Cultivation of Microalgae Using Compost as Nutrient Source for Biodiesel Production

by

Kinosraj a/l Kumaran

15352

Dissertation submitted in partial

fulfillment of the requirement for the

Bachelor of Engineering (Hons) Chemical

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Universiti Teknologi PETRONAS

32610 Bandar Seri Iskandar

Perak Darul Ridzuan

Malaysia

CERTIFICATION OF APPROVAL

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Approved by,

_____

(Dr. Lam Man Kee)

UNIVERSITI TEKNOLOGI PETRONAS

32610 BANDAR SERI ISKANDAR, PERAK DARUL RIDZUAN, MALAYSIA

September 2015

CERTIFICATION OF ORIGINALITY

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

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KINOSRAJ A/L KUMARAN

ABSTRACT

Cultivating microalgae for biodiesel production requires substantial amount of nutrient such as nitrogen and phosphorous. In this research project, it focuses on the use of low-cost compost or organic fertilizer as the main nutrient source for microalgae cultivation. It contains high value of nutrients without any contamination that can support microalgae growth. In this research, *Chlorella Vulgaris* species growth was investigated. The main objective of the experiment will be to study the effect of cultivation parameter such as amount of compost nutrients, duration of illumination, pH value and colour filter towards the growth of microalgae. The growth of the microalgae will be measured by using the spectrophotometer. After it have achieved stationary phase, the microalgae will be dried and biomass collection takes place. The last method will be lipid extraction using Soxhlet extractor and also transesterification reaction with Fatty Acid Methyl Ester (FAME) analysis. The analysis will be done by using gas chromatography-mass spectrometry analysis.

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TABLE OF CONTENTS

CERTIFICATION OF APPROVAL i

ABSTRACT iii

ACKNOWLEDGEMENT iv

LIST OF TABLES v

LIST OF FIGURES vi

CHAPTER 1: INTRODUCTION 1

1.1 Background 1

1.2 Problem Statement 3

1.3 Objectives 4

1.4 Scope of Study 4

CHAPTER 2: LITERATURE REVIEW 5

2.1 Introduction to microalgae 5

2.2 Cultivation of microalgae 11

2.3 Methods of Microalgae Cultivation 15

2.4 Microalgae Cultivation System 16

2.5 Compost as Nutrient provider for Microalgae Growth 20

2.6 Biodiesel Production 20

2.7 Biodiesel Production Technology 22

CHAPTER 3: METHODOLOGY 25

3.1 Pure Microalgae Strain and Culture Condition 25

3.2 Cultivating Microalgae with Compost . 25

3.3 Measurement of the Microalgae Growth . 26

3.4 Measurement of Nitrate Content in the medium 27

3.5 Microalgae Harvesting and Biomass Collection 27

3.6 Microalgae Lipid Extraction . . . 27

3.7 Transesterification reaction and Fatty Acid Methyl Ester (FAME) Analysis . . 28

3.8 Process Flow of the Research Project. . 29

3.9 Set up of the experiment . . . 30

3.10 Key milestones 32

CHAPTER 4: RESULT AND DISCUSSION . . . 33

4.1 Calibration and Preliminary Study . . 33

4.2 Effects of Amount of Nutrient and Comparison Type of Compost . . 36

4.3 Effects of initial pH 41

4.4 Effects of Colour filter . . . 43

CHAPTER 5: CONCLUSION AND RECOMMENDATION . 45

5.1 Conclusion 45

5.2 Recommendation 45

REFERENCES 47

APPENDICES 56

LIST OF TABLES

Table 2.1	Classification of microalgae	7
Table 2.2	Lipid content for different type of microalgae	9
Table 2.3	Average concentration according to the group	13
Table 2.4	Open and closed pond system comparison	18
Table 2.5	Comparative feature of microalgae cultivation approaches	20
Table 2.6	Different property between produced biodiesel from microalgae and biodiesel standard between diesel standard	22
Table 3.2	Project Gantt chart	32
Table 3.3	Key milestone	33

LIST OF FIGURES

Figure 2.1	Types of microalgae	8
Figure 2.2	Mechanism for the thermal decomposition for tryglycerides	24
Figure 2.3	Transesterification reaction of tryglyderide with alcohol	25
Figure 3.1	Microalgae lab set up	31
Figure 4.1	Calibration stage	34
Figure 4.2	Graph of microalgae dry weight vs optical density at 688nm	35
Figure 4.3	Five stages of microalgae growth	36
Figure 4.4	General pattern of microalgae growth in batch cultures	37
Figure 4.5	Effect of initial nutrient concentration of peat moss compost towards the growth of *chorella vulgaris.* other culture condition is: initial ph= 3.00, amount of seed= 100 ml and illumination for 24 hours continuously	38
Figure 4.6	Effect of initial nutrient of goat compost towards the growth of *chorella vulgaris.* Other culture condition is:initial ph= 3.00, amount of seed= 100 ml and illumination for 24 hours continuously	39
Figure 4.7	Specific growth and biomass productivity of *chorella vulgaris* in peat moss compost	39
Figure 4.8	Effects of initial ph value towards the growth of *chorella vulgaris.* Other culture condition is: amount of seed= 100 ml and illumination for 24 hours continuously	41
Figure 4.9	Specific growth and biomass productivity of *chorella vulgaris* in intial ph value	42
Figure 5.0	Effects of colour filter towards the growth of *chlorella vulgaris*. Other culture condition is: initial ph= 9.00, amount of seed= 100 ml and illumination for 24 hours continuously	43

Figure 5.1 Specific growth and biomass productivity of *chorella vulgaris* in different colour filter 44

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Fossil fuels are one-time energy gift to the human race which is very valuable. Once it is depleted, humankind has to find for other alternative. In 2008, the annual world primary energy consumption was estimated at around 11,295 million tonnes of oil equivalent (Brennan & Owenda, 2010). When all fossil fuels have been depleted, human kind will not be well-prepared to move on with the renewable energy. Since the beginning of the industrial era or time, mankind has been emitting large amounts of greenhouse gases (GHGs) and other radioactively active gases into the atmosphere as a result of fossil fuels usage. The atmospheric concentration of these polluting gases has been noticeably rising, with atmospheric CO_2 concentration currently close to 390 ppm (Tans, 2011). In order to achieve back environment and economic sustainability, fuel production processes are required to be not only renewable but also capable of sequestering CO_2 from the atmosphere.

There are a lot of source of renewable energy resources such as solar, wind, tidal, and biomass that can be used to generate energy as an alternative to fossil fuels. From all other energy, biomass is expected to become the most prominent renewable energy source with a four-fold increase to 23% of total world primary energy by the year of 2050 (Savacool BK &Drupady, 2011). Besides, biofuels are a very important and reliable substitute for fossil fuel with the privilege such as sustainability, environmental friendly and good adaptability. A biofuel is a fuel that is produced through contemporary biological processes which is through agriculture and anaerobic digestion. The alternative fuel that is gaining increased attention from researchers all around the world will be biodiesel. This is because one of the recent studies from U.S. Department of Energy showed that the production and use of

biodiesel, compared to petroleum diesel, resulted in a 78.5% reduction in carbon dioxide emissions.

There are different pathways for biodiesel production from feedstock, including pure vegetable oils, waste cooking oils, and animal fat but however, the limited supply of these feed stocks impedes or restricts the further expansion of biodiesel production (Canakci& J.Ven Gerpen, 2001). The best solution for this problem will be cultivation of microalgae for biodiesel production. The advantage of microalgae farms is that they can be built on land which does not have any agricultural value which means that they do not compete with food production. Other than that, the algae have special abilities due to their high metabolism. During photosynthesis process, microalgae are able to convert the sunlight and assimilate CO_2 in order to grow while producing oxygen and secondary metabolites. The main advantage of algae is that they can thrive or survive in a particularly high concentration of CO_2 of up to 10%. Microalgae is also well-known for its ability to produce two to three times more biomass than rooted plants over the same period of time and area due to fast multiplication process (Sihem Tebbani & Flipa Lopes, 2014).

1.2 Problem Statement

Microalgae usage for biofuel production has provided a viable alternative to replace or substitute fossil fuels. However, the technology to extract the oil from algae has a lot of barriers and challenges before it can be marketable and compete in the fuel market to be broadly deployed or used worldwide. The main challenges are strain identification and improvement which have significant impact on the oil productivity itself. Other than that, the crop protection, nutrient supply also is taken into consideration for algal biofuel. There is a lot of work that still need to be completed or developed although the potential is still blooming or rising for biofuel.

There are a lot of research have been done on biodiesel production by using algae. When choosing the appropriate cultivation system, many parameters must be observed: (A.Pandey, 2014)

- Biology of the microalgae
- The cost of land, energy, water, and nutrients.
- Local climate conditions
- Final product

Thus, this work aims to evaluate the laboratory cultivation of microalgae strain using nutrient from compost for biomass and biofuel production. The effect of various cultivation parameters such as amount of nutrients, illumination duration and pH value to the growth of microalgae will be systematically investigated.

1.3 Objective

The objectives include:

- To study the effect of cultivation parameters, such as amount of compost nutrients, type of compost, durations of illumination, pH value and amount of seed culture towards the growth of microalgae
- To harvest and to extract microalgae lipid from dried biomass
- To convert the microalgae lipid to biodiesel through transesterification

1.4 Scope of Study

This project focuses on the cultivation of microalgae for biodiesel production. The research work will be performed to determine the effectiveness of different culture parameters such as the amount of nutrient from compost, duration of illumination and pH value of the culture medium to the growth of microalgae. The amount of biomass yield (g/L) and the specific growth will be recorded throughout the experiments. Nevertheless, the effect of photobioreactor configuration, CO_2 flow rate and illumination intensity is not within the scope of the present work.

Lipid extraction will be performed on the dried microalgae biomass by using Soxhlet extractor method using mixed methanol-chloroform with volume ration of 2:1 as solvent. The lipid yield will be determined by gravitational method. The effect of different solvent towards the lipid extraction efficiency is not covered in the present study. Lastly, the lipid extracted will undergo transesterification reaction to convert into biodiesel.

CHAPTER 2

LITERATURE REVIEW

Literature review was done in this chapter to support the research in the present thesis. The literature review focuses on three main themes which is about microalgae, cultivation of microalgae as source for biodiesel production and last but not least about the compost which act as the nutrient source for the growth of microalgae.

2.1 Introduction to Microalgae

The term microalgae refer to the microscopic algae or oxygenic photosynthetic bacteria such as cyanobacteria which is also known as *Cyanophyceae* (Richmond, 2004). Microalgae represent a large group of different kind of organism from many types of phylogenetic groups from taxonomy divisions. In general, microalgae can be referred as plant like organism or living microorganism which are normally photosynthetic and aquatic but do not have stems, true roots, vascular tissue and have simple reproductive structures (Christi, 2007). These microalgae are distributed worldwide in the sea, freshwater and in wastewater mainly.

There are many kinds or types of microalgae:

- Green microalgae

These species is known as one of the largest group of microalgae with 7500 species. These microalgae contain or consist of chlorophyll and also large amount of proteins. Furthermore, these microalgae can produce starch and oil under stress condition. These microalgae exist in two species which is unicellular and multicellular organism. The most famous green algae species is *Chlorella* which is grown commercially all around the world by natural means. (Christi, 2007).

- Red microalgae

Red microalgae are a group which consist of 5000 species and lives in the tidal zone of the sea. Red microalgae multicellular are marine species microalgae (Christi, 2007).

- Diatoms microalgae

Diatoms are species with more than 100,000 species. Besides, diatom microalgae is unicellular microalgae which is well-known as it produces most of the biomass on earth. These diatom microalgae acts as indispensable food source or supply for the *zooplankton* in freshwater and seawater. Other than that, these microalgae have an attractive skeleton of silica which fits the microalgae like two halves of a sphere. Diatom microalgae are also known for its ability to produce oil which is stored in the cell and it contains oil in different amount according to the microalgae's buoyancy (Lundquist, 2007).

- Brown microalgae

These brown microalgae exist in multicellular have around 1500-2000 species. Brown algae which are like kelp are most likely to be found on the beach.These microalgae are also recognized by people as traditional sea weed (Lundquist, 2007).

- Gold microalgae.

Gold microalgae are a group of 1000 species of beautiful and colourful unicellular microalgae. These gold microalgae exist mainly in fresh water. Gold microalgae use flagella process for displacement (Christi, 2007).

- Yellow-Green microalgae

Yellow-Green microalgae are closely related to the brown microalgae but most of the species or to be specific around 600 species are unicellular and live in fresh water. *Nannochloropis* is a type of yellow-green microalgae which can produce large amount of oil as their food reserve. These microalgae are also fast growing microalgae which can be found in the sea. These two main factors made these type of microalgae highly suitable for production of biofuel (Christi, 2007).

- Blue microalgae.

Blue microalgae which are also known as cyanobacteria able to produce toxins in high concentration and can degrade the water quality. These microalgae can reserve food in the form of starch. One of the famous blue microalgae, *Spirulina* is cultivated for the use of dietary supplement (Christi, 2007).

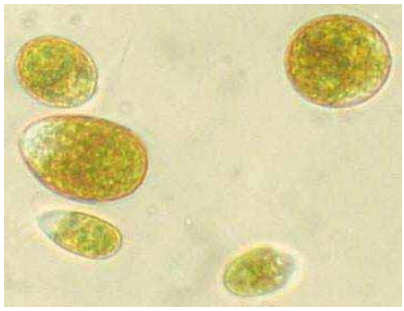
There are more than 50,000 species of microalgae exist world-wide compare to the main type of microalgae which is stated above. The table below shows brief detail about few main types of microalgae which present world-wide.

TABLE 2.1. Classification of Microalgae (Shalini Rajvanshi, 2012)

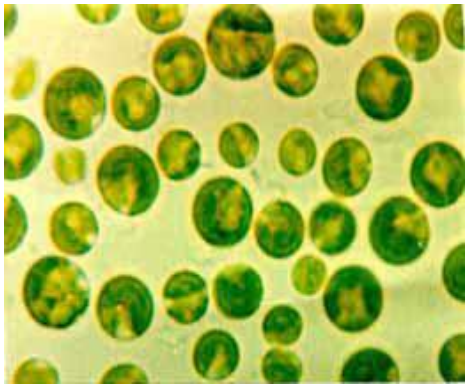
No	Name of microalgae	Known species	Storage material	Habitat
1	Diatoms (*Bacillariophyceae*)	100,000	Chyosolaminarin	Oceans
2	Green algae (*Chlorophyceae*)	8000	Starch,Triglyceride	Freshwater
3	Blue-green algae (*Cyanophyceae*)	2000	Starch ,Triglyceride	Different habitats
4	Golden algae	1000	Triglyceride and carboh	Freshwater

There are many usages of these microalgae however the main reason to cultivate microalgae is for biofuel production. It is estimated that total world commercial microalgae biomass production is about 10,000 tons per year (Benemann, 2008). Microalgae is also cultivated photosynthetically for nutritional product all around the world. The main microalgae for this purpose are *Spirulina, Chlorella, Dunaliella* and *Haematococcus* which is shows in Figure 2.1. The main producer for these microalgae is China and followed by Japan, Taiwan, USA, India and Australia and other small producer in other countries (Lundquist, 2007).

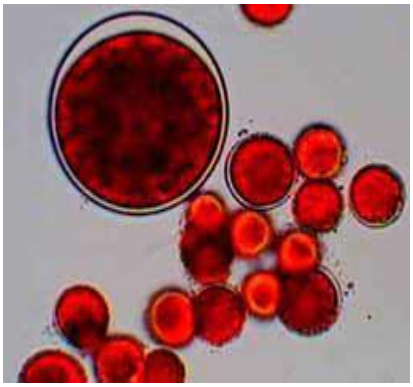
Spirulina (*Arthrospira platensis*)



Dunaliella salina



Chlorella vulgaris



Haematococcus pluvialis

FIGURE 2.1. Types of Microalgae (Benemann, 2002)

As discussed earlier, main reason for microalgae cultivation is for biofuel production. These microalgae which have the potential for accumulating or stored oil can be used used for biofuel or more specifically biodiesel production without competing for any land or bio diverse natural landscapes. The major or main requirement for commercial production of microalgae for this biodiesel production is the microalgae must be fast growing algae with high capability to produce a lot of lipid for oil extraction. The Table 2.2 shows that different potential of microalgae to produce lipid. The studies show that microalgae with high lipid typically have slower rate of growth compare to microalgae which have low amount of lipid (Kalpesh K. Sharma, 2012). There are a lot of challenges for microalgae production. One of the challenge is that microalgae will typically accumulate or able to reserve oil under stress condition in addition to slow growth. This means the ability of microalgae to thrive in extreme conditions should be taken into consideration for seeking efficient strains for biodiesel production.

TABLE 2.2. Lipid content for different type of microalgae (Benemann, 2002)

Microalgae Species	**Lipid content (% dry weight biomass)**
Ankistrodesmus sp.	24–31
Botryococcus braunii	25–75
Chaetoceros muelleri	33
Chlamydomonas reinhardtii	21
Chlorella emersonii	25–63
Chlorella minutissima	57
Chlorella protothecoides	14–57
Chlorella sorokiniana	19–22
Chlorella sp.	10–48
Chlorella vulgaris	5–58
Crypthecodinium cohnii	20–51
Dunaliella salina	6–25
Dunaliella primolecta	23
Dunaliella tertiolecta	16–71
Dunaliella sp.	17–67
Euglena gracilis	14-20
Ellipsoidion sp.	27
Haematococcus pluvialis	25
Isochrysis sp.	7–33
Monodus subterraneus	16
Monallanthus salina	20–22
Nannochloris sp.	20–56
Nannochloropsis oculata.	22–29
Nannochloropsis sp.	12–53

Pavlova salina	30
Prostanthera incisa	62
Prymnesium parvum	22-39
Pavlova lutheri	35
Phaeodactylum tricornutum	18–57
Scenedesmus obliquus	11–55
Skeletonema costatum	13–51
Scenedesmus dimorphus	16-40
Schizochytrium sp.	50-77
Thalassiosira pseudonana	20
Isochrysis galbana	7–40
Zitzschia sp.	45-47

2.2 Cultivation of Microalgae

Microalgae are unicellular microorganism which can be found in fresh water as well as marine environment. Microalgae have few main nutritional pre-requisite for growth which are carbon sources, energy, water and also inorganic nutrients. The carbon source for microalgae case can be CO_2, meanwhile the energy comes from the sun light. The responses from these microalgae to nutrients and cultivation environments can be used to manipulate the process to favour the production of biofuel from these microalgae (Benemann, 2002).

The conversion of light energy into chemical energy involves a two-step process known as the dark reaction and light reactions. Dark reaction is the process of carbon fixation reaction that can occur in the presence or absence of light, while light reactions needs illumination for the reaction to occur. The conversion of energy occurs the following reaction (Ho et al, 2011):

$$6CO_2 + 6\,H_2O + sunlight \longrightarrow C_6H_{12}\,0_6 + 6O_2 \qquad (1)$$

Nutrients are very important for the growth of microalgae. The nutrient absorption in the growth phase of microalgae can reach until 99% nitrogen intake (Fagerstone, 2012). There are many type of nutrients which can be found from different type of sources. However, sea water nutrient can be taken as reference point due to abundant type of nutrient. All these nutrients can be divided or summarized into three main groups which are I, II and III. The detail about this three group is also tabulated in the Table 2.3.

Group 1:

This group of nutrient have the exact same nutrient which presence in the sea water. These nutrients cannot be used up or depleted from the culture media due to absorption of microalgae for growth. These nutrients are very important for microalgae growth, therefore it need to be added to the medium.

Group 2:

This group of nutrient have quiet same concentration compare to sea water. This group also have nutrient from molybdenum and mainly selenium. However, these groups of nutrients have been shown to be not essential for microalgae growth. Therefore it is not important for microalgae growth.

Group 3:

This group of nutrient have nutrients mainly from silicon and some chrysophytes.

These nutrients are generally present at low concentrations in natural seawater, and because microalgae take up substantial amounts, concentrations vary widely. These nutrients important as it generate significant microalgae biomass.

Nitrate is the nitrogen source which is used abundantly for culture media. Ammonium can also be used as it is preferential form for many types of microalgae fur to reduced or less prior to amino and acid synthesis.

TABLE 2.3 Average Concentration of Nutrients According to the Group (Flávia Martins Franco De Oliveira1 and Maria Cristina Basílio Crispim, 2013)

Element	Average Molar
Group 1	
Na^+	4.7×10^{-1}
K^+	1.02×10^{-2}
Mg^{2+}	5.3×10^{-2}
Ca^{2+}	5.5×10^{-1}
Cl^-	2.8×10^{-2}
SO_4^{2-}	2.3×10^{-3}
HCO_3^-	4.2×10^{-4}
Group 2	
Br^-	8.4×10^{-4}
F^-	6.8×10^{-5}
IO_3^-	4.4×10^{-7}
Li^+	2.5×10^{-5}
Rb^+	1.4×10^{-6}
Sr^{2+}	8.7×10^{-5}
Ba^{2+}	1×10^{-7}
MoO_4^{2-}	1.1×10^{-7}
VO_4^{3-}	2.3×10^{-8}
Group 3	
NO^{3-}	3×10^{-5}
PO_4^{3-}	2.3×10^{-6}
Fe^{3+}	1×10^{-9}
Zn^{2+}	6×10^{-9}

Mn^{2+}	5×10^{-10}
Cu^{2+}	4×10^{-9}
Co^{2+}	2×10^{-11}
SiO_4^{4-}	1×10^{-4}
Ni^{2+}	8×10^{-9}

2.3 Methods of Microalgae Cultivation

There a lot of method for these microalgae cultivation. Researches from all around the world trying to find the most suitable way to cultivate these microalgae in order to grow more algae which can give high amount of oil for extraction.

Microalgae can be cultivated from different type of method namely as below:

2.3.1 Indoor/Outdoor

Indoor culture allows the control of temperature, illumination, nutrient level and other important parameters meanwhile outdoor cultivation system is cheap but it is very hard or difficult to grow microalgae.

2.3.2 Open/Closed

The main difference between open and closed is the open environment or also known as outdoor environment is more contaminated compare to closed which uses flasks, tubes, carboys and many more.

2.3.3 Axenic /Xenic

Axenic cultures are basically is not contaminated with any organism like bacteria but this culture is difficult to be achieved because it requires strict sterilization of the equipment used. Furthermore, this axenic method is very expensive. Meanwhile, non-axenic or can be also called as xenic cultivation is way cheaper. However, this type of cultivation has inconsistent quality.

2.3.4 Batch, Continuous, and Semi-Continuous

2.3.4.1 Continuous

This process is very efficient as it provides a consistent supply of high quality cells and highest rate of production for extended period.

2.3.4.2 Semi-Continuous

This process is quiet efficient and easier. However the quality of the cell is lower compare to the continuous process and less reliable.

2.3.4.3 Batch

This process is the easiest compare to the three method and quiet reliable. However, it is less efficient and quality may be inconsistent. Depending on the material used in cultivation of microalgae and the utilization of biomass, three different system can be distinguished (Becker,1994):

a) The first system is a system where selected microalgae strain is grown. The microalgae for this usually are utilized as food supplement due to clean process of growth.

b) The second system uses the sewage or industrial wastewater as culture medium for microalgae cultivation. The cultivation of microalgae for this method involves secondary removal which is BOD removal and also tertiary treatment which is nutrient removal for production of biomass based products mainly biofuel

c) The last system is cultivation of microalgae under closed system with sunlight or artificial light. These cultivation systems prefer microalgae grown in autotrophic media.

2.4 Microalgae Cultivation System

2.4.1 Open Pond System

These cultivation of microalgae in an open pond system has been used or practiced since early 1950's (Borowitzka, 1999) .Open pond system can be categorized or grouped into natural waters which are ponds, lakes or lagoons (S.Chinnasammy, 2012). The most typical system which is being used includes shallow and big ponds, circular tank and also raceway ponds. There are a lot of advantages of using these open ponds. One of the main advantage is open pond system is much easier to be built or constructed (Brennan & Owenda, 2010). It is also much easier to operate compare to the closed system. The major disadvantage of this system will be poor utilization of light and requirement of large areas for the pond. Other than that, these methods will create high competition between the microalgae if there is any fast growing heterotrophs as this is method is not under controlled environment.

Besides, the biomass production will be lower as open pond system is lack of proper stirring.

Another system which is under this open pond system is “raceway ponds”. These pond provides better circulation for these microalgae by using paddlewheels on regular frequency (Cat et al, 2013) . These ponds have continuous supply of CO_2 and nutrients with circulation.

The major drawbacks of these systems are the low production of microalgae. The contamination with bacterial strains and maintenance of optimum temperature are the main difficulty in large pond area (A.Pandey, 2014).

2.4.2 Closed Pond System

Another method for microalgae cultivation is in closed ponds. Closed ponds have a lot of advantages but the biggest concern will be on the cost which is quiet high with high production of microalgae. This is because in closed system all the important parameters such as the temperature, pH value of water and CO_2 supply is all controlled. The efficiency of these systems is very high. However, one of the major disadvantages of these closed ponds is it is difficult to construct, operate and very costly (Mahendra Pal Sharma, 2012). The table below shows the difference between and open pond system and closed pond system.

TABLE 2.4. Open and Closed Pond System Comparison (Christi,2007)

Cultivation Methods	**Advantages**	**Disadvantages**
Open pond system	Easier to construct	Poor light utilization by the cells
	Easier to operate	Poor light utilization by the cells
	Cheap	Poor diffusion of CO_2 to the atmosphere

Closed pond system	Process condition controlled	Difficult to construct
	Requires small area	Very costly
	Easier to harvest	Difficult to operate.

2.4.3 Photo-Bioreactor

Photobioreactor (PBR) is a container which allows light to pass through in order to make use of the light source.It can be operated in batch mode with a continuous stream of sterilized water to ensure the absence of bacteria with nutrients, air and carbon dioxide. Microalgae cultivation in a PBR is easier to be harvested if compared to the open system cultivation of microalgae. Besides, microalgae are well protected from all the outside pollutant inside this photo bioreactor. However, one of the major disadvantages in this system will be capital cost which is very high in term of industrial production to construct a photobioreactor (PBR) (Christi, 2007).

The first technology for closed pond system is flat plate photobioreactor. This flat plate photobioreactor gaines attention from all the researchers from world-wide because it have large surface area which is exposed to illumination (Ugwu, Aoyagi & Uchiyama, 2008). All the flat-plate photobioreactor are usually made of transparent material. The main reason is for maximum utilization of energy from solar light. These type of photobioreactors are very suitable for mass culture of microalgae (Hu et al.,1996; Richmond, 2000).

Tubular photobioreactors have straight, coiled or looped translucent tubing which are are arranged in different type of ways in order to maximize the sun light capture. A tubular photobioreactor which have a proper design is able to completely isolate the microalgae culture from being contaminated or polluted from external environment (A.Pandey,2014)

These tubular photobioreactor is used for outdoor mass culture of microalgae as this system has large illumination surface area. However, this system have its own disadvantage which is the poor oxygen build-up in the system. This problem always

happen when tubular photobioreactor is scaled up or constructed in bigger scale (Torzillo et al., 1986; Richmond et al., 1993; Molina et al., 2001) .The Table 2.5 shows a clear comparison between the types of system doe microalgae cultivation.

TABLE 2.5. Comparative Features of Microalgae Cultivation Approaches (Uqwu et al,2008)

Culture system	Prospects	Limitations
Open ponds	Relatively economical, easy to clean up after cultivation, good for mass cultivation of algae	Little control of culture conditions, difficulty in growl growing algal cultures for long periods,, poor productivity, occupy large land mass, limited to few strains of algae, cultures are easily contaminated.
Vertical-column photobioreactors	High mass transfer, good mixing with low shear stress, low energy consumption, high potentials for scalability, easy to sterilize, readily tempered, good for immobilization of algae, reduced photo inhibition and photo-oxidation	Small illumination surface area, their construction require sophisticated materials, shear stress to microalgae cultures, decrease of illumination surface area upon scale-up
Flat-plate photobioreactors	Large illumination surface area, suitable for outdoor cultures, good for immobilization of algae, good light path, good biomass productivities, relatively cheap, easy to dean up, readily tempered, low oxygen build up	Scale-up require many compartments and support materials, difficulty in controlling culture temperature, some degree of wall growth, possibility of hydrodynamic stress to some algal strains
Tubular photobioreactors	Large illumination surface area, suitable for outdoor cultures, fairly good biomass productivities, relatively cheap	Gradients of pH, dissolved oxygen and CO2along the tubes, fouling, some degree of wall growth, requires large land space

2.5 Compost as Nutrient Provider For Microalgae Growth

Compost means decayed organic material which used as plant fertilizer. In this microalgae cultivation system, compost is used as the source of nutrient for microalgae growth. The compost act as an alternative nutrient for the growth of microalgae. There are three major or main advantages by using this compost as a source of nutrient. Compost can be classified as low cost material and compost can also sustain the ecological outcomes besides give rapid growth rates for the microalgae cultivation (Flávia Martins Franco de Oliveira1 and Maria Cristina Basílio Crispim, 2013). The analysis on the compost or organic material shows that there are at least 15 types of nutrients in it. The lists are C, N, P, K, Ca, Mg, S, Fe, Zn, Mn, Cu, Al, Si, B and Mo (Sediyama,2011).Meanwhile, analysis derived from the waste water only shows 6 major nutrients which are C, N, P, K, Ca and Mg (Veras & Povinelli,2004).

2.6 Biodiesel Production

There are a lot of challenges which is associated with the decreasing fossil fuel reserves as energy sources which is extensively used for all kind of industries. Besides, the price of the heavy crude oil which always fluctuate in the market and also due to environmental concern , these encourages or drive the research on finding other alternative to fulfil the need. The most best solution is renewable energy. This is because renewable energy will not deplete and the emission of pollutant is less compare to crude oil. Several countries such as Brazil, United States, Germany, Australia and Italy are already utilizing biofuels especially bioethanol and biodiesel as their source of energy due to advancement of technology and the availability of feedstock in the respective countries (Yusuf et al, 2011).

The term biodiesel was originally initiated to represent the unmodified vegetable oils which could be replacement for diesel fuel (DF). The history starts back around 1880, when Rudolf Diesel designs a prototype of diesel engine. Later he received a German patent on 28 Feb 1892 and he able to demonstrate a running engine in 1897. The first demonstration of a small diesel engine operated on straight peanut which was called Arachis Hypogaea. After that a lot of evolution is made (Salvi & Panwar,2012).

Biodiesel can be produced from vegetable oil, animal oil, tallow and waste cooking oil and many more. However the most efficient production of biodiesel will be from microalgae. Microalgae was first cultured at Massachusetts Institute of Technology (MIT) during the early 1950s. The main purpose of the study of these microalgae is because of the energy shocks at that time. There are differences between biodiesel and diesel in the market. The Table 2.6 shows the property of the diesels.

TABLE 2.6. Different Property between Produced Biodiesel from Microalgae and Biodiesel Standard between Diesel Standard (M. Rakib Uddin,,Kaniz Ferdous etc al, 2013)

Properties	Produced biodiesel value	Biodiesel Standard	Diesel standard
Specific gravity, at 25°C	0. 792	0.88 (at 15.5°C)	0.85(at 15.5°C)
Kinematic viscosity (mm2s) at 40°C	3.000	1.9-6.0	1.3 — 4.1
FFA content (wt%)	0.94	-	-
Moisture content (%)	0.12	0.05% max.	0.161
Saponification value	194	-	-
Flash point (°C)	150	100 to 170	60 to 80
Iodine value	88	-	-
Cloud point (°C)	0	-3 to 12	-15 to 5
Pour point (°C)	-3	-15 to 10	-35 to -15
Yield (%)	79	-	-

2.7 Biodiesel Production Technology

Biodiesel production is the process of producing biofuel or biodiesel from chemical reaction transesterification. This process mainly involves the reaction of vegetables or animal fats and oil between short-chain alcohol which is normally methanol or ethanol (Van Gerpen & Shanks , 2014).

ASTM International or known as the American Society for Testing and Materials defines that biodiesel is a mixture of long-chain monoalkylic esters from fatty acids which are obtained from renewable resources to be utilized in diesel engines.

2.7.1 Direct Use and Blending

Vegetable oil can be mixed directly with diesel fuel to be used directly for running diesel engine. The blends with diesel fuel are indicated as ‘‘Bx’’, where ‘‘x’’ is the percentage of biodiesel in the blend. For example, ‘‘B5’’ indicates a blend with 5% biodiesel and 95% diesel fuel; in consequence, B100 indicates pure biodiesel.

The most advanced research or work had been done in South Arica with the sunflower oil. The results shows that at a certain point that it was not practical to substitute 100% vegetable oil for diesel fuel, but a blend of 20% vegetable oil and 80% diesel fuel was successful (Fukuda, Kondo,Noda ,2001)

The direct use of these vegetable oils and other blend of oils is considered to be not that efficient or satisfactory for diesel engines. The main problems for this direct use are high viscosity, acid composition , carbon deposits and lubricating oil thickening

2.7.2 Microemulsion

A microemulsion is known as a colloidal equilibrium dispersion of optically isotropic fluid microstructures with dimension stypically in the 1±150 nm range formed spontaneously from two normally immiscible liquids and one or more ionic or non-ionic amphiphiles (Schwab et al., 1987). Microemulsion is done to solve the main problem of direct use and blending which is the high viscosity of vegetable oils. Besides, microemulsion have low volumetric heating values than diesel fuels as the alcohol content gives latent heat of vaporization which cools down the combustion chamber (Fangrui Maa, Milford Hanna,1999). A microemulsion of methanol with vegetable oils can perform nearly as good as petroleum based diesel.

2.7.3 Thermal Cracking (Pyrolysis)

Pyrolysis is a process of converting one organic substance into another by process of heating in the presence of catalyst. Few years back when the microalgae production for generation of biofuel becomes a hot research topic, pyrolysis had drawn a lot of attention as a potential conversion method. This pyrolysis mechanism is complicated due to abundant of structures and possible reaction of mixed triglycerides. The mechanism for the thermal decomposition of triglyceride is showed in the figure below.

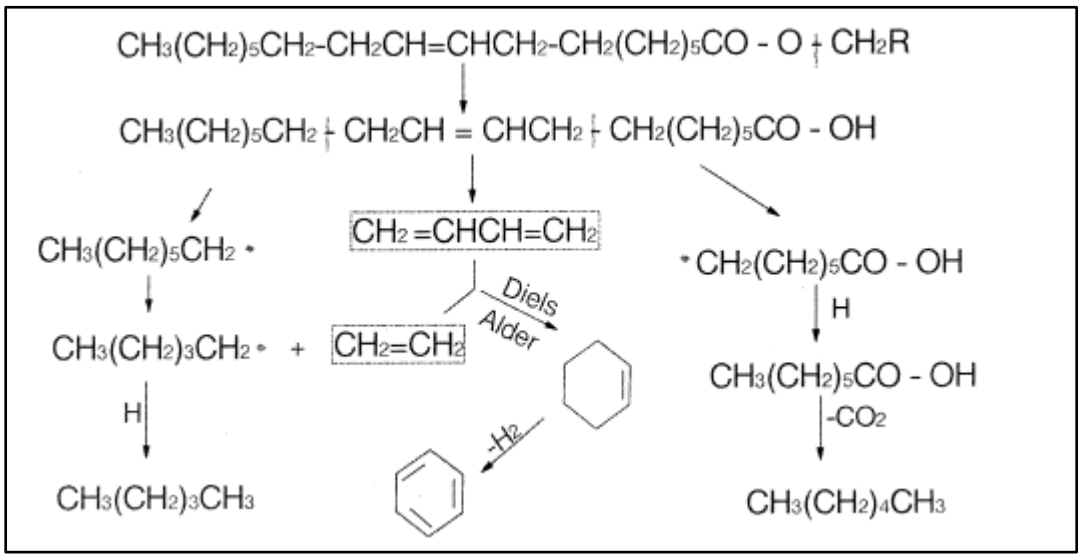


FIGURE 2.2. Mechanism For the Thermal Decomposition of Tryglycerides (Fangrui Maa, Milford Hanna,1999)

2.7.4 Transesterification

Transesterificantion is an important reaction which had been utilized extensively in all the industry world-wide since mayne years. Generally all the biodiesel uses same chemical process which is base catalysed transesterification. This is because this chemical process is the most economical as it requires low temperatures and pressures to produce a 98% conversion yield (Christi, 2007)

In the transesterificantion process, the triglyceride and alcohol is reacted together in the presence of catalyst which is usually strong alkaline, sodium hydroxide. The reaction froms mono-alky ester or in other words it is called biodiesel.

The Figure 2.3 below shows the chemical process for methyl ester biodiesel. The reaction is reversible action. This means the alcohol content must be in excess so that the reaction have complete conversion

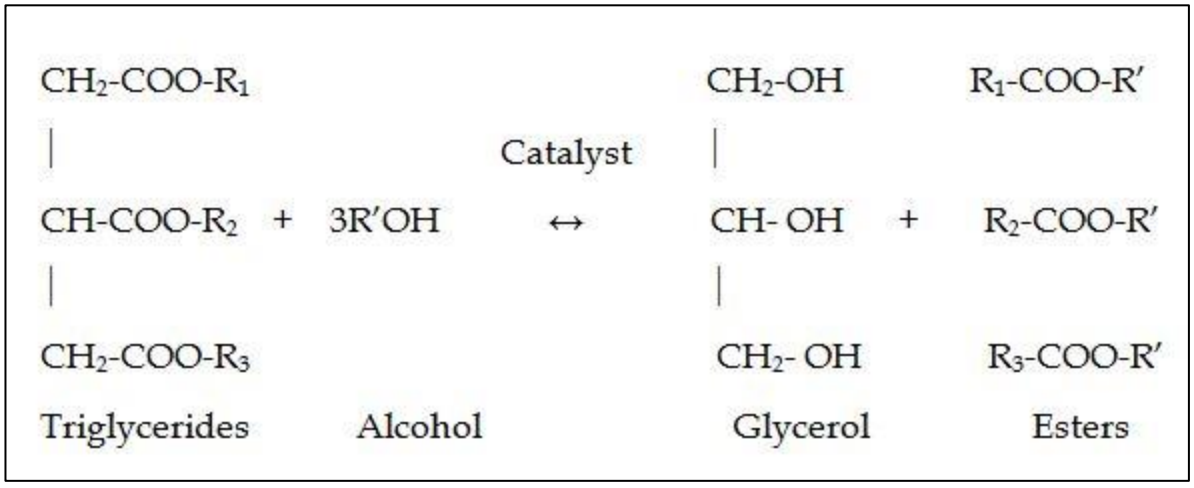


FIGURE 2.3. Transesterification Reaction of Tryglyderide with Alcohol (Fangrui Maa, Milford A. Hanna,1999)

CHAPTER 3

METHODOLOGY

This chapter outlines the general methodology of the study, research design and research instrument that will be involved in conduction the research project. The details of the methodology are further explained below.

3.1 Pure Microalgae Strain and Culture Condition

Chorella Vulgaris will be provided by Prof. Lee Keat Teong (School of Chemical Engineering, Universiti Sains Malaysia). The microalgae will be preserved and grown in Bold's Basal Medium (BBM) consisting of : (1) 10mL per litre of culture medium with the following chemicals: $NaNO_3$ (25g/L), $CaCL_2.2H_2O$ (2.5g/L), $MgSO_4.7H_2O$(7.5 g/L), K_2HPO_4 (7.5g/L) , KH_2PO_4(17.5g/L), NaCl(2.5g/L) and (2) 1mL per litre of culture medium with the following chemicals: EDTA anhydrous (50g/L), KOH(31g/L), $FeSO_4.7H_2O$ (4.98g/L), H_2SO_4(1mL), H_3BO_3(11.4 g/L), $ZnSO4_4.7H_2O$ (8.82/L), $MnCl_2.4H_2O$ (1.44 g/L), MoO_3(0.71 g/L) , $CuSO_4.5H_2O$ (1.57 g/L), $Co(NO_3)_2.6H_2O$ (0.49 g/L). The initial pH of the medium will be adjusted to 6.8. The seed culture will be grown in a 100 mL Erylenmeyer flask containing 50mL of culture medium, aeration with compressed air, surrounding temperature ranging from 25-28°C and illuminate with cool white fluorescent light (Philp TL-D 36W/865, light intensity of 60-70 µ mol m-2 s-1) continuously. (Man Kee Lam, 2012)

3.2 Cultivating Microalgae with Compost

Organic fertilizer will be used as the compost for the nutrient exraction for microalgae growth. The water from the photobioreactor will pass through the compost to absorb the nutrient from the compost and supply it to the microalgae in the photobioreactor, completing the entire process. (Man Kee Lam, 2012)

A 10 g of the fertilizer was immersed in 600 mL tap water and stirred for 24 h using magnetic stirrer. Non-soluble particulate solids were observed after the stirring process and were filtered using filter paper (Double Rings 101). The resulting organic fertilizer medium was dark-brown in colour with typical characteristics (Man Kee Lam, 2012)

Subsequently, a pre-determined volume of the organic fertilizer medium was introduced into a photobioreactor with 5 L of tap water (without sterilization) and the pH of the medium was adjusted according to pre-determined values. Then, C. vulgaris with initial cell concentration of 0.3×10^6 cells (around 10 mL from the seed culture) was introduced into the photobioreactor. The photobioreactor was aerated with compressed air continuously and illuminated with cool-white fluorescent light (Philip TL-D 36W/865, light intensity of 60–70 lmol m 2 s 1). (Man Kee Lam, 2012)

In the present study, two sets of experiments have been carried out. The first stage is to evaluate the effect of amount of nutrient towards the growth of *Chorella Vulgaris* in both type of compost . The following stage is to compare between plant based compost and animal based compost in order to select the compost which gives high growth rate of growth of *Chorella Vulgaris*. In this comparison, first experiment was done by using plant compost which is derived from peat most whereas the second experiment is done by animal compost which is derived from goat compost. For each set of experiment, different amount of nutrient were tested by using 5 Erlenmeyer in order to study the ability or potential of *Chorella Vulgaris* in using the nutrients provided for the growth.

3.3 Measurement of the Microalgae Growth

The microalgae growth is measured using the spectrophotometer (Shimadzu UV mini-1240). Sample around 10mL will be taken and be centrifuged at 10x1000g for 5 minute. The microalgae biomass will be dried in an oven for 100 °C for a day or to be exact 24 hours and the supernatant or the liquid denoting the liquid lying above will be poured back into the culture medium. All the samplings are performed in triplicate to ensure the the data is reliable and accurate. The data can correlate dry cell weights and optical density (Man Kee Lam, 2012)

3.4 Measurement of Nitrate Content in the Medium

A sample of 1 mL will be collected from the photobioreactor. Then the sample will be centrifuged at 10x1000 g for 5 minute. The spectrophotometer (Shimadzu UV mini-1240) is used to measure the optical density at 275 nm and 220 nm. After that, the absorbance reading at 275nm will be subtracted two times by the reading obtained at 220 nm in order to obtain the actual absorbance. Dry potassium nitrate (KNO_3) at different concentration will be used for calibration purpose. (Man Kee Lam, 2012)

3.5 Microalgae Harvesting and Biomass Collection

When the growth of microalgae had achieved the stationary phase, the air aeration to the culture medium will be stopped. Then, the microalgae will be left to settle down naturally at the bottom or in other word sedimentation process will occur for two days in the photobioreactor. The top layer is water with suspended microalgae cells and the bottom layer is microalgae biomass. The top layer will be slowly decanted and the microalgae biomass that is left at the bottom will be further dried in an oven at 80 °C for 24 hours. After that, the dried microalgae will be collected and sealed in an empty container for extraction of lipid. (Man Kee Lam, 2012)

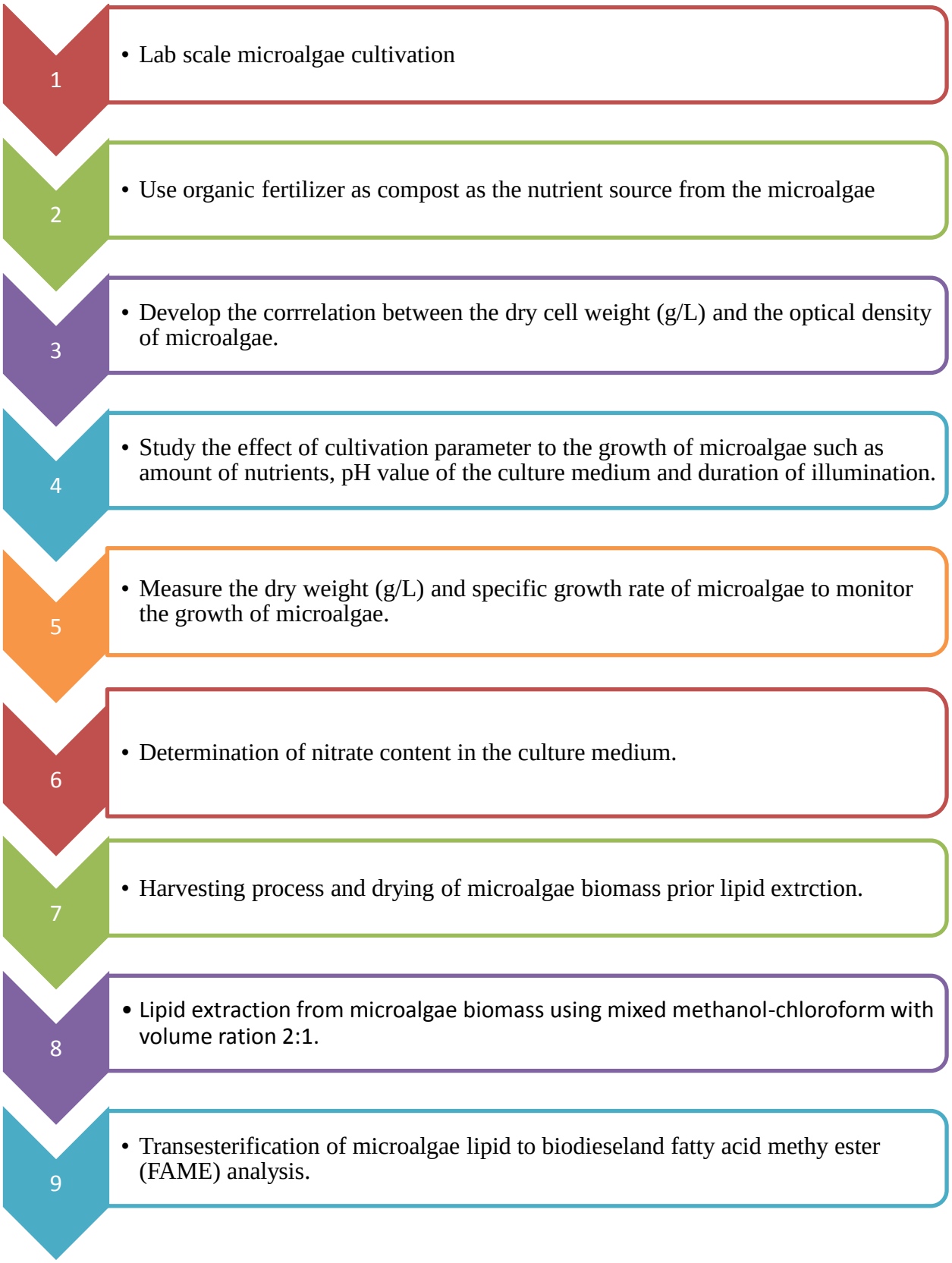
3.6 Microalgae Lipid Extraction

10 grams of dried *C.vulgaris* biomass will be placed in cellulose will be placed in a cellulose thimble and Soxhlet extractor will be used for extraction process. Methanol-chloroform with volume ratio of 2:1 will be used as solvent for the lipid extraction process. A total of 250 mL of solvent will be placed in the Soxhlet extractor and heat to 60-65 °C for a day or 24 hours. After that, the solvent will be evaporated in a rotary evaporator and the leftover lipid will be collected. The extraction is repeated twice using the same solvent for the residue that was left after the evaporation. All sampling will be conducted triplicate. (Man Kee Lam, 2012)

3.7 Transesterification reaction and Fatty Acid Methyl Ester (FAME) Analysis

1 gram of crude *C. vulagaris*, Methanol to lipid molar ration of 15:1 and 3 wt % of concentrated sulphuric acid as catalyst will be reacted for transesterification process. The reaction will be carried out in water bath shaker at 60 °C for around 3 hours. 1µ of the reaction product is subject to gas chromatography-mass spectrometry analysis after the reaction has completed. The gas chromatography-mass will be equipped with Flame Ionization Detector and Elite 5-MS column (30m x 0.25 mm x0.25mm) . The initial oven temperature is 65 °C which is held for 2 min and will be raised to 280 °C at ramping rate of 8°C/min and held at 280°C for 10 minute while the injector temperature is set to 250 °C. The compounds detected will be identified and quantified by using the NIST Mass Spectral Program. (Man Kee Lam, 2012)

3.8 Process Flow of the Research Project



3.9 Set-Up of the Experiment

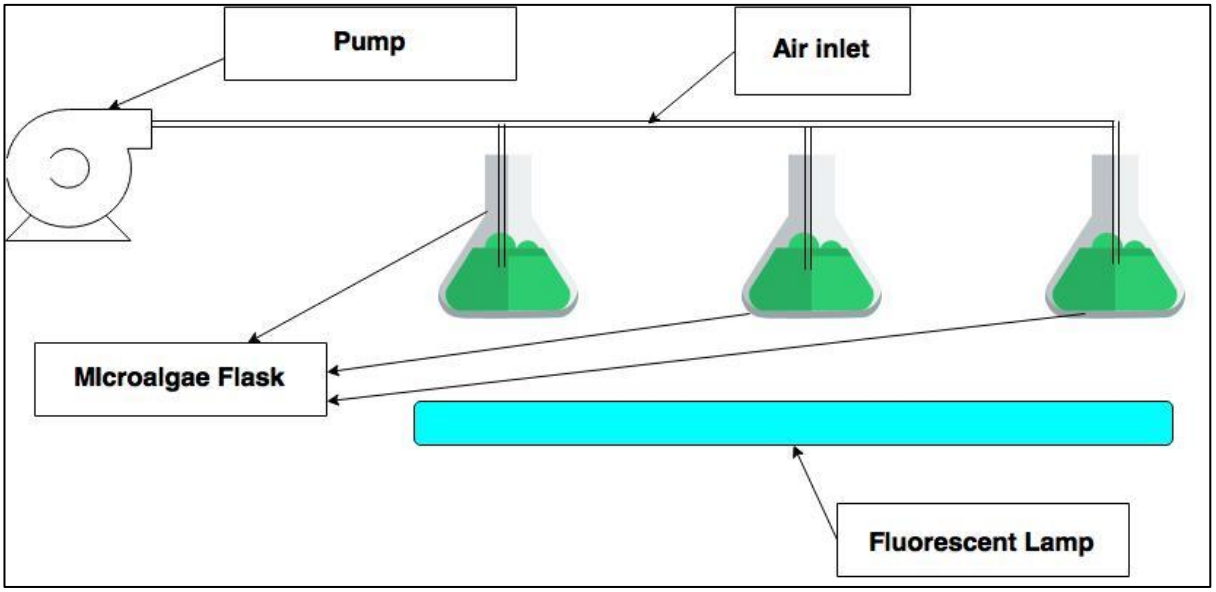


FIGURE 3.1. Microalgae Lab Setup

TABLE 3.2. Project Gantt Chart

Final Year Project	Final Year Project 1														Final Year Project 2													
Week(s)	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	15
Selection of Project Topic																													
Preliminary Research Work																													
Work on Extended Proposal and Submission																													
Proposal Defence																													
Continuation of Project Work																													
Work on Interim Report and Submission																													
Continuation of Project Work																													
Work on Progress Report and Submission																													
Continuation of Project Work																													
Submission of Technical Paper and Viva																													
Submission of Project Dissertation (Hardbound)																													

3.10 Key Milestone

TABLE 3.3 Key Milestone

FYP	Date Start	Date End	Period	Milestone
FYP 1	27/5/2015	27/5/2015	1 day	Select project title for FYP.
	5/7/2015	3/7/2015	4 weeks	Completed writing the extended proposal and submit to the supervisor.
	14/7/2015	15/7/2015	2 days	Present Proposal Defence
	16/7/2015	14/8/2015	6 weeks	Submit the Final Interim Report.

FYP	Date Start	Date End	Period	Milestone
FYP 2	7/9/2015	17/9/2015	11 days	Grow the *Chorella Vulgaris* in 5 L Bottle and perform Calibration Curve.
	18/9/2015	5/10/2015	18 days	Set up and test the Effect of Amount of Nutrient of Peat Moss Compost.
	6/10/2015	25/10/2015	20 days	Set up and test the Effect of Amount of Nutrient of Goat Compost.
	7/9/2015	9/11/2015	7 weeks	Submission of Progress Report
	26/10/2015	16/11/2015	20 days	Set up and test the Effects of Initial pH level and Colour Filter
	17/11/2015	4/12/2015	18 days	Set up and test the Effect of amount of Phosphate/ Effect of Amount of Seed
	5/12/2015	9/12/2015	5 days	Lipid Extraction and Biodiesel Production
	10/9/2015	17/12/2015	7 days	Submission of Soft Bound Dissertion and Viva
	12/12/2015	12/1/2016	4 weeks	Hard Bound Submission

CHAPTER 4

RESULT AND DISCUSSION

4.1 Calibration and Preliminary Study

In the preliminary study, a correlation between the optical density of *Chlorella Vulgaris* and biomass was pre-determined. The optical density was measured daily at 688nm by using spectrophotometer. This correlation is called calibration.

Calibration is a very important level or stage in almost all the measurements procedure. Calibration graph establish or develops the relationship between the output of the measurement system and the accepted values of calibration standards. The calibration normally involves the preparation of a set of standards containing known amount of analyte or substance which need to be measured, measuring the instrument response which is in term of Abs for UV-VIS spectrophotometer for each standard and finally develop a relationship between the instrument response and analyte concentration. This correlation is used to transform the measurement made on test samples into the amount of analyte sample. In this case the absorbance measurement is transformed into gram per litre.

There are actually 7 numbers of stages to perform this calibration as shown below:

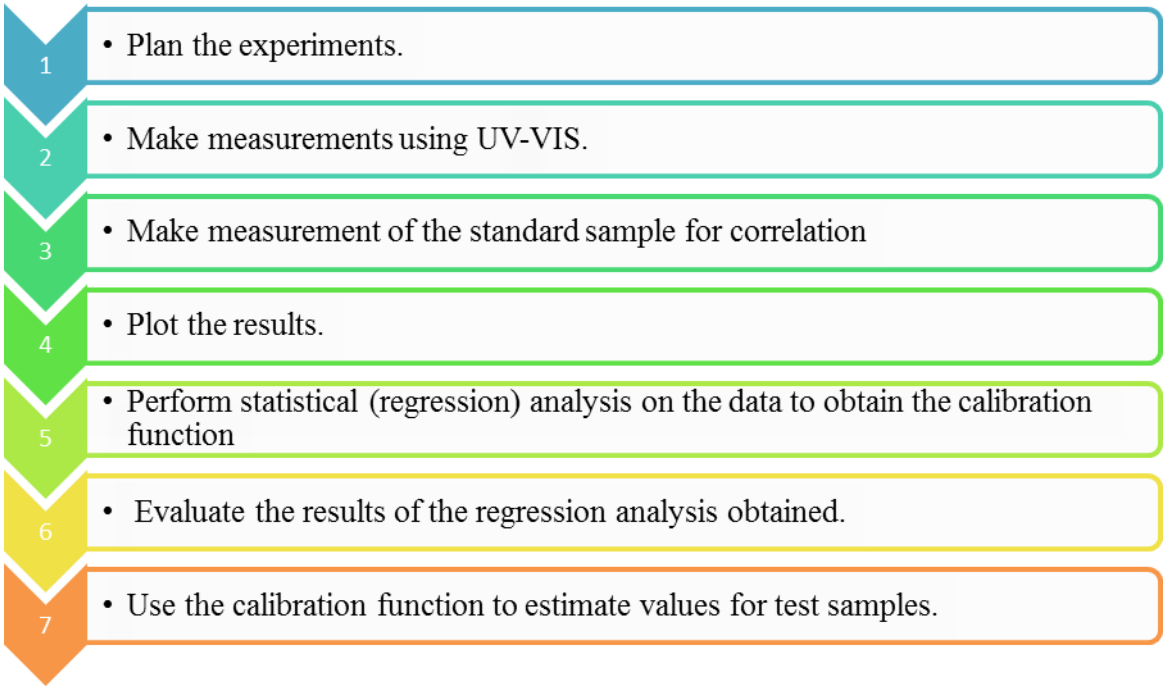


FIGURE 4.1 Calibration Stages

The optical density was measured daily at 688nm by using spectrophotometer. The 5ml sample was centrifuged at 10 × 1000g for 5 minutes and the supernatant was slowly decanted back into the culture medium. Then, the microalgae biomass was dried in an oven at 100 °C for 24 hours. The next day, the weight of the microalgae biomass will be measured and changed into gram per litre. The correlation obtained is shown in Figure 4.1.

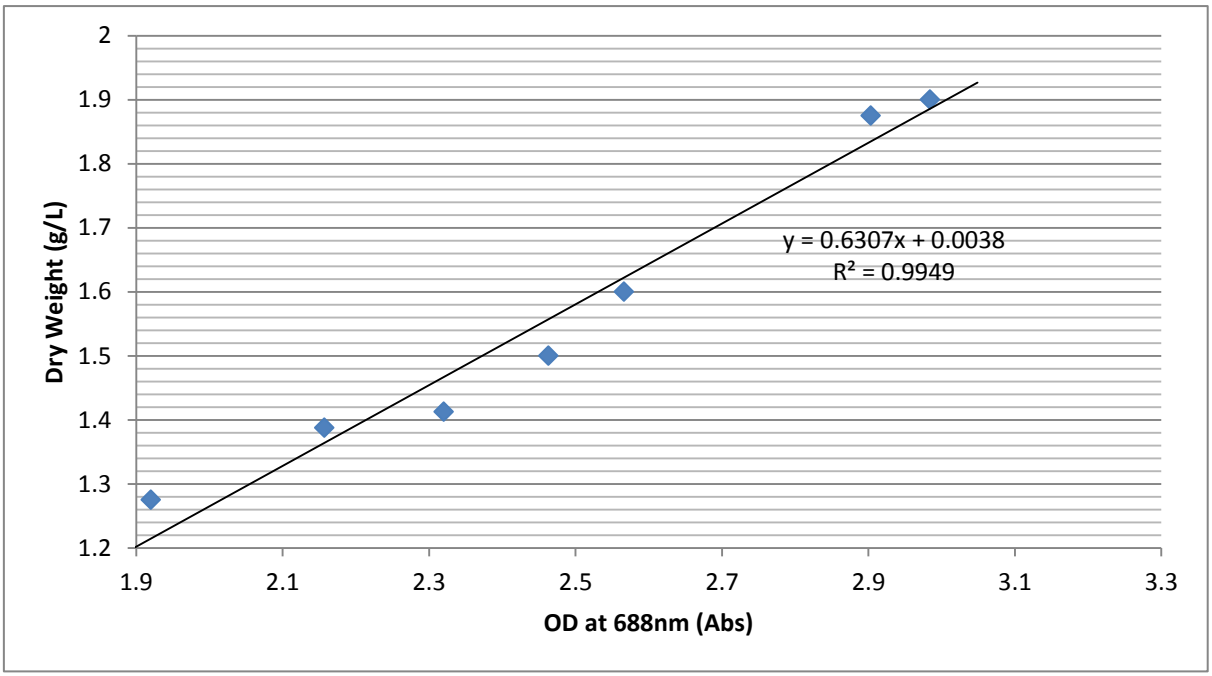


FIGURE 4.2 Graph of Microalgae Dry Weight Vs Optical Density at 688nm

The obtained linear correlation from figure 4.1 is as shown in the equation below:

Dry Weight (g/L) = $0.6307 \times OD_{688nm} + 0.0038$, $R^2 = 0.9949$

(Eqn 4.1)

The specific growth rate (***μ***) was measured by using below:

$$\mu(day^{-1}) = \frac{\ln(N_2/N_1)}{t_2 - t_1}$$

(Eqn 4.2)

where N_1 and N_2 are defined as biomass (g/L) at time t_1 and t_2 .

Meanwhile, the biomass productivity was measured using the equation:

$$Bimass\ productivty\ \left(\frac{\frac{g}{L}}{day}\right) = \frac{N_f - N_i}{Total\ cultivation\ time\ (day)}$$

(Eqn 4.3)

Where N_f is the final reading of biomass (g/L) and N_i is the initial biomass (g/L) reading

4.2 Effects of Amount of Nutrient and Comparison Type of Compost

The nutrient uptake by the microalgae is affected by overall composition of the nutrient that are available in the culture medium where nitrogen and phosphorus acts a important nutrients for the growth of microalgae. There is always a optimal or balanced ratio of N/P exist so that the growth of microalgae is at maximum (Kunikane, Kaneko & Maehara, 1984).

There are two main compost that is available which is animal compost and plant compost. The main difference between both compost is the nutrition content. Compost which is from plant contains low N-P-K (Nitrogen-Phosphorous-Potassium) values (Havens, K.E. et al. 1999) compare to the animal compost.

Figure 4.2 shows the the growth of *Chorella Vulgaris* in Peat Moss Compost whereas Figure 4.3 shows the growth rate of *Chorella Vulgaris* Goat Compost. The timeline for each each experiment was for 16 days. During this timeline, there are actually 5 stages of growth that the microalgae went through in the cultures. (Fogg and Thake, 2014). The five stages is as follows:

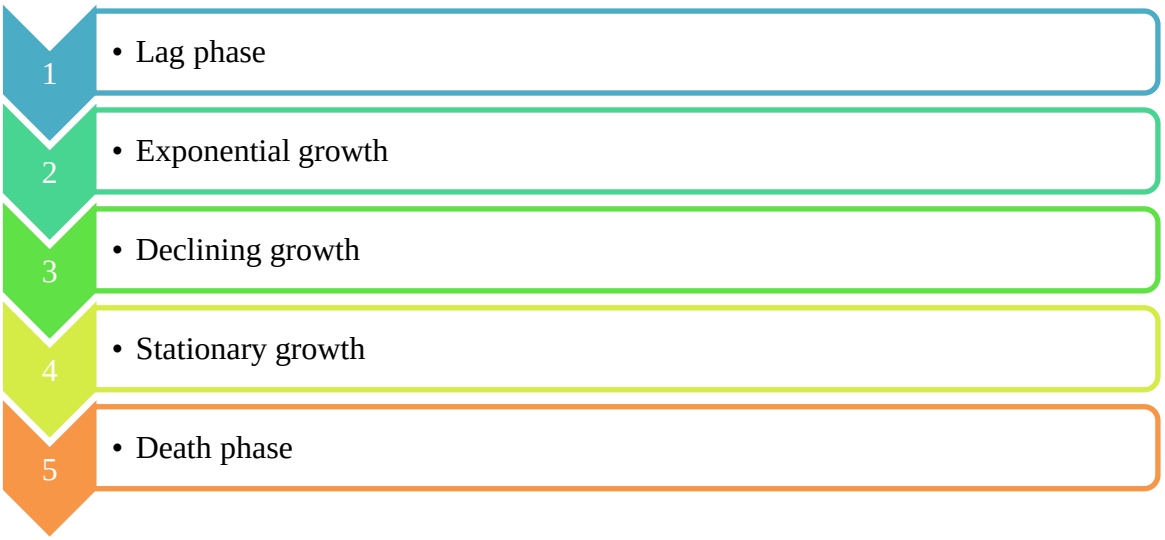


FIGURE 4.3 Five Stages of Microalgae Growth

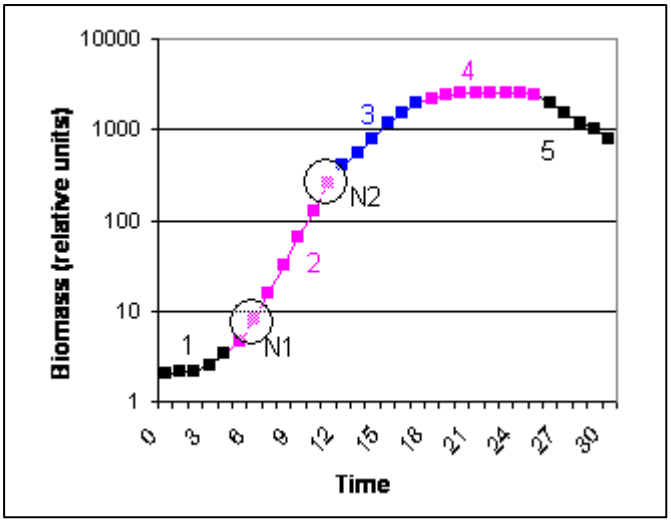


FIGURE 4.4. General Pattern of Microalgal Growth in Batch Cultures

1) Lag phase

Lag phase is a condition which the period of microalgae takes time to adapt to its new culture from its inoculum condition or source (Spencer, 2012). The lag phase duration depends on the condition of the source of the microalgae. If the sample is taken from a healthy exponentially growing culture, the microalgae will have short period of lag phase if compared to unhealthy growing culture of microalgae which will have longer time of la phase (Liu, J., Sun, Z., & Chen, F, 2014).

2) Exponential phase and calculating growth rates

The indication for increase in biomass over the time is measured by calculating the growth of the microalgae. This growth rate is calculated when the growth shows exponential phase where there are two extreme values. The duration of exponential phase depends on the availability of the nutrient in the medium, the capacity and the culturing condition of the microalgae.

3) Declining growth

When there is limitation which inhibits the reproduction of microalgae, this causes declining growth in microalgae. In this specific phase, microalgae often encounters very high exhaustion or deficiency of nutrient (Chinnasamy,S.,Bhatnagar,A., Claxton, R., & Das, K. C, 2010). When this

happens, the microalgae cannot grow exponentially. This proves the slight bend of the graph after rapid growth of the microalgae.

4) Stationary phase

Stationary phase is a phase where the microalgae growth stays stagnant without showing any exponential of decline growth. This means all the nutrient have been used up and the microalgae is just maintaining the growth achieved.

5) Death phase

This is the last phase of the growth of microalgae. After the stationary phase which is after the survival period, death phase arrives. The graph will shows decreasing values of growth.

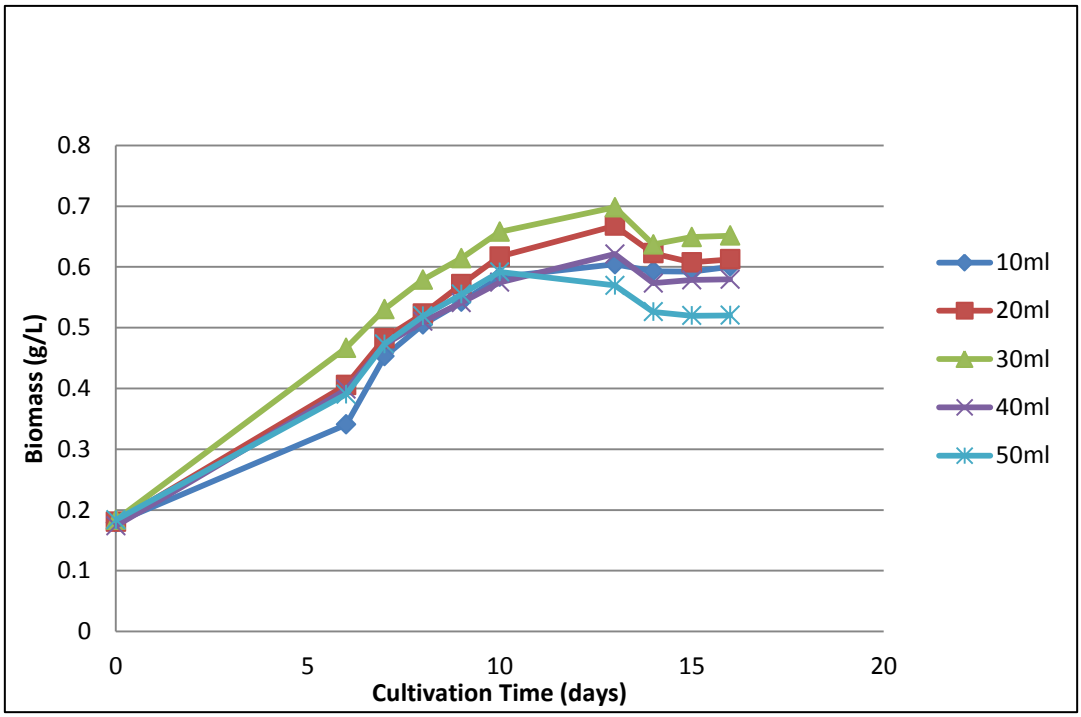


FIGURE 4.5. Effect of initial nutrient concentration of Peat Moss Compost towards the growth of *Chorella Vulgaris.* Other Culture Condition is :Initial pH= 3.00, Amount of seed= 100 mL and Illumination for 24 hours Continuously

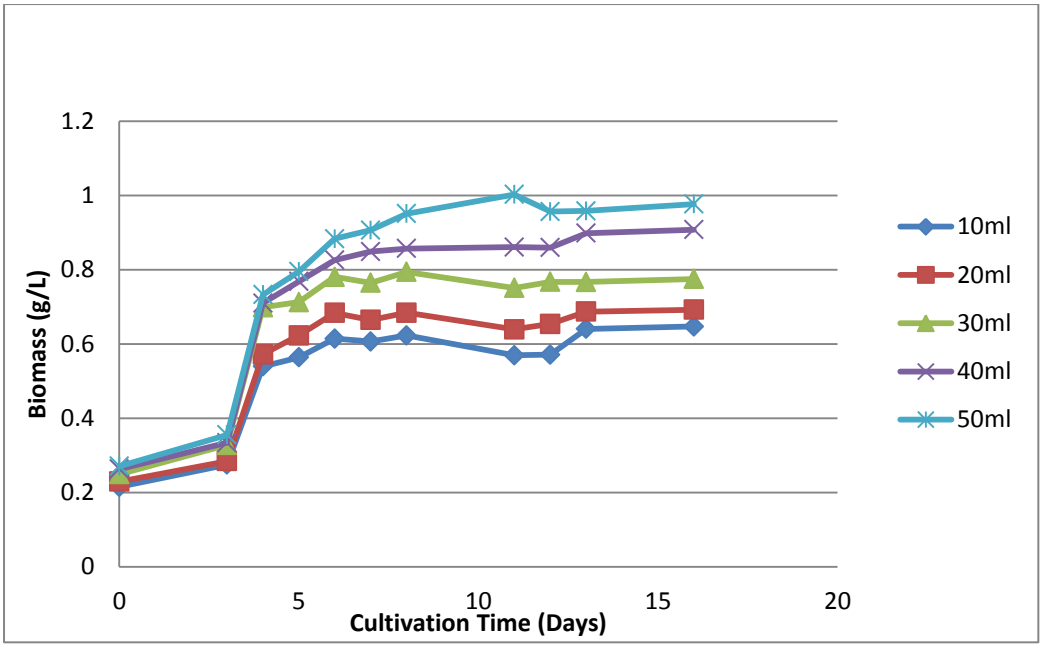


FIGURE 4.6. Effect of initial nutrient concentration of Goat Compost towards the growth of *Chorella Vulgaris*. Other Culture Condition is :Initial pH= 3.00, Amount of seed= 100 mL and Illumination for 24 hours Continuously

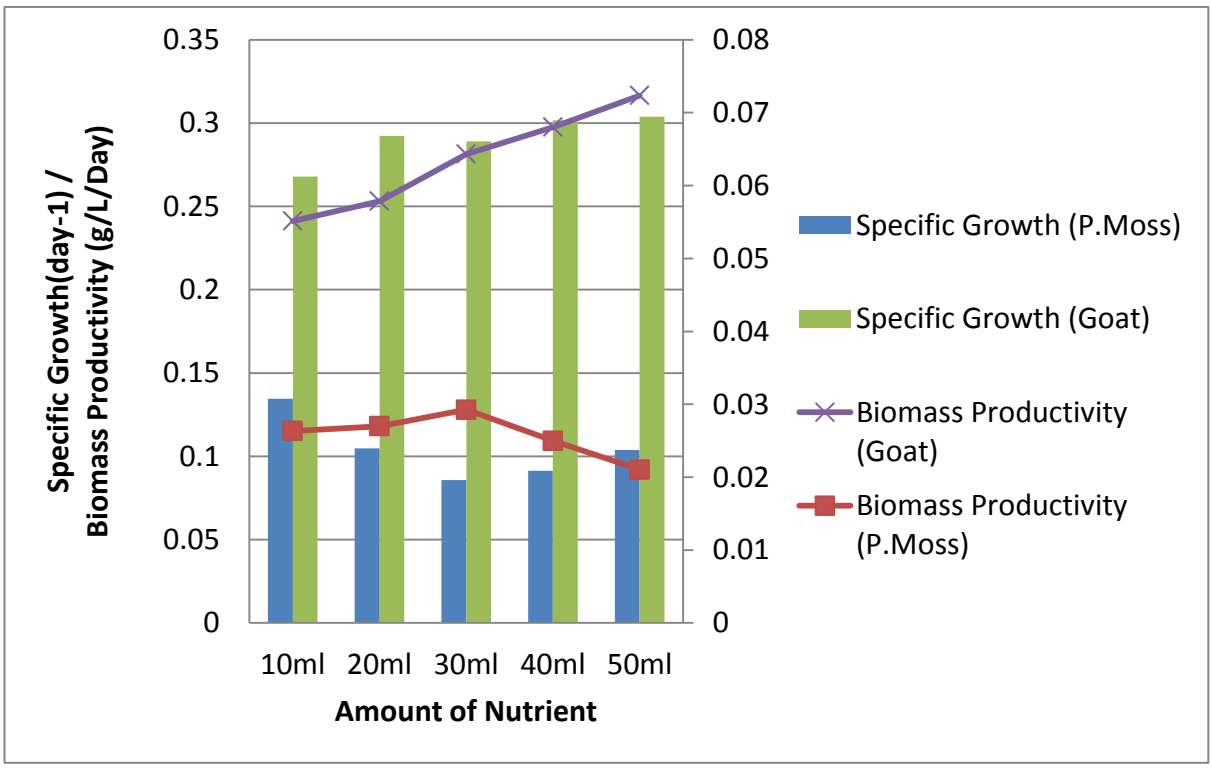


FIGURE 4.7. Specific Growth and Biomass Productivity of Chorella Vulgaris in Peat Moss Compost

From Figure 4.5, it shows that the highest growth for *Chorella Vulgaris* in Peat Moss Compost culture medium, was at 30ml nutrient, in which maximum biomass recorded was 0.67 g/L. It was observed that the growth of microalgae increase from 10ml to 30ml and started to decrease from 40ml-50ml. This shows the optimum nutrient level for Peat Moss Compost culture medium is 30ml. Although 40ml and 50ml has higher nutrient concentration than 30ml, however, it may also induce severe contamination to the cultivation medium and thus resulting to the lower growth rate of microalgae. It is also suspected there is presence of herbicide in the plant compost itself.

From Figure 4.6, it shows that the highest for *Chorella Vulgaris* in Goat Compost culture medium, is when the nutrient is 50ml in which maximum biomass recorded was 1.00 g/L. The growth rate increase from 10ml to 50ml. This shows that the optimum nutrient level for Goat Compost culture medium is 50ml. It can be concluded that, for this culture, the higher the nutrient level, the higher the growth of *Chorella Vulgaris.* This is maybe due to less contamination of compost compare to the Peat Moss Compost.

From both Figure 4.5 and Figure 4.6, we can conclude that Goat Compost provide a better culture medium as the growth of *Chorella Vulgaris* for Goat Compost is higher than the Peat Moss Compost. In other words, animal based compost is a better culture medium compare to plant based compost. This theory is further supported in Figure 4.7, in which the highest reading of biomass productivity of goat was 0.0513 g/L/day compare to peat moss which only shows 0.02105 g/L/day. From figure 4.5, we can also make a relation which the higher the specific growth of microalgae, the higher the amount of biomass produced.

4.3 Effects of Initial pH

One of the most important factor in cultivation of microalgae growth is the pH of the culture medium. Different pH can give effect to the microalgae growth in number of ways (Marshal1 & Orr 1948, Park et al. 1958). The distribution of carbon dioxide species can change and at extreme pH levels can cause physiological effects towards the microalgae (Hein, Morten Foldager Pedersen, Kaj Sand-Jensen,1995). Most of the microalgae can grow at in the neutral to alkaline range but however, there are some species of microalgae that can grow well in acidic condition as low as pH 1 (Raven ,1990) .This pH level factor is very essential for regulating the microalgae abundance and distribution (Salvi, B. L., & Panwar, N. L., 2012). The effect of pH level to the cultivation of microalgae is a very important or crucial data which will be very useful in the future when the flue gas is utilized for microalgae cultivation in an industrial scale where the suitable microalgae strains should be tolerable to the inconsistent concentration of CO_2 in the flue gas that results in pH variation (Tang, Han, Li, Miao & Zhong, 2011).

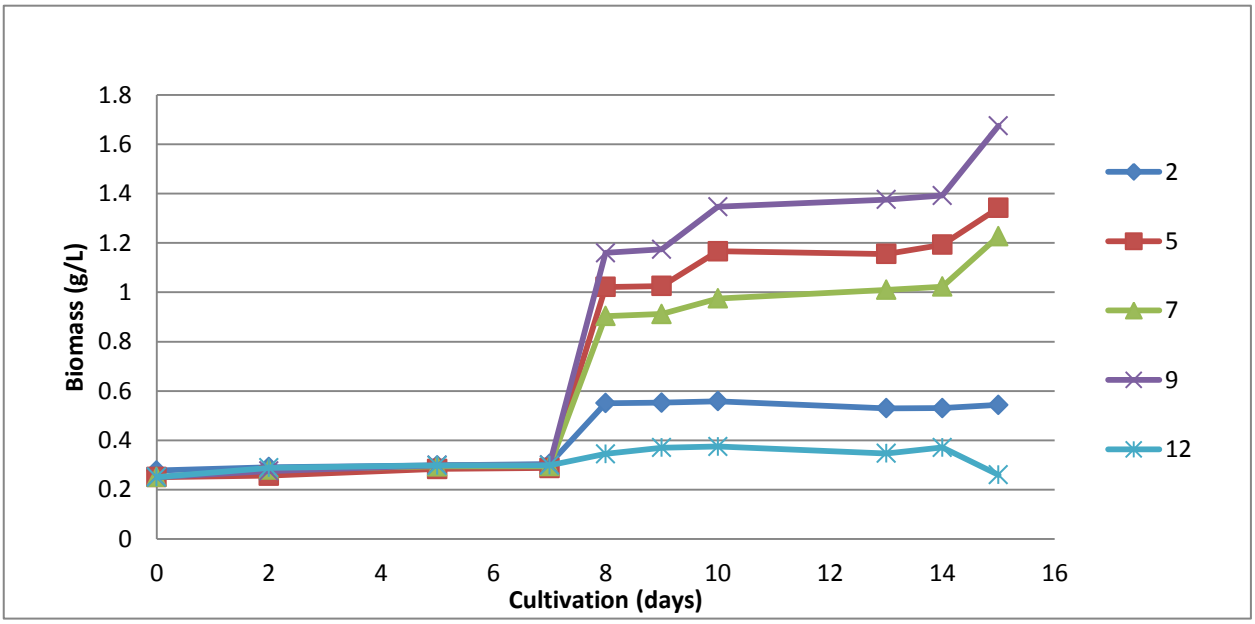


FIGURE 4.8. Effects of Initial pH Value towards the growth of *Chorella Vulgaris.* Other Culture Condition is : Amount of seed= 100 mL and Illumination for 24 hours Continuously

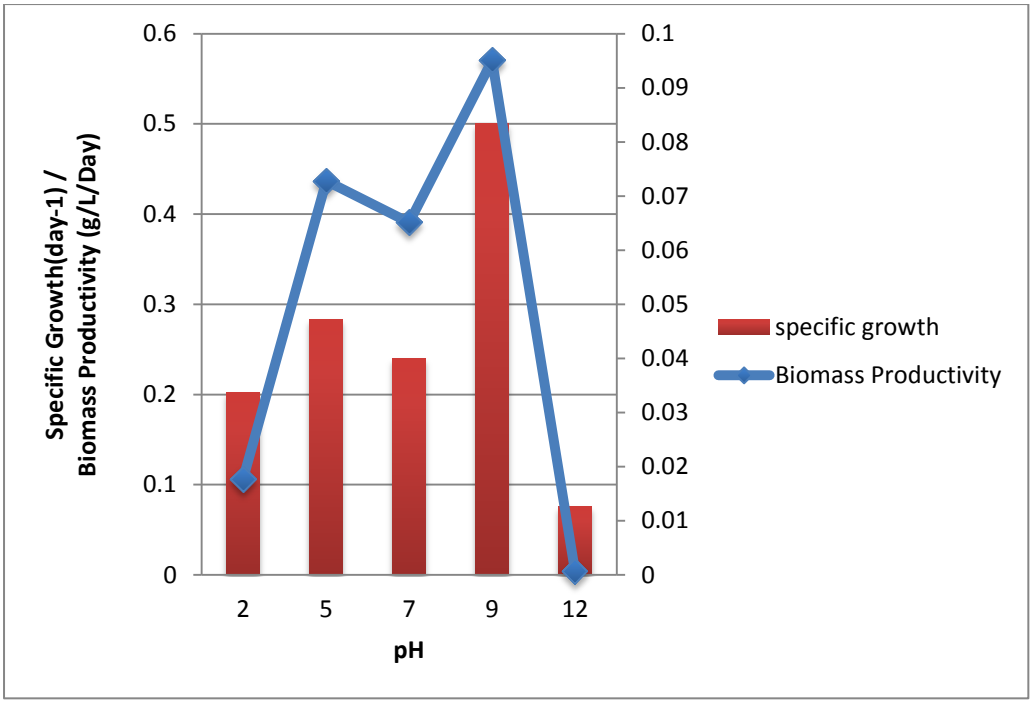


FIGURE 4.9 Specific Growth and Biomass Productivity of *Chorella Vulgaris* in intial pH value

Figure 4.8 shows effect of intial pH value of the culture medium towards the growth of *Chlorella Vulgaris.* Based on the result that we obtained, *Chlorella Vulgaris* showed a similar trend and almost a linear growth at intial pH of 5, 7 and 9 with maximum biomass obtained ranging from 1.20 to 1.60 g/L after 15 days of cultivation. For lower initial pH of the culture medium, the microalgae did not exhibit a satisfactory growth. This case is also same for the highest pH culture medium. The growth of microalgae at pH 2 and 12 was stagnant and showed no significant increment of biomass produced even after 15 days of cultivation. From Figure 4.6, the sample with initial pH of 9 recorded the highest specific growth which is at 0.50 day^{-1}. From these results, it was found that *Chlorella Vulgaris* in the present study favoured the culture medium at initial pH of 9 as it recorded the highest biomass productivity. This result also indicates that *Chlorella Vulgaris* used in this study can adapt very well to highpH medium which is an advantage to inhibit other contaminants such as fungus that exist in the culture medium and enable the microalgae to sustain the growth naturally.

4.4 Effects of Colour Filter

Different colour filter or colour spectra has been shown to be capable of stimulating the growth of various microalgae which a further studies have suggested an effect of light wavelength have effects on the synthesis of some intracellular molecules (Mohsenpour, Richards B, Willoughby N, 2012)

In the present study, the effect of different colour filter is tested. There were 5 colour chosen for these experiments which were yellow, purple, green, blue and red. Table below shows the light intensity and wave length of the colour filter.

TABLE 4.1. Light Intensity and Wavelength for Each Colour

Colour	Light Intensity (cd)	Wavelength (nm)
Purple	0.42	455
Green	0.68	577
Red	1.12	780
Orange	2.53	622
Blue	0.37	492

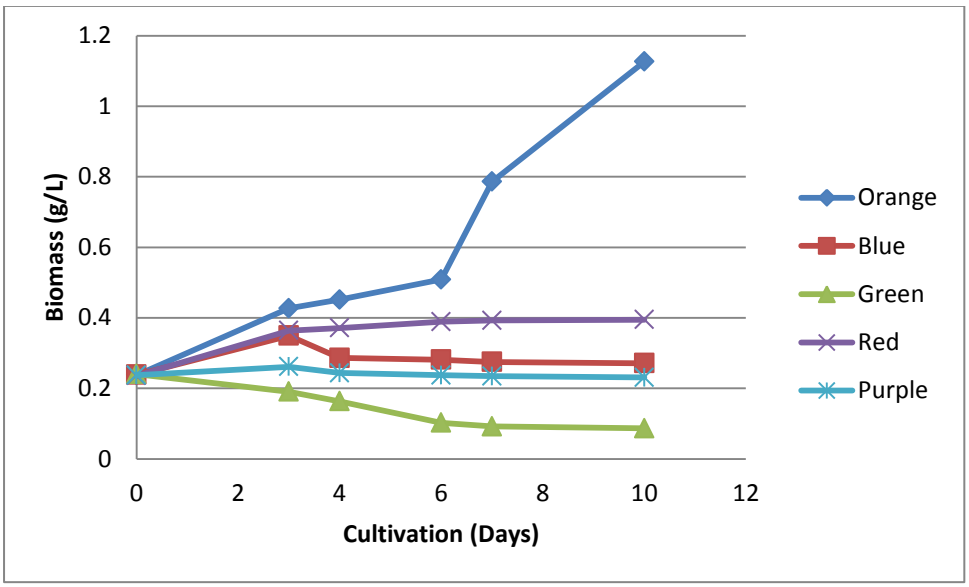


FIGURE 5.0. Effects of Colour Filter towards the growth of *Chlorella Vulgaris*. Other Culture Condition is :Initial pH= 9.00, Amount of seed= 100 mL and Illumination for 24 hours Continuously

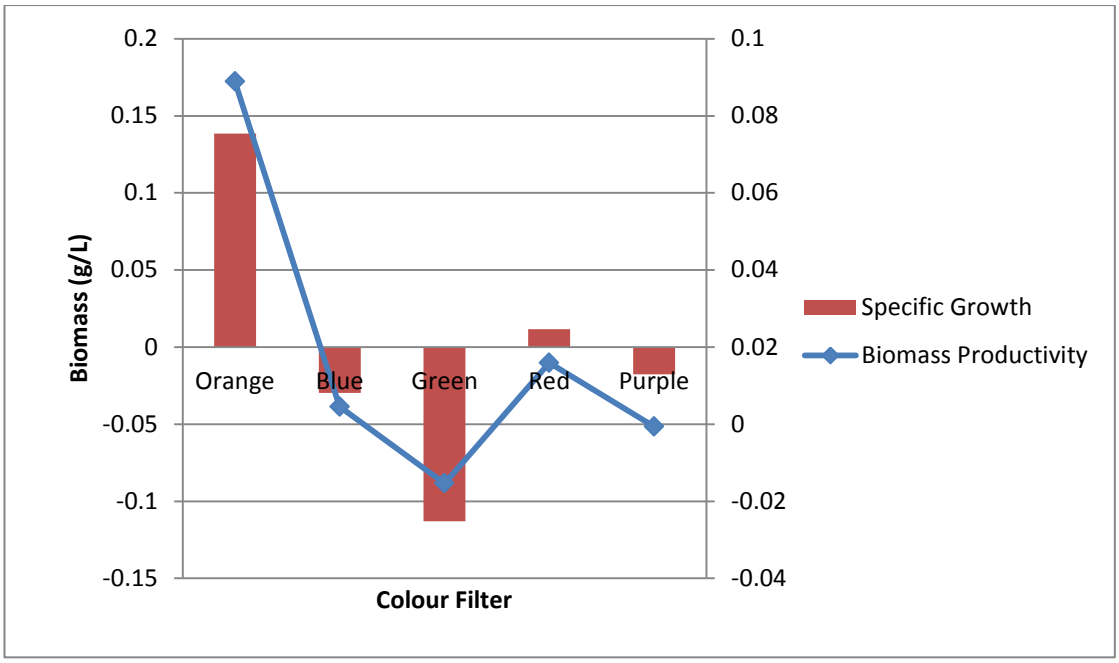


Figure 5.1. Specific Growth and Biomass Productivity of Chorella Vulgaris in different colour filter

From Figure 5.0, it can be observed that sample with orange colour filter recorded the highest biomass at 1.12 g/L compare to other colours which were recorded at lower biomass. This concludes that different wavelength gives different effect towards the growth of microalgae. Orange filter shows the highest biomass productivity because the wavelength of orange filter is in the range of wavelength of chlorophyll absorbance around 600-680nm. Other than that, the higher light intensity also boost up the growth of the microalgae.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The potential for utilization of microalgae for biodiesel compare to other sources is very high. An integrated development of algae by using engineering process is needed to increase the efficiency of this production of biodiesel from microalgae. This experiment is very important to initiate the usage or production of biodiesel in Malaysia itself in a large scale. Biodiesel can be a great alternative to the diesel. Furthermore, this biodiesel will be the solution for the oil price which is rising tremendously. These experiments can discover or maximize the yield of biodiesel from the microalgae.

5.2 Recommendations

One of the recommendations for this project will be to use wastewater as nutrient source. For the purpose of environmental sustainability in microalgae biofuel production, wastewater can be used as an alternative nutrient source to cultivate microalgae while at the same time purifying the wastewater. Hence, by using wastewater to cultivate microalgae provides mutual benefit of producing biofuel and removing nitrogen and phosphorus as well as organic carbon from the wastewater. Second recommendation will be to use solar energy or outdoor cultivation. This will let the microalgae to grow naturally and save the usage of electric for uv light. Last but not least, maybe different type of microalgae species can be used such as *nanochloropsis sp* which have high resistance and easier to be handled.

One of the recommendations for this project will be to have more parameters to test the amount of nutrient that effects the growth of *Chorella Vulgaris*. This is because testing amount of nutrient itself is not enough because sometime different types of culture have different condition to give the best result. Example some culture gives good result if the air is not aerated and be given real sun light compare to artificial illumination. In other words, few parameters have to be done in outdoor. This can give better comparison between the results.

REFERENCES

Acién, F. G., Fernández, J. M., & Molina-Grima, E. (2014). Chapter 14 - Economics of Microalgae Biomass Production. In A. P.-J. L. C. R. Soccol (Ed.), Biofuels from Algae (pp. 313-325). Amsterdam: Elsevier.

Azachi, M., Sadka, A., Fisher, M., Goldshlag, P., Gokhman, I., & Zamir, A. (2002). Salt induction of fatty acid elongase and membrane lipid modifications in the extreme halotolerant alga Dunaliella salina. Plant Physiology, 129(3), 1320-1329. doi: 10.1104/pp.001909

Banerjee, A., Sharma, R., Chisti, Y., & Banerjee, U. C. (2002). Botryococcus braunii: A renewable source of hydrocarbons and other chemicals. Critical Reviews in Biotechnology, 22(3), 245-279. doi: 10.1080/07388550290789513

Barupal, D. K., Kind, T., Kothari, S. L., Lee, D. Y., & Fiehn, O. (2010). Hydrocarbon phenotyping of algal species using pyrolysis-gas chromatography mass spectrometry. BMC Biotechnology, 10. doi: 10.1186/1472-6750-10-40

Bigelow, N. W., Hardin, W. R., Barker, J. P., Ryken, S. A., MacRae, A. C., & Cattolico, R. A. (2011). A comprehensive GC-MS Sub-microscale assay for fatty acids and its applications. JAOCS, Journal of the American Oil Chemists' Society, 88(9), 1329-1338. doi: 10.1007/s11746-011-1799-7

Biller, P., Ross, A. B., Skill, S. C., Lea-Langton, A., Balasundaram, B., Hall, C., Llewellyn, C. A. (2012). Nutrient recycling of aqueous phase for microalgae cultivation from the hydrothermal liquefaction process. Algal Research, 1(1), 70-76. doi: 10.1016/j.algal.2012.02.002

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. Canadian journal of biochemistry and physiology, 37(8), 911-917.

Bölling, C., & Fiehn, O. (2005). Metabolite profiling of Chlamydomonas reinhardtii under nutrient deprivation. Plant Physiology, 139(4), 1995-2005. doi: 10.1104/pp.105.071589

Brennan & Owende. "Biofuels from Microalgae. Renewable and Sustainable Energy Reviews". 2009.

Cha, T. S., Chen, J. W., Goh, E. G., Aziz, A., & Loh, S. H. (2011). Differential regulation of fatty acid biosynthesis in two Chlorella species in response to nitrate treatments and the potential of binary blending microalgae oils for biodiesel application. Bioresource Technology, 102(22), 10633-10640. doi: 10.1016/j.biortech.2011.09.042

Chen, M., Tang, H., Ma, H., Holland, T. C., Ng, K. Y. S., & Salley, S. O. (2011). Effect of nutrients on growth and lipid accumulation in the green algae Dunaliella tertiolecta. Bioresource Technology, 102(2), 1649-1655. doi: 10.1016/j.biortech.2010.09.062

Chinnasamy, S., Bhatnagar, A., Claxton, R., & Das, K. C. (2010). Biomass and bioenergy production potential of microalgae consortium in open and closed bioreactors using untreated carpet industry effluent as growth medium. Bioresource Technology, 101(17), 6751-6760. doi: 10.1016/j.biortech.2010.03.094

Christi, Y., "Biodiesel from Microalgae," Biotechnology Advances, Vol. 25, 2007, pp. 294-306.

Collet, P., Spinelli, D., Lardon, L., Hélias, A., Steyer, J.-P., & Bernard, O. (2014). Chapter 13 - Life-Cycle Assessment of Microalgal-Based Biofuels. In A. P.-J. L. C. R. Soccol (Ed.), Biofuels from Algae (pp. 287-312). Amsterdam: Elsevier.

Collet, P., Spinelli, D., Lardon, L., Hélias, A., Steyer, J.-P., & Bernard, O. (2014). Chapter 13 - Life-Cycle Assessment of Microalgal-Based Biofuels. In A. P.-J. L. C. R. Soccol (Ed.), Biofuels from Algae (pp. 287-312). Amsterdam: Elsevier.

Dalmas Neto, C. J., Sydney, E. B., Assmann, R., Neto, D., & Soccol, C. R. (2014). Chapter 7 - Production of Biofuels from Algal Biomass by Fast Pyrolysis. In A. P.-J. L. C. R. Soccol (Ed.), Biofuels from Algae (pp. 143-153). Amsterdam: Elsevier.

Davis, R., Aden, A., & Pienkos, P. T. (2011). Techno-economic analysis of autotrophic microalgae for fuel production. Applied Energy, 88(10), 3524-3531. doi: 10.1016/j.apenergy.2011.04.018

Doan, T. T. Y., & Obbard, J. P. (2012). Enhanced intracellular lipid in Nannochloropsis sp. via random mutagenesis and flow cytometric cell sorting. Algal Research, 1(1), 17-21. doi: 10.1016/j.algal.2012.03.001

Doan, T. T. Y., Sivaloganathan, B., & Obbard, J. P. (2011). Screening of marine microalgae for biodiesel feedstock. Biomass and Bioenergy, 35(7), 2534-2544. doi: 10.1016/j.biombioe.2011.02.021

Droop, M. R. (1954). A note on the isolation of small marine algae and flagellates for pure cultures. J Mar Biol Assoc, 33, 511-514.

Durrett, T. P., Benning, C., & Ohlrogge, J. (2008). Plant triacylglycerols as feedstocks for the production of biofuels. Plant Journal, 54(4), 593-607. doi: 10.1111/j.1365-313X.2008.03442.x

Elliott, D. C., Hart, T. R., Schmidt, A. J., Neuenschwander, G. G., Rotness, L. J., Olarte, M. V., . . . Holladay, J. E. (2013). Process development for hydrothermal liquefaction of algae feedstocks in a continuous-flow reactor. Algal Research, 2(4), 445-454. doi: 10.1016/j.algal.2013.08.005

Fang, S.-C. (2014). Chapter 3 - Metabolic Engineering and Molecular Biotechnology of Microalgae for Fuel Production. In A. P.-J. L. C. R. Soccol (Ed.), Biofuels from Algae (pp. 47-65). Amsterdam: Elsevier.

Fang, S.-C. (2014). Chapter 3 - Metabolic Engineering and Molecular Biotechnology of Microalgae for Fuel Production. In A. P.-J. L. C. R. Soccol (Ed.), Biofuels from Algae (pp. 47-65). Amsterdam: Elsevier.

Francisco, É. C., Neves, D. B., Jacob-Lopes, E., & Franco, T. T. (2010). Microalgae as feedstock for biodiesel production: Carbon dioxide sequestration, lipid production and biofuel quality. Journal of Chemical Technology and Biotechnology, 85(3), 395-403. doi: 10.1002/jctb.2338

Gavrilescu, M., & Chisti, Y. (2005). Biotechnology - A sustainable alternative for chemical industry.

Greenwell, H. C., Laurens, L. M. L., Shields, R. J., Lovitt, R. W., & Flynn, K. J. (2010). Placing microalgae on the biofuels priority list: A review of the technological challenges. Journal of the Royal Society Interface, 7(46), 703-726. doi: 10.1098/rsif.2009.0322

Griffiths, M. J., & Harrison, S. T. L. (2009). Lipid productivity as a key characteristic for choosing algal species for biodiesel production. Journal of Applied Phycology, 21(5), 493-507. doi: 10.1007/s10811-008-

Havens, K.E. et al. 1999. Algal responses to experimental nutrient addition in the littoral community of a subtropical lake. Freshwater Biology 42:329-344

Ho, S. H., Chen, C. Y., & Chang, J. S. (2012). Effect of light intensity and nitrogen starvation on CO 2 fixation and lipid/carbohydrate production of an indigenous microalga Scenedesmus obliquus CNW-N. Bioresource Technology, 113, 244-252. doi: 10.1016/j.biortech.2011.11.133

Hosseini Tafreshi, A., & Shariati, M. (2009). Dunaliella biotechnology: Methods and applications. Journal of Applied Microbiology, 107(1), 14-35. doi: 10.1111/j.1365-2672.2009.04153.x

Hu, Q., Sommerfeld, M., Jarvis, E., Ghirardi, M., Posewitz, M., Seibert, M., & Darzins, A. (2008). Microalgal triacylglycerols as feedstocks for biofuel production: Perspectives and advances. Plant Journal, 54(4), 621-639. doi: 10.1111/j.1365-313X.2008.03492.x

Huerlimann, R., de Nys, R., & Heimann, K. (2010). Growth, lipid content, productivity, and fatty acid composition of tropical microalgae for scale-up production. Biotechnology and Bioengineering, 107(2), 245-257. doi: 10.1002/bit.22809

Iersel, S., & Flammini, A. (2010). Algae-based Biofuels: Applications and Co-products, FAO, No. 44.

Imahara, H., Minami, E., & Saka, S. (2006). Thermodynamic study on cloud point of biodiesel with its fatty acid composition. Fuel, 85(12-13), 1666-1670. doi: 10.1016/j.fuel.2006.03.003

Johnson, M. K., Johnson, E. J., MacElroy, R. D., Speer, H. L., & Bruff, B. S. (1968). Effects of salts on the halophilic alga Dunaliella viridis. Journal of Bacteriology, 95(4), 1461-1468.

Kagan, J., & Bradford, T. (2009). Biofuels 2010: Spotting the Next Wave.

Krisnangkura, K. (1986). SIMPLE METHOD FOR ESTIMATION OF CETANE INDEX OF VEGETABLE OIL METHYL ESTERS. JAOCS, Journal of the American Oil Chemists' Society, 63(4), 552-553.

Lamers, P. P., Van De Laak, C. C. W., Kaasenbrood, P. S., Lorier, J., Janssen, M., De Vos, R. C. H., . . . Wijffels, R. H. (2010). Carotenoid and fatty acid metabolism in light-stressed Dunaliella salina. Biotechnology and Bioengineering, 106(4), 638-648. doi: 10.1002/bit.22725

Lang, I., Hodac, L., Friedl, T., & Feussner, I. (2011). Fatty acid profiles and their distribution patterns in microalgae: A comprehensive analysis of more than 2000 strains from the SAG culture collection. BMC Plant Biology, 11. doi: 10.1186/1471-2229-11-124

Lepage, G., & Roy, C. C. (1986). Direct transesterification of all classes of lipids in a one-step reaction. Journal of Lipid Research, 27(1), 114-120.

Li, Y., Horsman, M., Wu, N., Lan, C. Q., & Dubois-Calero, N. (2008). Biofuels from microalgae. Biotechnology Progress, 24(4), 815-820. doi: 10.1021/bp.070371k

Liang, Y., Sarkany, N., & Cui, Y. (2009). Biomass and lipid productivities of Chlorella vulgaris under autotrophic, heterotrophic and mixotrophic growth conditions. Biotechnology Letters, 31(7), 1043-1049. doi: 10.1007/s10529-009-9975-7

Liu, J., Huang, J., Sun, Z., Zhong, Y., Jiang, Y., & Chen, F. (2011). Differential lipid and fatty acid profiles of photoautotrophic and heterotrophic Chlorella zofingiensis: Assessment of algal oils for biodiesel

Liu, J., Sun, Z., & Chen, F. (2014). Chapter 6 - Heterotrophic Production of Algal Oils. In A. P.-J. L. C. R. Soccol (Ed.), Biofuels from Algae (pp. 111-142). Amsterdam: Elsevier.

Mandal, S., & Mallick, N. (2009). Microalga Scenedesmus obliquus as a potential source for biodiesel production. Applied Microbiology and Biotechnology, 84(2), 281-291. doi: 10.1007/s00253-009-1935-6

Mata, T. M., Martins, A. A., & Caetano, N. S. (2010). Microalgae for biodiesel production and other applications: A review. Renewable and Sustainable Energy Reviews, 14(1), 217-232. doi: 10.1016/j.rser.2009.07.020

Matthew, T., Zhou, W., Rupprecht, J., Lim, L., Thomas-Hall, S. R., Doebbe, A Schenk, P. M. (2009). The metabolome of Chlamydomonas reinhardtii following induction of anaerobic H2 production by sulfur depletion. Journal of Biological Chemistry, 284(35), 23415-23425. doi: 10.1074/jbc.M109.003541

Mendoza, H., Martel, A., Jiménez Del Río, M., & García Reina, G. (1999). Oleic acid is the main fatty acid related with carotenogenesis in Dunaliella salina. Journal of Applied Phycology, 11(1), 15-19. doi: 10.1023/A:1008014332067

Mitra, D., van Leeuwen, J., & Lamsal, B. (2012). Heterotrophic/mixotrophic cultivation of oleaginous Chlorella vulgaris on industrial co-products. Algal Research, 1(1), 40-48. doi: 10.1016/j.algal.2012.03.002

Man Kee Lam, Keat Teong Lee (2012), Potential of using organic fertilizer to cultivate Chlorella vulgaris for biodiesel production, Applied Energy, 303-308:Elsveir.

Pandey, A., Lee, D.-J., Chisti, Y., & Soccol, C. R. (2014). Preface. In A. P.-J. L. C. R. Soccol (Ed.), Biofuels from Algae (pp. xi-xii). Amsterdam: Elsevier.

Pérez, A., Casas, A., Fernández, C. M., Ramos, M. J., & Rodríguez, L. (2010). Winterization of peanut biodiesel to improve the cold flow properties. Bioresource Technology, 101(19), 7375-7381. doi: 10.1016/j.biortech.2010.04.063

Pinzi, S., Leiva, D., Arzamendi, G., Gandia, L. M., & Dorado, M. P. (2011). Multiple response optimization of vegetable oils fatty acid composition to improve

biodiesel physical properties. Bioresource Technology, 102(15), 7280-7288. doi: 10.1016/j.biortech.2011.05.005

Rad, F. A., Aksoz, N., & Hejazi, M. A. (2011). Effect of salinity on cell growth and β-carotene production in Dunaliella sp. isolates from Urmia Lake in northwest f Iran. African Journal of Biotechnology, 10(12), 2282-2289.

Ramos, M. J., Fernández, C. M., Casas, A., Rodríguez, L., & Pérez, A. (2008). Influence of fatty acid composition of raw materials on biodiesel properties. Bioresource Technology, 100(1), 261-268. doi: 10.1016/j.biortech.2008.06.039

Research Council, N. (2012). Sustainable Development of Algal Biofuels in the United States.

Richardson, J. W., Johnson, M. D., & Outlaw, J. L. (2012). Economic comparison of open pond raceways to photo bio-reactors for profitable production of algae for transportation fuels in the Southwest. Algal Research, 1(1), 93-100. doi: 10.1016/j.algal.2012.04.001

Rodolfi, L., Zittelli, G. C., Bassi, N., Padovani, G., Biondi, N., Bonini, G., & Tredici, M. R. (2009). Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. Biotechnology and Bioengineering, 102(1), 100-112. doi: 10.1002/bit.22033

Rosenberg, J. N., Oyler, G. A., Wilkinson, L., & Betenbaugh, M. J. (2008). A green light for engineered algae: redirecting metabolism to fuel a biotechnology revolution. Current Opinion in Biotechnology, 19(5), 430-436. doi: 10.1016/j.copbio.2008.07.008

Salvi, B. L., & Panwar, N. L. (2012). Biodiesel resources and production technologies – A review. Renewable and Sustainable Energy Reviews, 16(6), 3680-3689. doi: http://dx.doi.org/10.1016/j.rser.2012.03.050

Sarin, A., Arora, R., Singh, N. P., Sarin, R., Malhotra, R. K., & Kundu, K. (2009). Effect of blends of Palm-Jatropha-Pongamia biodiesels on cloud point and pour point. Energy, 34(11), 2016-2021. doi: 10.1016/j.energy.2009.08.017

Sheehan, J., Dunahay, T., Benemann, J., & Roessler, P. (1998). A look back at the US Department of Energy's Aquatic Species Program: biodiesel from algae. Close-out Report.

Shen, Y., Yuan, W., Pei, Z., & Mao, E. (2010). Heterotrophic culture of Chlorella protothecoides in various nitrogen sources for lipid production. Applied Biochemistry and Biotechnology, 160(6), 1674-1684. doi:

Show, K.-Y., & Lee, D.-J. (2014). Chapter 9 - Production of Biohydrogen from Microalgae. In A. P.-J. L. C. R. Soccol (Ed.), Biofuels from Algae (pp. 189-204). Amsterdam: Elsevier.

Singh, A., Nigam, P. S., & Murphy, J. D. (2011). Renewable fuels from algae: An answer to debatable land based fuels. Bioresource Technology, 102(1), 10-16. doi: 10.1016/j.biortech.2010.06.032

Singh, R., Bhaskar, T., & Balagurumurthy, B. (2014). Chapter 11 - Hydrothermal Upgradation of Algae into Value-added Hydrocarbons. In A. P.-J. L. C. R. Soccol (Ed.), Biofuels from Algae (pp. 235-260). Amsterdam: Elsevier.

Singh, R., Bhaskar, T., & Balagurumurthy, B. (2014). Chapter 11 - Hydrothermal Upgradation of Algae into Value-added Hydrocarbons. In A. P.-J. L. C. R. Soccol (Ed.), Biofuels from Algae (pp. 235-260). Amsterdam: Elsevier.

Šťávová, J., Stahl, D. C., Seames, W. S., & Kubátová, A. (2012). Method development for the characterization of biofuel intermediate products using gas chromatography with simultaneous mass spectrometric and flame ionization detections. Journal of Chromatography A, 1224, 79-88. doi: 10.1016/j.chroma.2011.12.013

Sydney, E. B., Novak, A. C., de Carvalho, J. C., & Soccol, C. R. (2014). Chapter 4 - Respirometric Balance and Carbon Fixation of Industrially Important Algae. In A. P.-J. L. C. R. Soccol (Ed.), Biofuels from Algae (pp. 67-84). Amsterdam: Elsevier.

Tan, Y., & Lin, J. (2011). Biomass production and fatty acid profile of a Scenedesmus rubescens-like microalga. Bioresource Technology, 102(21), 10131-10135. doi: 10.1016/j.biortech.2011.07.091

Ugwu, C. U., Aoyagi, H., & Uchiyama, H. (2008). Photobioreactors for mass cultivation of algae. Bioresource Technology, 99(10), 4021-4028. doi: http://dx.doi.org/10.1016/j.biortech.2007.01.046

Waltz, E. (2009). Biotech's green gold? Nature Biotechnology, 27(1), 15-18. doi: 10.1038/nbt0109-15

Waltz, E. (2013). Algal biofuels questioned. Nat. Biotechnol., 31.

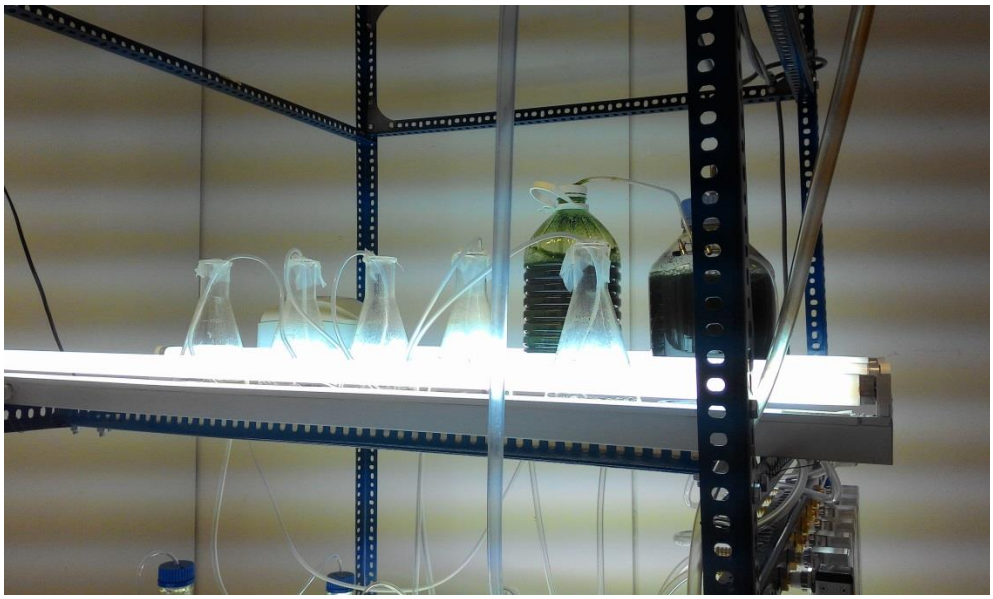
Yeh, K. L., & Chang, J. S. (2012). Effects of cultivation conditions and media composition on cell growth and lipid productivity of indigenous microalga Chlorella vulgaris ESP-31. Bioresource Technology, 105, 120-127. doi: 10.1016/j.biortech.2011.11.103

Yen, H.-W., Hu, I. C., Chen, C.-Y., & Chang, J.-S. (2014). Chapter 2 - Design of Photobioreactors for Algal Cultivation. In A. P.-J. L. C. R. Soccol (Ed.), Biofuels from Algae (pp. 23-45). Amsterdam: Elsevier.

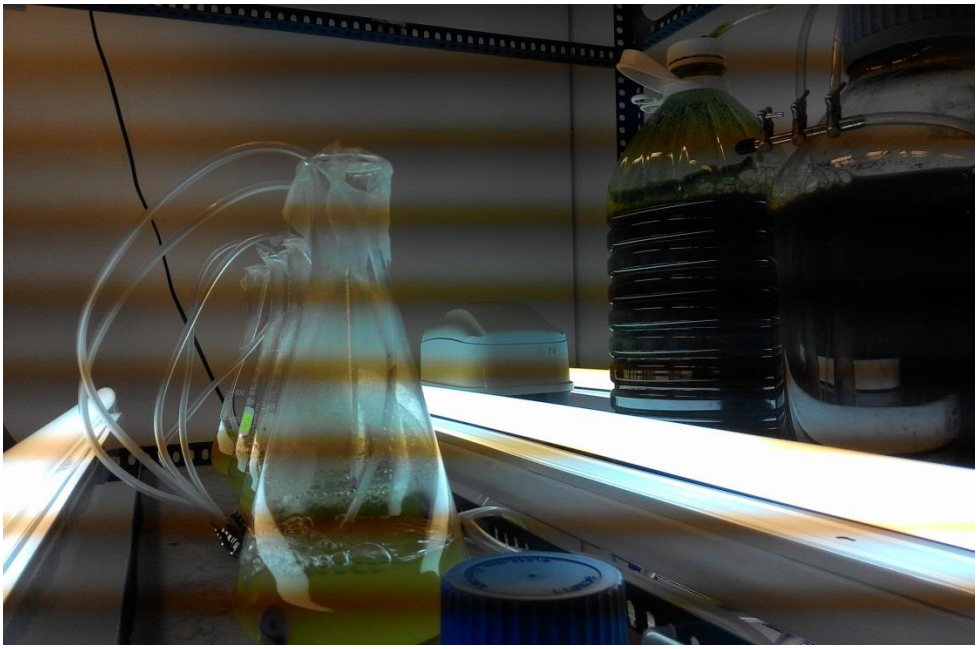
Yen, H.-W., Hu, I. C., Chen, C.-Y., & Chang, J.-S. (2014). Chapter 2 - Design of Photobioreactors for Algal Cultivation. In A. P.-J. L. C. R. Soccol (Ed.), Biofuels from Algae (pp. 23-45). Amsterdam: Elsevier

APPENDICES

Appendix 1: Figure shows the experiment being done.

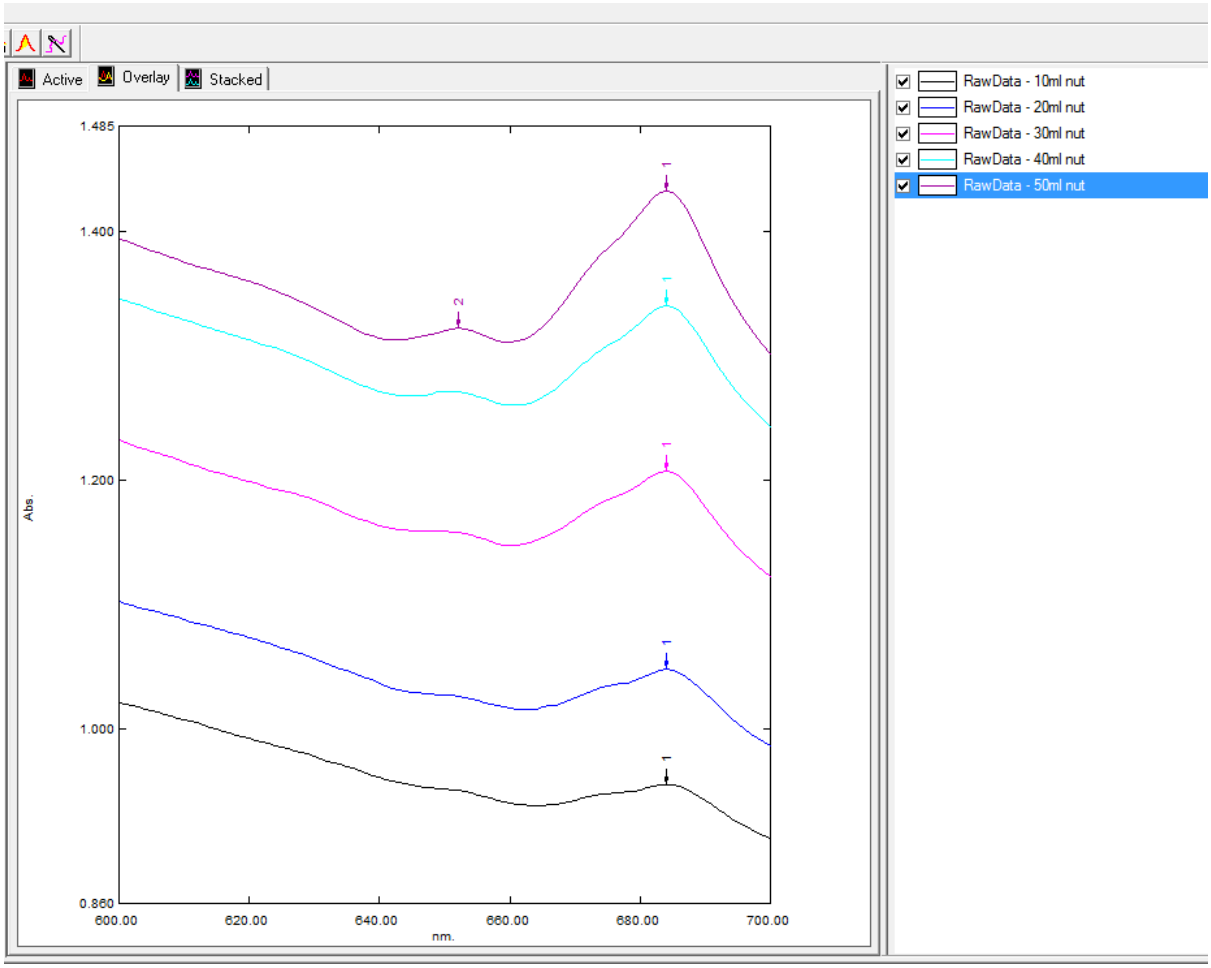


Appendix 2: Figure shows the set up of the experiment.



Appendix 3: Figure shows experiment being done with few samples

Appendix 4: Figure shows experiment effects of colour filter



Appendix 4: Sample of Data Extracted from UV-VIS Spectorophotometer.