# PRODUCTION OF BIODIESEL FROM MICRALGAE USING ULTRASOUND

VIKINESH A/L MARIAPPAN

Thesis submitted in fulfillment of the requirements
for the award of the degree in Chemical Engineering

Faculty of Chemical and Natural Resources Engineering
UNIVERSITI MALAYSIA PAHANG

FEBRUARY 2013

## SUPERVISOR'S DECLARATION

I hereby declare that I have checked this thesis and from my point of view, this thesis fulfills in terms of scope and quality for the award of Degree of Bachelor Chemical Engineering in Biotechnology.

| | | |
|---|---|---|
| Signature | : | _____ |
| Name of Supervisor | : | DR. AZILAH AJIT @ABD AZIZ |
| Date | : | 1ST FEBRUARY 2013 |

## STUDENT'S DECLARATION

I declare that this report is my own work except for the quotations and summaries which have been duly acknowledged.

| | | |
|---|---|---|
| Signature | : | _____ |
| Name | : | VIKINESH A/L MARIAPPAN |
| Date | : | 1ST FEBRUARY 2013 |

*I dedicate this entire work to my family especially to my beloved parents (Mr. Mariappan a/l Kuppan and Mrs. Ratha a/p Karuppannan ) whose patient and support have facilitated my study, and made my life enjoyable. I also dedicate this work to my supervisor and co-supervisor (Dr. Azilah Ajit and Dr. Ahmad Ziad Sulaiman) for their patience in guiding me to complete the work and thesis.*

## ACKNOWLEDGEMENT

First and foremost, I would like to express my sincerest gratitude to my supervisor ,Dr Azilah Ajit and to my co-supervisor Dr Ahmad Ziad Sulaiman for the encouragement, guidance, critics, and friendship in overseeing the progress of my Projek Sarjana Muda (PSM) from initial until the completion of this thesis. Without their support, this thesis would not been completed as presented here. I believe that all their advices and comments as for the benefit of this research.

I also would like to thanks the entire lab assistants especially Mr. Mohd Zaki and Mr Mohd Anuar for their assistance in handling the equipments in the laboratory. I also would like to thanks the science officers from Fist laboratory for their continues support to provide equipments during the project. My sincere appreciation also extends to all my colleagues and others who have provided assistance at various occasions. Their views and teaches are very useful indeed. Unfortunately, it is not possible to list all of them in a limited space.

Last but not least, I would like to express my deepest gratitude to all my family members.

**PRODUCTION OF BIODIESEL FROM MICROALGAE USING ULTRASOUND**

## ABSTRACT

Biodiesel is a clean-burning environmentally friendly fuel. Since the combustion of biodiesel releases carbon dioxide into the air that is taken out of the air by plants, it is called carbon neutral. In fact, it releases less greenhouse gas than diesel and it is biodegradable so it does not cause great harm to the environment if there is a spill. The ultrasound has been proved that low intensity of sonication can improve the fermentation process instead of high intensity sonication. The objective of the study is to investigate the production of lipid from microalgae by applying ultrasound, determine kinetic parameters and determine the rate of production of lipid. *Nannochloropsis sp.* will be used in this study and cultured in f/2 medium. The cell will be treated with ultrasound where the culture will be pump to ultrasound and recycled back to the medium. The impact of different power and regiment of ultrasound sonication will be investigated. Duty cycles of 10%, 20% and, 40% will be used. The outcome is ultrasound can enhance the production of lipid from microalgae *Nannochloropsis sp.* The optimum power and intensity is selected by observing the cell reduction. The optimum intensity for this project is 10.2 watt/cm$^2$. *Nannochloropsis sp.* gives a better result in 10% of duty cycle with the intensity of 10.2 watt/cm$^2$. The power and the intensity is determined after observe the absorbance after sonication at various power and amplitude. The 20% and 40% sonication did not give a good result because it seems to give a intense condition for the microalgae to grow.

**PRODUCTION OF BIODIESEL FROM MICROALGAE USING ULTRASOUND**

## ABSTRAK

Biodiesel adalah bahan api pembakaran bersih yang mesra alam. Pembakaran biodiesel mengeluarkan karbon dioksida ke udara ini dibawa keluar dari udara oleh tumbuh-tumbuhan, ia dipanggil neutral karbon. Malah, ia mengeluarkan gas rumah hijau berbanding diesel dan ia terbiodegradasikan supaya ia tidak menyebabkan kemudaratan yang besar kepada alam sekitar jika terdapat tumpahan. Ultrasound telah dibuktikan bahawa intensiti rendah sonikasi boleh meningkatkan proses penapaian dan bukannya sonikasi intensiti yang tinggi. Objektif kajian ini adalah untuk menyiasat pengeluaran lipid dari mikroalga dengan menggunakan ultrasound, menentukan parameter kinetik dan menentukan kadar pengeluaran lipid. *Nannochloropsis sp*. akan digunakan dalam kajian ini dan dikulturkan dalam medium f / 2. Sel akan dirawat dengan ultrasound dimana sel yang dikulturkan akan dipam ke ultrasound yang disetkan ke dalam bekas dan dialirkan kembali ke kelalang kon dua liter. Kesan kuasa yang berbeza dan rejimen sonikasi ultrasound akan disiasat. Kitaran tugas sebanyak 10%, 20%, dan 40% akan digunakan. Keputusannya yabng jelas adalah ultrasound boleh meningkatkan pengeluaran lipid dari mikroalga *Nannochloropsis sp.* Kuasa optimum dan intensiti dipilih dengan memerhatikan pengurangan sel. Keamatan optimum untuk projek ini adalah 10.2 watt/cm$^2$. *Nannochloropsis sp.* memberikan hasil yang lebih baik dalam 10% kitar sonikasi dengan keamatan sebanyak 10.2 watt/cm$^2$. Kuasa dan keamatan ditentukan selepas melihat keserapan selepas sonication pada kuasa dan amplitud pelbagai. Kitar sonikasi 20% dan 40% tidak memberikan hasil yang baik kerana ia seolah-olah memberi satu keadaan sengit untuk mikroalga untuk berkembang.

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| cm | Centimetre |
| $CO_2$ | Carbon Dioxide |
| FAME | Fatty Acid Methyl Ester |
| hr | Hour |
| L | Litre |
| m | Metre |
| mg | Milligram |
| ml | Millilitre |
| min | Minute |
| N | Nitrogen |
| NaOH | Sodium hydroxide |
| Kg | Kilogram |
| P | Phosphorus |
| rpm | Revolutions per minute |
| s | Second |
| Si | Silicon |
| TAGs | Triglycerides |
| V | Volume |
| W | Power |
| % | Percentage |
| ℃ | Celsius Degree |
| µ | Specific growth rate |

# CHAPTER 1

# INTRODUCTION

## 1.1 Background of Proposed Study

As energy demands increase and oil reserves begin to weaken in their stability, the need for a reliable renewable fuel source increase. There are many options such as bioethanol, biodiesel, and green diesels have the capability of providing a fuel source. Microalgae have become a source alternative energy in this modern world. Microalgae are microscopic algae, typically found in freshwater and marine systems.

It uses photosynthesis process to survive and important for life on earth. Microalgae can be used as a potential oil source due to its faster growth rates, high oil content and the ability to be harvested frequently over a long period of time (Haag and A.L, 2007). It produces storage lipids in the form of triacyglycerols (TAGs) (Balasubramanian et al, 2011). Biodiesel can be synthesized from triacyglycerols (TAGs) through transesterification reaction by using acid or base and methanol. Transesterification is the

reaction of a fat or triacyglycerols (TAGs) with an alcohol to produce esters and glycerin.

Ultrasound is sound of frequency greater than 20 kHz. Wave frequency is the number of repetitions (or cycles) per second of a defined vibration state at a fixed location in space. Yusuf Chisti (2003) said that ultra-sonication is commonly related with damaging the cells but evidence is emerging for beneficial effects of controlled sonication on conversions catalyzed by live cells.

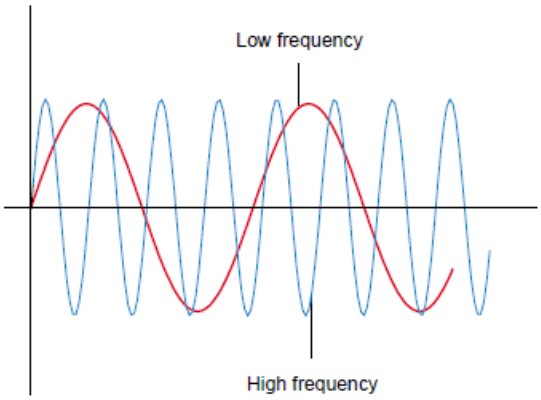


**Figure 1.1** A Low And A High Frequency Wave

Source (Yusuf Chisti, 2003)

In this project, *Nannochloropsis sp* strain is selected for the experiment. After that, it involves the culturing of microalgae at a correct and optimum medium nutrient. Then, the cultured microalgae are set into two liters of conical flask and ultrasound is introduced. It is estimated the lipid production will increase. The amount of lipid is

compared with and without ultrasound. Finally, the lipid obtained is compared with control growth to calculate and observe the changes. From Table 1.1, we can see that microalgae basically are an efficient source for biodiesel production.

**Table 1.1** Comparison of microalgae with other biodiesel feed stocks

| Plant source | Seed oil content (% oil by wt in biomass) | Oil yield (L oil/ha year) | Land use ($m^2$ year/kg biodiesel) | Biodiesel productivity (kg biodiesel/ha year) |
|---|---|---|---|---|
| Corn/Maize (*Zea mays* L.) | 44 | 172 | 66 | 152 |
| Hemp (*Cannabis sativa* L.) | 33 | 363 | 31 | 321 |
| Soybean (*Glycine max* L.) | 18 | 636 | 18 | 562 |
| Jatropha (*Jatropha curcas* L.) | 28 | 741 | 15 | 656 |
| Camelina (*Camelina sativa* L.) | 42 | 915 | 12 | 809 |
| Canola/Rapeseed (*Brassica napus* L.) | 41 | 974 | 12 | 862 |
| Sunflower (*Helianthus annuus* L.) | 40 | 1070 | 11 | 946 |
| Castor (*Ricinus communis*) | 48 | 1307 | 9 | 1156 |
| Palm oil (*Elaeis guineensis*) | 36 | 5366 | 2 | 4747 |
| Microalgae (low oil content) | 30 | 58,700 | 0.2 | 51,927 |
| Microalgae (medium oil content) | 50 | 97,800 | 0.1 | 86,515 |
| Microalgae (high oil content) | 70 | 136,900 | 0.1 | 121,104 |

Source (Teresa M. Mata et al, 2010)

## 1.2 Problem Statement

It is known that in normal microalgae growth, the microalgae are capable of producing certain amount of lipid for biodiesel production. During the normal microalgae growth, for it to produce certain amount of lipid it is taking too much time. So, it is predicted that by introducing the ultrasound during the microalgae growth, we are able to get higher amount of lipid for biodiesel for the same amount of substrate. The specific growth rate of *Nannochloropsis sp*. is quite slow compared to other strain.

## 1.3 Research Objectives

The objectives of the production of biodiesel from algae using ultrasound by are:

1.3.1: To determine kinetic parameter of microalgae.

1.3.2: To identify and determine the rate of production of lipid by algae by using ultrasound.

## 1.4 Research Questions

The proposed study is constituted to find answers of the following questions:

1.4.1: How the production of lipid from algae changes when ultrasound is used?

1.4.2: What are suitable the power used for algae biomass production?

1.4.3: What are the suitable conditions of ultrasound to enhance lipid production?

## 1.5 Scope of Proposed Study

In order to achieve the objectives of the study, the scope of the proposed study are to culture the *Nannochloropsis sp* .in f/2 medium to grow at optimal condition. Furthermore, the lipid production by ultrasound is done by manipulating the sonication regiment. The sonication regiment can be manipulated by changing the duty cycle and the intensity. The intensity is calculated by using, $I = P/A$. The duty cycle and power of ultrasound is manipulated in order to enhance lipid production.

## 1.6 Expected Outcome

The ultrasound can increase the rate of production of lipid from microalgae. So that, the lipid obtained can be changed into a better renewable energy; biodiesel.

## 1.7 Significance of Proposed Study

The significance of the producing biodiesel from microalgae using ultrasound is to increase the biodiesel / lipid production and to improve the fermentation process of microalgae.

## 1.8 Conclusion

Lipid is extracted from the microalgae and the production of the lipid is increased by manipulating the suitable power and duty cycle of sonication. Since biodiesel is extracted from algae, it is a clean energy, renewable, non toxic and sustainable alternative to petroleum based fuels, and it is able to reduce toxic emissions when is burned in a diesel engine. Hence biodiesel can be a better replacement for fossil fuel.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Microalgae

Microalgae are prokaryotic or eukaryotic photosynthetic microorganisms that can grow rapidly and live in harsh conditions due to their simple multicellular structure as shown in the Figure 2.1. Microalgae are present in all existing earth ecosystems, not just aquatic but also terrestrial. It represents a big diversity of species living in a wide range of environmental conditions. Microalgae reproduction occurs mainly by vegetative cell division, although sexual reproduction can occur in many species under appropriate growth conditions. Microalgae are the most primitive form of plants but the mechanism of photosynthesis in microalgae is similar to that of higher plants (Scott et al. , 2010)

The Figure 2.1 shows the basic overview of photosynthetic pathway of a microalgae. Precursor fatty acids are synthesized de novo in the chloroplast, using either

carbon fixed during photosynthesis, or from an exogenous supply of organic carbon; the exact nature of what enters the chloroplast is unknown in algae (dashed line). Free fatty acids are exported from the chloroplast and then converted to TAGs in the endoplasmic reticulum (ER), where they bud off into oil bodies in the cytosol.

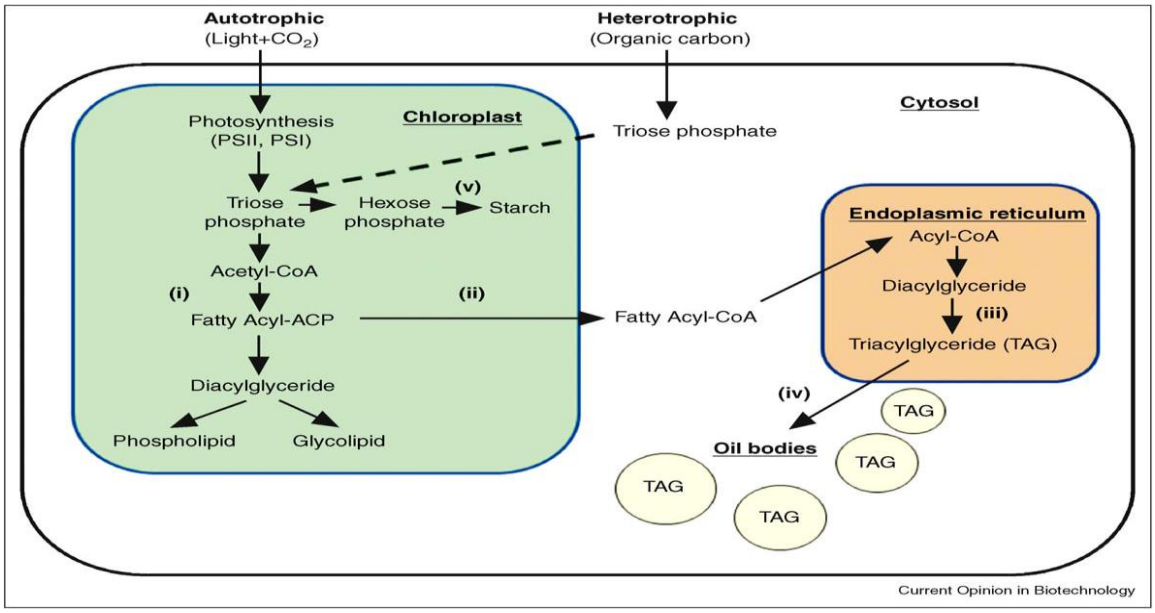


**Figure 2.1** Basic overview of the pathway of carbon capture and lipid biosynthesis. Key: (i) = acetyl-CoA carboxylase (ACCase) and fatty acid synthase (FAS); (ii) = fatty acid thioesterases and acyl-CoA synthetases; (iii) = TAG biosynthesis enzymes, including acyl-CoA:diacylglycerol acyltransferase (DGAT); (iv) = oil body formation; and (v) = ADP-glucose pyrophosphorylase and starch synthase.

Source (Scott et al., 2010)

Microalgae consist of four major groups which are diatoms, green algae, blue green algae, and golden brown algae. Diatoms are among the most common and widely distributed groups of algae in existence. This group tends to dominate the phytoplankton of the oceans, but is commonly found in fresh and brackish water habitats. The cells are golden-brown because of the presence of high levels of fucoxanthin, which is a

photosynthetic accessory pigment. Meanwhile in green algae, it is often referred to as chlorophytes. These are also quite abundant, especially in freshwater. They can occur as single cells or as colonies. In blue green algae, they have an organization and structure which is similar with bacteria. The function of these algae is fixation on nitrogen from atmosphere. Finally is golden brown alga which is similar to diatoms which respects to pigments and biochemical composition. Their usual reproduction is asexual by cell division and some species of this group have flagellation morphology.

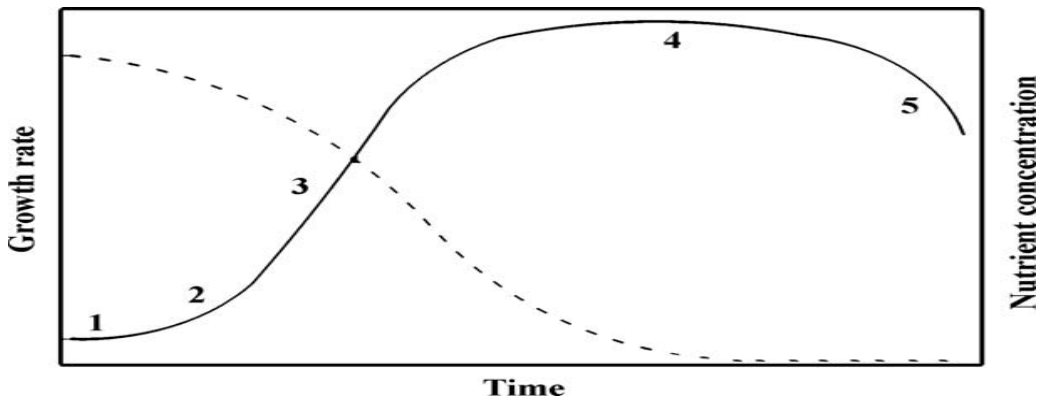


**Figure 2.2** Schematic representation of algae growth rate in batch culture (solid line) and nutrients concentration (dashed line) 1. Lag phase,2. Exponential growth phase, representing the maximum growth rate, 3. Linear growth phase, 4. Stationary growth phase, 5. Decline or death phase.

Source (Mata et al., 2010).

According to Debirmas (2011), microalgae can be the best option for production of microalgae because:

1. Algae are the fastest growing plants in the world. Microalgae have much faster growth rates than other crops.
2. The cost of harvesting of microalgae is cheaper than other crops.

3. Microalgae are capable of fixing $CO_2$ in the atmosphere, thus help the reduction of increasing atmospheric $CO_2$ levels which are now considered a global problem.
4. Microalgae are easily biodegradable and they can be chemically treated easily.
5. Algae cultivation is not complex where they can grow practically in every place where there is enough sunshine.

### 2.1.1 Selection of Microalgae for Biodiesel

The Table 2.1 shows the lipid productivity of different strain. The strain is chosen according to their availability Some strain seems not to be available in the selected area. Hence, that is why raw material availability is taken into consideration.

**Table 2.1** Lipid content and productivities of different microalgae species

| Marine and freshwater microalgae species | Lipid content (% dry weight biomass) | Lipid productivity (mg/L/day) | Volumetric productivity of biomass (g/L/day) | Areal productivity of biomass (g/m$^2$/day) |
|---|---|---|---|---|
| *Ankistrodesmus* sp. | 24.0–31.0 | – | – | 11.5–17.4 |
| *Botryococcus braunii* | 25.0–75.0 | – | 0.02 | 3.0 |
| *Chaetoceros muelleri* | 33.6 | 21.8 | 0.07 | – |
| *Chaetoceros calcitrans* | 14.6–16.4/39.8 | 17.6 | 0.04 | – |
| *Chlorella emersonii* | 25.0–63.0 | 10.3–50.0 | 0.036–0.041 | 0.91–0.97 |
| *Chlorella protothecoides* | 14.6–57.8 | 1214 | 2.00–7.70 | – |
| *Chlorella sorokiniana* | 19.0–22.0 | 44.7 | 0.23–1.47 | – |
| *Chlorella vulgaris* | 5.0–58.0 | 11.2–40.0 | 0.02–0.20 | 0.57–0.95 |
| *Chlorella* sp. | 10.0–48.0 | 42.1 | 0.02–2.5 | 1.61–16.47/25 |
| *Chlorella pyrenoidosa* | 2.0 | – | 2.90–3.64 | 72.5/130 |
| *Chlorella* | 18.0–57.0 | 18.7 | – | 3.50–13.90 |
| *Chlorococcum* sp. | 19.3 | 53.7 | 0.28 | – |
| *Crypthecodinium cohnii* | 20.0–51.1 | – | 10 | – |
| *Dunaliella salina* | 6.0–25.0 | 116.0 | 0.22–0.34 | 1.6–3.5/20–38 |
| *Dunaliella primolecta* | 23.1 | – | 0.09 | 14 |
| *Dunaliella tertiolecta* | 16.7–71.0 | – | 0.12 | – |
| *Dunaliella* sp. | 17.5–67.0 | 33.5 | – | – |
| *Ellipsoidion* sp. | 27.4 | 47.3 | 0.17 | – |
| *Euglena gracilis* | 14.0–20.0 | – | 7.70 | – |
| *Haematococcus pluvialis* | 25.0 | – | 0.05–0.06 | 10.2–36.4 |
| *Isochrysis galbana* | 7.0–40.0 | – | 0.32–1.60 | – |
| *Isochrysis* sp. | 7.1–33 | 37.8 | 0.08–0.17 | – |
| *Monodus subterraneus* | 16.0 | 30.4 | 0.19 | – |
| *Monallanthus salina* | 20.0–22.0 | – | 0.08 | 12 |
| *Nannochloris* sp. | 20.0–56.0 | 60.9–76.5 | 0.17–0.51 | – |
| *Nannochloropsis oculata.* | 22.7–29.7 | 84.0–142.0 | 0.37–0.48 | – |
| *Nannochloropsis* sp. | 12.0–53.0 | 37.6–90.0 | 0.17–1.43 | 1.9–5.3 |
| *Neochloris oleoabundans* | 29.0–65.0 | 90.0–134.0 | – | – |
| *Nitzschia* sp. | 16.0–47.0 | | | 8.8–21.6 |
| *Oocystis pusilla* | 10.5 | – | – | 40.6–45.8 |
| *Pavlova salina* | 30.9 | 49.4 | 0.16 | – |
| *Pavlova lutheri* | 35.5 | 40.2 | 0.14 | – |
| *Phaeodactylum tricornutum* | 18.0–57.0 | 44.8 | 0.003–1.9 | 2.4–21 |
| *Porphyridium cruentum* | 9.0–18.8/60.7 | 34.8 | 0.36–1.50 | 25 |
| *Scenedesmus obliquus* | 11.0–55.0 | – | 0.004–0.74 | – |
| *Scenedesmus quadricauda* | 1.9–18.4 | 35.1 | 0.19 | – |
| *Scenedesmus* sp. | 19.6–21.1 | 40.8–53.9 | 0.03–0.26 | 2.43–13.52 |
| *Skeletonema* sp. | 13.3–31.8 | 27.3 | 0.09 | – |
| *Skeletonema costatum* | 13.5–51.3 | 17.4 | 0.08 | – |
| *Spirulina platensis* | 4.0–16.6 | – | 0.06–4.3 | 1.5–14.5/24–51 |
| *Spirulina maxima* | 4.0–9.0 | – | 0.21–0.25 | 25 |
| *Thalassiosira pseudonana* | 20.6 | 17.4 | 0.08 | – |
| *Tetraselmis suecica* | 8.5–23.0 | 27.0–36.4 | 0.12–0.32 | 19 |
| *Tetraselmis* sp. | 12.6–14.7 | 43.4 | 0.30 | – |

Source (Mata et al, 2010)

From the Table 2.1, we can see that *Botryococcus braunii* contains highest lipid content however its lipid productivity is very low. The aim of the study is to find the strain that have faster growth rate. Furthermore, *Botryococcus braunii* has been found unsuitable for biodiesel production, because its hydrocarbons have a chain length greater

than C30, while vegetable oils currently used for biodiesel are mainly C16 and C18. (Griffiths et al. 2009). The suitable microalgae would be *Nannochloropsis*. This is because, a research shows that the characteristics of fatty acid from *Nannochloropsis* is suitable for biodiesel and it has low viscosity (Miri Koberg,2011) . In addition, it also has high lipid productivity. Furthermore, *Nannochloropsis* can be obtained from University Malaysia Pahang. So, this shows that *Nannochloropsis* will be a suitable for this project.

According to Chojnacka et al.,(2004) microalgae can be culture through:

1. Photo autotrophically, using light as an only energy source that is converted to chemical energy through photosynthetic reactions.
2. Heterotrophically, utilizing only organic compounds as carbon and energy source.
3. Mixotrophically, performing photosynthesis as the main energy source, though both organic compounds and $CO_2$ are essential.
4. Photoheterotrophycally, also known as photo assimilation, describes the metabolism in which light is required to use organic compounds as carbon source. The photo heterotrophic and mixotrophic metabolisms are not well distinguished, in particular they can be defined according to a difference of the energy source required to perform growth and specific metabolite production.

Liam Brennan and Philip Owende (2011) justified that photoautotrophic production is the only method which is technically and economically feasible for large-scale production of algae biomass.

### 2.1.2 Water selection

To have a constant medium so that there will be no change in the medium nutrient, artificial sea water will be used. The artificial sea salt concentration is 17.5 g/l (Illman et al., 2000)

### 2.1.3 Selection of Medium

There are many medium for microalgae culture such as DYIY medium, f/2 medium, Bold Basal medium, D medium, and BG – 11 medium. Among the major considerations to grow microalgae are pH, concentration of major nutrients, nitrogen source, possible organic or growth factors for enrichment and micro nutrient composition (Shilpakar et al, 2010). *Nannochloropsis* will grow better in f/2 medium because of the optimum nutrients for its metabolism (Chiu et al., 2009). This shows that the f/2 medium should be the preferred choice for the laboratory culture (Phukan et al.,2011).

## 2.2 Microalgae for Biodiesel

Biodiesel refers to a vegetable oil or animal fat-based diesel fuel consisting of long-chain alkyl (methyl, propyl or ethyl) esters. Biodiesel is typically made by chemically reacting lipids vegetable oil or animal fat with an alcohol producing fatty

acid esters. In this project we considering lipid from microalgae. Oil productivity of many microalgae greatly exceeds the oil productivity of the best producing oil crops (Demirbas and Fatih Demirbas 2011). The lipid from microalgae is known as triacyglycerols which is also known as TAG. This triacyglycerols is reacted with alcohol to produce esters and glycerol. This process is known as transesterification. Debirmas MF (2010) stated that the biodiesel transesterification reaction is :

$$\text{Triglycride} + 3\ \text{methanol} \underset{\text{Catalyst}}{\rightleftharpoons} \text{glycerine} + 3\ \text{methyl esters} \qquad (1.1)$$

Demirbas and Fatih Demirbas (2011) justified that an excess of methanol is used to force the reaction to favor the right side of the equation 1. The excess methanol is later recovered and reused. The algae that are used in biodiesel production are usually aquatic unicellular green algae because this type of algae is characterized by high growth rates, high population densities can double its biomass in less than 24 hour and have huge lipid contents ( Demirbas and Fatih Demirbas 2011). Hence, this high yield, high density biomass is ideal for intensive agriculture and may be an excellent source for biodiesel production.

Mustafa Balat (2011) has done a research in biodiesel and has said that non-edible plant oils have been found to be promising crude oils for the production of biodiesel. In addition to that, Mustafa Balat (2011) also said that microalgae have long been recognized as potentially good sources for biofuel production because of their high oil content and rapid biomass production.

Biodiesel can be used alone, or blended with petro diesel. Biodiesel can also be used as a low carbon alternative to heating oil. Blends of biodiesel and conventional hydrocarbon-based diesel are products most commonly distributed for use in the retail diesel fuel marketplace. B100 is referred as 100% biodiesel meanwhile B20 referred to 20% biodiesel and 80% of petrol diesel. According to biodiesel standard published by the American Society for Testing Materials (ASTM), biodiesel from microalgae oil is similar in properties to the standard biodiesel, and is also more stable according to their flash point values (Demirbas and Fatih Demirbas 2011). Biodiesel produced from algae is a new sustainable energy source substituted for petroleum diesel. Producing biodiesel from algae has been touted as the most efficient way to make biodiesel fuel.

## 2.3 Ultrasound

Ultrasound, or sound of frequency greater than 20 kHz, is inaudible to the human ear. Although this limit varies from person to person, it is approximately 20 kilohertz (20,000 hertz) in healthy, young adults. The production of ultrasound is used in many different fields, typically to penetrate a medium and measure the reflection signature or supply focused energy. Similar to ultrasonic cleaning, biological cells including bacteria can be disintegrated. This has uses in biological science for analytical or chemical purposes and in killing bacteria in sewage. High power ultrasound can disintegrate corn slurry and enhance liquefaction and saccharification for higher ethanol yield in dry corn milling plants. This is because high power ultrasounds produce cavitations that facilitate particle disintegration or reactions. Cavitation is a two-

step phenomenon. The first step is the formation of vapor bubbles, and the second step is the collapse or implosion of the vapor bubbles. According to Zhaofeng Liang and his co-researches (2006) the first stage of cavitation is called stable cavitation and usually does not cause damage meanwhile full cavitation will sound like rocks flowing through the piping system and results in surface damage and called transient cavitation. According to Yusuf Chisti (2003) ultrasonication is generally associated with damage to cells but evidence is emerging for beneficial effects of controlled sonication on conversions catalyzed by live cells. Furthermore, Yusuf Chisti (2003) also stated that ultrasound has the potential for enhancing mass transfer within a cell. Recent studies have shown the low frequency ultrasonic irradiation can enhance penetrability of cell membrane and accelerate substance exchange because the cell may be hurt in some ways and make cell membrane flaw (Chuanyun et al., 2003). Ultrasonics can also be delivered via an ultrasonic horn, which is a popular method not for cleaning but for cell disruption, emulsification,and homogenizing of biological matter, where cavitation drives the actions (Parag Kanthale et al., 2008). Figure 2.3 shows different types of ultrasound horn for sonication process.

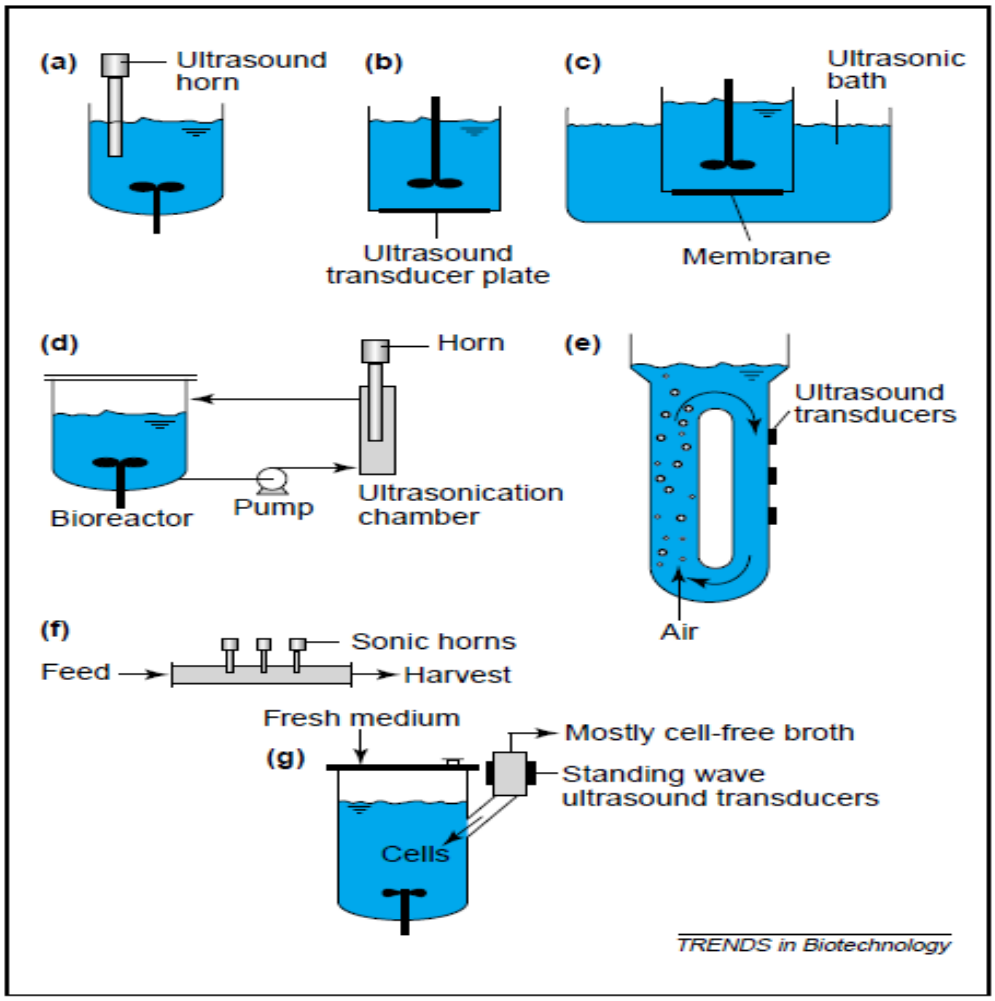


**Figure 2.3** Varies kind of ultrasonic device (a) Stirred bioreactor with an ultrasound horn; (b) stirred reactor with flat-plate ultrasound transducer; (c) ultrasound transmission from an ultrasonic bath through a membrane or plate sonocoupling; (d) external ultrasound irradiation of broth in a recycle bioreactor; (e) airlift sonobioreactor; (f) tubular flow sonobioreactors; (g) high-density cell culture with ultrasonic retention of cells using a standing wave. All devices except (g) can be used for continuous or intermittent sonication.

Source (Yusuf Chisti, 2003)

In this project, since we want to control the temperature, type D device is used so that only the sound is transmitted and other parameters such as temperature is controlled. Suitably controlled ultrasonication has shown beneficial effects on the metabolic

performance of live systems (Yusuf Chisti, 2003). Furthermore, A.Z. Sulaiman et al (2011) also stated that, intermediate ultrasound can increase the biomass production and ethanol production in fermentation process.

## 2.4 Influence of culture conditions (Nitrogen and Phosphorus concentration)

Phototrophic organisms use light energy to obtain most of their nutritional needs from inorganic compounds. Essential element include nitrogen (N), phosphorus (P), iron (Fe) and in some cases silicon (Si). Each microalgae species tends to have its own optimum nutrient concentration. Nutrients limitation can influence the lipid content in the microalgae cell.

Lipid and fatty acid accumulation in microalgae typically occurs during a period of environmental stress. Most microalgae responds to N-limitation by increasing their lipid content ( Illman ,2000). It was reported that N-deficient cultures will develop higher percentage lipid content than N-sufficient cultures (Hidayat, 2008).Phosphorus is involved in the production of enzymes, phospholipids and energy-supplying compounds such as AMP, ADP and ATP (Mohermani, 2005). Increasing in the concentration of $NaH_2PO_4$ in the culture increases the microalgae cell growth rate, but exceeding the optimum $NaH_2PO_4$ concentration in the culture decreased cell growth rate (Hidayat, 2008).

# CHAPTER 3

# RESEARCH METHODOLOGY

## 3.1 Method Overview

This research focus on the inoculation of microalgae and ultrasound regiment on *Nannochloropsis*. At initial stage *Nannochloropsis* is inoculated with f/2 medium. Then, the grown microalgae is cultured in 2000 ml conical flask. The relevant materials and methods are explained in this chapter.

## 3.2 Chemicals & Materials

The minerals that will be used for *Nannochloropsis* fermentation are:

$NaNO_3$, $NaH_2PO_4.H_2O$ , $Na_2SiO_3.9H_2O$, $Na_2C_{10}H_{14}O_8N_2.H_2O$ ($Na_2EDTA$), $CoCl_2.6H_2O$, $CuSO_4.5H_2O$, $FeCl_3.6H_2O$, $MnCl_2.4H_2O$, $Na_2MoO_4.2H_2O$, $ZnSO_4.7H_2O$, Thiamin HCl,

Vitamin H (Biotin), $B_{12}$ (Cyanocobalamin), NaCl, methanol, chloroform, distilled water, sodium hydroxide.

500 ml Erlenmeyer flask, 2000 ml Erlenmeyer flask, aluminium foil, ultrasound device, oven, petri dish, inoculums, spatula, weighing machine, autoclave, filter 0.2 µm PTFE, syringe.

### 3.3 Medium Preparation

For medium preparation, in 1000 ml of water, 39g of an artificial sea salt will be added. The f/2 medium consists of (g/L): $NaNO_3$ 75, $NaH_2PO_4.H_2O$ 5 , $Na_2C_{10}H_{14}O_8N_2.H_2O$ ($Na_2EDTA$) 4.36 , $CoCl_2.6H_2O$ 0.01, $CuSO_4.5H_2O$ 0.01 , $FeCl_3.6H_2O$ 3.15, $MnCl_2.4H_2O$ 0.18 , $Na_2MoO_4.2H_2O$ 0.006 , $ZnSO_4.7H_2O$ 0.022 , Thiamin HCl 0.1, Vitamin H (Biotin) 0.0005 , $B_{12}$ (Cyanocobalamin) 0.0005 , NaCl 37 .

**Table3.1** N+P solution

| Nutrients | Quantity (g/L) |
|---|---|
| $NaNO_3$ | 75 |
| $NaH_2PO_4.H_2O$ | 5 |

**Table 3.2** Trace Elements

| Nutrients | Quantity (g/L) |
|---|---|
| $Na_2C_{10}H_{14}O_8N_2.H_2O$ ($Na_2EDTA$) | 4.36 |
| $CoCl_2.6H_2O$ | 0.01 |
| $CuSO_4.5H_2O$ | 0.01 |
| $FeCl_3.6H_2O$ | 3.15 |
| $MnCl_2.4H_2O$ | 0.18 |
| $Na_2MoO_4.2H_2O$ | 0.006 |
| $ZnSO_4.7H_2O$ | 0.022 |

**Table 3.3** Vitamin Solution

| Nutrients | Quantity (g/L) |
|---|---|
| Thiamin HCl | 0.1 |
| Vitamin H (Biotin) | 0.0005 |
| $B_{12}$ (Cyanocobalamin) | 0.0005 |

For 1 liter medium 2ml of volume is taken from each solution and the artificial seawater with 39 g of salt is added to 30% of solution.

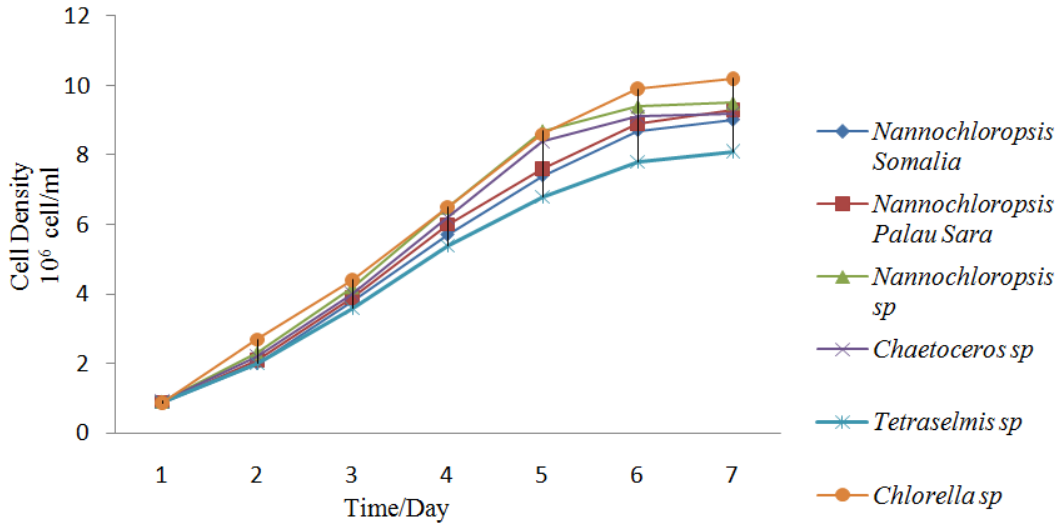


**Figure 3.1** Biomass Growth Profiles of Strains of Microalgae

Source (Ding Gong Tao and Jailani Salihon,2009)

### 3.4 Inoculation of Microalgae

*Nannochloropsis sp.* is obtained from University Malaysia Pahang. The medium is prepared for 500 ml and agar is added with 5g for 500 ml of medium. Then it is autoclaved for 15 minutes at 121 °C. After that, the laminar flow hood is cleaned with ethanol and a Bunsen burner also needed. The medium is then poured in petri dish. Microalgae sample is streak in the petri dish. The petri dish is then sealed with parafilm. For this experiment, there will be fourteen petri dish samples to minimize the error.

### 3.5 Culturing Of Microalgae

The microalgae are cultured in 2000 ml Erlenmeyer flask using f/2 medium (Ratanaporn Leesing, 2011). The culture medium is prepared for 2000 mL and 78g of artificial sea salt is added to make the condition in saline. The pH is set to 7. Then, it is autoclaved for 15 minute at 121 °C. The microalgae are cultured in a conical flask with aeration and with light intensity of 2000 lux. The calculation of lux is by seeing the lumen/watt and watt value on bulb. Then, lumen/watt is multiple with watt to get lumen value. The lumen is then divided by the area of the box to get lux.

$$\frac{\text{lumen}}{\text{watt}} \times \text{watt} = \text{lumen} \tag{3.1}$$

$$\frac{lumen}{A^2} = lux \tag{3.2}$$

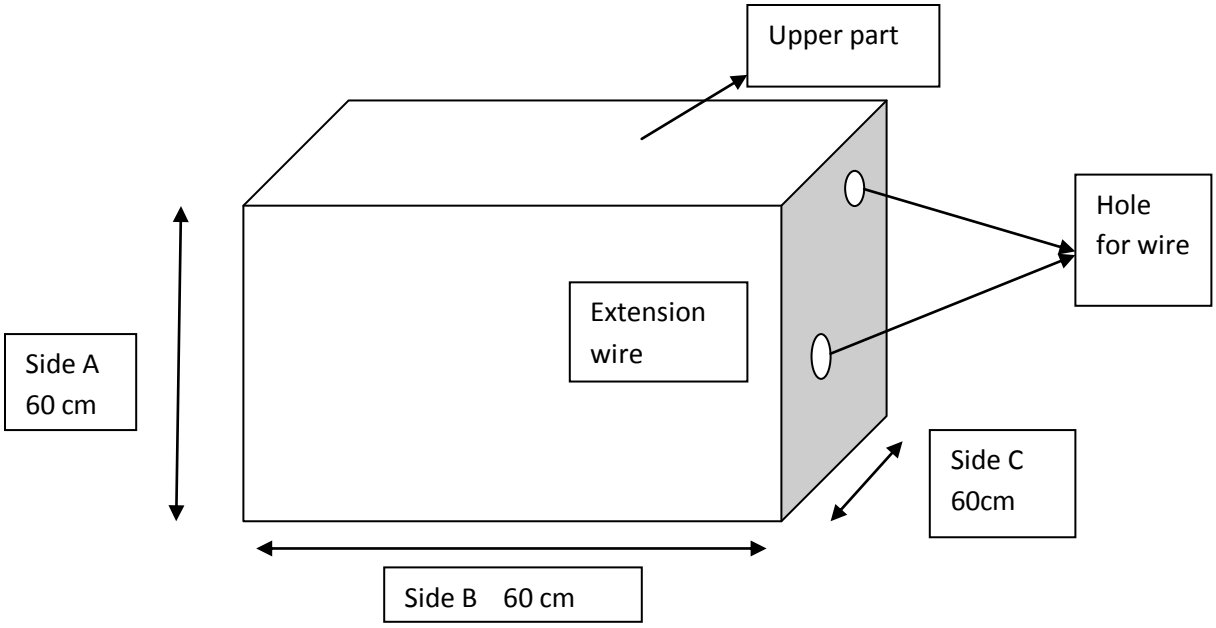


**Figure 3.2** Culture Box Outer View

Side A and side C have a fluorescent lamp each side.

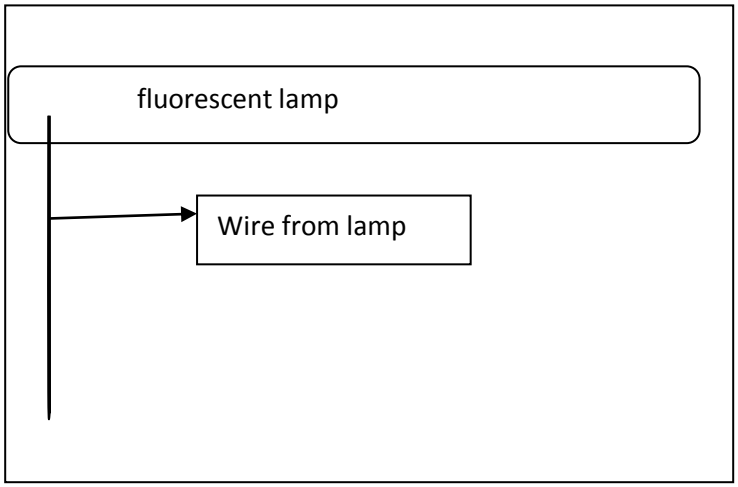


**Figure 3.3** Inside Box View

This box is made from plywood to create an artificial condition for the microalgae to grow with sufficient light source and aeration.

### 3.6 Power Determination

Nine types of amplitude is set to determine which power is suits for treatment.20%, 30%, 40%,50 % 60%, 70%, 80%,90%, 100% of amplitude is set. The OD was measured at 665 nm (OD 665) using a UV-vis spectrophotometer (Hanhua Hu and Kunshan Gao , 2006). The time of the exposure for continues sonication is set for 5 minute. Accoding to Miri and the coworkers (2011), they had used sonication for 5 minute to disrupt the microalgae cell. So, the amplitude which make less cell reduction is used as the optimum power. Each sample was sonicated for 5minute continuously. The samples were allowed to stand for 30 min, after which 4 mL of the suspension was taken from 1 cm below the surface and the OD665 was measured.

$$cell\ reduction = \frac{OD\ (665)\ before\ ultrasonicaton - OD\ (665)\ after\ ultrasonicaton}{OD\ (665)\ before\ ultrasonicaton} \qquad (3.3)$$

### 3.7 Treatment of microalgae with ultrasound

The microalgae are treated with ultrasound. The time units of seconds were used in setting the duty cycle. Duty cycles of 10%, 20% (1 s sonication, 5 s rest period) and 40% (2 s sonication, 5 s rest period) were used (Sulaiman et al., 2011). The intensity of the sonication is calculated by using I = P/A where A ($cm^2$) was the area of the sono trode tip. After various power is tested ,the optimum power is chosen for the duty cycle

by considering cell viability. According to Ahmad Ziad Sulaiman and his research members (2011) found that 20% duty cycle induced cell growth.


**Figure 3.4** Ultrasonic Processor Q500

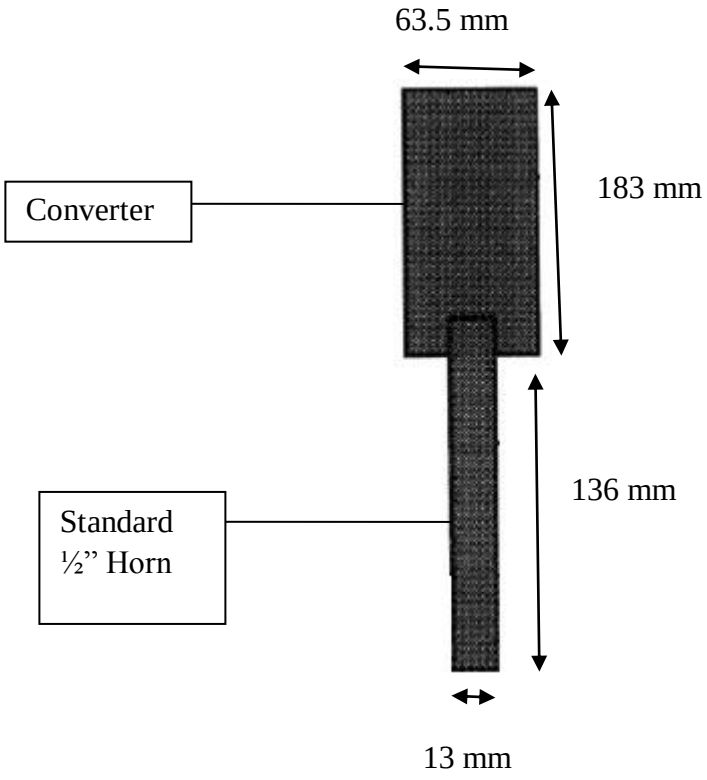


**Figure 3.5** Ultrasonic Dimension in mm

**Table 3.4** Generator Specification

| Specification | Description |
|---|---|
| Weight | 15 lbs. (6.8 Kg) |
| Dimensions | 8"W x 15.25"L x 8.5"H<br>203 mm x 387 mm x 216 mm |
| Output Voltage | 1000 V rms (max.) |
| Output Frequency | 20 KHz |

**Table 3.5** Converter Specification

| Specification | Description |
|---|---|
| Weight | 2 lbs. (900 g) |
| Dimensions | 7.25" L x 2.5" Dia. (183 mm x 63.5 mm) |
| Materials | Aluminum Alloy |

**Table 3.6** Standard ½" Horn Specification

| Specification | Description |
|---|---|
| Weight | 0.75 lbs. (340 g) |
| Dimensions | 5.375" L x .5" Dia. (136 mm x 13 mm) |
| Materials | Titanium Alloy |

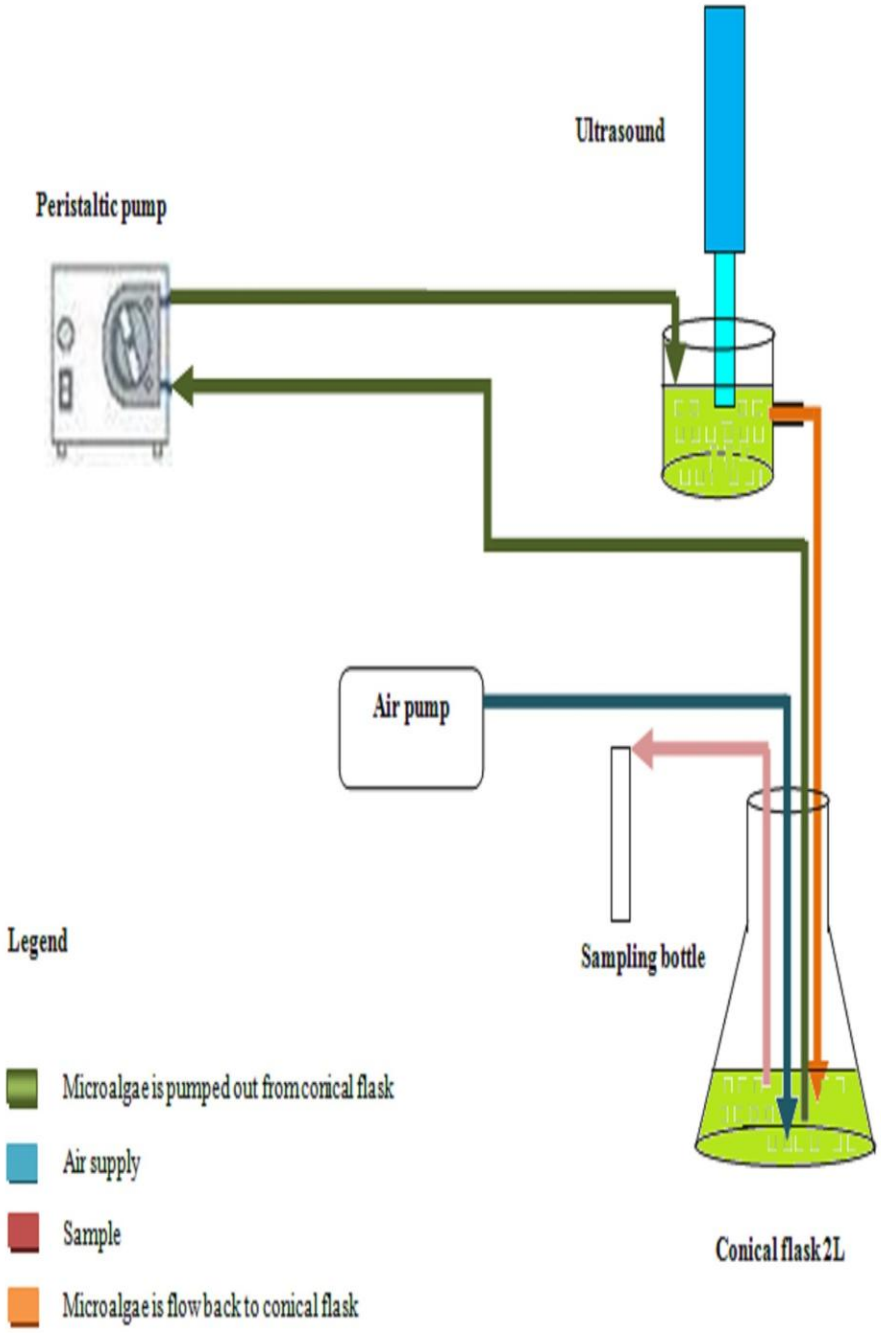


**Figure 3.6** Ultrasound Set Up

### 3.8 Analytical Method

#### 3.8.1. Biomass Determination

The cultured sample is taken for 100 ml. It is then were harvested by centrifugation at 8000 rpm for 10 min. The cells were washed twice with 5ml distilled water after centrifugation. The pellet was dried at 60 ℃ for 5 hour. The dry weight of the algal biomass was determined gravimetrically and growth was expressed in terms of dry weight.

#### 3.8.2. Growth Monitoring

Cell density was measured turbid metrically at 665 nm and converted from an appropriate calibration curve to dry mass (DW). All cultures were initiated with an OD665 of about 0.1. OD665 was monitored every 24 hour until the stationary phase was reached (Hanhua Hu and Kunshan Gao , 2006).

#### 3.8.3. Extraction of Lipid (Modified Bligh And Dyer Method)

Cultures transfer to a centrifuge tube 50ml to be centrifuged at 8,000 rpm for 10 minutes. The supernatant will removed by pipette. The pellet will be dried by dried to get the weight. Then sample will be suspended with 4ml of distilled water, then 10ml of

methanol and 5 ml of chloroform will be added, resulting in a 10:5:4 ratio of methanol: chloroform: water. At this ratio, all solvents will be miscible and form one layer; homogenizer will be used to break the cell wall for 2 hour. Then after extraction , 5 ml of water and 5ml of chloroform are added which results in a 10:10:9 ratio of methanol: chloroform: water. Tube will be centrifuged for 10 minutes at 8,000 rpm. At this solvent ratio, two layers will be formed, a water methanol upper layer and chloroform lower layer. The chloroform lower layer which contains the extracted lipids will be then removed by Pasteur pipette and placed into a pre-weighed vial. After the first extraction, 10ml of additional chloroform will be added to conduct a second extraction. The additional 10ml of chloroform again results in a 10:10:9 methanol: chloroform:water ratio and two layers are formed. The tube will be centrifuged at 8,000 rpm for 10 minutes, and the lower chloroform layer will be removed by Pasteur pipette and placed into the same pre-weighed vial. Repeat this extraction step again and then 30ml of chloroform dissolved with lipid can be collected. The chloroform will be evaporated by heating in a 55°C water bath. After 1 hour in a 105°C oven, vials will be weighed again. The weight difference represents weight of lipids extracted from the culture sample. Percentage of lipid content can be determined by measuring the dry weight of the culture sample at the same time as the lipid analysis.

### 3.8.4. Nitrate Determination

Hach Programs is touched. Program 353 N, Nitrate MR selected and started. A round sample cell with 10 mL of sample is filled. The contents of one NitraVer 5 Nitrate

Reagent Powder Pillow (the prepared sample) are added and the cell is capped. A one minute reaction period is selected and the cell is shake well until the timer beeps. Then, the timer icon is touch again. A five minute reaction period will begin. When the timer beeps a second round sample cell if filled with 10 ml of blank. The blank is placed in cell holder. Zero is selected, prepared sample is placed in cell holder and the reading is observed.

### 3.9. Kinetic and yield parameters

The specific growth rate was calculated by the equation:

$$\mu = \frac{1}{t}\ In\ (\frac{Xm}{Xo}) \qquad (3.4)$$

where $X_m$ and $X_0$ are the concentrations of biomass at the end and at the beginning of a batch run, respectively, and t is the duration of the run.

The lipid productivity was calculated by the equation:

$$v = \frac{C_L}{t} \qquad (3.5)$$

where $C_L$ in the concentration of lipids at the end of the batch run and t is the duration of the run.

The yield of the microalgae lipids was calculated by the equation:

$$Y\ (\%) = \frac{W_L}{W_{DA}} \qquad (3.6)$$

where $W_L$ and $W_{DA}$ are the weights of the extracted lipids and of the dry algae biomass, respectively.

# CHAPTER 4

# RESULT AND DISCUSSION

## 4.1 Controlled Growth

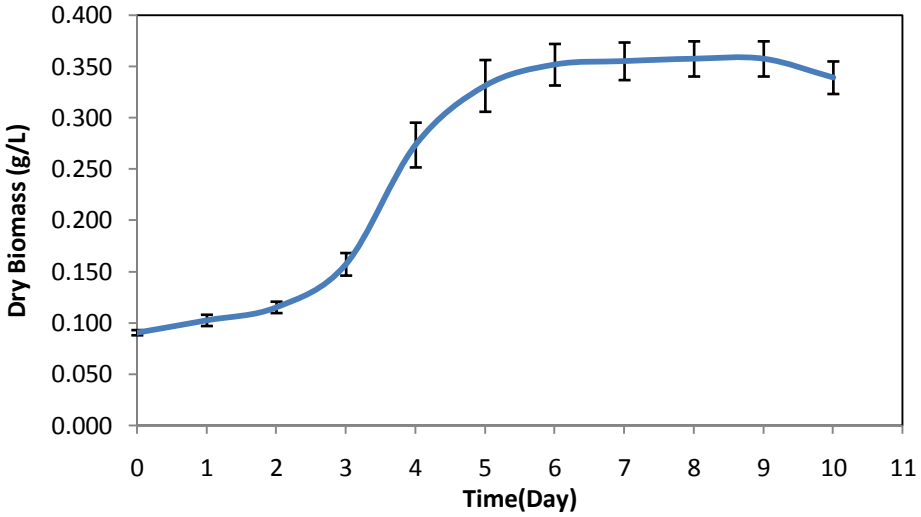


**Figure 4.1** Dry Biomass versus Time (Control)

During lag phase, which is from zero until third day, *Nannochloropsis sp.* adapts themselves to growth conditions. It is the period where the individual microalgae are

maturing and not yet able to divide. During the lag phase of the microalgae growth cycle, synthesis of RNA, enzymes and other molecules occurs.

Exponential phase or known as log phase which is from $4^{th}$ until $6^{th}$ day, is a period characterized by cell doubling (Mata et al., 2010).The number of new *Nannochloropsis sp.* appearing per unit time is proportional to the present population. If growth is not limited, doubling will continue at a constant rate so both the number of cells and the rate of population increase doubles with each consecutive time period. For this type of exponential growth, plotting the natural logarithm of cell number against time produces a straight line. The slope of this line is the specific growth rate of the organism, which is a measure of the number of divisions per cell per unit time (Mata et al., 2010).

The actual rate of this growth depends upon the growth conditions, which affect the frequency of cell division events and the probability of both daughter cells surviving. Exponential growth cannot continue indefinitely, however, because the medium is soon depleted of nutrients and enriched with wastes.

The stationary phase from seventh day until ninth is due to a growth-limiting factor; this is mostly of inhibitory products such as organic acids. An awkward but unfortunately widespread explanation is that the stationary phase results from a situation in which growth rate and death rate have the same values (newly formed cells per time = dying cells per time) (Mata et al., 2010).

At death phase, also known as endogenous phase, microalgae are undergoing photo inhibition. It emphasizes clonality, asexual binary division, the short development time relative to replication itself, the seemingly low death rate, the need to move from a dormant state to a reproductive state or to condition the media, and finally, the tendency of lab adapted strains to exhaust their nutrients. Furthermore for *Nannochloropsis sp* growth it needs nitrate source to grow. From the nitrate analysis it shows that there is depletion on the tenth day of culture.

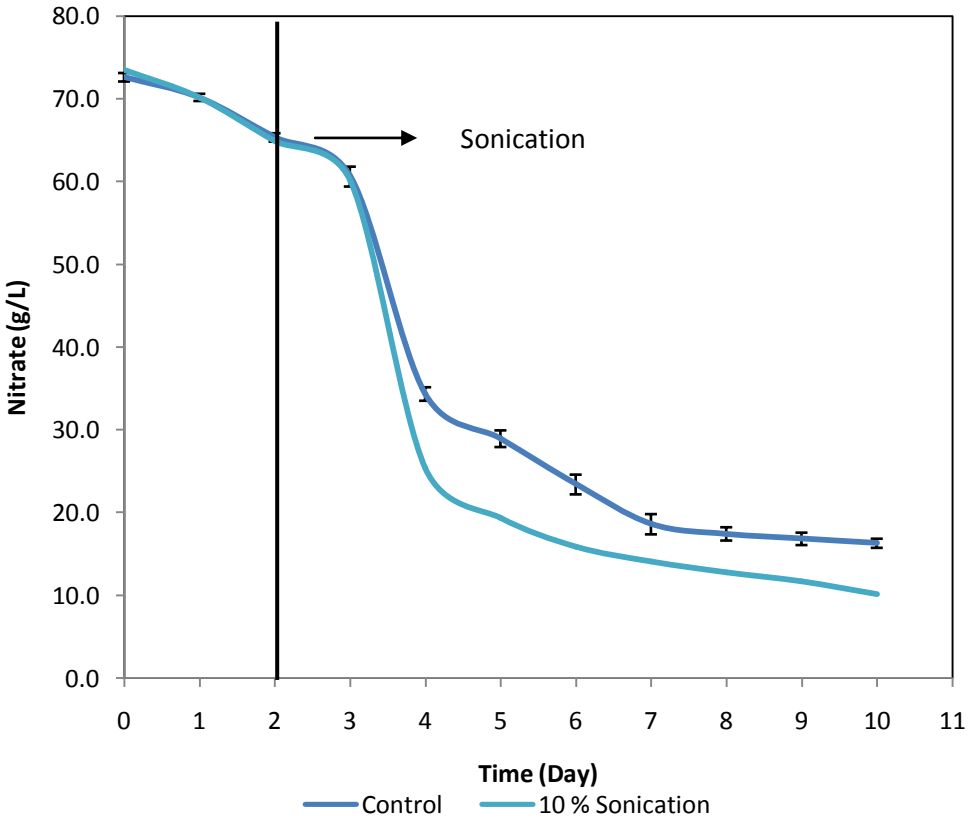


**Figure 4.2** Nitrate Concentrations versus Time

Nitrate is a source where responsible for the *Nannochloropsis sp*. growth. In the f/2 medium the source of nitrate is through the mixing of sodium nitrate. According to this graph, nitrate consumption is very low in the first three days due to the adaption of microalgae to the new environment. After it enters the log phase the nitrate consumption increases rapidly as it had adapted to the new environment and started to producing new cells drastically. But as time moves on, during seventh day until tenth day the nutrient had depleted and making the microalgae to enter to its stationary phase and then followed by endogenous phase on tenth day. This graph proved that nitrate is responsible for the microalgae growth. As for the 10% sonication, the nitrate consumption seems to be quite similar with the control growth before pulsed sonication applied. After pulsed sonication introduced, the nitrate consumption seems to be higher than the control one. Ultrasonication is known to improve interfacial mass transfer. At a low power of sonication and at a low duty cycle, the mass transfer between the cell and the nutrient had been increased. This is may be due to, when sonication is introduced the cell wall and the cell membrane of the *Nannochloropsis sp*. becomes permeable and increase the transfer of nutrient. When nutrient uptake increases, it will naturally increase the growth of the microalgae.

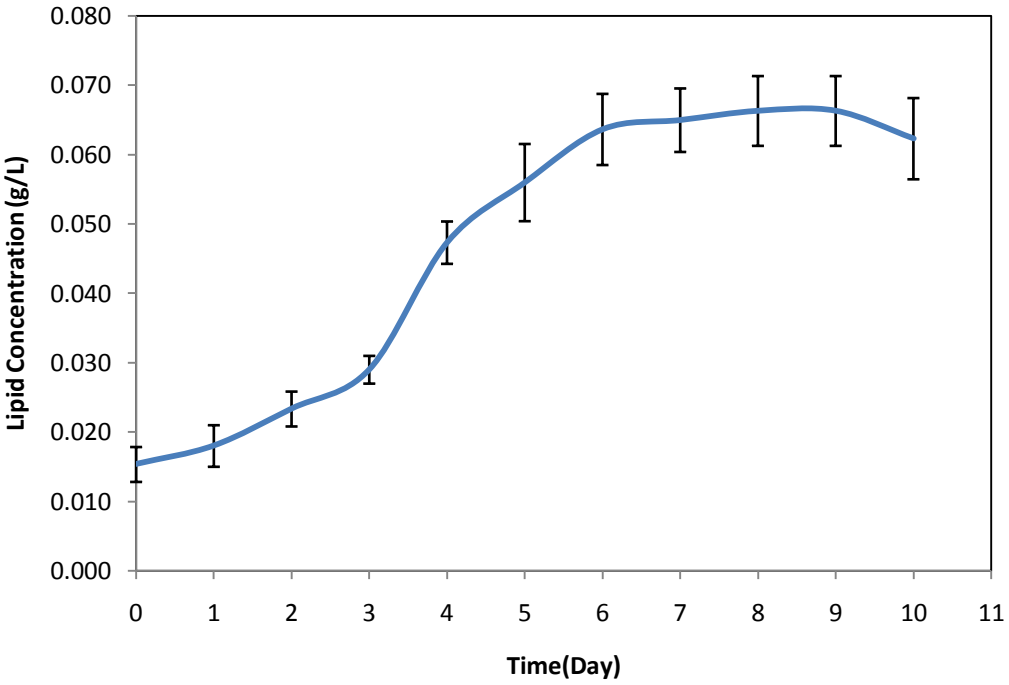


**Figure 4.3** Lipid Concentration versus Time (Control)

During lag phase, the lipid production is slow because lipid is produced is growth associated product. As culture time increase, where lag phase changed to log phase on forth day, the lipid produced increase dramatically because *Nannochloropsis sp.* had adapoted to the culture medium and the lipid produced increase. The lipid at the log phase which is at forth day is 0.046g/L.

At the stationary phase *Nannochloropsis sp.* produce 0.064 g/L of lipid. The lipid produced is known as triacyglycerols (TAGs). The triacyglycerols is produced from the *Nannochloropsis sp*. metabolism. Since in this experiment light is used as the photosynthesis source for *Nannochloropsis sp*. it utilizes it in Photosysytem I and

Photosystem II to produce Triose Phosphate. It will then convert into Acetyl Coenzyme then to fatty Acyl-ACP with the help of acetyl-CoA carboxylase (ACCase).

After that, the product will be released in cytoplasm to as fatty Acyl-CoA and will enter endoplasmic reticulum as Ayl-Coa. Then, it will converted into diacylglyceride . Finally, it will become triacyglycerols (TAGs) and will be released as oil bodies in cytoplasm. Hence, the lipid produced is an intracellular product.

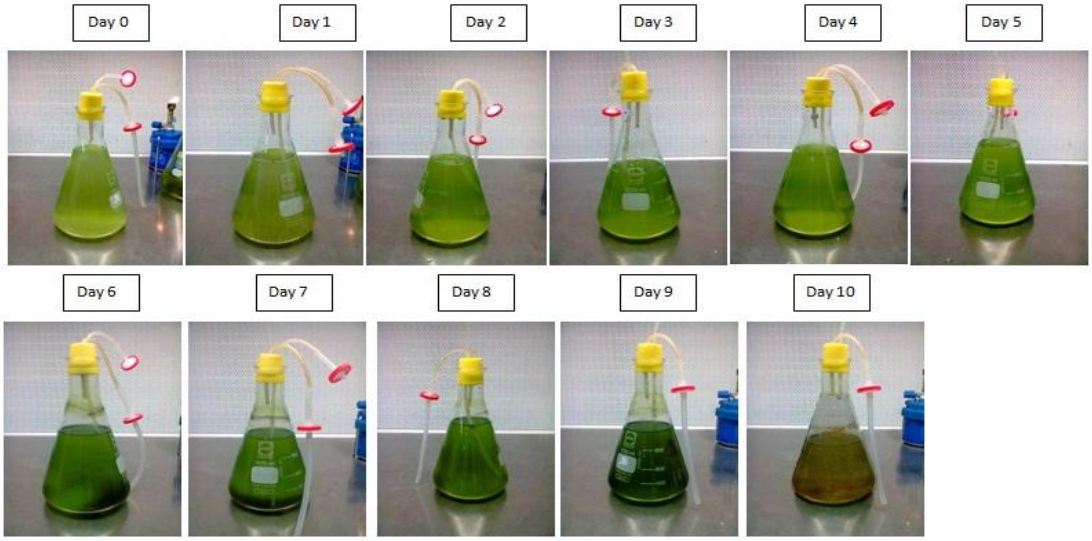


**Figure 4.4** Turbidity of *Nannochloropsis sp.*

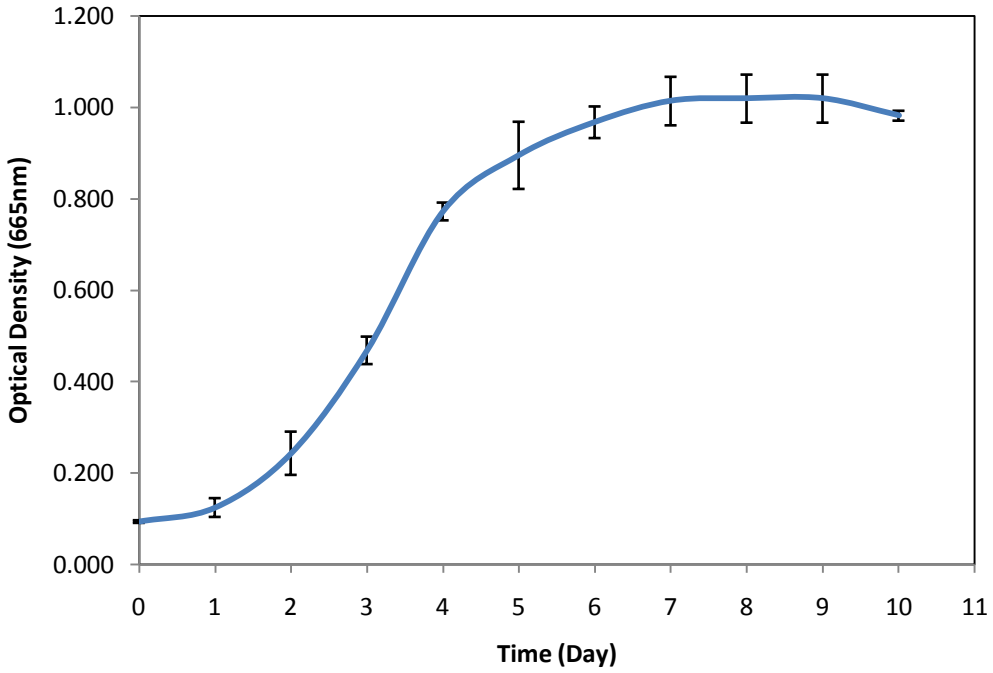


**Figure 4.5** Optical Density versus Time (Control)

From Figure 4.4, the colour of the cultured *Nannochloropsis sp.* is changing from a very light green into a dark green and changed into a brownish color in its endogenous phase. This is because, as culture time increase the cell produce is also increases. This makes the turbidity of the microalgae changes according to time. On the other hand, if the microalgae die the colour of the culture will become brownish as time goes on and finally all the microalgae will be precipitated under the conical flask. For this study, the wavelength of 665 nm is chosen due to the chlorophyll a present in the *Nannochloropsis sp.* which will give a better response during the absorbance test (Hanhua Hu and Kunshan Gao , 2006).

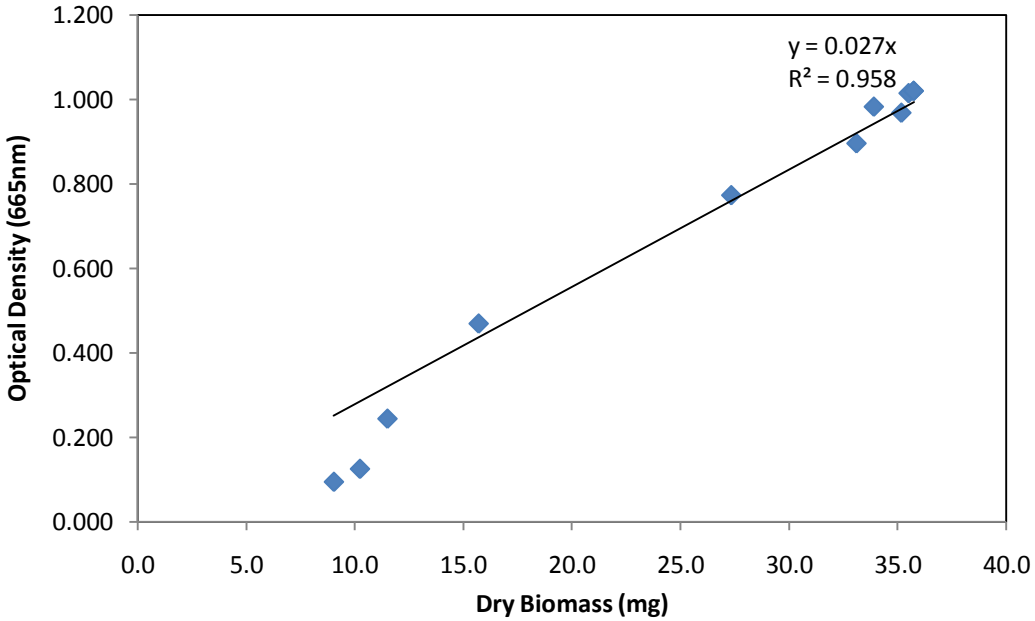


**Figure 4.6** Optical Density versus Dry Biomass

Optical density versus dry biomass graph is plotted and the regression obtained is 0.953. From this graph, dry biomass can be known by taking absorbance reading and substitute in this formula: x=y/0.026, where y is optical density.

## 4.2 Effect of Power

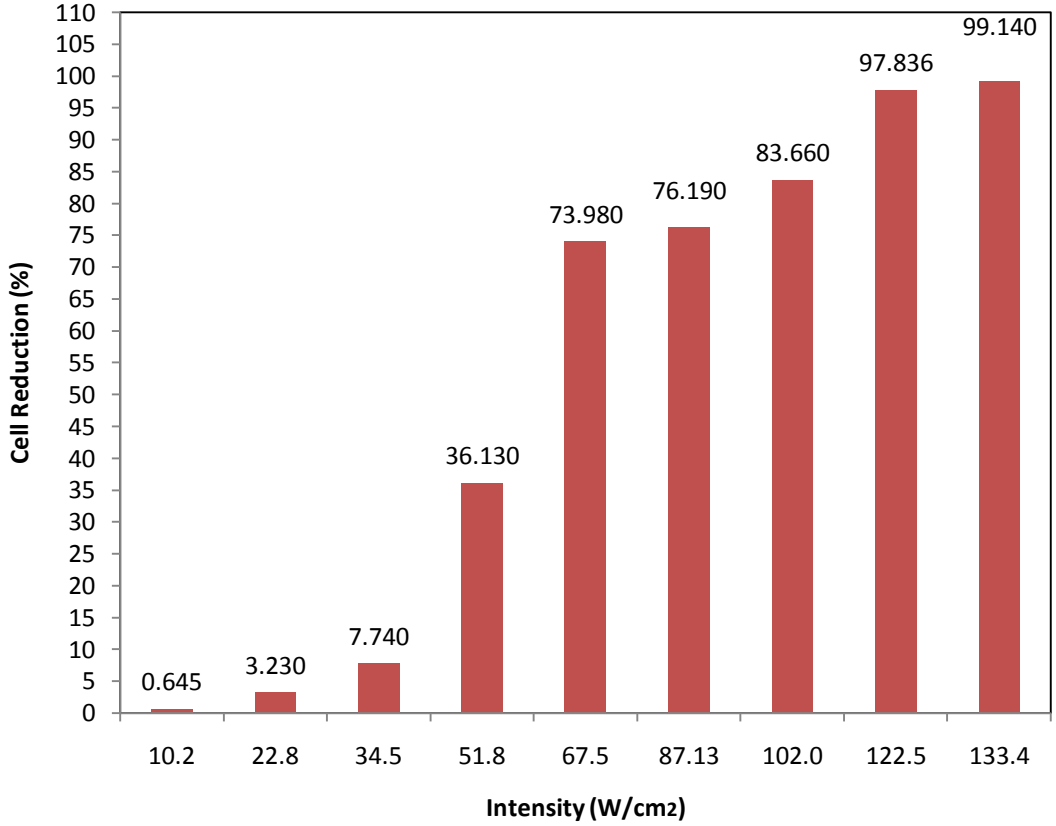


**Figure 4.7** Cell Reduction versus Intensity

Sonication power is measured in Watts. Amplitude is a measurement of the excrusion of the tip of the horn. The wattage displayed is the energy required to drive the radiating face of a horn. As the resistance to the movement of the horn increases, additional power will be delivered by the power supply to ensure that the excursion at the horn tip remain constant. Hence, the displayed wattage reading will be varied according to the load. However, the amplitude remains same. In the experiment the desired amplitude is 20 %. This is because the intensity delivered by the selected amplitude is 10.2 W/cm$^2$ where it causes the lowest cell reduction to *Nannochloropsis*

*sp*. By applying sonication, the cells which are alive will float meanwhile the death cell will precipitate and settle down. This is because, green algal have an organelle known as vacuole, when the ultrasound vibrate at a higher rate the gas vesicle collapse and burst. So, the buoyancy capability of the cell will be lost. In addition, the gas vesicle in green algal present in a small amount so small amplitude will cause lass damage to cell (Pradeep Rajasekhar et al., 2012). Hence, when using high amplitude the cell reduction increases rapidly. This is because, higher amplitude offers a high sonication power and causes high intensity which disrupts the cell wall of the *Nannochloropsis sp.*

Cell walls often represent the dominant component of the extracellular matrix and represent the largest or a significant percentage of the photosynthetically fixed carbon of the algae. Walls are typically fibrous composites of microfibrillar polysaccharides embedded in matrix polysaccharides and proteoglycans. According to Zhaofeng Liang and his co-researches (2006) the first stage of cavitation is called stable cavitation and usually does not cause damage meanwhile full cavitation will sound like rocks flowing through the piping system and results in surface damage and called transient cavitation. This shows that during the stable cavitation there is no collapse of bubble violently in the medium. The stable cavitation is just to disperse the cell and mildly mix the solution without any damage to the cell.

On the other hand, when apply high intensity there will be trasnsient cavitation where microbubbles form at various nucleation sites in the medium and grow during the rarefaction phase of the sound wave. Then, in the compression phase, the bubbles implode and collapsing bubbles release a violent shock wave that propagates through the

medium (Yususf, 2003). This will cause the disruption of *Nannochloropsis sp.* and makes it to precipitate and settle down. Using a high intensity of sonication also leads to increase in temperature. This is because, the excessive energy released by the horn is converted into heat energy which is then increase the medium temperature. Heat also can be a main factor for microalgae disruption. The heat generation can be prevented by using pulse. The main function of using pulse during the sonication is to prevent temperature build up. *Nannochloropsis sp*. needs an optimum temperature to grow which is between 26 °C to 27 °C. So it is proved that high intensity of sonication leads to *Nannochloropsis sp.* death meanwhile low intensity of sonication causes less reduction to the cell.

### 4.3 Comparison of Sonication and Controlled Growth

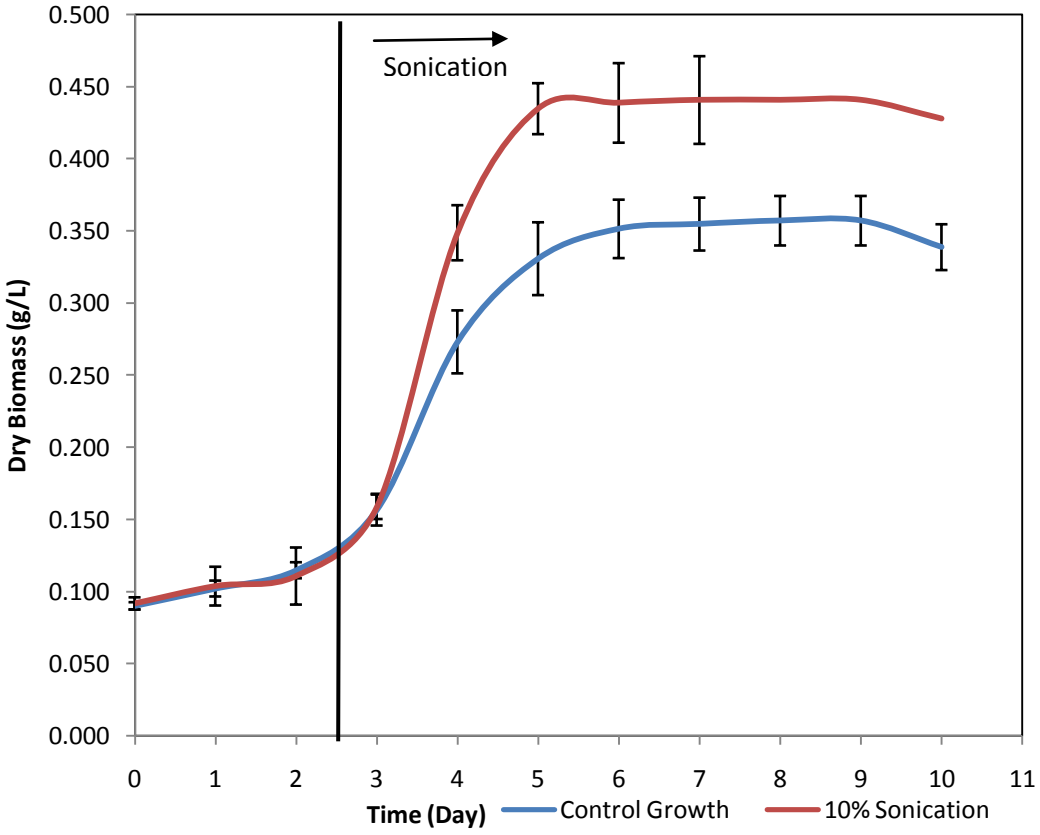


**Figure 4.8** Dry Biomass versus Time (Comparison of 10% Sonication and Control Growth Biomass )

From the figure above, 10% sonication proved to increase the biomass production of Nannochloropsis sp. This is because the 10% sonication is a very low doasage of sonication which actually wakes up the weak or dormant cell to be active and increase the permeability of cell. When this happens, it will actually increase the uptake of nutrient and the metabolism of the Nannochloropsis sp. will also eventually increase. Since the metabolism of the microalgae increased the biomass growth will elevate and become higher than the normal growth. Hence, it is proved the 10% sonication will

improve the Nannochloropsis sp. fermentation. Here, from the graph,on the ninth day 1.23 fold is obtained after 10% sonication is used. At the endogenous phase the nitrate consumed is used to repair the damaged cell rather than producing new cell.

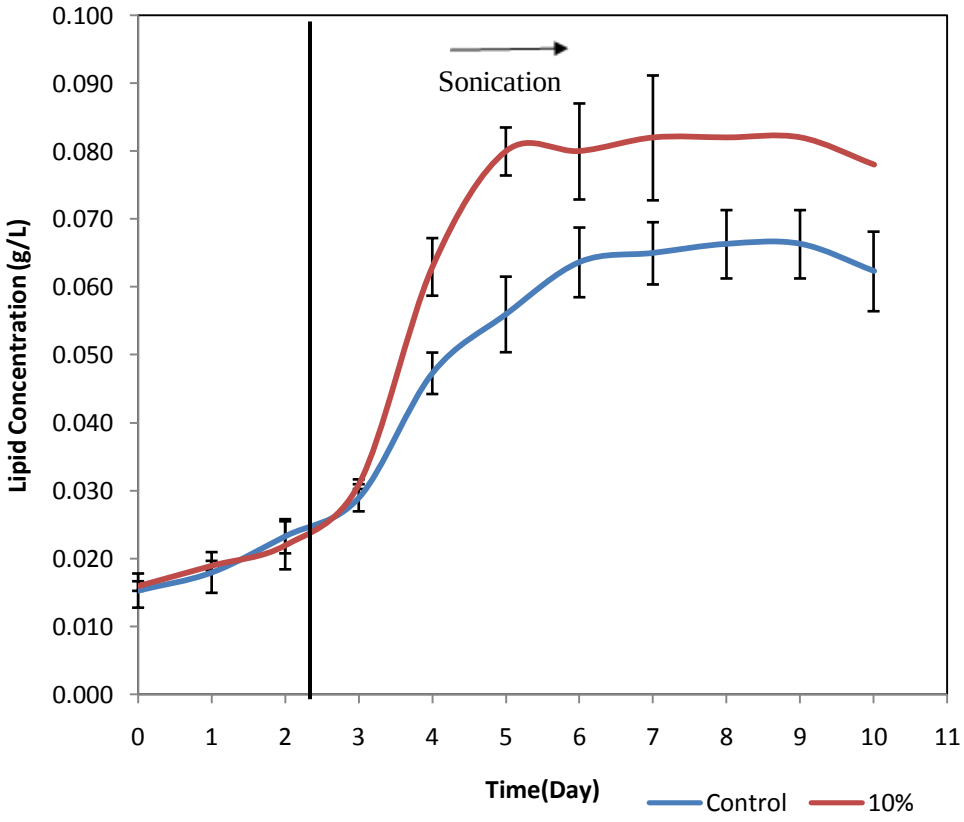


**Figure 4.9** Lipid Concentrations versus Time (Comparison of 10% Sonication and Control Growth Lipid Production)

During the control growth the maximum lipid produced is only 0.066 g/L. The production of the lipid from *Nannochloropsis sp*. is improved by introducing 10% sonication during its log phase until endogenous phase. The maximum lipid produced from the sonication is 0.081 g/L. From this growth, 1.23 fold is obtained. *Nannochloropsis sp.* seems to give a good response during the 10 % duty cycle. The 10

% sonication did not rupture the cell but it increases the permeability of the cell and the cell is “awakened” during the sonication. This will cause the cell which is inactive to be active and continue its metabolism. This is reason why the nutrient uptake of 10 % sonication increase tremendously after the sonication is introduced.

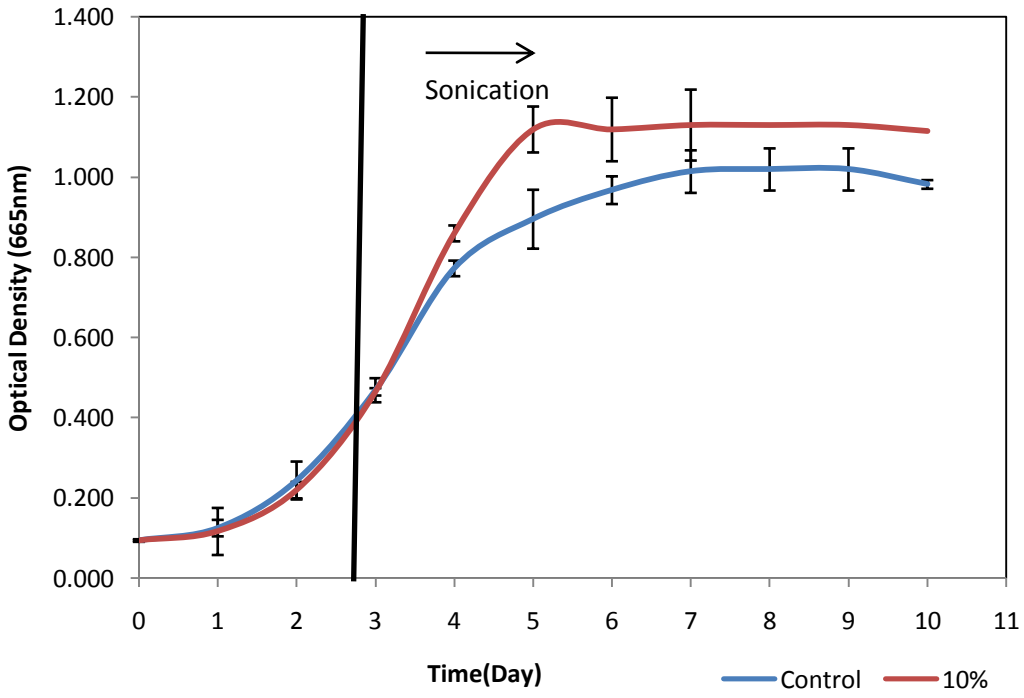


**Figure 4.10** Optical Density versus Time (Comparison of 10% Sonication and Control Growth Absorbance)

Since the production of new cell is increased with 10% sonication the colour of the culture become more even darker than the control growth. This is because the absorbance is measured by the production of chlorophyll of cell. The more chlorophyll is produced shows that the more cells have been produced. Hence the absorbance reading also will give a higher reading than the control growth.

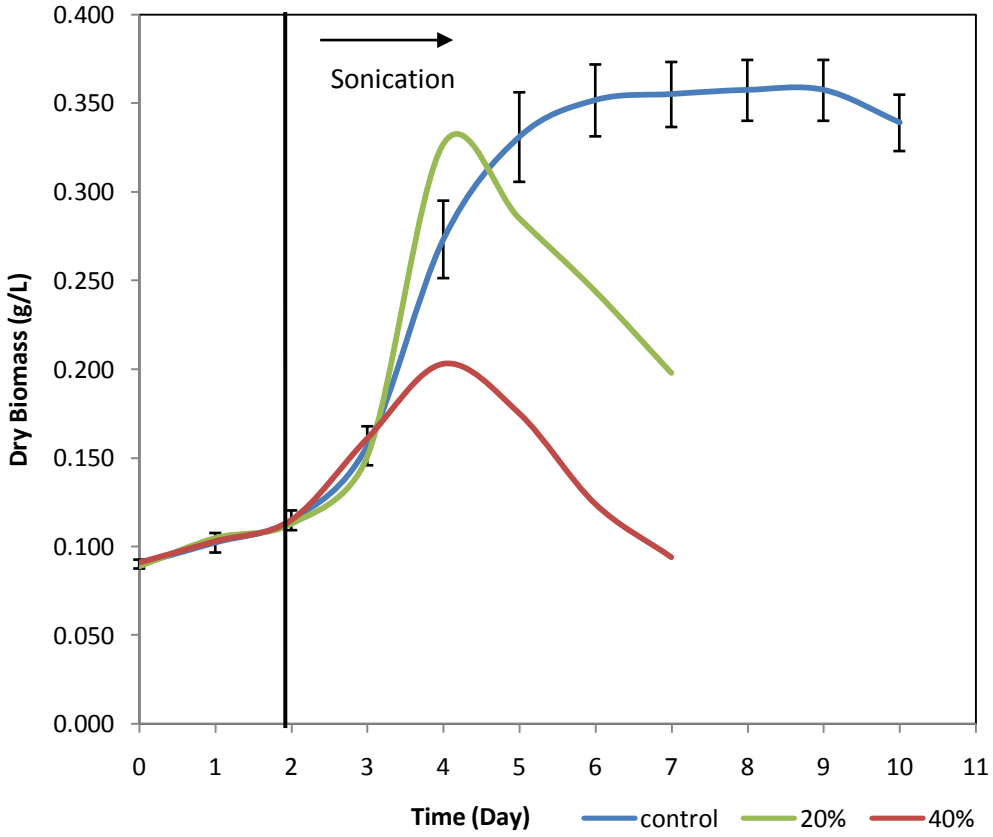


**Figure 4.11 Dry** Biomass versus Time (Comparison of 20%, 40% Sonication and Control Growth Biomass)

According to the figure, 20% and 40 % sonication is actually killing the *Nannochloropsis sp*. At first, when the *Nannochloropsis sp*. is induced with 20 % sonication on forth day it gives a higher biomass production compared to normal growth. This is because the cell which is inactive had been awakened at a higher rate with intense sonication. This is the reason why the cell dies at the following days because the gas vesicle that present in the cell had been destroyed and this makes the buoyancy capability of a cell decrease. Furthermore, intense sonication can make the cell to undergo shock and causing it to die. In 40 % sonication, the *Nannochloropasis sp*. did not give a good response at all since it starts to die in the first day of sonication.

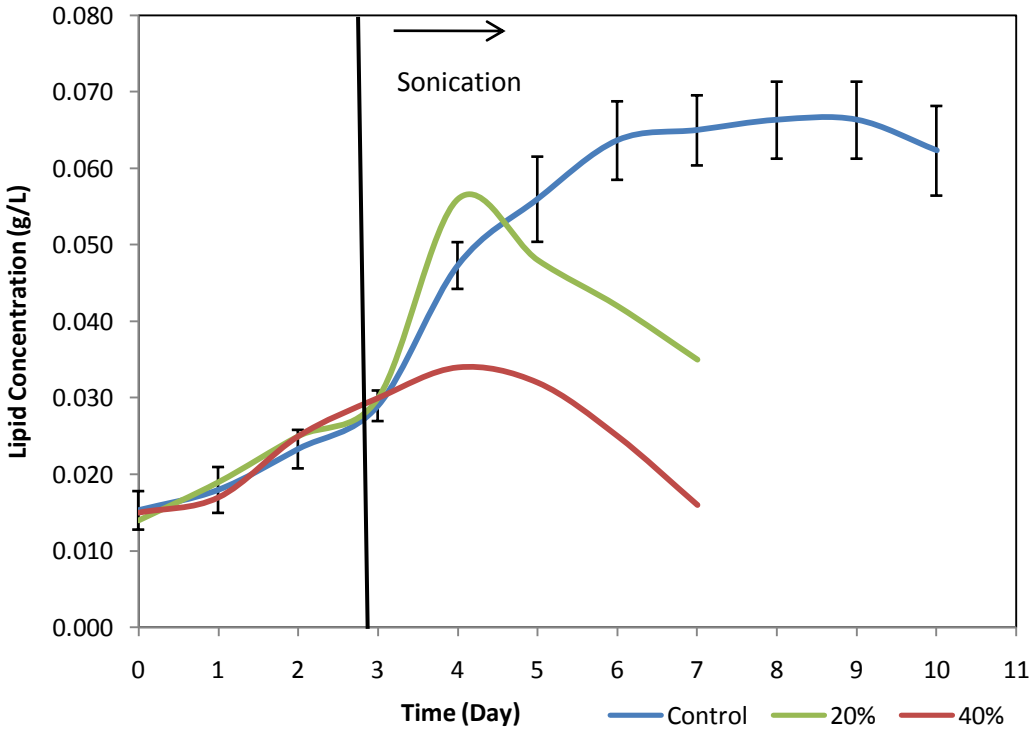


**Figure 4.12** Lipid Concentrations versus Time (Comparison of 10% Sonication and Control Growth Lipid Production)

From the result, it shows that 20% sonication improves the *Nannochloropsis sp.* fermentation at the first day of sonication. Unfortunately, it starts to die on the second day of sonication. This is because, the cell is disturbed at an intense vibration where at the first day it is able to withstand the disturbance but on the following day it was unable to digest the vibration to its fermentation process. For the 40% sonication *Nannochloropsis sp*. did not give good response at lipid production because the cell is disturbed at a very intense sonication of duty cycle. This makes the cell wall and the cell membrane of the cell to rupture thus causing it to die when sonication is used. Furthermore, the gas vesicle that present in the cell had been broken due to the sonication.

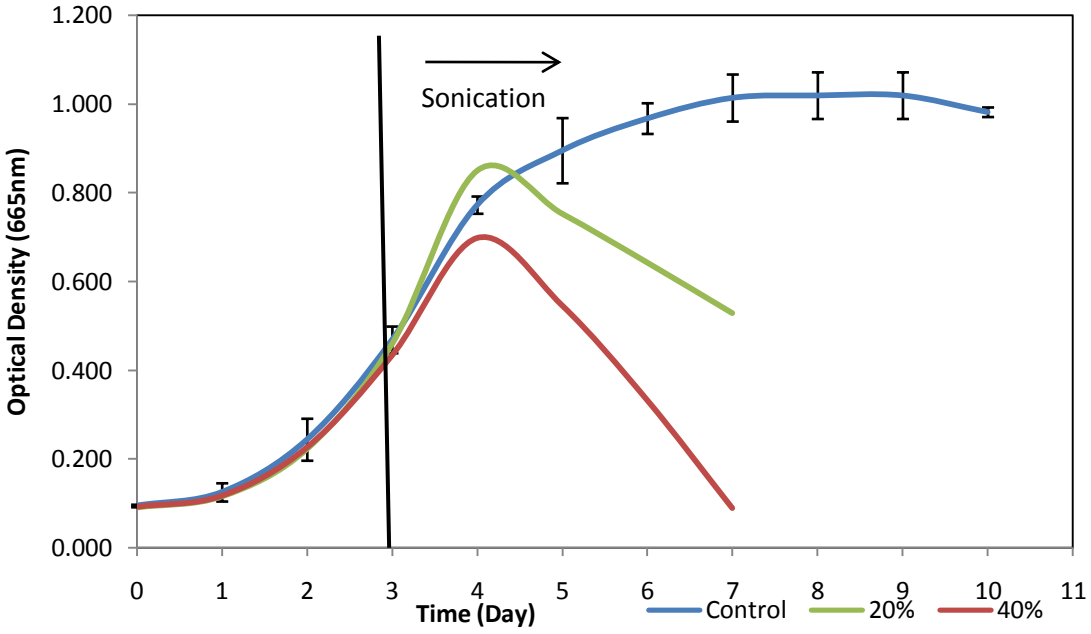


**Figure 4.13** Optical Density versus Time Comparison of 20%, 40% Sonication and Control Growth Absorbance

From the Figure 4.13, it shows that in 20% sonication *Nannochloropsis sp.* grew well because the absorbance in the 20 % sonication is higher the control growth. Meanwhile in 40 % sonication the cell was not able to withstand the intense vibration thus it starts to die at the first day of sonication.

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**Table 4.1** Comparison of Fermentation Kinetics.

| Kinetic parameter | Control | 10 % Sonication | 20% Sonication | 40% Sonication |
|---|---|---|---|---|
| Specific growth rate, $day^{-1}$ $\mu = \frac{1}{t} \ In\ (\frac{Xm}{Xo})$ | 0.812 | 0.855 | 0.809 | 0.732 |
| Lipid productivity, $g\ L^{-1}\ day^{-1}$ $v = \frac{C_L}{t}$ | 0.079 | 0.119 | 0.107 | 0.044 |
| Maximum dry biomass, g, $W_L$ | 0.357 | 0.441 | 0.327 | 0.203 |
| Maximum extracted lipid , g, $W_{DA}$ | 0.066 | 0.082 | 0.056 | 0.034 |
| Lipid yield $Y\ (\%) = \frac{W_{DA}}{W_L}$ | 18.49 | 18.59 | 17.13 | 16.75 |
| Final lipid productivity, $P_l$ | 0.0249 | 0.0336 | 0.0156 | $4.286\times10^{-4}$ |

From the Table 4.1, it can be concluded that 10% sonication gives a better result than the control growth and other two sonication

# CHAPTER 5

# CONCLUSION AND RECOMMENDATION

## 5.1 Conclusion

From this project it can be concluded that duty cycle at a low intensity can improve the *Nannochloropsis sp.* fermentation and increase the lipid synthesis for biodiesel production. The sonication intensity of 10.2 watt/cm$^2$ with duty cycle of 10 % where one second of sonication and ten second of rest periods gives better result compared to other two duty cycle. This is because the cell is being awaken gently and mixed mildly with the f/2 medium. This makes the cell to survive the sonication and gives a better lipid production where 1.23 fold is obtained. In the 20 % duty cycle where one second sonication and five second without sonication makes the cell to survive for only one day of sonication but gives a poor result on the following days of sonication. For 40 % duty cycle, two second of sonication and five second of rest destroy the cell and give a very poor result compared to 20 % duty cycle. Hence low intensity of

sonication with low duty cycle improves *Nannochloropsis sp.* fermentation for lipid synthesis which contributes for biodiesel production.

## 5.2 Recommendation

Recommendation from this project is, the other kind of species of microalgae is tested with the duty cycle. Flow cell of ultrasound is used to improve the set up. So a more controlled condition of sonication can be obtained. One of the effective approaches to increase the microalgae oil content is to obtain the genetically modified species by way of genetic engineering, which is to produce a high-yielding transgenic microalgae strain with the selective advantage which would enable it to grow in highly selective environments so that it can be grown in open culture systems whilst still remaining relatively free of contamination by other algae and protozoa. A set up room for microalgae cultivation is prepared since the culture box is only enough for few two litre conical flask. Flow cell ultrasound is used with bioreactor which is placed in a set up room to give a maximum controlled condition for the microalgae to grow.

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## APPENDIX A

## RAW RESULT

**Table A.1** Dry Biomass for Control

| Time (Day) | Sample 1 (mg) | Sample 2 (mg) | Sample 3 (mg) | Average Dry Biomass (mg) | Concentration (g/L) |
|---|---|---|---|---|---|
| 0 | 9.0 | 9.3 | 8.8 | 9.0±0.252 | 0.090±0.003 |
| 1 | 10.2 | 10.8 | 9.7 | 10.2±0.551 | 0.102±0.006 |
| 2 | 11.4 | 12.1 | 11.0 | 11.5±0.557 | 0.115±0.006 |
| 3 | 15.7 | 16.8 | 14.6 | 15.7±1.100 | 0.157±0.011 |
| 4 | 26.9 | 29.7 | 25.4 | 27.3±2.183 | 0.273±0.022 |
| 5 | 33.8 | 35.2 | 30.3 | 33.1±2.524 | 0.331±0.025 |
| 6 | 35.8 | 36.8 | 32.9 | 35.2±2.026 | 0.352±0.020 |
| 7 | 35.9 | 37.1 | 33.5 | 35.5±1.833 | 0.355±0.018 |
| 8 | 36.0 | 37.3 | 33.9 | 35.7±1.716 | 0.357±0.017 |
| 9 | 36.0 | 37.3 | 33.9 | 35.7±1.716 | 0.357±0.017 |
| 10 | 34.5 | 35.1 | 32.1 | 33.9±1.587 | 0.339±0.016 |

**Table A.2** Lipid Concentration for Control

| Time (Day) | Sample 1 (mg) | Sample 2 (mg) | Sample 3 (mg) | Average Lipid mass (mg) | Concentration (g/L) |
|---|---|---|---|---|---|
| 0 | 1.5 | 1.8 | 1.3 | 1.5±0.3 | 0.015±0.003 |
| 1 | 1.8 | 2.1 | 1.5 | 1.8±0.3 | 0.018±0.003 |
| 2 | 2.3 | 2.6 | 2.1 | 2.3±0.3 | 0.023±0.003 |
| 3 | 2.9 | 3.1 | 2.7 | 2.9±0.2 | 0.029±0.002 |
| 4 | 4.8 | 5.0 | 4.4 | 4.7±0.3 | 0.047±0.003 |
| 5 | 5.7 | 6.1 | 5.0 | 5.6±0.6 | 0.056±0.006 |
| 6 | 6.5 | 6.8 | 5.8 | 6.4±0.5 | 0.064±0.005 |
| 7 | 6.6 | 6.9 | 6.0 | 6.5±0.5 | 0.065±0.005 |
| 8 | 6.7 | 7.1 | 6.1 | 6.6±0.5 | 0.066±0.005 |
| 9 | 6.7 | 7.1 | 6.1 | 6.6±0.5 | 0.066±0.005 |
| 10 | 6.0 | 6.9 | 5.8 | 6.2±0.6 | 0.062±0.006 |

**Table A.3** Absorbance for *Nannochloropsis sp*

| Time (Day) | Absorbance (665nm) | | | Average |
|---|---|---|---|---|
| | Sample 1 | Sample 2 | Sample 3 | |
| 0 | 0.093 | 0.098 | 0.093 | 0.095±0.003 |
| 1 | 0.115 | 0.149 | 0.112 | 0.125±0.021 |
| 2 | 0.224 | 0.298 | 0.210 | 0.244±0.047 |
| 3 | 0.458 | 0.503 | 0.446 | 0.469±0.030 |
| 4 | 0.768 | 0.794 | 0.756 | 0.773±0.019 |
| 5 | 0.857 | 0.980 | 0.849 | 0.895±0.073 |
| 6 | 0.975 | 0.998 | 0.930 | 0.968±0.035 |
| 7 | 0.988 | 1.075 | 0.979 | 1.014±0.053 |
| 8 | 0.990 | 1.080 | 0.988 | 1.019±0.053 |
| 9 | 0.990 | 1.080 | 0.988 | 1.019±0.053 |
| 10 | 0.985 | 0.991 | 0.970 | 0.982±0.011 |

**Table A.4** Effect of Power to Cell

| Amplitude ( %) | Power (Watt) | Intensity (W/cm2) | Absorbance (665nm) | Cell Reduction (%) |
|---|---|---|---|---|
| 0 | 0 | 0 | 0.4650 | 0 |
| 20 | 13 | 10.2 | 0.4620 | 0.645 |
| 30 | 29 | 22.8 | 0.4500 | 3.230 |
| 40 | 44 | 34.5 | 0.4290 | 7.740 |
| 50 | 66 | 51.8 | 0.2970 | 36.130 |
| 60 | 86 | 67.5 | 0.1210 | 73.980 |
| 70 | 111 | 87.13 | 0.1100 | 76.190 |
| 80 | 130 | 102.0 | 0.0760 | 83.660 |
| 90 | 156 | 122.5 | 0.0010 | 97.836 |
| 100 | 170 | 133.4 | 0.0040 | 99.140 |

**Table A.5** Biomass Result For 10% Sonication

| Days | Dry Biomass (mg) | Concentration (g/L) | Optical Density (665nm) |
|---|---|---|---|
| 0 | 9.2 | 0.092 | 0.094 |
| 1 | 10.4 | 0.104 | 0.117 |
| 2 | 11.1 | 0.111 | 0.220 |
| 3 | 15.9 | 0.159 | 0.465 |
| 4 | 34.9 | 0.349 | 0.860 |
| 5 | 43.5 | 0.435 | 1.119 |
| 6 | 43.9 | 0.439 | 1.119 |
| 7 | 44.1 | 0.441 | 1.130 |
| 8 | 44.1 | 0.441 | 1.130 |
| 9 | 44.1 | 0.441 | 1.130 |
| 10 | 42.8 | 0.428 | 1.110 |

**Table A.6** Biomass Result For 20% Sonication

| Days | Dry Biomass (mg) | Concentration (g/L) | Optical Density (665nm) |
|---|---|---|---|
| 0 | 8.9 | 0.089 | 0.091 |
| 1 | 10.5 | 0.105 | 0.116 |
| 2 | 11.3 | 0.113 | 0.223 |
| 3 | 15.1 | 0.151 | 0.460 |
| 4 | 32.7 | 0.327 | 0.850 |
| 5 | 28.5 | 0.285 | 0.753 |
| 6 | 24.4 | 0.244 | 0.643 |
| 7 | 19.8 | 0.198 | 0.529 |

**Table A.7** Biomass Result For 40% Sonication

| Days | Dry Biomass (mg) | Concentration (g/L) | Optical Density (665nm) |
|---|---|---|---|
| 0 | 9.1 | 0.091 | 0.093 |
| 1 | 10.3 | 0.103 | 0.118 |
| 2 | 11.5 | 0.115 | 0.227 |
| 3 | 16.1 | 0.161 | 0.434 |
| 4 | 20.3 | 0.203 | 0.698 |
| 5 | 17.5 | 0.175 | 0.546 |
| 6 | 12.4 | 0.124 | 0.333 |
| 7 | 9.4 | 0.094 | 0.090 |

**Table A.8** Lipid Concentration For 10% Sonication

| Days | Lipid Weight (mg) | Lipid Concentration (g/L) |
|---|---|---|
| 0 | 1.6 | 0.016 |
| 1 | 1.9 | 0.019 |
| 2 | 2.2 | 0.022 |
| 3 | 3.1 | 0.031 |
| 4 | 6.3 | 0.063 |
| 5 | 8.0 | 0.080 |
| 6 | 8.0 | 0.080 |
| 7 | 8.2 | 0.082 |
| 8 | 8.2 | 0.082 |
| 9 | 8.2 | 0.082 |
| 10 | 7.8 | 0.078 |

**Table A.9** Lipid Concentration For 20% Sonication

| Days | Lipid Weight (mg) | Lipid Concentration (g/L) |
|---|---|---|
| 0 | 1.4 | 0.014 |
| 1 | 1.9 | 0.019 |
| 2 | 2.5 | 0.025 |
| 3 | 3.0 | 0.030 |
| 4 | 5.6 | 0.056 |
| 5 | 4.8 | 0.048 |
| 6 | 4.2 | 0.042 |
| 7 | 3.5 | 0.035 |

**Table A.10** Lipid Concentration For 40% Sonication

| Days | Lipid Weight (mg) | Lipid Concentration (g/L) |
|---|---|---|
| 0 | 1.5 | 0.015 |
| 1 | 1.7 | 0.017 |
| 2 | 2.5 | 0.025 |
| 3 | 3.0 | 0.030 |
| 4 | 3.4 | 0.034 |
| 5 | 3.2 | 0.032 |
| 6 | 2.5 | 0.025 |
| 7 | 1.6 | 0.016 |

**Table A.11** Nutrient analysis

| Sample 1 (g/L) | Sample 2 (g/L) | Sample 3 (g/L) | Average (g/L) | 10% sonication (g/L) |
|---|---|---|---|---|
| 72.5 | 73.2 | 72.2 | 72.6 | 73.5 |
| 70.4 | 69.7 | 70.5 | 70.2 | 70.2 |
| 65.3 | 64.9 | 65.9 | 65.4 | 64.9 |
| 60.7 | 59.4 | 61.8 | 60.6 | 60.1 |
| 35.3 | 33.8 | 34.0 | 34.4 | 25.4 |
| 29.1 | 27.9 | 29.9 | 29.0 | 19.4 |
| 23.8 | 22.1 | 24.4 | 23.4 | 15.9 |
| 18.9 | 17.3 | 19.7 | 18.6 | 14.1 |
| 17.4 | 16.7 | 18.3 | 17.5 | 12.8 |
| 16.9 | 16.1 | 17.6 | 16.9 | 11.7 |
| 16.3 | 15.8 | 16.9 | 16.3 | 10.2 |

## APPENDIX B

## PICTURES

**Figure B.1** Weighing Scale


**Figure B.2** Centrifuge


**Figure B.3** UV- Spectrophotometer

**Figure B.4** Homogenizer


**Figure B.5** Autoclave

**Figure B.6** Culture Box