from Table 4.4 is then plotted into graph as shown in Figure 4.7 below. The graph in Figure 4.7 shows that the growth rate calculated using experimental data of *S. quadricauda* is increased when the amount of nitrogen supply to the alga is increased as well as for the growth rate obtained from the Monod Model. Thus, we can conclude that Monod Model can be used to predict the growth curve of *S. quadricauda* in nitrogen limitation environment.

4.4.3 Biomass Concentration and Lipid Yield of *S. Quadricauda*

The biomass concentration of *S. quadricauda* was also calculated by weighing the dry weight of the *S. quadricauda* using the test tube. The result obtained from the biomass concentration shows that the highest biomass is collected where the nitrogen supply is 100% which is 0.3007 g/L of biomass.

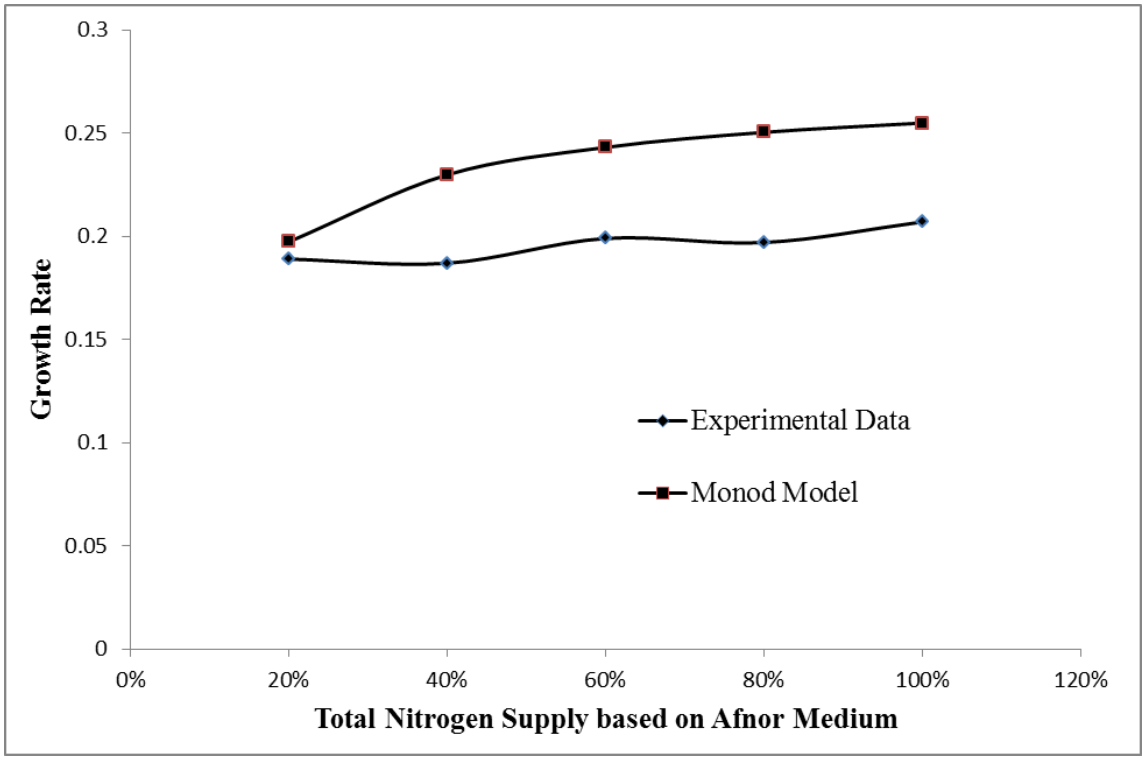

Figure 4.7: Comparison of Growth Rate for S. Quadricauda using experimental data and Monod Model

However, the highest lipid content for *S.quadricauda* is at 20% of nitrogen supply which is 0.0401 g/L. The rest of the results for the biomass concentration and lipid content are shown in Table 4.5.

Table 4.5: Biomass Concentration and Lipid Yield of S. Quadricauda at different amount of nitrogen supply

	Nitrogen Content (%)	m1	m2	Δm	Yield (g/L)
Biomass	20	10.4089	10.6795	0.2706	0.2706
	40	10.2773	10.5515	0.2742	0.2742
	60	10.2095	10.4896	0.2801	0.2801
	80	10.2989	10.5866	0.2877	0.2877
	100	10.231	10.5317	0.3007	0.3007
Lipid	20	10.3007	10.3408	0.0401	0.0401
	40	10.2917	10.3314	0.0397	0.0397
	60	10.4122	10.4503	0.0381	0.0381
	80	10.3306	10.3684	0.0378	0.0378
	100	10.3917	10.4278	0.0361	0.0361

For determination of lipid yield, Bligh and Dyer Method (1959) had been used by using the help of solvent which is the mixture of methanol and chloroform in the ratio 2:1 of the volume. This method shows that the highest lipid yield is produced by the alga that has been put in unfavorable condition which is in the nitrogen limitation condition (20% N supply). This had cause the alga to become in stress condition and converting the carbon and protein stored inside their body into lipid. However due to not enough nitrogen for growth, the lowest biomass concentration of alga is at the nitrogen limitation condition which is at 20% of nitrogen supply to the alga as shown in Table 4.5.

4.4.4 Lipid Content of *S. Quadricauda*

The lipid content of *S. quadricauda* was also calculated by dividing the lipid yield with the biomass concentration of the alga. The results for the lipid content in dry weight % of *S. quadricauda* at different amount of nitrogen supply is shown in Table 4.6 below.

Table 4.6: Lipid content of S. quadricauda at different amount of nitrogen supply

Nitrogen Content (%)	Lipid Content (wt%)
20	14.82
40	14.48
60	13.60
80	13.14
100	12.00

Based on the Table 4.6, we can observe that the highest lipid content for *S. Quadricauda* is at 20% of nitrogen supply which is 14.82%. Although that at nitrogen limitation, the alga has the lowest amount of biomass concentration, the amount of lipid yield is still high thus making the lipid content for *S. Quadricauda* at nitrogen limitation is prove to be the highest compared to the other condition of nitrogen supply to the alga.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This dissertation reports the experimental investigation into the growth of microalga *S. Quadricauda*. Several conclusions that can be made are:

- The optimum amount of flowrate for the air supply for cultivating microalgae *S. quadricauda* in cylindrical photobioreactor of 5L working volume is at 4.0 L/min. However, if cultivating *S. quadricauda* higher than 4.0 L/min will cause decrease in the growth curve and biomass concentration of *S. quadricauda.*
- The highest lipid yield by *S. quadricauda* is 0.0401 g/L which is at 20% nitrogen supply to the microalga which the microalga is grow under nitrogen limitation condition.
- The biomass concentration of *S. quadricauda* is the lowest when the microalga is put under nitrogen limitation condition which is 0.3007 g/L.
- The lipid content of *S. quadricauda* is the highest which is 14.82% of dry weight which obtained when the microalga is under nitrogen limitation environment.

5.2 Recommendations

There are several recommendations that can be implemented to this project for further researches which are:

- Cultivating *S. quadricauda* in larger volume with proper agitation system because the strain is too heavy and tends to settle down which effects the growth of the microalgae.
- More studies on increasing the lipid content of *S. quadricauda* need to be carried on such as manipulating the carbon dioxide supply and the temperature.

All in all, this species of microalgae, *S. quadricauda* has high potential for producing higher lipid content which can then be converted into biofuels, as the main alternative renewable energy in the future.

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