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Biodiesel Production from Consortium of Microalgae

A Thesis Submitted to the School of Graduate Studies of Addis Ababa Institute of Technology, in Partial Fulfillment of the Requirements for the Degree of Master of Science in Chemical Engineering (Environmental Engineering Stream)

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This is to certify that the thesis prepared by Birhanu Ayalew, entitled: Biodiesel Production from algal biomass and submitted in partial fulfillment of the requirements for the Degree of Master of Science (Environmental Engineering) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abstract

The aim of the research work is to determine and analyze the factors that affect the yield of biodiesel production from algal biomass and to optimize the processing conditions. Microalgae oil was extracted from dried and grinded consortium microalgae through soxhlet extraction method and the physicochemical properties were determined. Design Expert 7.0.0 software application was used to statistically analyze data obtained from experimental work. The factors that affect the biodiesel yield were investigated. The density, viscosity, acid value, saponification value and free fatty acids were recorded as 0.94g/ml, 41.85mm^2/s, 4.63mgKOH/g of oil, 201.72mg/g of oil, and 2.32% respectively.

Alkali catalytic methanol transesterification method was employed to produce biodiesel from the oil and to improve the physicochemical properties of the oil. Temperature is found to be the factor that highly affects the yield of biodiesel. An optimum yield of 89.61% (44.8ml) biodiesel was obtained at reaction temperature of 53.27^{0}c, 1.99% catalyst and 6.18 alchol to oil molar ratio. The physicochemical properties of the optimum obtained biodiesl were determined and the results were compared with the ASTM and EN standards. The physicochemical properties were recorded as density (0.89g/ml), viscosity (5.5mm^2/s), acid value (0.78mgKOH/g of oil), moisture content (0.026%w/w), ash content (0.022%), free fatty acid (0.39%).

The results showed that the fuel properties are within the ASTM and EN standards and it suggests the potential of algal oil as a feedstock for biodiesel industry which could be exploited as an alternative source of fuel.

***Key Words**: Biodiesel, Algal Biomass, Soxhlet Extraction, Transesterification & Physicochemical Properties.*

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List of Abbreviations

ANOVA	Analysis of Variance
ASTM	American Society for Testing and Materials
AV	Acid Value
CIEs	Compression Ignition Engines
EN	European Committee for Standardization
EPA	Environmental Protection Agency
FAME	Fatty Acid Methyl Ester
FFA	Free Fatty Acid
GHG	Green House Gases
GW	Global Warming
NHP	Non-Hydratable Phospholipids
PBR	Photo Bio Reactors
SG	Specific Gravity
SV	Saponification Value
TAG	Triacylglycerols

1. INTRODUCTION

1.1 Background of the Study

Global energy crisis, unstable prices of petroleum products, environmental concerns arising from oil spillage and noxious gaseous emissions into the atmosphere are the major problems of conventional fossil energy source (Barnwal and Sharma, 2005; Amish *et al.,* 2009). Therefore, the search for alternative sources of new, sustainable and renewable energy such as biomass, solar, hydro, wind has become necessary. The demand for petroleum is on the increase daily, possibly due to the increase in industrialization, world population and the quest for better living standard. The escalated demand along with depletion of existing resources is fueling the escalation of fuel price. Besides the ever growing price of petroleum, the world is also anguishing from its emission related problems such as global warming, ozone layer depletion and the consequence of climate change: drought, and flooding (Klass, 1998). The rise in prices of petroleum fuel and increasing threat to the environment, have generated an international interest in developing alternative, non-petroleum; renewable fuels that have the potential to solve many of the current social problems and concerns (Demirbas, 2006).

On the other hand, most of the needed services that enhance our standard of living are energy dependent, thus their optimal delivery can be achieved through sufficient energy. This realization made many countries to investigate possibilities of using alternative fuels to petroleum and its derivatives (Carrareto *et al.,* 2004). The essential minimum requirement for biofuels to be more sustainable alternative for fossil fuels is that they should be produced from renewable raw materials and that their use has a lower negative environmental impact (Janulis, 2008). Biofuels are generally divided into primary and secondary. Primary biofuels such as fuel wood are used in an unprocessed form primarily for heating and cooking. Secondary biofuels such as bioethanol and biodiesel are produced by processing biomass and are able to be used in vehicles and various industrial processes. The secondary biofuels again categorized into three generations: first, second and third generation biofuels on the basis of different parameters, such as the type of processing technology, type of feedstock or their level of development (Nigam, 2010). First generation biofuels are bioethanol or butanol which are obtained by fermentation of starch from

wheat, barley, corn, potato or sugars from sugarcane, sugar beet, etc. and biodiesel by transesterification of oil crops such as rapeseed, soybeans, sunflower, palm, coconut, used cooking oil, animal fats, and so on. Second generation biofuels are bioethanol and biodiesel produced from conventional technologies but based on novel starch, oil and sugar crops such as j*atropha*, cassava from lignocellulosic materials such as straw, wood, and grass. Third generation biofuels are biodiesel and bioethanol from microalgae and seaweeds, hydrogen from green microalgae and microbes.

Although biofuel processes have a great potential to provide a carbon-neutral route to fuel production, first generation production systems have considerable economic and environmental limitations. The most common concern related to the current first generation biofuels is that as production capacities increase, so does their competition with agriculture for arable land used for food production. The increased pressure on arable land currently used for food production can lead to severe food shortages, in particular for the developing world where already more than 800 million people suffer from hunger and malnutrition (Schenk *et al.,* 2008). In addition, the intensive use of land with high fertilizer and pesticide applications and water use can cause significant environmental problems (Schenk *et al.,* 2008). The advent of second generation biofuels is intended to produce fuels from lignocellulosic biomass, the woody part of plants that do not compete with food production. Sources include agricultural residues, forest harvesting residues or wood processing waste such as leaves, straw or wood chips as well as the non-edible components of corn or sugarcane. However, converting the woody biomass into fermentable sugars requires costly technologies involving pre-treatment with special enzymes, meaning that second generation biofuels cannot yet be produced economically on a large scale (Brennan, 2010).

Therefore, third generation biofuels derived from microalgae are considered to be a viable alternative energy resource that is devoid of the major drawbacks associated with first and second generation biofuels (Nigam, 2010; Horsman, 2008). Microalgae are able to produce 15–300 times more oil for biodiesel production than traditional crops on an area basis. Furthermore compared with conventional crop plants which are usually harvested once or twice a year,

microalgae have a very short harvesting cycle (≈1– 10 days depending on the process), allowing multiple or continuous harvests with significantly increased yields (Schenk *et. al.*,2008).

Liquid biofuels made from biomass are attracting interest worldwide. Industrialized countries consider biofuels as a way of reducing GHG emissions from the transport sector and diversifying energy sources. Developing countries, on the other hand, consider biofuels as a way to stimulate rural development, create jobs, and save foreign exchange. However, both groups view biofuels as a means of increasing energy security. These concerns taken together and highlighted by recent surges in the world oil price have endorsed biofuel as an alternative.

In recent years, global warming, world oil supply and energy demand have all played a part in the push for alternatives to petroleum-based fuels. The Intergovernmental Panel on Climate Change (IPCC) affirms that during the 20th century, the Earth's average temperature increased by 0.6°C and will continue to increase anywhere from 1.5°C to 4.5°C by the year 2100 (McCarl & Bruce, 2005). This increase in global temperature is enough to cause flooding in coastal regions and makes storms like Hurricane Katrina a more common occurrence (Weart & Spencer, 2003). The major force in rising global temperatures is anthropogenic carbon dioxide emissions, which accounts for 80% of all greenhouse gases produced (McCarl & Bruce, 2005).

In contrast to the hydrogen alternative, biodiesel is a more immediate option as a renewable fuel source. Biodiesel contains no sulfur or aromatics, and use of biodiesel in a conventional diesel engine results in substantial reduction of unburned hydrocarbons, carbon monoxide and particulate matter (Biodiesel Emissions). Biodiesel consists of alkyl esters of long chain fatty acids that are derived from oils and fats produced by organisms (Nation Biodiesel Board, 2006). There are a number of animal fats and plants that can be used to produce biodiesel. Animal fats that are usually used include lard, yellow grease, and tallow (Guschina *et al.,* 2006). Plants that are typically grown for biodiesel include corn, cottonseed, peanut, rapeseed, and soybean (Fangriu, 1999). Currently, the supply of biodiesel from animal fats and plant oils is not enough to completely support the world's energy needs. It is also not economically feasible to rely completely on plants and animal fats for biodiesel.

Biodiesel is an alternative fuel from agricultural products that can be used directly in any existing unmodified diesel engine. It can be produced easily from common feed stocks (lipid sources such as: vegetable oils, animal fats and recyclable cooking oils) by transesterification in which oil or fat is reacted with a monohydric alcohol in the presence of a catalyst. Furthermore it can be blended with diesel fuel at any proportion without major changes in infrastructure and many other potential benefits. Unlike fossil diesel, pure biodiesel is biodegradable, nontoxic and essentially free of sulfur and aromatics (Roth, 2007).

The production and use of biodiesel has increased significantly in many countries around the world using numerous feedstock sources. Unfortunately, it is in nascent status in many African countries. Over the past decade, consumption of transport fuels in Sub-Saharan Africa has increased at a rate of about 7% per year in line with increased economic activity (Mulugetta Yadeta, 2008).

Ethiopia, imports its entire petroleum fuel requirement and the demand for petroleum fuel is rising rapidly due to a growing economy and expanding infrastructure. The annual consumption of petroleum fuels amounts to more than 1.2 million tones. Import of fuel grew strongly by 26.6 percent and reached USD 1.7 billion largely owing to the continued rise in international oil price and increased volume. Consequently, the share of fuel import in total imports of goods in 2010/11 rose to 20.1 percent from 16 percent in 2009/10. Export earning of the fiscal year 2010/11 was 2,534,685,400 and the import cost of petroleum for the same fiscal year was 1,648,800,000 which shows 65% of the country's export earnings are used to pay for the import of petroleum products which accounts 20% of the total imports (2010/11 annual report of National Bank of Ethiopia*).* Therefore, significant improvements to the trade balance are needed to stimulate the required economic development.

1.2 Statement of the Problem

Fossil fuels are the major energy sources but consumption of these fuels is leading to disastrous effects such as air pollution. They are non-renewable sources of energy as they are derived from pre-historic fossils and are no longer available if once used. Fossil fuel consumption is the major contributor to the increasing concentration of carbon dioxide (CO_2) in the atmosphere, a key cause of global warming which in turn reduces agricultural production and causes other biological and social problems (Schneider *et al.,* 2000). Fossil fuel source is limited and now days the world petroleum reserves are depleting at a faster rate and their environmental challenges are increasing (Demirbas, 2003).

The other impacts in using fossil fuel are fluctuation of oil price, instability of world political condition, problems on aquatic life by oil spill, acid rain, and air quality deterioration (Amish *et al.,* 2009). It produces many pollutants including nitrogen oxides, sulfur oxides, hydrocarbons, dust, soot, smoke, and other suspended matter. These pollutants can cause serious health problems including asthma, irritation of the lungs, bronchitis, pneumonia, decreased resistance to respiratory infections, and early death (Schneider *et al.,* 2000). Nitrogen oxides and hydrocarbons emitted from the burning of fossil fuels combine in the atmosphere to form ground-level ozone, which is the major constituent of smog. Human exposure to ozone can produce shortness of breath and, over time, permanent lung damage. It can also reduce crop yields. In addition, nitrogen oxides and sulfur oxides are important constituents of acid rain, which destroys lakes and rivers, diminishes crop yields, and deteriorates buildings. Besides, the ozone layer is being worn out due to the release of greenhouse gases from the fuels. Hence, Ozone holes are being created from which harmful UV rays enter the earth surface that affect human life causing diseases like cancer (Dunn, 2001).

Climate scientists predict that if carbon dioxide levels continue to increase, the planet will become warmer in the next century. Increases in temperature will most likely result in a variety of impacts including sea-level rise, extreme weather events, and an increased frequency of droughts in inland agricultural zones (Klass, 1998).

Therefore, assessing sustainable renewable energy source is crucial at present, due not only to combat the fuel supply uncertainty and price fluctuations, but also becoming global environmental concern. It's each country's responsibility to seek for environmentally benign energy sources that are supporter to the global deeds to reduce GHG and air pollutions. A number of countries have successfully applied the use of biodiesel made from plant seed oils or animal fats, purely or by blending with petroleum diesel, and developed appropriate technologies to exploit the resources specific to their agro-climatic conditions. (Turkenburg, 2000).

Ethiopia, endowed with diverse ecological varieties that have an ample potential for biofuel production (both alcohols and biodiesel), is little benefited from its wealth. Castor oil, jatropha, palm oil, neem, microalgae and others are among some of non-edible naturally occurring biodiesel feed stocks in the country. Most of the indigenous plants are not yet exploited or even not identified. Therefore, identifying the feasible and reliable oil bearing plant for biodiesel from indigenous species is essential for finding solution to the aforementioned problems and to overcome the trade imbalance that the country is facing so as to stimulate the required economic development.

1.4 Objectives

1.4.1 General Objective

The general objective of this research is to study biodiesel production from algal biomass using methanol with alkali catalyst.

1.4.2 Specific Objectives

The specific objectives of this research are

i. To extract crude oil from the algal biomass by soxhlet extraction method
ii. To analyze the physicochemical properties of the extracted algal oil
iii. To optimize the process parameteres temperature, alcohol-to-oil molar ratio, and catalyst concentration on the process of biodiesel production (transesterification process)
iv. To determine physical and chemical properties of the biodiesel produced such as: density, kinematic viscosity, acid value, saponification value and free fatty acid by titration and empirical formulas.

1.3 Significance of the Study

This study has great significance in terms of assuring the production of an alternative form of energy that is environmentally friendly from algal biomass which is a non edible & can be abundantly cultivated and grow on lands not suitable for higher plants, using waste waters discharged from industries & households. In addition, the study will be used as a reference material for anyone who is interested to carry out further study & produce biodiesel from consortium of microalgae.

1.5 Scope of the Study

The scope of this research is to investigate the possibility of biodiesel production from consortium of microalgae. There will be two major steps to get the final product; Microalgal oil extraction using soxhlet extraction method and conversion of the oil into biodiesel using transesterification method. The transeasterification process will be carried out by varying the parameters such as reaction temperature, alcohol-to-oil molar ratio, and catalyst concentration to identify their effects on the response parameter which is biodiesel yield. Based on the final product yield the optimum parameters will be determined. Finally, the biodiesel produced from the algal biomass will be analyzed for its physicochemical properties such as kinematic viscosity, flash point, acid value, saponification value and specific gravity.

2. LITERATURE REVIEW

2.1 Introduction

Concerns about shortage of fossil fuels, increasing crude oil price, energy security and accelerated global warming have led to growing worldwide interests in renewable energy sources such as biofuels. An increasing number of developed and rapidly developing nations see biofuels as a key to reducing reliance on foreign oil, lowering emissions of GHG, mainly carbon dioxide and methane and meeting rural development goals (Koh, Ghazoul, 2008).

Biofuels are fuel products produced from biomass. In the general sense, biofuel refers to an energy source or carrier that is derived from recently living material. Biofuel can be solid (i.e., wood, crops and their residues, portions of municipal wastes, etc.), liquid (raw plant oils, waste cooking oils, as well as refined biodiesel, ethanol and other alcohols, pyrolysis oils, and biobased liquid hydrocarbons), and gaseous (biomethane, biogas, landfill gas, hydrogen derived from biomass).

Biodiesel, a biofuel which is comprised of mono-alkyl esters of long chain fatty acids derived from vegetable oils (both edible and non edible), animal fats, and mixtures thereof, is produced by transesterification, with glycerol being produced as a co-product. It is the name for a variety of ester based fuels (fatty ester) generally defined as mono-alkyl ester made from renewable biological resources & designated as B_{100}. This renewable source is as efficient as petroleum diesel in powering unmodified diesel engine. Today's diesel engines require a clean burning, stable fuel operating under a variety of conditions. Biodiesel is miscible with petro diesel in all ratios and can be used either as B_{100} (neat) or in a blend with petroleum diesel. A blend of 20 % biodiesel with 80 % petro diesel, by volume, is termed "B_{20}". A blend of 2 % biodiesel with 98 % petro diesel is "B_2" and so on (Van Gerpen, 2004).

The main advantages of using biodiesel fuels as 100 % alkyl esters of vegetable oil and animal fat or biodiesel blends (up to 20 % blend to the diesel fuel) are producing less smoke and particulate matter, improves lubricity which in turn minimizes engine wear and increases engine

life, results in higher cetane numbers that makes combustion smooth and produces lower carbon monoxide and hydrocarbon emissions (Diwani, 2009).

The cost of raw materials for biodiesel production accounts for large percent of the direct biodiesel production costs required. Thus, one way of reducing the biodiesel production costs is to use the less expensive raw material containing fatty acids such as animal fats, non edible oils, and waste cooking oil, plant and by products of the refining vegetable oils (Meher, 2006). Algae seem to be one of the very effective feedstock for biodiesel production as it is non edible, thus does not compete with food materials and has no effect on food scarcity; it can be grown on land and in water unsuitable for other plant growth. Some species are able to grow in high salinity, effluent (industrial & municipal waste water effluents), and can be grown in ponds independent of soil (Christi, 2007).

2.2 Diesel Production Problems

The transportation and energy sectors are the major anthropogenic sources responsible for more than 20% and 60% of the global GHG emissions respectively (European Environmental Agency, 2004). Agriculture is the third largest anthropogenic source, representing about 9% of GHG emissions, where the most important gases are nitrous oxide (N_2O) and methane (CH_4) (European Environmental Agency, 2007). It is expected that with the development of new growing economies, such as India and China, the global consumption of energy will rise and lead to more environmental damage (International Energy Agency, 2007).

GHG contributes not only to global warming (GW) but also to other impacts on the environment and human life. Oceans absorb approximately one-third of the CO_2 emitted each year by human activities and as its levels increase in the atmosphere, the amount dissolved in oceans will also increase turning the water pH gradually to more acidic. This pH decrease may cause the quick loss of coral reefs and of marine ecosystem biodiversity with huge implications in ocean life and consequently in earth life (Ormerod *et al.,* 2002).

As GW is a problem affecting different aspects of human life and the global environment, not only a single but a host of solutions is needed to address it. One side of the problem concerns the

reduction of crude oil reserves and difficulties in their extraction and processing, leading to an increase of its cost (Laherrere, 2005). This situation is particularly acute in the transportation sector, where currently there are no relevant alternatives to fossil fuels. To find clean and renewable energy sources ranks as one of the most challenging problems facing mankind in the medium to long term. The associated issues are intimately connected with economic development and prosperity, quality of life, global stability, and require from all stakeholders tough decisions and long term strategies. For example, many countries and regions around the world established targets for CO_2 reduction in order to meet the sustainability goals agreed under the Kyoto Protocol. Presently many options are being studied and implemented in practice, with different degrees of success, and in different phases of study and implementation. Examples include solar energy, either thermal or photovoltaic, hydroelectric, geothermal, wind, biofuels, and carbon sequestration, among others (Dewulf *et al.,* 2006). Each one has its own advantages and problems depending on the area of application.

2.3 Algae: A Renewable Energy Source

Algae are plant-like organisms that are photosynthetic and aquatic with simple reproductive structures, but do not have true roots, stems, leaves, or vascular tissue. Algae are distributed worldwide in the sea, freshwater, and wastewater. Most algae are microscopic while some are quite large, exceeding fifty meters in length. The unicellular forms are known as microalgae whereas multicellular forms are known as macro algae. Macro algae are large multicellular algae that often grow in ponds. The largest macro algae, called seaweed, is a kelp plant that can grow to be more than 100 feet long (Wen, 2011).

Microalgae range from a few microns to hundreds of microns and exist individually, in chains, or in flocks in marine and/or freshwater systems. Microalgae contribute around 40 to 50 percent of the oxygen in the atmosphere and simultaneously consume carbon dioxide to grow photoautotrophically without additional carbon sources. The production of biofuel from microalgae has gained considerable attention due to the fact that they can be converted into several different types of renewable biofuels such as green diesel, jet fuel, methane biogas, ethanol, and butanol (Johnson & Wen, 2008).

Their photosynthetic mechanism is similar to land based plants, but due to a simple cellular structure, and submerged in an aqueous environment where they have efficient access to water, CO_2 and other nutrients, they are generally more efficient in converting solar energy into biomass.

Compared with traditional crops, they have a high areal productivity, a relatively high oil and protein content, and do not depend on arable land and freshwater. Today, there is a strong interest in lipid production using microalgae. Microalgae are theoretically capable of producing much more lipids than any conventional crop and are, therefore, attractive as a potential source of biodiesel. Microalgae are the fastest growing photosynthesizing organisms. They can complete an entire growing cycle every few days (Metting and Pyne, 1986).

Microalgae consume large amounts of nitrogen, phosphate, and carbon dioxide that are converted into biomass, which makes algae attractive for carbon dioxide mitigation and reduction of pollution and toxic chemicals (Park *et al*., 2011). Carbon dioxide fixation is regulated by temperature, light intensity, and the concentration of the carbon dioxide. Generally more carbon dioxide can be fixated at moderate temperatures and with increasing light intensity. Carbon dioxide mitigation is considered one of the primary benefits of algal biofuel production since it can be used to combat global warming (Ge *et al*., 2011).

Some species of microalgae can produce more than 50% of their dry mass as triacylglycerides or long chain hydrocarbons that can be converted to biodiesel and jet fuel. Algae can accumulate 20-75% of lipids as part of their dry mass. Most algae species produce triacylglycerides and alkanes from what is known as the fatty acid biosynthetic pathway (Niehaus *et al*., 2011).

2.4 Wastewater Nitrogen and Phosphorous as Microalgae Nutrients

There is a unique opportunity to both treat wastewater and provide nutrients to algae using nutrient-rich effluent streams. By cultivating microalgae, which consume polluting nutrients in

municipal wastewater, and abstracting and processing this resource, then the goals of sustainable fuel production and wastewater treatment can be combined (Andersen, 2005).

The nutrients in waste water can be utilized by algae, which provide the co-benefit of producing biofuels and removing nitrogen and phosphorus as well as organic carbon from the waste water. (Mostafa and Ali, 2009). Wastewater treatment using algae has many advantages. It offers the feasibility to recycle these nutrients into algae biomass as a fertilizer and thus can offset treatment cost. Oxygen rich effluent is released into water bodies after wastewater treatment using algae (Becker, 2004).

2.5 Algae as a Biodiesel Feedstock

Algae are recognized as one of the oldest life-forms (Falkowski, 1997). They are primitive plants (thallophytes), i.e. lacking roots, stems and leaves, have no sterile covering of cells around the reproductive cells and have chlorophyll as their primary photosynthetic pigment (Lee, 1990). The most important classes of algae are: green algae (Chlorophyta), red algae (Rhodophyta) and diatoms (Bacillariophyta). Algae can either be autotrophic or heterotrophic; the former require only inorganic compounds such as CO_2, salts and a light energy source for growth; while the latter are non photosynthetic therefore require an external source of organic compounds as well as nutrients as an energy source. Some photosynthetic algae are mixotrophic, i.e. they have the ability to both perform photosynthesis and acquire exogenous organic nutrients **(Lee, 1990).** For autotrophic algae, photosynthesis is a key component of their survival, whereby they convert solar radiation and CO_2 absorbed by chloroplasts into adenosine triphosphate (ATP) and O_2 the usable energy currency at cellular level, which is then used in respiration to produce energy to support growth (Zilinskas,1994).

In recent years, microalgae have gained attention as a possible solution to some imminently critical issues, including the role of an alternative fuel source, and as a potential source of many bioactive compounds intended for human consumption. Microorganisms bear multiple advantages over traditional energy crops. Microalgae are a very effective feedstock for biodiesel production. Some algal species have been found to have very high oil contents, ranging up to and sometimes beyond 75% dry weight (Chisti, 2007).

Microalgae are capable of producing between 10-30 times the amounts of oil per year than other high oil producing plants; they have a higher growth rate than other crops, a shorter maturity rate, a higher biomass production rate than other cash crops, as well as using far less land than conventional crops (Lee, 2009). Furthermore, with microalgae there is no competition for land space that could be used for food crops. For example, using corn as a feedstock for making ethanol creates a negative competition between human and animal consumption and fuel production (Huang, 2010). Microalgae also have the potential to counteract a portion of the greenhouse effect and water pollution, because through photosynthesis, they have the ability to fix carbon dioxide produced by industrial plants. Some microalgae also fix nitrogen and absorb other contaminants such as heavy metals and phosphorous (Gouveia, 2009).

2.5.1 Algae Oil Extraction

Once the biomass has been harvested, the following step is to dry and extract the oil. Drying the biomass after it has been harvested is a vital step, partially because it is the most energy intensive segment of the feedstock production process. Drying of the biomass can be important because depending on the climate, there is the possibility of having it spoil. There are various ways to dry the harvested algae such as spray drying, drum drying, freeze-drying, and sun drying. Little research has been conducted into evaluating the best possible methods of drying algae on a large scale with biodiesel production in mind. However, spray drying, freeze-drying, and drum drying have been shown to produce adequate dried product of Dunaliella algae in attempts to isolate carotene (Ben-Amotz and Avron, 1987). When trying to isolate high value products, spray drying is often the method of choice; however, there is the risk of causing deterioration of pigments or other components. In laboratories, freeze-drying is commonly used although it is too expensive to be used on a large scale (Molina e*t al.*, 2003).

Extraction of materials from the dried biomass can either be done mechanically, chemically, or through some combination of the two. Many of the cell disruption techniques used for other organisms can also be applied to algae (Belarbi *et al*., 2000). Doing extraction chemically typically entails using solvents, such as hexane, and can be used with or without cell disruption first. Other possible solvents that might be used include ethanol, chloroform, and diethyl ether. However, one major drawback when using solvents is that certain side products may become

denatured by the solvent, leading to difficult or expensive decontamination procedures. Also, when solvents like hexane are used on a large scale, there are associated risks of fire and explosion that would need to be taken into consideration (Greenwell *et al*., 2009).

Three methods of oil extraction processes from dried algae biomass are identified. These are solvent extraction, mechanical extraction and ultrasonic cell disruption. All the three methods destroy the algae cell walls, allowing access to the oils inside it. The methods are discussed below (Amin *et al*., 2010).

2.5.1.1. Oil Extraction Using Solvent

Solvent extraction with n-hexane can produce about high yield by weight of oil per kilogram of the microalgae biomass. To extract oil using solvent, dried microalgae biomass are crushed at the required size. The crushed biomass and hexane at a ratio of 1:5 (w/v) respectively are fed to soxhlet apparatus to extract oil. The extraction temperature is near to the boiling point of hexane (Amin *et al.*, 2010).

2.5.1.2 Mechanical Methods of Oil Extraction

Two mechanical methods that have been studied at a large scale are cell homogenization and bed milling. Cell homogenization involves fluid being forced through an orifice. The change in diameter causes a high liquid shear, which can disrupt the algal cell walls. Bed milling uses vessels packed with small glass beds that are agitated at high speeds, which leads to disruption of the cells. Both methods depend on the strength of the algal cell walls, which will have impact when selecting the species of algae for biodiesel production. Additionally, with bed milling, the degree of disruption depends on the residence time of the algae in the system (Greenwell *et al.*, 2009). Mechanical methods of extraction are chosen in most cases to avoid contamination of products that might occur through chemical extraction (Chisti and Moo-Young, 1986).

2.5.1.3 Ultrasonic Cell Disruption Methods of Oil Extraction

Ultrasonication, the use of sound to agitate particles, is another approach that seems particularly promising to disrupt algal cells. This method exposes the algae to high intensity ultrasonic waves, which create tiny cavitations bubbles around the cells. The collapse of the bubbles emits

shockwaves, shattering the cell walls. The destroyed cells release the oils inside into the solution. (Aladetohun *et al*., 2006). Though, such a method can only handle small amounts of biomass at a time, so it is not suitable for large scale extraction (Dunstan *et al.,* 1992).

2.6 Biodiesel Production Processes

2.6.1 Transesterification Process

The viscosities of vegetable oils and microalgae oils are usually higher than that of diesel oils (Fuls *et al*., 1984). Hence, they cannot be applied to engines directly. The transesterification of microalgae oils will greatly reduce the original viscosity and increase the fluidity (Chisti, 2007). Transesterification is the reaction of a fat or oil with an alcohol (in the presence of catalyst) to form esters and glycerol as a by-product (Agarwal, 2001; Henning, 2004; Martinez-Herrera, 2001). It involves stepwise conversions of Triglyceride, which are esters of glycerol with long-chain acids commonly called fatty acids, to diglyceride, monoglyceride, and, finally to glyceride producing 3 mol of ester in the process (Freedman, 1984). Since the reaction is reversible, excess alcohol is required to shift the equilibrium to the product side. Among the alcohols that can be used in the transesterification process are methanol, ethanol, propanol, butanol and amyl alcohol. Methanol and ethanol are utilized most frequently, because of their low cost and physical and chemical advantages (Freedman, 1984). The reactions are often catalyzed by an acid or a base, since the alcohol is sparingly soluble in the oil phase. The catalyst promotes an increase in solubility to allow the reaction to proceed at a reasonable rate.

The most common catalysts used are strong mineral bases such as sodium hydroxide and potassium hydroxide. There are also some other catalysts used such as acid, enzymes and non-ionic base catalysts but alkali-catalyzed transesterification is faster than that catalyzed by the same amount of an acidic catalyst transesterification (Gerpen, 2004). The transesterification process has been widely used to reduce the viscosity of triglycerides, thereby enhancing the physical properties of renewable fuels to improve engine performance.

The biodiesel production process is comprised of the following steps:

2.6.1.1 Raw Material Treatment

Nowadays, most of the biodiesel is produced by the alkali-catalyzed process. Feed stocks with high free fatty acid will react undesirably with the alkali catalyst thereby forming soap. The maximum amount of free fatty acids acceptable in an alkali-catalyzed system is below 2.5 wt. % FFA. If the oil or fat feedstock has FFA content over 2.5 wt. %, a pretreatment step is necessary before the transesterification process.

The raw materials, which can be vegetable oils, animal fats, or recycled greases, used in the production of biodiesel contain triglycerides, free fatty acids, water, and other contaminants in various proportions. Some crude vegetable oils contain phospholipids that need to be removed in a degumming step. (Gerpen and Dvorak, 2002).

Liu *et al.,* (2008) compared the different degumming methods, such as membrane filtration, hydration, acid micelles degumming, supercritical extraction, etc. The characteristics of the raw oils should be investigated before choosing the suitable degumming method because different degumming methods have their advantages and disadvantages (Liu *et al.,* 2008). The oil can be separated through membrane filtration according to the average molecular weight or the particle size of phospholipids. Although degumming method can solve the problem, its process involves two steps with the use of an organic solvent. Therefore, this method has no superiority on cost because of its complicated processing (Carvalho *et al.,* 2006; Souza, 2008).

In the hydration process, because of phospholipids hydrophilicity, hot water can be added into the oil with stirring. The phospholipids solubility will be significantly reduced, so it could be separated from the oil by natural settlement. The hydration method features a simple process, easy operation, and high yields refining, but non-hydratable phospholipids cannot be removed by this method, as reported by Verleyen *et al.,* (2002). For removing non-hydratable phospholipids (NHP) critic acid or phosphoric acid can be added into the oil which is heated to 70 ^{0}C, this is called the special micelles degumming method.

The free fatty acids can be removed in a refining step and excess free fatty acids can be removed as soaps in a later pretreatment step (Demirbas and Kara, 2006). Next, in order to determine the percentage of FFA in the oils or fats, titration is performed. As described above, if the percentage of FFA is over 2.5 wt. %, pretreatment is necessary to reduce the content of FFA. This step also determines the amount of catalyst required in the neutralization step.

- **Pretreatment of Acidic Feed Stocks**

Many pretreatment methods have been proposed for reducing the high free fatty acid content of the oils, including steam distillation, extraction by alcohol (Turkay & Civelekoglu,1991), and esterification by acid catalysis (Zhang *et al.*, 2008). However, steam distillation for reducing high free fatty acids requires a high temperature and has low efficiency. Because of the limited solubility of free fatty acids in alcohol, extraction by alcohol method needs a large amount of solvent and the process is complicated.

Compared with the two former methods, esterification by acid-catalysis makes the best use of the free fatty acids in the oil and transforms it into biodiesel. The catalysts can be homogeneous acid-catalysts or solid acid-catalysts. Compared with the former one, solid acid-catalysts offer some advantages for eliminating separation, corrosion, toxicity, and environmental problems, but the reaction rate is slower (Zhang *et al.*, 2008; Serio *et al.* ,2005).

An alternative approach to reduce the FFA is to use iodine as a catalyst to convert free fatty acids into biodiesel. An obvious advantage of this approach is that the catalyst (iodine) can be recycled after the esterification reaction. Liu *et al.*, (2008) found through orthogonal tests, under the optimal conditions (i.e. iodine amount: 1.3 wt. % of oils; reaction temperature: 80 ^{0}C; ratio of methanol to oils: 1.75:1; reaction time: 3 h) that the FFA content can be reduced to < 2%.

Another new method of pretreatment is to add glycerol into the acidic feedstock and heat it to a high temperature of about 200 ^{0}C, normally with a catalyst such as zinc chloride. The glycerol will react with the FFA to form monoglycerides and diglycerides. Then the FFA level will become low and biodiesel can be produced using the traditional alkali-catalyzed transesterification method. The advantage of this approach is that no alcohol is needed during the pretreatment and the water formed from the reaction can be immediately vaporized and vented

from the mixture. However, the drawbacks of this method are its high temperature requirement and relatively slow reaction rate (Gerpen, 2004).

- **Oil Heating**

To speed up the reaction as well as remove any water residue, the algal oil should be boiled at 105-110 ^{o}C for at least 30 min (Kandpal, 1995; Satyanarayana, 2010). Heating should be stopped if there is no more water bubbles in the oil. When water is present, particularly at high temperatures, it can hydrolyze the triglycerides to diglycerides and form a free fatty acid (Gerpen, 2004). It is important to stir the oil as it is heated. This will result in a more even heating and reduce the temperature of oil exposed directly to the heating element. After the entire water residue is removed from the oil it should be cooled to a temperature a little lower than boiling temperature of methanol.

- **Mixing of Catalyst and Alcohol**

In general, there are three categories of catalysts used for biodiesel production: alkalis, acids, and enzymes. Enzyme catalysts have become more attractive recently since it can avoid soap formation and the purification process is simple to accomplish. However, they are less often used commercially because of the longer reaction times and higher cost. To reduce the cost, some researchers developed new biocatalysts in recent years. An example is so called whole cell biocatalysts which are immobilized within biomass support particles. An advantage is that no purification is necessary for using these biocatalysts.

Compared with enzyme catalysts, the alkali and acid catalysts are more commonly used in biodiesel production. The alkali and acid catalysts include homogeneous and heterogeneous catalysts. Due to the low cost of raw materials, sodium hydroxide and potassium hydroxide are usually commercially used as alkali homogeneous catalysts. These materials are the most economic because the alkali-catalyzed transesterification process is carried out under a low temperature and pressure environment, and the conversion rate is high with no intermediate steps. However, the alkali homogeneous catalysts are highly hygroscopic and absorb water from air during storage. They also form water when dissolved in the alcohol reactant and affect the yield. Therefore, they should be properly handled.

On the other hand, some heterogeneous catalysts are solid and it could be rapidly separated from the product by filtration, which reduces the washing requirement (Dennis *et al.,* 2010). In addition, solid heterogeneous catalysts can stimulatingly catalyze the transesterification and esterification reaction that can avoid the pre-esterification step, thus these catalysts are particularly useful for those feed stocks with high free fatty acid content.

The alcohol materials that can be used in the transesterification process include methanol, ethanol, propanol, butanol, and amyl alcohol. Among these alcohols, methanol and ethanol are used most frequently. Methanol is especially used because it makes the separation of the methyl easter from the glycerol easy. Ma and Hanna (1999) reported that methanol can react with triglycerides quickly and the alkali catalyst is easily dissolved in it. However, due to its low boiling point, there is a large explosion risk associated with methanol vapors which are colorless and odorless. Both methanol and methoxide are extremely hazardous materials that should be handled carefully. It should be ensured that one is not exposed to these chemicals during biodiesel production (Qian *et al*., 2008). The purpose of mixing alcohol methanol and the catalyst (NaOH) is to make the reaction for the two substances to form methoxide.

- **Transesterification Reaction**

In the transesterification reaction, the reactants initially form a two phase liquid system. Hence the reaction is diffusion controlled and poor diffusion between the phase results in a slow rate. Therefore in order to speed up the reaction between the mixture of methoxide and the oil, a mixing mechanism should be there. Experiment conducted by (Knothe, 2004) reveals that transesterification vegetable oil into butyl ester was function of mixing intensity. Best reaction rate in this experiment is observed for a rotational speed above 200 rpm. However this mixing effect is most significant during the slow rate region of the reaction. As the single phase is established, mixing becomes insignificant and the reaction rate is primarily influenced by the reaction temperature. Many researchers (Bernard Freedman, 1986; Amin, *et al*., 2010) have conducted lots of experiment so as to find an optimum temperature. For an optimum yield of ethyl ester the reaction should be close enough to the boiling temperature of ethanol.

- **Draining of Glycerol**

After the transesterification reaction, one must wait for the glycerol to settle to the bottom of the container. This happens because glycerol is heavier than biodiesel. The settling will begin immediately, but the mixture should be left a minimum of eight hours (preferably 12) to make sure all of the glycerol has settled out. There will be a difference in viscosity and color between the two liquids. Glycerol looks very dark compared to the yellow biodiesel. The viscosity difference is large enough between the two liquids that the difference in flow from the drain can be seen.

- **Biodiesel Washing**

The purpose is to wash out the remnants of the catalyst and other impurities. There are three main methods:

- Water wash only (a misting of water over the fuel, draining water off the bottom)
- Air bubble wash (slow bubbling of air through the fuel)
- Air/water bubble wash (with water in the bottom of the tank, bubbling air through water and then the fuel)

Which method works the best is dependent on the quality of the fuel. The mostly used method is a combination of water washing and air bubble washing. Warm water, usually from 49-60ºC (Gerpen, 2004), is misted above the fuel. The use of warm water prevents precipitation of saturated fatty acid esters and retards the formation of emulsions with the use of a gentle washing action. The amount of wash water should equal from 25-100% of the volume of oil (Alamu, 2007; Center, 2004) and can be drained throughout the washing process.

After the water is drained, the air washing process can start. At this point, the biodiesel is usually a pale yellow color. Air should be bubbled through the biodiesel mixture for approximately 8 hours. The bubbling should be just enough to agitate the biodiesel surface. A final drain of accumulated contaminants is done immediately after the air bubble wash is finished. During the washing process, gentle agitation is required to avoid the emulsion.

- **Biodiesel Drying**

After the biodiesel is washed, it should be dried until it is crystal clear. This can be done by letting the biodiesel sit uncovered in a sunny location for a few days, or it may be heated to about 49 ^{0}C for a few hours. Another popular technique is recirculating the biodiesel from the bottom of the drying tank through a shower head or sprayer suspended above the top of the open tank. This increased contact with air will dry biodiesel in about an hour, depending on humidity. It should have a pH of close to 7, or chemically neutral and it should have no alcohol left in it (Center, 2004).

2.7 Possible Ways of Transesterification Reaction

Transesterification is the chemical conversion of the oil into its corresponding fatty ester. It's one of the most common methods used to reduce oil viscosity in the biodiesel industry. A catalyst is usually used to improve the reaction rate and yield. Because the reaction is reversible, excess alcohol is used to shift the equilibrium to the product side (Demirbas, 2008).

2.7.1 Homogeneous Base and Acid Catalyzed Transesterification

The base catalyzed transesterification reaction commonly uses alkali catalysts such as NaOH, CH_3ONa, CH_3OK and KOH. In the alkali catalytic methanol transesterification method, the catalyst is dissolved into methanol by vigorous stirring in a small reactor. The oil is transferred into a biodiesel reactor and then the catalyst/alcohol mixture is pumped into the oil. A successful transesterification reaction produces two liquid phases: ester and crude glycerol (Vyas *et al.*, 2009). It is reported to be very sensitive to the purity of the reactant. Free fatty acid (FFA) content should not exceed beyond a certain limit. It has been found that the alkaline-catalyzed transesterification process is not suitable to produce esters from unrefined oils. In order to prevent saponification during the reaction, FFA and water content of the feed must be below 2 wt. % and 0.05 wt. %, respectively. Because of these limitations, only pure vegetable oil feeds are appropriate for alkali-catalyzed transesterification without extensive pre-treatment.

The liquid acid-catalyzed transesterification process (also known as Esterification process) is not much popular as the base-catalyzed process. It uses strong acids as catalysts to convert a high

FFA content feedstock reacted with alcohols to its corresponding esters. Homogeneous acid catalyzed reaction is slower than the homogeneous base-catalyzed reaction. However, the performance of the acid catalyst is not strongly affected by the presence of FFAs in the feedstock. In fact, acid catalysts can simultaneously catalyze both esterification and transesterification. Thus, a great advantage with acid catalysts is that they can directly produce biodiesel from low-cost lipid feed stocks. For acid-catalyzed systems, sulfuric acid, hydrochloric acid, phosphoric acid, and organic sulfonic acids, have been used by different researchers (Demirbas, 2008).

2.7.2 Heterogeneous Acid and Base Catalyzed Transesterification

Homogeneous catalysts showed greater performance toward transesterification to obtain biodiesel. The use of heterogeneous catalysts could be an attractive solution. Heterogeneous catalysts can be separated more easily from reaction products. Undesired saponification reactions can be avoided by using heterogeneous catalysts. They enable the transesterification of vegetable oils or animal fats with high contents of FFAs, such as deep-frying oils from restaurants and food processing. Bio-diesel synthesis using solid catalysts could potentially lead to cheaper production costs because of reuse of the catalyst and the possibility for carrying out both transesterification and esterification simultaneously (Demirbas, 2008).

2.7.3 Enzymatic Transesterification

Enzymatic transesterification using lipase looks attractive and encouraging for reasons of easy product separation, minimal wastewater treatment needs, easy glycerol recovery and the absence of side reactions. Practical use of lipase in pseudo homogenous reaction systems presents several technical difficulties such as contamination of the product with residual enzymatic activity and economic cost. In order to overcome this problem, the enzyme is usually used in immobilized form so that it can be reused several times to reduce the cost and also to improve the quality of the product. When free enzymes are used in a bio-diesel process, the enzymatic activity can be partially recovered in the glycerol phase. However, the build-up of glycerol limits the possible number of reuses (Demirbas, 2008). Compared to chemical approach, enzymatic approach for

biodiesel production offers more advantages but cost of lipase is the major issue for the industrialization of lipase-mediated biodiesel production.

2.7.4 Microwave Assisted Transesterification

An alternative energy stimulant, ‘‘microwave irradiation’’, can be used for the production of the alternative energy source, bio-diesel. Microwave irradiation activates the smallest degree of variance of polar molecules and ions such as alcohol with the continuously changing electrical field. The changing electrical field, which interacts with the molecular dipoles and charged ion, causes these molecules or ions to have a rapid rotation and heat, is generated due to molecular friction . The preparation of biodiesel using microwave offers a fast, easy route to this valuable biofuel with advantages of a short reaction time, a low oil/ methanol ratio, an ease of operation a drastic reduction in the quantity of by-products, and all with reduced energy consumption. Aside from the great advantages of microwave-assisted reactions, there are also a few drawbacks. Microwave synthesis is not easily scalable from laboratory small-scale synthesis to industrial multi kilogram production. The most significant limitation of the scale up of this technology is the penetration depth of microwave radiation into the absorbing materials, which is only a few centimeters, depending on their dielectric properties. The safety aspect is another reason for rejecting microwave reactors in industry (Knothe *et al.*, 2005).

2.7.5 Ultrasound Assisted Transesterification

Ultrasound has proven to be a very useful tool in enhancing the reaction rates in a variety of reacting systems. It has successfully increased the conversion, improved the yield, changed the reaction pathway, and/or initiated the reaction in biological, chemical, and electrochemical systems. Ultrasonic assisted transesterification method presents advantages such as shorter reaction time and less energy consumption than the conventional mechanical stirring method, efficient molar ratio of methanol to triglycerides, and simplicity (Knothe *et al.*, 2005).

2.7.6 Two-Step Acid - Base Catalyzed Transesterification

This process involves a two-step process for the production of biodiesel from oils having high FFA. Initially, the acid catalyzed esterification reaction takes place to convert the FFAs present in the oil to esters. After the reduction of FFA, transesterification reaction was carried out, in

which the pre-treated oil reacts with methanol using conventional base catalysts. This method of biodiesel production alleviates the draw backs of the esterification process -requires longer time reaction to come to completion and transesterification- problem of soap formation process (Wang *et al.,* 2001).

2.8 Advantages and Disadvantages of Biodiesels

- **Advantages of Biodiesel as Diesel Fuel**

The advantages of biodiesel as a fuel are its renewability, higher combustion efficiency, lower sulfur and aromatic content (Ma and Hanna, 1999; Knothe *et al.*, 2006), higher cetane number, and higher biodegradability (Zhang *et al.*, 2003). The main advantages of biodiesel include its domestic origin, its potential for reducing a given economy's dependency on imported petroleum, and high flash point (Knothe *et al.*, 2005).

Availability and Renewability of Biodiesel

Biodiesel is the only alternative fuel in which low-concentration biodiesel-diesel blends run on conventional unmodified engines. It can be stored anywhere that petroleum diesel fuel is stored. Biodiesel can be made from domestically produced, renewable oil seed crops such as soybean, rapeseed, and sunflower. The risks of handling, transporting, and storing biodiesel are much lower than those associated with petrodiesel. Biodiesel is safe to handle and transport because it is as biodegradable as sugar and has a high flash point compared to petroleum diesel fuel. Biodiesel can be used alone or mixed in any ratio with petroleum diesel fuel. The most common blend is a mix of 20% biodiesel with 80% petroleum diesel, or B_{20}.

Lower Emissions from Biodiesel

The combustion of biodiesel alone provides over a 90% reduction in total unburned hydrocarbons and a 75 to 90% reduction in polycyclic aromatic hydrocarbons. Biodiesel further provides significant reductions in particulates and carbon monoxide than petroleum diesel fuel (Laforgia *et al.*, 1994; Cardone *et al.*, 1998).

Fuel characterization data show some similarities and differences between biodiesel and petro diesel fuels (Shay, 1993). The sulfur content of petro diesel is 20 to 50 times that of biodiesels. The use of biodiesel to reduce N_2O is attractive for several reasons. Biodiesel contains little nitrogen, as compared with petro diesel. The N_2O reduction is strongly dependent upon initial N_2O concentrations and only slightly dependent upon temperature, where increased temperature increases N_2O reduction. In addition, biodiesel contains trace amounts of sulfur, so SO_2 emissions are reduced in direct proportion to the petro diesel replacement.

Biodiesel has demonstrated a number of promising characteristics, including reduction of exhaust emissions (Dunn, 2001). Vegetable oil fuels have not been accepted because they are more expensive than petroleum fuels. With recent increases in petroleum prices and uncertainties concerning petroleum availability, there is renewed interest in vegetable oil fuels for compression ignition engines (CIEs) or diesel engines. Alternative fuels for CIEs have become increasingly important due to increased environmental concerns and for various several socioeconomic reasons. In this sense, vegetable oils and animal fats represent a promising alternative to conventional diesel fuel (Dorado *et al.*, 2003).

One of the most common blends of biodiesel contains 20 volume percent biodiesel and 80 volume percent conventional diesel (EPA, 2002). The use of blends of biodiesel and diesel oil are preferred in engines in order to avoid some problems related to the decrease of power and torque and to the increase of NOx emissions (a contributing factor in the localized formation of smog and ozone) that occurs with an increase in the content of pure biodiesel in a blend (Schumacher *et al.*, 1996). Emissions of all pollutants except NOx appear to decrease when biodiesel is used.

The use of biodiesel in a conventional diesel engine dramatically reduces the emissions of unburned hydrocarbons, carbon dioxide, carbon monoxide, sulfates, polycyclic aromatic hydrocarbons, nitrated polycyclic aromatic hydrocarbons, ozone-forming hydrocarbons, and particulate matter. The net contribution of carbon dioxide from biomass combustion is small (Carraretto *et al.*, 2004). Reductions in net carbon dioxide emissions are estimated at 77 to 104 g/MJ of diesel displaced by biodiesel (Tan *et al.*, 2004). These reductions increase as the amount

of biodiesel blended into the diesel fuel increases. The greatest emissions reductions are seen with biodiesel. The exhaust emissions of commercial biodiesel and petrodiesel were studied in a 2003 model year heavy-duty 14 L six-cylinder diesel engine with EGR (Knothe *et al.*, 2006). The commercial biodiesel fuel significantly reduced PM exhaust emissions (75 to 83%) compared to the petrodiesel base fuel. However, NOx exhaust emissions increased slightly with commercial biodiesel compared to the base fuel. The chain length of the compounds had little effect on NOx and PM exhaust emissions, while the influence was greater on HC and CO, the latter being reduced with decreasing chain length. Non-saturation in the fatty compounds causes an increase in NOx exhaust emissions (Knothe *et al.*, 2006).

Biodegradability of Biodiesel

Biodiesel fuels can be used as a renewable energy source to replace conventional petroleum diesel. When degradation is caused by biological activity, especially by enzymatic action, it is called biodegradation. Biodegradability of biodiesel has been proposed as a solution for the waste problem. Biodegradable fuels such as biodiesels have an expanding range of potential applications and they are environmentally friendly. Therefore, there is growing interest in degradable diesel fuels that degrade more rapidly than conventional disposable fuels. In recent years biodiesel has become more attractive because of its environmental benefits and the fact that it is made from renewable resources (Ma and Hanna, 1999).

Biodiesel is non-toxic and degrades about four times faster than petro diesel. Its oxygen content improves the biodegradation process, leading to a decreased level of quick biodegradation. In comparison with petro diesel, biodiesel shows better emission parameters. It improves the environmental performance of road transport and reduces greenhouse emissions (mainly of carbon dioxide).

As biodiesel fuels are becoming commercialized, their existence in the environment is an area of concern since petroleum oil spills constitute a major source of contamination of the ecosystem (Peterson *et al.*, 1995). Among these concerns, water quality is one of the most important issues for living systems. It is important to examine the biodegradability of biodiesel fuels and their biodegradation rates in natural waterways in case they enter the aquatic environment in the

course of their use or disposal. Chemicals from biodegradation of biodiesel can be released into the environment. With the increasing interest in biodiesel, the health and safety aspects are of utmost importance, including determination of their environmental impacts in transport, storage, or processing (Ma and Hanna, 1999).

The biodegradability of several biodiesels in the aquatic environment shows that all biodiesel fuels are readily biodegradable. In one study, after 28day all biodiesel fuels were 77 to 89% biodegraded; diesel fuel was only 18% biodegraded (Zhang, 2003). The biodegradability data of petroleum and biofuels are presented in Table 2.1. In 28-d laboratory studies, heavy fuel oil had a low biodegradation of 11% due to its higher proportion of high-molecular-weight aromatics. Gasoline is highly biodegradable (28%) after 28 d. Vegetables oils and their derived methyl esters (biodiesels) are rapidly degraded to reach a biodegradation rate of between 76 and 90% (Zhang *et al.*, 2003). In their studies Zhang *et al.* (1998) have shown that vegetable oils are slightly less degraded than their modified methyl ester.

Table 2.1 Biodegradability data of Petroleum and biofuels (Demirbas, 2008)

Sr.No.	Fuel sample	Degradation in 28 d (%)
1.	Gasoline	28
2.	Heavy fuel	11
3.	Refined rapeseed oil	78
4.	Refined soybean oil	76
5.	Rapeseed oil methyl ester	88
6.	Sunflower seed oil methyl ester	90

- **Disadvantages of Biodiesel as Diesel Fuel**

The major disadvantages of biodiesel are its higher viscosity, lower energy content, higher cloud point and pour point, higher nitrogen oxide (NOx) emissions, lower engine speed and power, engine compatibility, and high price.

Table 2.2 shows the fuel ASTM standards of biodiesel and petroleum diesel fuels. Important operating disadvantages of biodiesel in comparison with petro diesel are cold start problems, lower energy content, higher copper strip corrosion, and fuel pumping difficulty from higher viscosity. This increases fuel consumption when biodiesel is used instead of pure petro diesel, in

proportion to the share of the biodiesel content. Taking into account the higher production value of biodiesel as compared to petro diesel, this increase in fuel consumption raises in addition the overall cost of application of biodiesel as an alternative to petro diesel. Biodiesel has a higher cloud point and pour point compared to conventional diesel (Prakash, 1998). Neat biodiesel and biodiesel blends increase nitrogen oxide (NOx) emissions compared with petroleum-based diesel fuel used in an unmodified diesel engine (EPA, 2002). Biodiesels on average decrease power by 5% compared to diesel at rated loads (Demirbas, 2006).

Table 2.2 ASTM standards of biodiesel and petro diesel fuels (Demirbas, 2008)

Property	Test method	ASTM D975 (petrodiesel)	ASTM D6751 (biodiesel, B100)
Flash point	D 93	325 K min	403 K
Water and sediment	D 2709	0.05 max %vol	0.05 max %vol
Kinematic viscosity (at 313 K)	D 445	1.3-4.1 mm^2/s	1.9-6.0 mm^2/s
Sulfated ash	D 874	-	0.02 max %wt
Ash	D482	0.01 max %wt	-
Sulfur	D 5453	0.05 max %wt	-
Cetane number	D 613	40 min	47 min
Aromaticity	D 1319	35 max %vol	-

2.9 Main Factors Affecting the Yield of Biodiesel

- **Alcohol Quantity**

Many researchers recognized that one of the main factors affecting the yield of biodiesel is the molar ratio of alcohol to triglyceride. Theoretically, the ratio for transesterification reaction requires 3 mol of alcohol for 1 mol of triglyceride to produce 3 mol of fatty acid ester and 1 mol of glycerol. An excess of alcohol is used in biodiesel production to ensure that the oils or fats will be completely converted to esters and a higher alcohol triglyceride ratio can result in a greater ester conversion in a shorter time (Dennis *et al*., 2010). On the other hand, an excessive amount of alcohol makes the recovery of the glycerol difficult, so that the ideal alcohol/oil ratio has to be established empirically, considering each individual process. Freedman *et al.*, 1984, studied the effect of molar ratio (from 1:1 to 6:1) on ester conversion with vegetable oils. Soybean, sunflower, peanut and cottonseed oils behaved similarly and achieved highest

conversions (93–98%) at a 6:1 molar ratio. However, increasing the molar ratio beyond the 6:1 may interfere with separation of glycerol because there is an increase in glycerol solubility. When glycerin remains in solution, it will drive the equilibrium back to the left, lowering the yield of esters (Gupta, 2008).

- **Reaction Temperature**

Temperature clearly influences the reaction and yield of the biodiesel product. A higher reaction temperature can decrease the viscosities of oils and result in an increased reaction rate, and a shortened reaction time. However, Leung and Guo (2006) and Eevera *et al.,* (2009) found that when the reaction temperature increases beyond the optimal level, the yield of the biodiesel product decreases because a higher reaction temperature accelerates the saponification reaction of triglycerides.

Nakpong *et al.,* 2010, studied the transesterification of jatropha curcas oil with methanol to oil ratio of 6:1, 1% NaOH catalyst, at four different temperatures 60, 50, 40, and 32^{o}C. The results indicate that methanolysis could occur at 32°C, but it would be incomplete, even if the reaction time were extended to 40 minutes. For the same reaction time, the methyl ester content increased with temperature. The methanolysis proceeded to completion in 40 minutes at 60°C; yielding a methylester content of 98.6% w/w. similar result is also reported by other researchers (Freedman, 1984; Matthew, 2008).

- **Catalyst**

Catalyst type and concentration can affect the yield of the biodiesel product. As mentioned before, the most commonly used catalyst for the reaction is sodium hydroxide. However, Freedman *et al*., (1984) found that sodium methoxide was more effective than sodium hydroxide probably because upon mixing sodium hydroxide with methanol a small amount of water will be produced, which will affect the product yield because of the hydrolysis reaction (Guo, 2005). This is the reason why the catalyst should be added into the methanol first and then mixed with the oil.

2.10 Comparison between Biodiesel Production from Algae & Vegetables

Quantifying the land use changes associated with intensive biofuel feedstock production relies upon many assumptions (Chisti, 2007), but it is clear that the accelerated cultivation of terrestrial plant biomass for biofuels will have an exceptionally large land footprint (Table 2.3). For example, the United States has the fourth largest absolute biodiesel potential of the 119 countries studied by Johnston and Holloway (Johnston and Holloway, 2007). However, recent work has suggested that the projected year 2016 demand for corn ethanol alone would require 43% of all U.S. land used for corn production in 2004 (Chisti, 2007).

A related study concluded that the annual corn production needed to satisfy one half of all U.S. transportation fuel needs would require an area equivalent to more than eight times the U.S. land area that is presently used for crop production (Chisti, 2007). Other land-based crops would require less cropland, based on their oil content: oil palm (24% of current cropland area), coconut (54%), jatropha (77%), canola (122%) and soybean (326%) (Chisti, 2007). Moreover, recent work indicates that the ability of countries to grow terrestrial crops explicitly for the production of biofuels such as ethanol and biodiesel is significantly overestimated (Johnston and Holloway, 2007), contributing to concerns that these biofuels are not feasible options for providing a significant fraction of global fuel demand.

Table 2.3 Comparison of estimated biodiesel production efficiencies from vascular plants & microalgae (Source: Emad A.Shalaby, Algal biomass and Biodiesel production)

Biodiesel Feedstock	**Area needed to meet global oil Demand (10^6 hectares)**	**Area required as a percent of total global land**	**Area required as a percent of total arable global land**
Cotton	15000	101	757
Soybean	10900	73	552
Mastard seed	8500	57	430
Sunflower	5100	34	258
Rapeseed/canola	4100	27	207
Jatropha	2600	17	130[a]
Oil palm	820	5.5	41
Microalgae($10g/m^3$/day, 30%TAG)	410	2.7	21[b]
Microalgae($50g/m^3$/day,50%TAG)	49	0.3	25[b]

[a]Jatropha is mainly grown on marginal land

[b]Assuring that microalgae ponds and bioreactors are located on non-arable land

2.11. Fuel Property Measurement

Replacement of existing fuels with new fuel formulations requires understanding the critical fuel properties to insure that the new fuels can be used. Discussed in this section are some key fuel properties as well as the methods to measure these properties. The properties discussed are specific gravity, kinematic viscosity, flash point, boiling point (distillation test), cetane number, cloud point, pour point, and copper strip corrosion.

Specific Gravity

The specific gravity is a relative measure of the density of a substance. It is defined as the ratio of the density of the substance, ρ, to a reference density, ρ_{ref}. The equation for the specific gravity (SG) is $SG = \rho/\rho_{ref}$. The most common reference density used in the measurement of specific gravity is the density of water at 4˚C, which corresponds to a reference density of 1 g/cc. The higher the specific gravity of a biofuel indicates the heavier the biofuel is.

Flash Point

The flash point is defined as the "lowest temperature corrected to a barometric pressure of 101.3kPa (760 mm Hg), at which application of an ignition source causes the vapors of a specimen to ignite under specified conditions of test." This test, in part, is a measure of residual alcohol in the B100.

The flash point is determined by heating a sample of the fuel in a stirred container and passing a flame over the surface of the liquid. If the temperature is at or above the flash point, the vapor will ignite and an easily detectable flash can be observed. The flash need not correspond to a sustained flame. The "fire point" is sometimes used to designate the fuel temperature that will produce sufficient vapor to maintain a continuous flame.

The flash point is a determinant for flammability classification of materials. The typical flash point of pure methyl esters is > 200 ° C, classifying them as "non-flammable". However, during production and purification of biodiesel, not all the methanol may be removed, making the fuel flammable and more dangerous to handle and store if the flash point falls below 130ºC. Excess methanol in the fuel may also affect engine seals and elastomers and corrode metal components.

Cetane Number

Perhaps the most important measure of ignition characteristics of diesel and/or biodiesel fuels is the cetane number, since it directly pertains to ignition within compression ignition engines. Thus, it is the approximate equivalent of octane rating for gasoline (petrol).

Cetane number is a measurement of the combustion quality of diesel fuel during compression ignition. It is a measure of a fuel's ignition delay, the time period between the start of injection and the first identifiable pressure increase during combustion of the fuel. In a particular diesel engine, higher cetane fuels will have shorter ignition delay periods than lower cetane fuels. In short; the higher the cetane number, the more easily the fuel will combust in a compression setting (such as a diesel engine).

Acid Number

It is "The quantity of base, expressed as milligrams of potassium hydroxide per gram of sample, required to titrate a sample to a specified end point." The acid number is a direct measure of free fatty acids in B_{100}. The free fatty acids can lead to corrosion and may be a symptom of water in the fuel.

Cloud Point

The cloud point is the temperature at which a cloud of wax crystals first appears in a liquid upon cooling. Therefore, it is an index of the lowest temperature of the fuel's utility under certain applications. The cloud point is a critical factor in cold weather performance for all diesel fuels. Operating at temperatures below the cloud point for a diesel fuel can result in fuel filter clogging due to the wax crystals.

3. MATERIALS AND METHODS

3.1 Materials

The major raw materials used during the experiment for biodiesel production were algae oil, methanol (analytical grade) and sodium hydroxide catalyst, physicochemical property test study chemicals as; potassium hydroxide, hydrochloric acid, phosphoric acid, phenolphthalein, sodium chloride and distilled water. The algal oil was obtained by soxhlet extraction of dried algal biomass using hexane as a solvent. The chemicals were purchased and obtained from Neway Private Limited Company, BaleZaf chemical import company and from chemical engineering laboratory; in Addis Ababa institute of technology.

3.1.1. Algae Harvesting and Drying

The Wet algal biomass in which Scenedesmus is the dominant one were harvested using a 60 micro filter from Addis Ababa University Institute of Technology which was cultivated in a green house by Ato Abreham (PhD fellow in Environmental Engineering Stream) for water treatment purpose. 5 kg of wet biomass was obtained in two rounds and 9 kg of wet biomass was obtained from Addis Ababa waste water treatment plant which is found in Kality. The Wet biomass was freeze dried using a freeze drier and weighed. From the two sources a total of 2.5 kg of the dry biomass was obtained. The obtained dry biomass was grounded to a 0.5micrometer mesh size sieve. The powdered algal biomass was used for oil extraction using soxhlet apparatus.

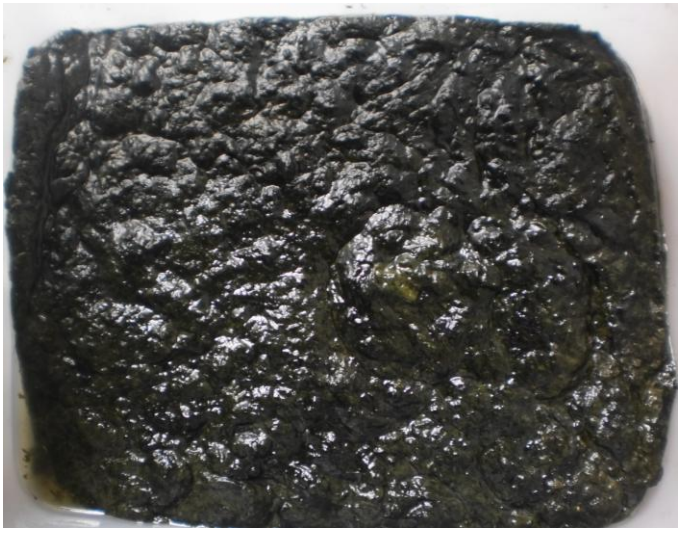


Figure 3.1 wet algal biomass

3.2 Experimental Methods

All experimental works except freeze drying of the wet algal biomass were done in Chemical Engineering Department, analytical laboratory. Freeze drying of the wet algal biomass was done in Addis Ababa University Science Faculty, Food Science Department Laboratory and Ethiopian Health and Nutrition Research Institute.

a) Arat Kilo Food Science Laboratory b) Ethiopian Health and Nutrition Research Institute

Figure 3.2 Freeze drying

The general experimental setup is shown in figure 3.3 below. The algae biomass is collected from the two sources by filtering using a 60 micrometer filter and dried in a freeze dryer. Oil was

extracted from the dried and ground algae using soxhlet extraction method with a solvent n-hexane. After the oil obtained is refined and analyzed for its physicochemical properties, it was subjected to a chemical reaction/ transesterification with methoxide (a mixed solution of methanol and sodium hydroxide). After the transesterification reaction is completed the crude biodiesel was separated from the glycerol using a separator funnel and the methanol was distilled to be recycled. Hot water was applied to remove the impurities from the biodiesel and was dried in an oven to get a pure biodiesel. Finally, the obtained biodiesel was checked for its physico chemical properties and compared with the international standard.

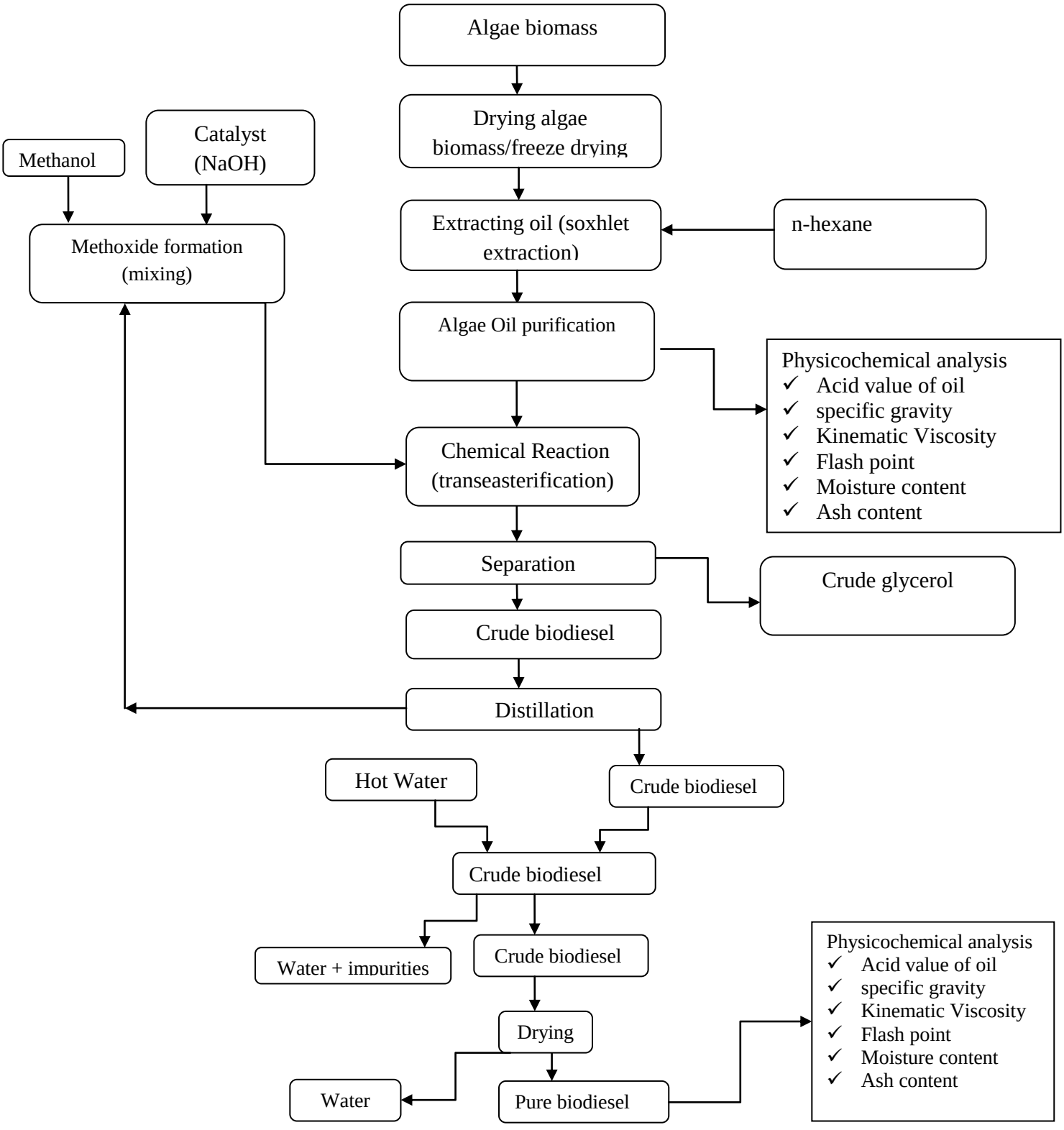


Figure 3.3 General experimental setup

3.2.1 Algal Oil Extraction Process

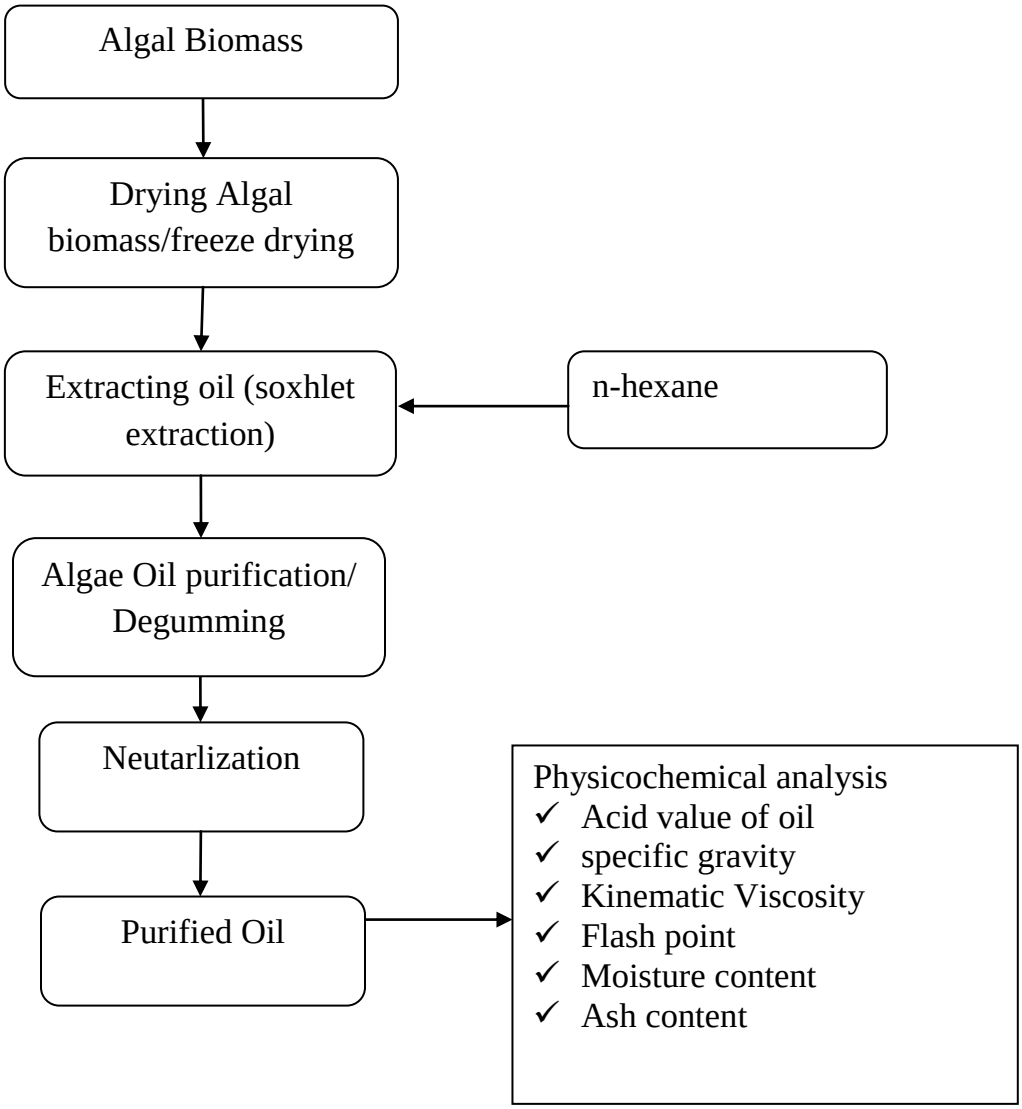


Figure 3.4 oil extraction processes

To increase the surface area and thereby the oil extraction efficiency, the dried algae biomass is ground by mortar and pestle to a 0.5 micrometer sieve size. The oil was extracted by solvent extraction method. The extraction method used is known as soxhlet extraction. A 20 g sample of dried and grounded algal biomass was placed in a cellulose thimble, filling it two-thirds to three-fourths full. The thimble was placed inside the soxhlet apparatus and a plug of glass wool was

placed on top of the sample to prevent spillage. The thimble is made from thick filter paper, which is loaded in the main chamber of soxhlet extractor. The 500ml round-bottomed flask is filled with 100 ml of the solvent hexane and the soxhlet extractor is placed onto the flask containing the extraction solvent. The soxhlet is then equipped with a condenser that is connected with a chiller. The temperature of the chiller was set at 10^{O}C. The outlet of the fist condenser was connected to the inlet of the second condenser and the outlet from the second condenser was recycled back to the chiller. The solvent is heated to reflux.

The extraction was carried out at 72^{0}C (a little bit above the boiling temperature of hexane) for 9h in a water bath heated by a thermostat (set-up is shown in Figure 3.5). The solvent hexane forms vapors, which travels up a distillation arm, and floods into the chamber housing the thimble of solid. The condenser ensures that any solvent vapor that cools drips down into the chamber housing the solid material. The chamber containing the solid material slowly fills with warm solvent. Some of the desired compound will then dissolve in the warm hexane. When the soxhlet chamber is almost full, the chamber is automatically emptied by the siphon side arm, with hexane running back to the distillation flask.

This cycle was repeated for varying time. During each cycle, a portion of the oil is dissolved in hexane. After many such cycles, desired oil was concentrated in the distillation flask. After extraction, hexane was removed, yielding the extracted compound. The insoluble portion of the algae remains in the thimble. The extracted oil and hexane was separated by fractional distillation and hexane condenser was used to recover hexane. The oil obtained from each run were mixed and prepared for physicochemical properties analysis.

a) Dried algal biomass

b) Ground dry algal biomass

Figure 3.4 Dried and powdered algal biomass

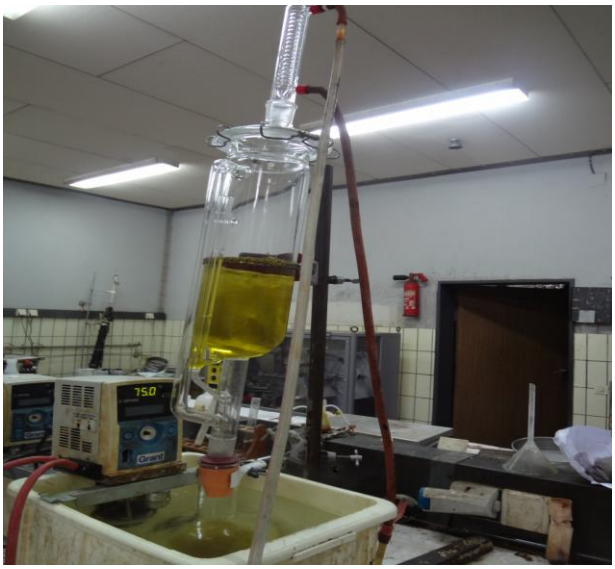


Figure 3.5 algal oil extractions (soxhlet extraction)

- Oil Content Determination

To determine or calculate the oil content of the powdered algal biomass, the following method was employed

$$\text{Oil content of the grinded algae}(\%) = \frac{\text{Weight of oil obtained or extracted}}{\text{Weight of dried algae used}} \times 100\% \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.1)$$

3.2.2 Physicochemical Properties of Extracted Oil

The oil was analyzed for physicochemical properties such as specific gravity, kinematic viscosity, acid value, percentage of FFA content, and moisture content. These parameters directly or indirectly may affect the quality of the biodiesel.

Determination of Specific Gravity (SG)

To determine the specific gravity of the oil, a 50 ml sample that is heated to a temperature of 25^0C was filled into graduated cylinder. Densimeter was inserted into the graduated cylinder to measure the SG of the oil and the reading was taken. On the other hand the specific gravity or relative density of the oil can be determined by taking the ratio of the mass of the 50ml oil at t^0C to the mass of an equal volume of water at 20 ^{0}C. Usually it is expressed as "sp gr at t°C/20°C".

Determination of Kinematic Viscosity

A digitalized Sine-Wave (SV-10, 2011, Australia) vibro viscometer was employed to determine the viscosity of the oil. The sample filled in the viscometer cup was kept in a 40 ^{0}C constant temperature water bath for 30 minutes. The vibro viscometer tip was inserted in the cup containing the sample and the reading was recorded for a fixed volume of liquid. The reading of the vibro viscometer was dynamic viscosity therefore the value had to be corrected to find the kinematic viscosity using the following equation

$$\text{Kinematic Viscosity of oil } (\nu) = \frac{\text{Dynamic viscosity of oil } (\eta)}{\text{Density of oil } (\rho)} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots (3.2)$$

Where:

η = dynamic viscosity of the oil

ρ = density of the oil

ν = kinematic viscosity

Determination of Acid Value

Acid value or acid number is "the quantity of base, expressed as milligrams of potassium hydroxide per gram of sample, required to titrate a sample to a specified end point." The acid number is a direct measure of free fatty acids in B100. The free fatty acids can lead to corrosion and may be a symptom of water in the fuel (Gerpen, 2004).

To determine the acid value of oil a titration solution of 0.1N of KOH in distilled water was prepared. 2g of oil was added to a beaker and heated at 70^{o}C for 3 minutes. Then, 20ml of anhydrous ethanol (99.5%w/w), 20ml of diethylether and 5 drops of phenolphthalein were added into the titration beaker that contains sample oil. Then oil sample was mixed thoroughly with a mixture of 20ml of ethanol, 20ml of diethylether and 5 drops of phenolphthalein. Finally, titration solution, 0.1N of KOH was being added 1 drop at a time until the first color (until pinkish color appeared) change was observed. Once the color change was observed, the titration volume (ml) was recorded and the recorded volume was used to calculate the acid value using the equation;

$$AV = \frac{M\ x\ N\ xV}{W} \quad \text{..... (3.3)}$$

Where; N= normality of aqueous solution of KOH

V= Volume of titrant used for titration (ml)

M = Molecular weight of KOH

W = the weight of oil sample

Free Fatty Acid(FFA) Determination

The free fatty acid, FFA of the oil was calculated empirically from the acid value previously determined using the equation

$$\%FFA = \frac{AV}{2} \quad \text{..... (3.4)}$$

Where: %FFA = percentage of free fatty acid, AV = Acid Value of the oil

Saponification Value of Oil

Saponification value (SV) is expressed as the number of milligram of KOH required to saponify 1g fat. A 0.5mol/l potassium hydroxide in anhydrous ethanol (99.5%w/w) and a titration volume of 0.5mol/l hydrochloric acid solution in distilled water was prepared to determine the saponification value of the oil. Then a 2g oil sample and a 25ml solution of 0.5mol/l potassium hydroxide in ethanol were added in a 250ml conical flask and were heated at a temperature of 70 ^{o}C for 30 minutes. Then the heated mixture was cooled immediately and 5 drops of phenophtaline was added as indicator. Before the test liquid solidified the sample was titrated with 0.5mol/l hydrochloric acid solution. As the color change observed the titration was stopped and the value was recorded. The same procedure was also performed using a blank level test (without addition of the oil sample) in parallel with the above activity. The saponification value was then calculated using the equation

$$\text{Saponification value, mg KOH/g} = \frac{\text{MW x N x } (Vb - Vs)}{\text{w}} \quad \text{……………………. (3.5)}$$

Where, MW= molecular weight of KOH

N = normality of HCL solution

V_b = volume of HCl solution used in blank

V_s= volume of HCl solution used in the sample

W = weight of oil used

Determination of Moisture Content

A dish was weighed with and without oil. The dish with oil was dried in an oven at 105 ^{o}C for 7hr, weighing each 2hr till constant weight is obtained and finally the weight was taken and compared with the initially recorded weight. The percentage weight in the oil was calculated using the formula;

$$\text{Moisture Content} = \frac{\text{W1} - \text{W2}}{W1}\text{X100\%} \quad \text{…………………………………………...… (3.6)}$$

Where; W_1 = Original weight of the sample

W_2 = Weight of the sample after drying

Ash Content Determination

Furnace was used to determine the ash content of the oil. A burning cup containing 20g oil was placed in a furnace for 4 hour which was set at a temperature of 550 ^{0}C. Then after burning the residue sample was weighted and ash content was calculated.

$$\text{Ash Content } \% \left(\frac{w}{w}\right) = \frac{\text{mass of oil after burning}}{\text{initial mass of oil}} x100\% \quad \text{.................................... (3.7)}$$

3.2.3 Purification of Crude Oil

- **Degumming**

This step was used to remove phosphorus compounds of crude oil using a phosphoric acid and hot distilled water. The oil was heated to 70 ^{o}C. 3% Distilled water (v/v of oil) which was heated to 80 ^{o}C and 2% phosphoric acid (v/v of oil) were mixed with the heated oil. The mixtures were stirred at speed of 200 rpm for 1 hour at a temperature of 70 ^{o}C. The impurities were separated using a centrifuge at a speed of 800 rpm for 20 minutes.

- **Neutralization**

After determining the free fatty acid (FFA) of oil, the free fatty acid was neutralized by 0.05N of NaOH. Neutralization was done by heating the oil at 70 ^{o}C. The mixture of oil and NaOH solution were stirred at 200 rpm at a temperature of 70 ^{o}C for 1 hour. The mixture was washed with a distilled water to remove a trace NaOH and produced soap. Finally, trace water was removed in an oven drying at a temperature of 105 ^{o}C.

3.2.4 Transesterification Process/ Conversion to Biodiesel

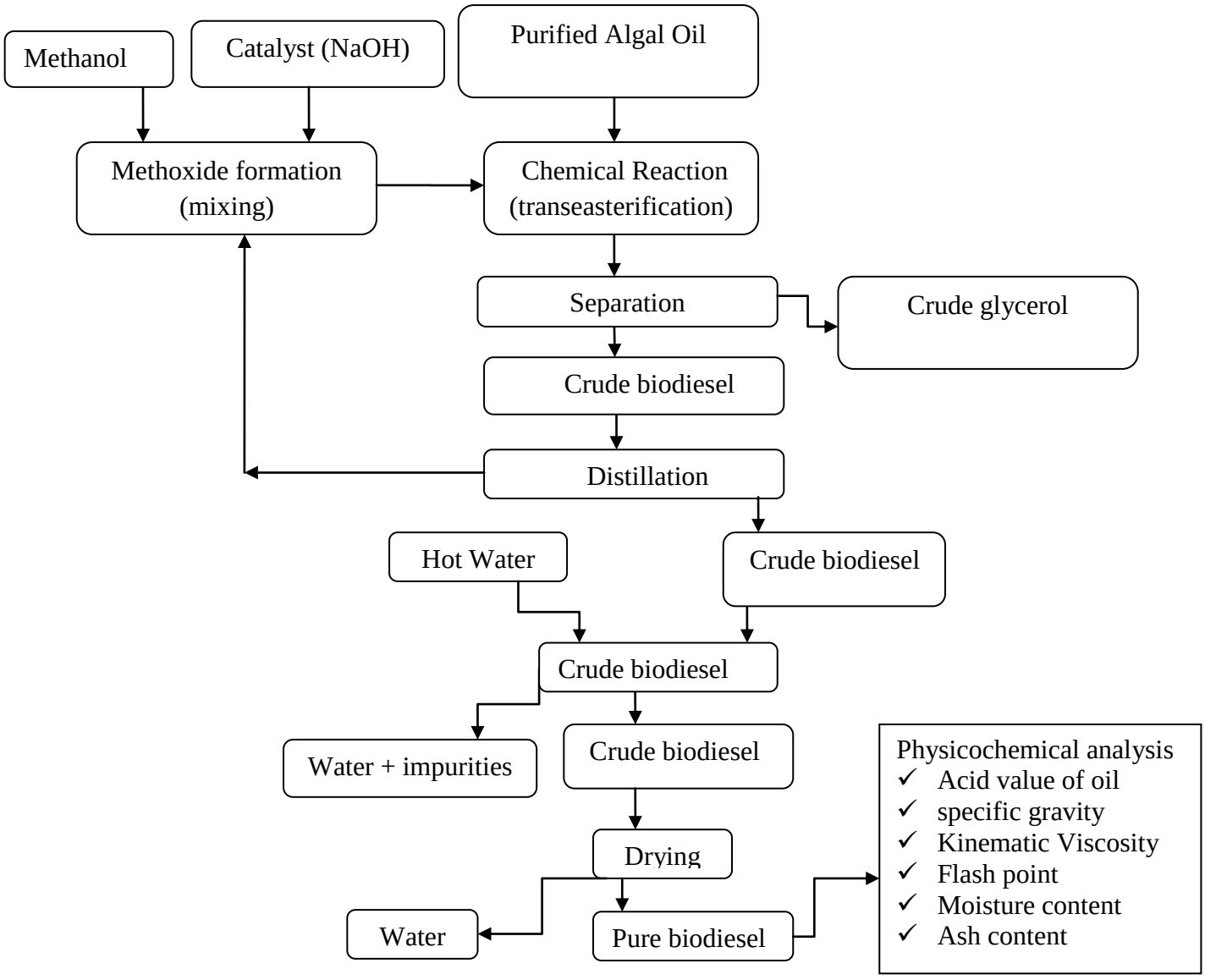


Figure 3.5 biodiesel production processes

Once the oil is extracted, purified and the level of free fatty acid reached to the required level, it must be processed further to convert it into biodiesel. Raw fats and oils (glycerides) cannot be used directly in diesel engines without modifications to the fuel systems. The glycerides can contaminate the lubricating oil, induce the formation of carbon deposits in the engine, and affect engine durability, among other issues. Although other methods exist (pyrolysis, microemulsion, and blending of oils) current emphasis is on the process known as transesterification

(alcoholysis). In transesterification, fats or oils are reacted with an alcohol to produce esters and glycerol in a one step reaction. In this research the alkali catalytic transesterification methods was applied to convert the purified oil to biodiesel. In the alkali catalytic methanol transesterification method, the catalyst (NaOH) and alcohol (methanol) are mixed together through vigorous stirring in a small reactor prior to the reaction with glycerides. This mixture is then fed to a reactor, where it is then combined with raw oils and continuously stirred for 2 h at a speed of 500rpm. A successful transesterification reaction produces two liquid phases: ester and crude glycerine. Next, the mixture of glycerin, biodiesel, and unreacted methanol is fed to a separator.

Biodiesel and methanol are separated from the glycerin byproduct by use of gravity settling. Crude glycerine, the heavier liquid, will collect at the bottom after several hours of settling. Phase separation can be observed within 10 min and can be complete within 2 h of settling. Complete settling can take as long as 20 h. After settling is complete, water is added at the rate of 5.5% by volume of the methyl ester of oil and then stirred for 5 min, and the glycerine is allowed to settle again. The washing process is continued until the ester layer becomes clear. After settling, the aqueous solution is drained and hot distilled water is added at 28% by volume of oil for the final washing. Finally, biodiesel and methanol are purified through distillation to allow for collection of pure biodiesel. The resulting biodiesel fuel when used directly in a diesel engine will burn up to 75% cleaner than petroleum D2 fuel. (Ma and Hanna, 1999; Demirbas, 2003; Demirbas, 2008).

Figure 3.6 Transesterification reaction & Separation of biodiesel from glycerol

3.2.5 Design of Experiment

A three variable Box-Behnken design for response surface methodology was used to study the combined effects of catalyst concentration, methanol to oil molar ratio and reaction temperature on the amount of biodiesel yield over three levels. The range and levels of the variables used for optimization are shown in Table 3.1.

Process variables revised were reaction temperature, molar ratio of methanol to oil and weight percentage of catalyst. The reaction time and mixing intensity were fixed at 2 hours and 500 rpm respectively for all experimental runs.

The Box-Behnken design is suitable for the exploration of quadratic response surfaces and generates a second degree polynomial model, which in turn is used in optimizing a process using a small number of experimental runs. This design requires an experimental number of runs according to:

$$N= k^2+ k + C_p \quad \text{..... (3.8)}$$

Where k is the factor number which is three in this case and C_p is the number of replications at the center point which is also three in this case. The design which was developed using Design Expert ® 7.0.0 (Stat-ease, Inc. Minneapolis, USA), resulted in 15 experimental runs as shown in

Table 3.2. The 15 experimental runs were randomized to maximize the effects of unexplained variability in the observed responses due to extraneous factors. The levels of the independent variables as shown in Table 3.1 were selected based on the operating limit of biodiesel production process conditions and previous research works.

The upper temperature level (70°C) is near to the theoretical boiling point of methanol, and catalyst concentration of 3 % by weight of oil was based on literature data (Nakpong, 2010). The lower molar ratio (3:1) was the theoretically declared maximum yield giving molar ratio (Gerpen, 2004). To avoid systematic errors, the order in which the runs were made was randomized. The relation between the coded and actual values is described as follows:

$$x_i = (X_i - X_o) / \Delta X_i \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3.9)$$

Where x_i and X_i are the coded and actual values of the independent variable respectively.

X_o is the actual value of the independent variable at the center point, and

ΔX_i is the step change of X_i

A second degree polynomial was fitted to the experimental data using the statistical package design Expert ® 7.0.0 to estimate the response of the dependent variable and predict the optimal point. The second degree polynomial was expressed as follows:

$$Y= b_0+b_1X_1+b_2X_2+b_3X_3+b_{12}X_1X_2 + b_{13}X_1X_3+b_{23}X_2X_3+b_{11}X_1^2+b_{22}X_2^2+b_{33}X_3^2$$

Where Y is predicted response, X_1, X2 & X_3 are independent variables, b_o is offset term, b_1, b_2, b_3 are linear effects, b_{11}, b_{22}, b_{13} are interaction terms.

The independent variables and levels used for base catalyzed transesterification and the order for conducting the 15 experimental runs was presented in table 3.1 and table 3.2 respectively.

Table 3.1 coded and actual levels of the factors for three factor Box-Behnken design

Independent Variables	**Symbols**	**Unit**	**Coded & Actual Levels**		
			-1	**0**	**+1**
Amount of catalyst	A	%	1	2	3
Reaction temperature	B	^oC	40	55	70
Methanol to oil ratio	C	-	3	6	9

Table 3.2 Box-Behnken experimental design matrix

Run	Factors			Response
	Temperature (^{0}C)	Catalyst (%) (wt/wt)	Methanol to oil ratio	
1.	55	2.00	6	
2.	40	2.00	3	
3.	55	2.00	6	
4.	55	1.00	9	
5.	70	2.00	3	
6.	55	3.00	9	
7.	55	1.00	3	
8.	40	3.00	6	
9.	55	2.00	6	
10.	70	2.00	9	
11.	70	1.00	6	
12.	70	3.00	6	
13.	40	2.00	9	
14.	55	3.00	3	
15.	40	1.00	6	

3.2.5.1 Feed Material Requirement for the transesterification process

For each experimental run of the transesterification process, 50ml of purified oil was used. The amount of catalyst and methanol required was calculated as follows:

Amount of Methanol required

The amount of methanol required when the molar ratio of methanol to oil is 3:1

$$\frac{mole\ of\ methanol}{mole\ of\ oil} = 3 \quad \text{..... (3.10)}$$

$$\frac{\text{Given mass of methanol/Molecular mass of methanol}}{Given\ mass\ of\ oil / Molecular\ mass\ of\ oil} = 3$$

$$\frac{Density\ of\ methanol * Volume\ of\ methanol\ /\ Molecular\ mass\ of\ methanol}{Density\ of\ oil * Volume\ of\ oil\ /\ Molecular\ mass\ of\ oil} = 3$$

$$\frac{\frac{0.791g}{ml} * Volume\ of\ methanol\ /\ 32.04g/mol}{\frac{0.94g}{ml} * 50ml\ /\ 912g/mol} = 3$$

Volume of methanol = 6.26ml

Similarly,

Volume of methanol required when the molar ratio of methanol to oil is 6:1and 9:1 was calculated and obtained as 12.52ml & 18.79ml respectively.

Amount of Catalyst required

The amount of catalyst required when catalyst weight to oil ratio is 1%

$$\frac{Mass\ of\ catalyst\ (NaOH)}{Mass\ of\ oil}\ x\ 100\% = 1\% \qquad \text{..... (3.11)}$$

$$\frac{Mass\ of\ catalyst\ (NaOH)}{Density\ of\ oil * Volume\ of\ oil}\ x\ 100\% = 1\%$$

$$\frac{Mass\ of\ catalyst\ (NaOH)}{\frac{0.94g}{ml} * 50ml}\ x\ 100\% = 1\%$$

Mass of catalyst (NaOH) = 0.47g

Similarly, the amount of catalyst required when catalyst weight to oil ratio is 2% & 3% was calculated & obtained as 0.94g & 1.41g respectively.

3.2.5.2 Statistical Analysis

The design expert software was used for the analysis of variance (ANOVA). Response surface method of Box Behnken was used to generate surface plots using the fitted equation obtained from the regression analysis, holding one of the independent variables constant. Experimental values of conversion of the oil to biodiesel at the design points at different parameters was obtained and from those values the yield was calculated and recorded on table 4.4. The response of the transesterification process which is biodiesel yield was calculated as ;

$$Biodiesel\ Yield(in\ \%) = \frac{Weight\ of\ Biodiesel\ obtained}{Weight\ of\ Oil\ used} x100\% \quad \text{…..…… (3.12)}$$

4. RESULTS AND DISCUSSION

4.1 Oil Extraction and Characterization

4.1.1 Oil Extraction

From the 2.5kg grounded algal mass, 798.4g of oil was obtained. After the oil was extracted and separated from the solvent (hexane), 850ml were obtained. The oil content of the powdered algal biomass was calculated using equation 3.1.The result showed that 32% of the dried mass of the sample (the algae) is oil.

4.2 Characterization of Algal Oil

4.2.1 Density of the Oil

The specific gravity was measured by densimeter and obtained to be 0.94g/ml. From the simple empirical formula explained in equation 3.2, the density of the oil was obtained to be 0.94g/ml =940kg/m^3.

4.2.2 Kinematic Viscosity of the Oil

The dynamic viscosity of the oil was measured using Sine-Wave (SV-10, 2011, Australia) vibro viscometer at 40^0c and recorded as 39.34m.pa.s = $39.34 * 10^{-3}$ kg/m.s. The kinematic viscosity can be calculated using the equation 3.3 and the result was obtained to be 41.85mm^2/s. The result shows that the oil obtained is highly viscous & needs transesterification so as to minimize its viscosity.

4.2.3 Acid Value of the Oil

The acid value of the oil was determined using the method stated under section 3.2.2. The results were recorded in table 4.1 as shown below.

Table 4. 1: Titration results for Acid value test

Run number	Mass of oil (g)	Titration volume (V_{KOH}) (ml)	Color change	Acid Value (mg KOH/g oil)	FFA
1.	2	1.69	Yellow to pink	4.74	2.37
2.	2	1.61	Yellow to pink	4.52	2.26
3.	2	1.65	Yellow to pink	4.63	2.32
Average		1.65		4.63	2.32

As it can be shown in table 4.1 the titration volume at which the first color change observed on average was 1.65 ml. The acid vlaue of the oil was calculated using equation 3.3 and the result was obtained to be 4.63mg KOH/g oil

4.2.4 Free Fatty Acid of the Oil

The free fatty acid of the oil is half of the acid value of the oil. Using equation 3.4 the free fatty acid is calculated and obtained to be 2.32%. The result shows that the oil needs pretreatment to reduce the amount of free fatty acidso as to overcome soap formation during transesterification reaction.

4.2.5. Saponification Value of Oil

The procedure used to determine saponification value of oil was stated in section 3.2.2. The titration results for saponification test are illustrated in Table 4.2.

Table4. 2: Titration results for Saponification value test

Run Number	Mass of oil (g)	N_{HCl}	V_{bHCl}	V_{sHCl}	V_{bHCl}- V_{sHCl}	Saponification Value (mgKOH/g oil)
1	2	0.46	44.3	28.6	15.7	202.58
2	2	0.46	43.5	27.7	15.8	203.87
3	2	0.46	43	27.6	15.4	198.7
Average value						**201.72**

The saponification value was then calculated using equation 3.4 & gives an average saponification value of 201.72mgKOH/g of oil.

4.2.6 Moisture Content of the Oil

Moisture content of the oil was determined using equation 3.6 and it is obtained to be 0.5%. The result shows that further removing of the moisture is required to avoid soap formation in transesterification reaction.

4.2.7 Ash content of the Oil

Using equation 3.7 the ash content of the oil was determined to be 0.045%. The physicochemical properties of the oil are summarized in table 4.3 below.

Table 4.3 Summary of physicochemical properties of the oil

S.n.	Physicochemical property	Units	Value	Remark
1.	Density	kg/m^3	940	
2.	Kinematic Viscosity	mm^2/s	41.85	
3.	Acid value	mg KOH/g oil	4.63	
4.	Free fatty acid	%	2.31	
5.	Saponification value	mg KOH/g	201.72	
6.	Moisture content	%	0.5	
7.	Ash content	%	0.045	

4.3 Biodiesel Production and Analysis of Effects of the Parameters

4.3.1 Transesterification Process

The transesesterification process was carried out using the method discussed in section 3.3.4. The actual results obtained from the 15 runs were recorded in table 4.4 and used to analyze the variance.

4.3.2 Statistical Analysis

The design expert software was used for the analysis of variance (ANOVA). Response surface method of Box Behnken was used to generate surface plots using the fitted equation obtained from the regression analysis, holding one of the independent variables constant. Experimental values of conversion of the oil to biodiesel at the design points at different parameters was

obtained and from those values the yield was calculated and recorded on table 4.4. The response of the transesterification process which is biodiesel yield was calculated using equation 3.12;

Table 4.4 Actual and predicted values of biodiesel yield

Run	Factors			Biodiesel(ml)		% of Biodiesel Yield		Residuals
	Temperature	Catalyst	Methanol to oil ratio	Actual	Predicted	Actual	Predicted	
1.	55	2.00	6	45	44.66667	90	89.33333	0.333333
2.	40	2.00	3	29	28.3125	58	56.625	0.6875
3.	55	2.00	6	44	44.66667	88	89.33333	-0.66667
4.	55	1.00	9	35	34.6875	70	69.375	0.3125
5.	70	2.00	3	29	28.9375	58	57.875	0.0625
6.	55	3.00	9	33	32.5625	66	65.125	0.4375
7.	55	1.00	3	32	32.4375	64	64.875	-0.4375
8.	40	3.00	6	32	32.375	64	64.75	-0.375
9.	55	2.00	6	45	44.66667	90	89.33	0.333333
10.	70	2.00	9	24	24.6875	48	49.375	-0.6875
11.	70	1.00	6	29	28.625	58	57.25	0.375
12.	70	3	6	25	24.75	50	49.5	0.25
13.	40	2.00	9	34	34.0625	68	68.125	-0.0625
14.	55	3.00	3	33	33.3125	66	66.625	-0.3125
15.	40	1.00	6	29.5	29.75	59	59.5	-0.25

The predicted model equation that correlates the response to the process variables interms of actual value was given below.

Final Equation Interms of Coded Factors:

$$yield = +44.67 - 2.19 * A - 0.31 * B + 0.37 * C - 1.62 * A * B - 2.50 * A * C - 0.75 * B * C - 10.02 * A^2 - 5.77 * B^2 - 5.65 * C^2$$

Where, A-temperature

B-catalyst

C-molar ratio of methanol to oil

Final Equation Interms of Actual Factors:

$$yield = -161.07870 + 5.30324 * temperature + 30.22917 * catalyst + 11.20833 * molar\ ratio - 0.10833 * temperature * catalyst - 0.055556 * temperature * molar\ ratio - 0.25000 * catalyst * molar\ ratio - 0.044537 * temperature^2 - 5.77083 * catalyst^2 - 0.62731 * molar\ ratio^2$$

Table 4.5 Analysis of variance /ANOVA/ for response surface quadratic model

Source	Squares	df	Mean square	F value	P-value Prob>F	Significance
Model	617.83	9	68.65	131.80	<0.0001	significant
A	38.28	1	38.28	73.50	0.0004	
B	0.78	1	0.78	1.50	0.2752	
C	1.13	1	1.13	2.16	0.2016	
AB	10.56	1	10.56	20.28	0.0064	
AC	25.00	1	25.00	48.00	0.0010	
BC	2.25	1	2.25	4.32	0.0922	
A^2	370.77	1	370.77	711.88	<0.0001	
B^2	122.96	1	122.96	236.09	<0.0001	
C^2	117.69	1	117.69	225.97	<0.0001	
Residual	2.60	5	0.52			
Lack of Fit	1.94	3	0.65	1.94	0.3583	Not significant
Pure Error	0.67	2	0.33			
Cor Total	620.43	14				

As Table 4.5 shows, the model F-value of 131.80 implies the model is significant. There is only a 0.01% chance that a " Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, AB, AC, A^2, B^2 and C^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

As it can be seen from p-values of the model coefficients, the value of temperature in both linear and quadratic is much less than 0.001. This indicated that temperature is the most significant factor in determining the model than the rest. To minimize error, all of the coefficients were considered in the design. The low lack of fit from the ANOVA analysis indicated that the model does indeed represent the actual relationships of reaction parameters, which are well within the selected ranges (Table 4.5). The Lack of Fit F-value of 3 implies its insignificance relative to the pure error. Non-significant lack of fit is good because we want the model to fit. The actual values versus predicted values obtained using the developed correlation is plotted in the figure below.

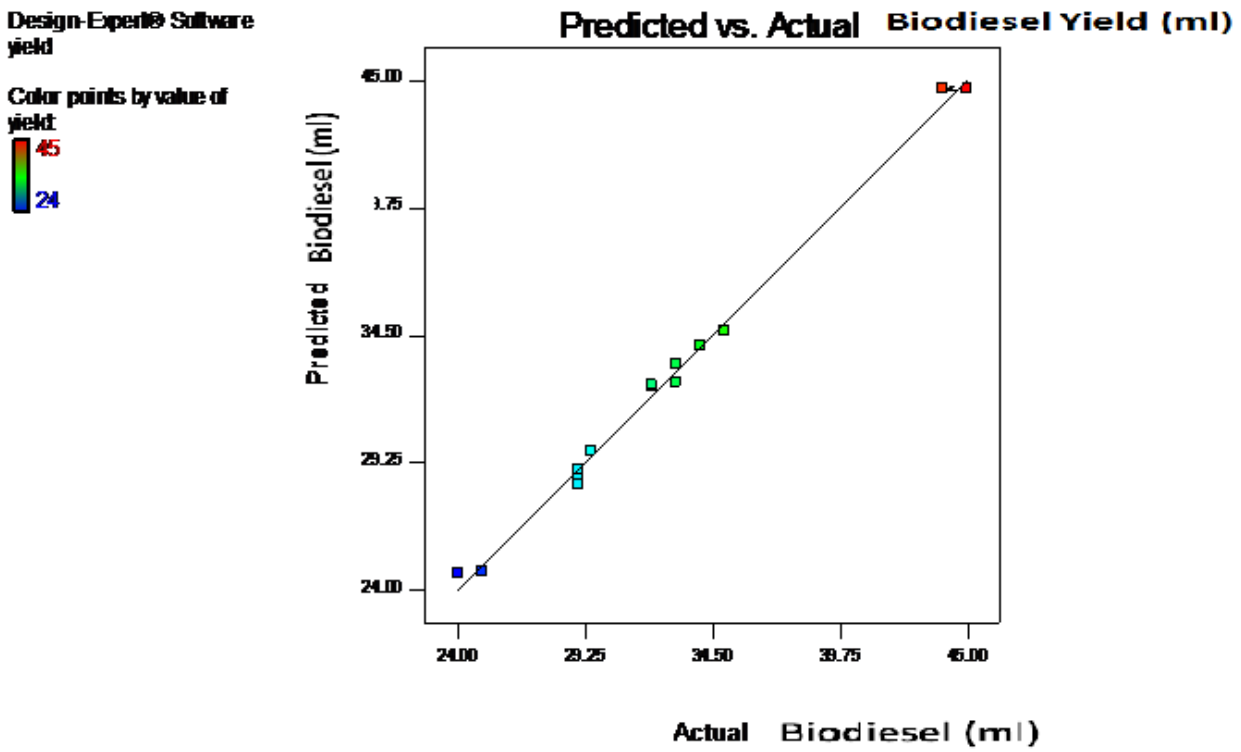


Figure 4.1 Actual vs. predicted value of biodiesel

As Figure 4.1 above shows the plot contains a line of unit slope which is the line of perfect fit, with points corresponding to zero error between predicted values and actual. This plot therefore clarifies the performance of the correlation in an evident way. Hence, the regression model equation granted a very accurate description of the experimental data, in which all the points are very close to the line of perfect fit. This outcome indicates that the design expert software was successful in creating the correlation between the three process variables to the biodiesel yield.

4.3.3 Effects of Individual Process Variables on Biodiesel Yield

The transesterification process was significantly affected by individual process variables. The effect of each process variable on the yield of biodiesel is discussed below.

a) Effect of Temperature

As it can be shown below in Figure 4.2 when the temperature increase the viscosity of the oil decrease and the reaction is facilitated which leads to the increase on the yield of biodiesel. But after the temperature reached to an optimum value the yield start to decline. The increase in the

methyl ester content up to an optimum temperature might have been due to the decrease of viscosity of the oil with an elevation of reaction temperature, which results in an increase in the solubility of the oil in the methanol, leading to an improvement in the contact between the oil and the methanol. Hence, the reaction was faster at a higher temperature. But at a maximum reaction temperature the transesterification process yield was too low. This might be due to the fact that the required methanol to oil ratio doesn't kept in the reactor because at higher temperature (temperature near or above the boiling point of methanol) the methanol is evaporated and not fully recovered by the reflux condenser. In addition at higher temperature the saponification is higher and this hinders the separation of glycerol.

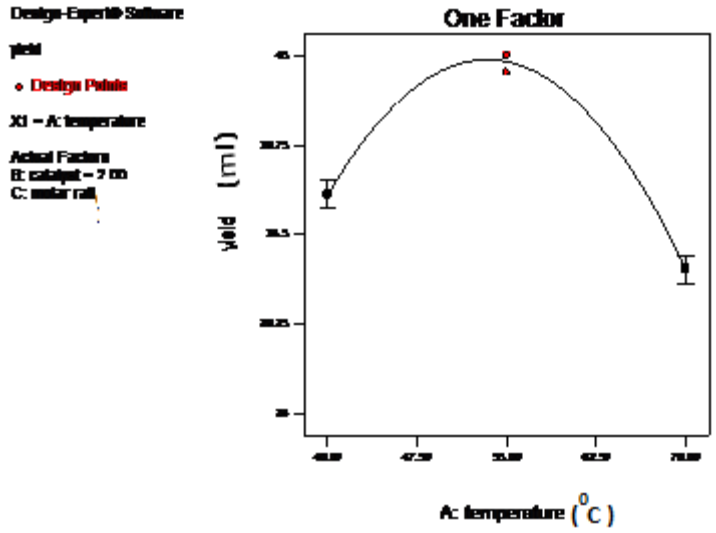


Figure 4.2 Effect of temperature on biodiesel yield

b) Effect of Methanol to Oil Molar Ratio

Stoichiometrically, the ratio for transesterification reaction requires 3 mol of methanol for 1 mol of triglyceride to produce 3 mol of fatty acid ester and 1 mol of glycerol, but in practice, a higher molar ratio is required in order to drive the reaction towards completion and produce more FAME as products (Dennis *et al*., 2010). The results obtained in this study are in agreement with Dennis. As shown in Figure 4.3, the methanol to oil ratio showed positive influence to the yield

of methyl ester, but the yield started to decrease as the ratio increased. The decrease in the yield contrary to increase in molar ratio may be due to the separation problem resulted from an increase in glycerol solubility due to the excessive methanol. Higher ratio of methanol used could also minimize the contact of triglyceride molecules on the catalyst's active sites which could decrease the catalyst activity.

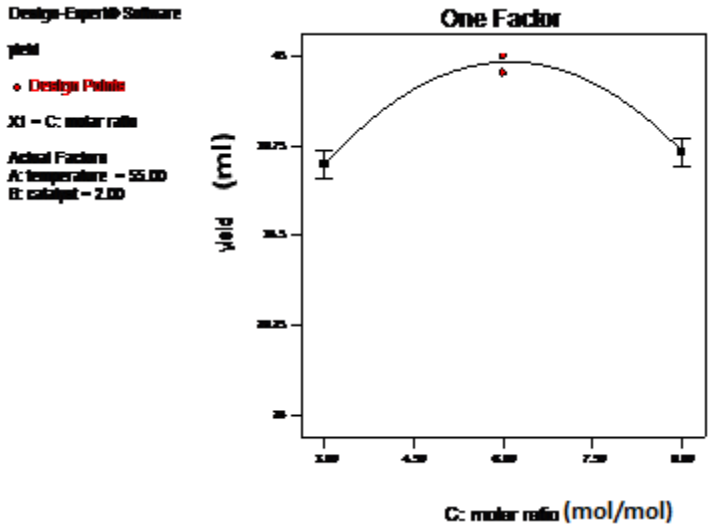


Figure 4.3 Effect of ethanol to oil molar ratio on biodiesel yield

c) Effect of Catalyst Concentration

From Figure 4.4 below, it can be seen that the biodiesel yield was increased as the catalyst concentration increased up to a certain level. This means that the biodiesel yield was influenced positively by the amount of catalyst up to a certain concentration. This might be due to the reason that when the catalyst amount was improved, the active site of the catalyst was increased; thus, the transesterification reaction was accelerated and biodiesel yield was increased. But increasing the catalyst beyond this concentration (optimum amount) led to a decrease in biodiesel yield. The decrease in biodiesel yield as the catalyst concentration increased may be due to the formation of soap and emulsion at higher catalyst concentration which hinders the separation of glycerol from biodiesel.

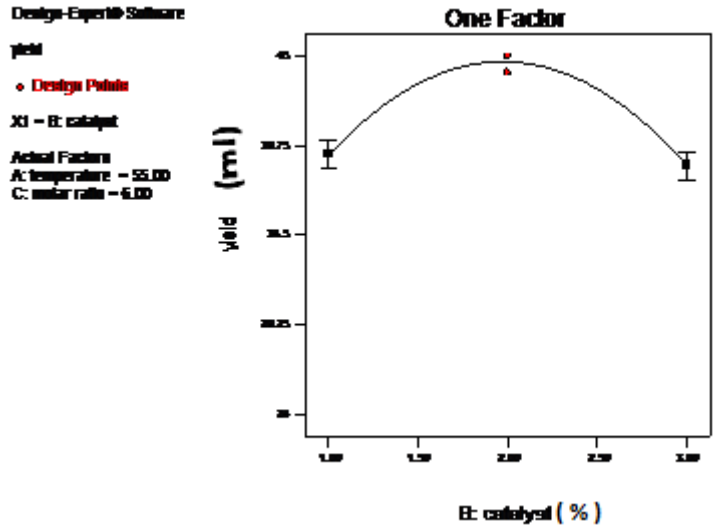
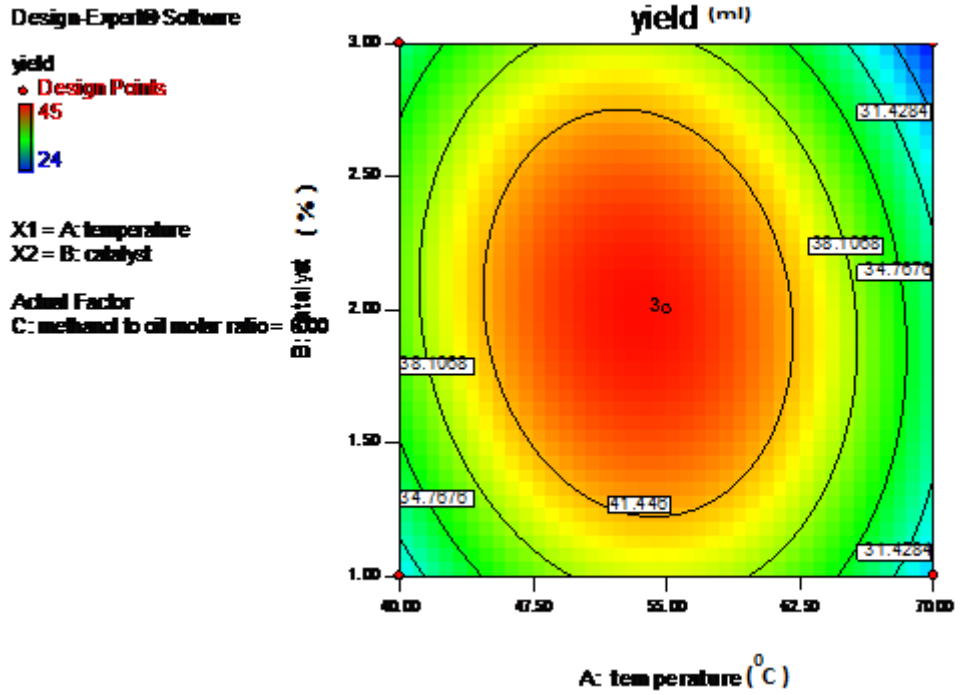


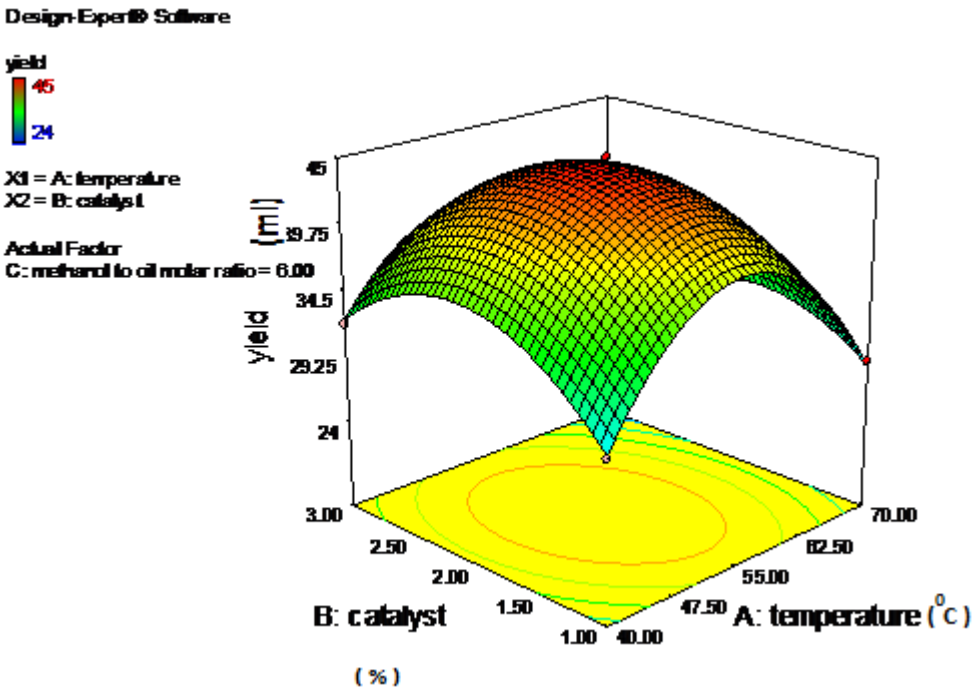
Figure 4.4 Effect of catalyst on biodiesel yield

4.3.4 Effects of Interaction between Process Variables on Biodiesel Yield

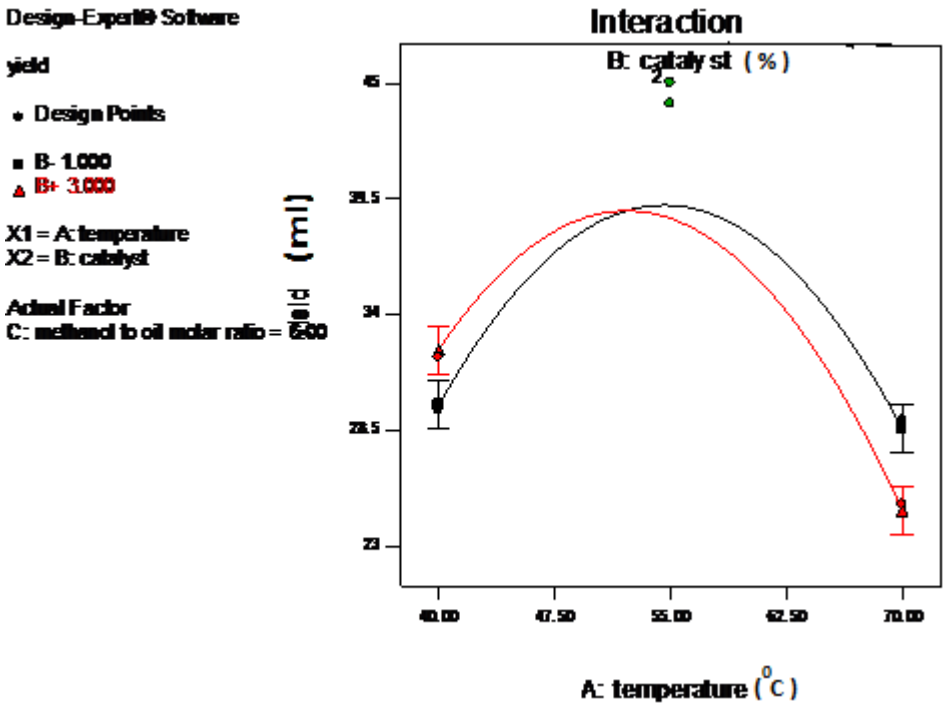
The 3D surface and the Contour plots were drawn to show the interaction effect of the process variables or independent variables on the biodiesel yield. Each contour curve presented the effect of two variables on the methyl ester yield, holding the third variable at constant level (at zero level). However, the interaction factor also must be considered as the individual effect plot does not give information regarding the significant interaction involved


Design-Expert® Software
yield
Design Points
45
24
X1 = A: temperature
X2 = B: catalyst
Actual Factor
C: methanol to oil molar ratio = 6.00
yield (ml)
B: catalyst (%)
A: temperature (°C)
3.00
2.50
2.00
1.50
1.00
40.00
47.50
55.00
62.50
70.00
31.4284
38.1068
34.7676
38.1068
34.7676
41.445
31.4284

a)


Design-Expert® Software
yield
45
24
X1 = A: temperature
X2 = B: catalyst
Actual Factor
C: methanol to oil molar ratio = 6.00
yield (ml)
45
39.75
34.5
29.25
24
3.00
2.50
2.00
1.50
1.00
B: catalyst (%)
40.00
47.50
55.00
62.50
70.00
A: temperature (°C)

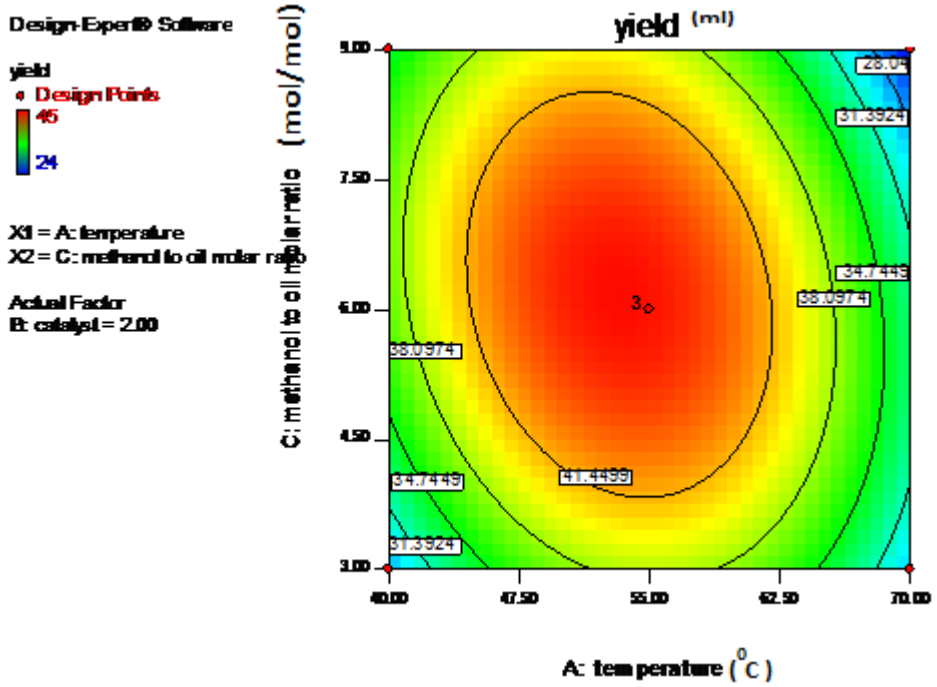
b)

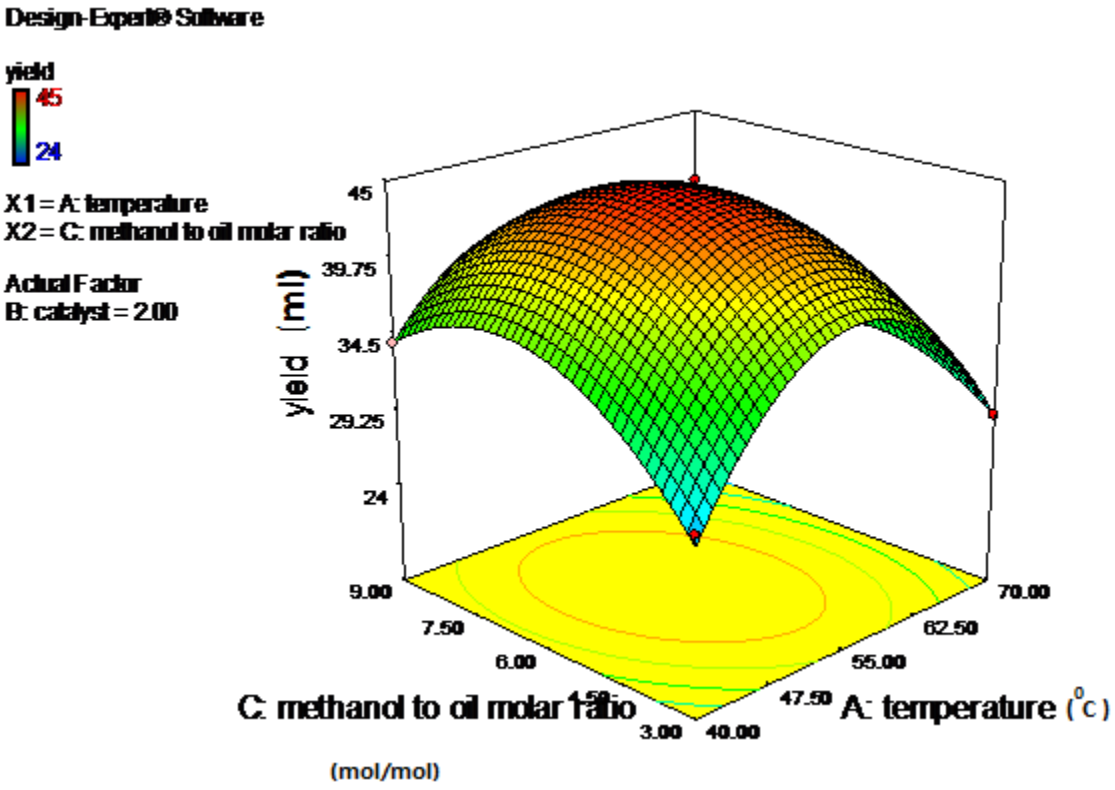


c)

Figure 4.5 Effect of temperature & catalyst on biodiesel yield

From Figure 4.5., it can be seen that the yield of the biodiesel increased for an increase in temperature up to the optimum degree at a lower catalyst concentration but as both the temperature and the catalyst increase the cumulative effect led to a decrease in the yield. This could be due to the fact that only the temperature was enough to make the reaction go forward.


Design-Expert® Software
yield
Design Points
45
24
X1 = A: temperature
X2 = C: methanol to oil molar ratio
Actual Factor
B: catalyst = 2.00
yield (ml)
C: methanol to oil molar ratio (mol/mol)
A: temperature (°C)
28.05
31.3924
34.7449
38.0974
41.4499
3


Design-Expert® Software
yield
45
24
X1 = A: temperature
X2 = C: methanol to oil molar ratio
Actual Factor
B: catalyst = 2.00
yield (ml)
45
39.75
34.5
29.25
24
9.00
7.50
6.00
3.00
C: methanol to oil molar ratio
(mol/mol)
40.00
47.50
55.00
62.50
70.00
A: temperature (°C)

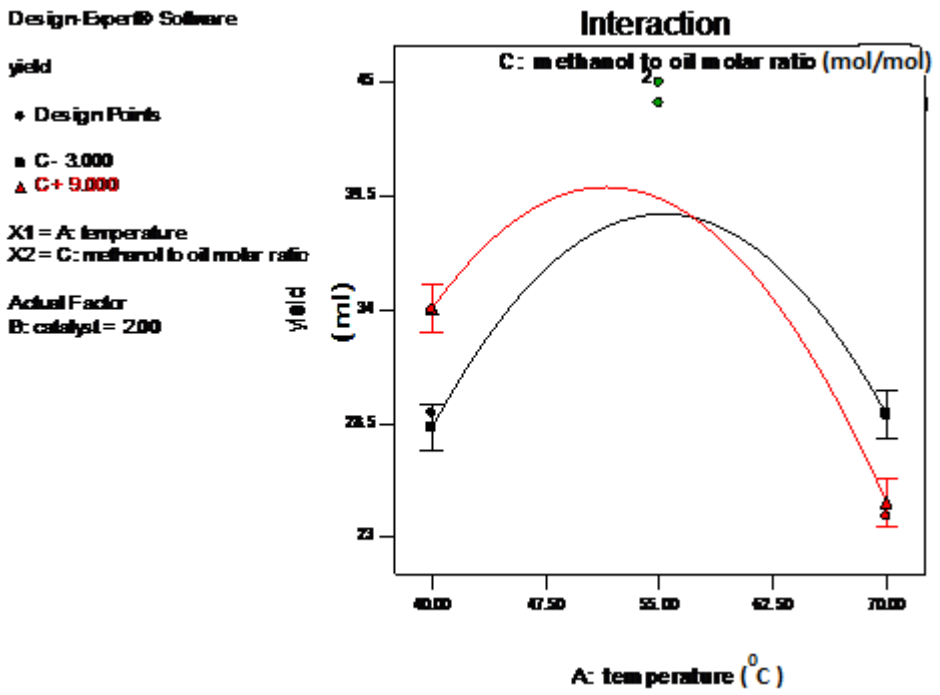
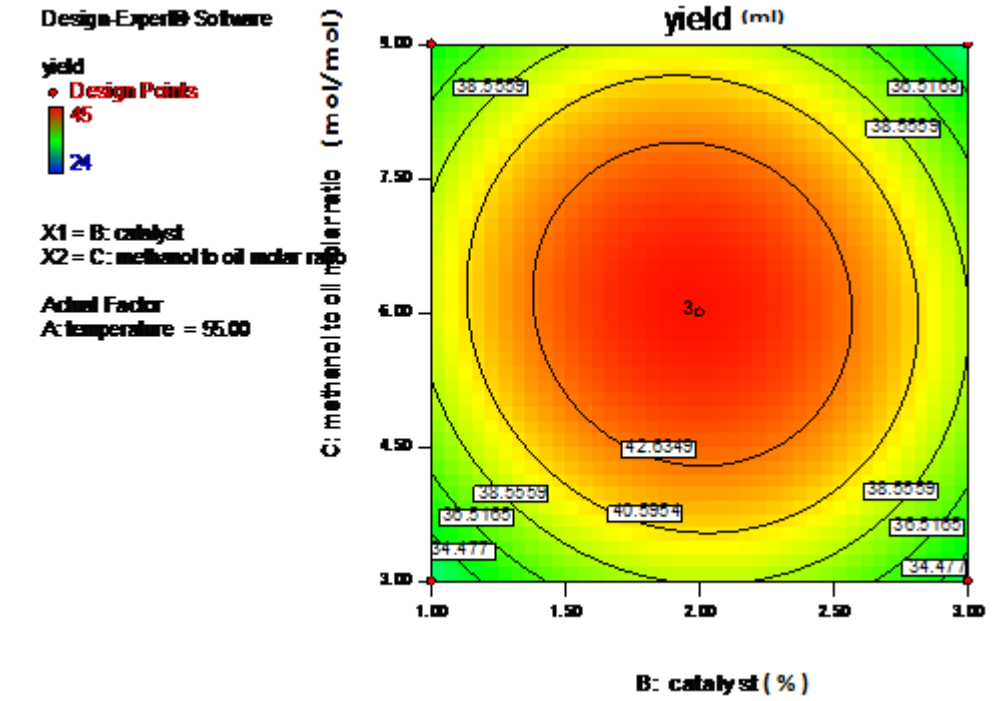
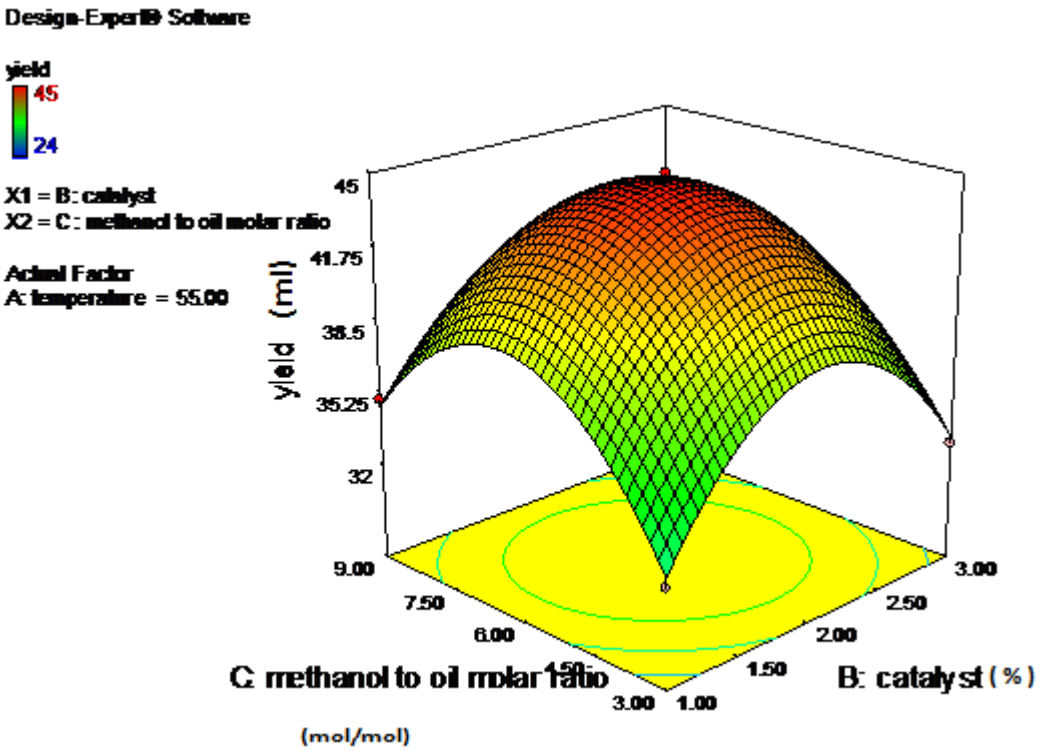


Figure 4.6 Effect of temperature & methanol to oil molar ratio on biodiesel yield

Figure 4.6 shows the effect of temperature and methanol to oil molar ratio on biodiesel yield when the catalyst concentration is 2%. As the temperature of the reaction increased at lower oil to molar ratio, the yield of biodiesel increased. The biodiesel yield also increases as the methanol to oil ratio increased to an optimal level and decreases thereafter.


Design-Expert® Software
yield
Design Points
45
24
X1 = B: catalyst
X2 = C: methanol to oil molar ratio
Actual Factor
A: temperature = 55.00
yield (ml)
C: methanol to oil molar ratio (mol/mol)
B: catalyst (%)
42.6349
40.6954
38.6559
36.6164
34.477
1.00
1.50
2.00
2.50
3.00
3.00
4.50
6.00
7.50
9.00


Design-Expert® Software
yield
45
24
X1 = B: catalyst
X2 = C: methanol to oil molar ratio
Actual Factor
A: temperature = 55.00
yield (ml)
45
41.75
38.5
35.25
32
C: methanol to oil molar ratio
(mol/mol)
9.00
7.50
6.00
3.00
B: catalyst (%)
1.00
1.50
2.00
2.50
3.00

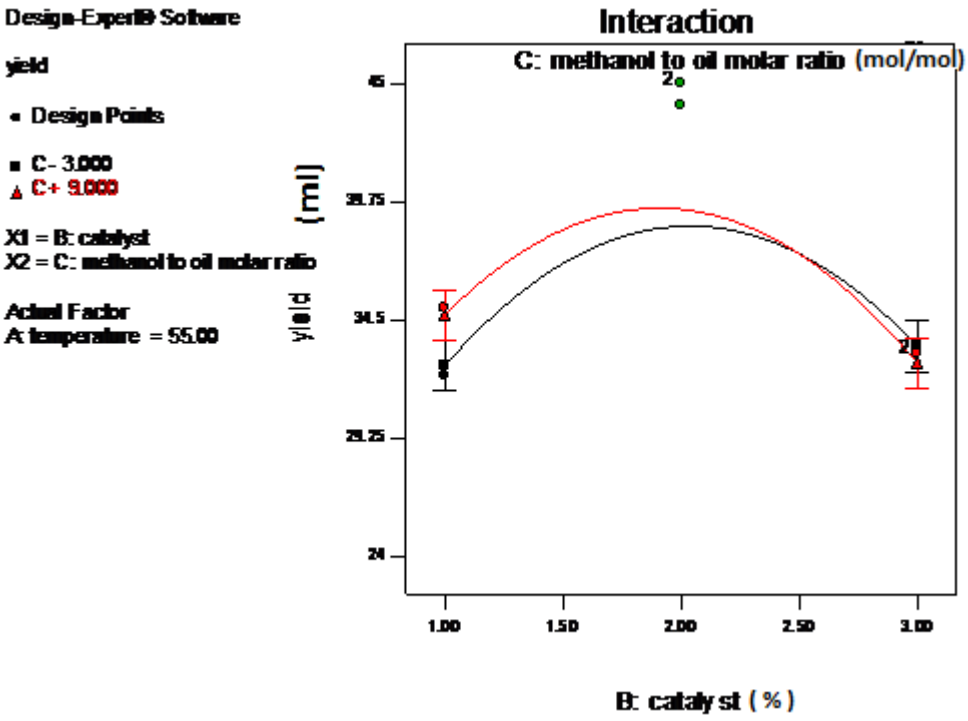


Figure 4.7 Effect of methanol to oil molar ratio & catalyst on biodiesel yield

From Figure 4.7., it can be seen that the biodiesel yield increase when the catalyst concentration increase. But after an optimal concentration level, the yield starts to decrease. Similarly when the methanol to oil ratio increased at first, the yield also increased. However after the methanol to oil ratio reached an optimal level the yield started to decline.

The increase of yield to an optimal level as the catalyst increases could be due to the increase on miscibility of methanol and triglyceride in the oil which facilitates the transesterfication reaction. However for further increase of catalyst the yield started to decline this could be due to the formation of soap which hinders the separation of glycerol from biodiesel. On a similar manner, when the methanol to oil ratio increased the yield also increased while a further increase in the molar ratio reduced the biodiesel yield.

4.4 Optimization of Process Variables

The optimization function in Design Expert 7.0.0 software was employed for the optimization of process variables. The goal of the three variables temperature, catalyst concentration and methanol to oil molar ratio was set in range and the goal of the response was set to maximize. Accordingly, the optimum result predicted was 44.8064 ml at the process variable of reaction temperature 53.27^{0}C, catalyst concentration of 1.99% and methanol to oil ratio of 6.18.

Table 4.6 optimization of process variables

Constraints name	Goal	Lower limit	Upper limit	Lower weight	Upper weight	Importance
Temperature	in range	40	70	1	1	3
Catalyst	in range	1	3	1	1	3
Methanol to oil molar ratio	in range	3	9	1	1	3
Yield	Maximize	24	45	1	1	3

Solution						
Solutions number	**Temperature**	**Catalyst**	**Molar ratio**	**Yield**	**desirability**	**Remark**
1	53.27	1.99	6.18	44.8064	0.991	Selected

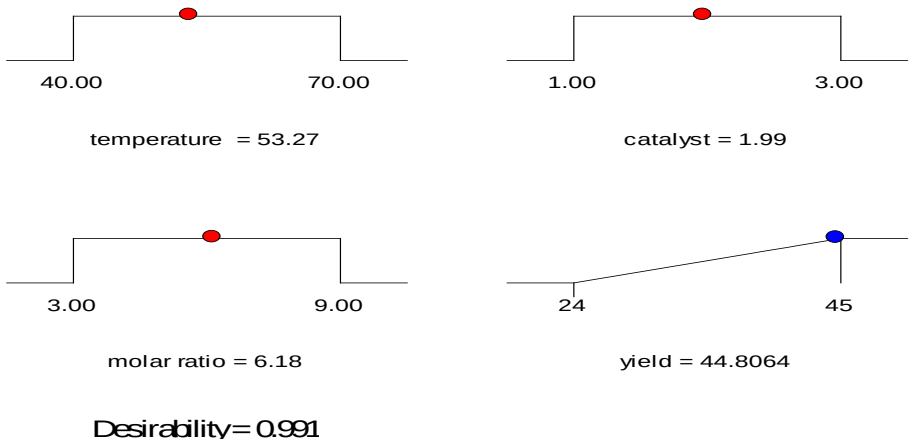


Figure 4.8 Optimization of process variables

To check the optimum process value experiment was conducted at the specified parameters. The yield obtained through experiment at a temperature of 53.3, catalyst concentration of 1.99 and methanol to oil molar ratio of 6.18 was 43.9ml which is near to the value obtained using the design expert software.

4.5 Physicochemical Properties of Biodiesel

The physicochemical analysis is conducted for the optimum biodiesel obtained. Determination of density, kinematic viscosity, acid value, free fatty acid, saponification value, moisture content, ash content and flash point of biodiesel were made according to the procedures mentioned under section 3.3.2.

4.5.1 Density of Biodiesel

The density of biodiesel was determined in a similar procedure as determination of density of oil in section 4.2.1. The specific gravity was measured by densimeter and obtained to be 0.89g/ml. From the simple empirical formula stated in equation 3.1 the density of the biodiesel was calculated and obtained to be 0.89 g/ml =890kg/m^3. When the result is compared with the ASTM D6751, which is 875–900 kg/m^3 for biodiesel, the value is acceptable.

4.5.2 Kinematic Viscosity of Biodiesel

Viscosity of biodiesel was determined on a similar manner as oil viscosity determination procedures that were explained in Sections 4.2.2. Density of the biodiesel was 890kg/m^3. Kinematic viscosity of biodiesel was found to be 5.5mm^2/s. Thus, transesterification reaction reduced density and kinematic viscosity.

4.5.3 Acid Value of Biodiesel

similar procedure as that of algae oil acid value determination was employed to determine the acid value of the biodiesel. The titration volume at which the first color change observed was 0.28 ml. the acid vlaue of the biodiesel was calculated using the equation

AV= 0.78mg KOH/g oil

4.5.4 Moisture Contents of Biodiesel

Determination of moisture contents of biodiesel was done in similar manner with that of oil moisture content determination as discussed in Sections 3.2.2. Moisture content of the biodiesel was determined using equation 3.6 and recorded as 0.026%

4.5.5 Ash Content of Biodiesel

The same procedure was employed in Determination of ash contents of biodiesel as it was discussed in ash content determination of algae oil. Using equation 3.7 the ash content of the biodiesel was calculated and obtained to be 0.022%.

4.6 Comparison of Biodiesel Physicochemical Properties against Standards

Table 4.7 physicochemical properties of biodiesel against standards

Physicochemical Properties	Unit	Biodiesel values (Measured Values)	ASTM D 6751-02	EN14214	Test Methods
Density@20^{o}C	Kg/m^3	890	875-900	860-900	
Kinematic Viscosity @40 ^{o}C	(mm^2/s)	5.5	1.9-6.0	3.5-5.0	ASTM D 445
Moisture Content	(%w/w)	0.026	<0.03		
Ash Content	(%w/w)	0.022	<0.03	<0.02	ASTM D 482
Acid Value	(mgKOH/g)	0.78	≤ 0.8	≤ 0.5	ASTM D 664

The density of the oil was reduced from 0.94g/ml to 0.89g/ml after the transesterification process and the result is within the limit of both the ASTM and EN14214. The kinematic viscosity measured for the biodiesel was found to be 5.5mm^2/s. The kinematic viscosity of the original oil at 40 ^{0}C was 41.85mm^2/s. As the result indicates, the methanolysis significantly reduces the kinematic viscosity of the primary algal oil by approximately one-tenth of its initial value. The results are still within the limit of the ASTM specification i.e. (1.9 – 6.0 mm^2/s).

5. Conclusions and Recommendations

5.1 Conclusions

Algae are more promising feed stocks to their wide spread availability and higher oil yields. Using microalgae to produce biodiesel will not compromise production of food, fodder and other products derived from crops. In this research the experimental oil yield obtained from the consortium microalgae was about 32% of its dry weight. For the extraction of oil, soxhlet method was used. Extracted oil was further used for biodiesel production by transesterification process. Three factors at three levels were considered in the alkali catalytic transesterification process. The individual and interaction effects of the three factors on biodiesel yield were analyzed and optimization of the process variables was made using design expert 7.0.0 software. From the experiment performed it was obtained that an optimal biodiesel yield of 89.61% (44.8ml) at reaction temperature of 53.27^0C, amount of catalyst 1.99% and methanol to oil ratio of 6.18.

The obtained biodiesel were tested for its physicochemical properties to check whether it complies with the ASTM and EN standards or not. The result showed that the fuel properties are within the ASTM and EN standards. The density, kinematic viscosity, acid value, and free fatty acids were recorded as 0.89g/ml, 5.5mm^2/s, 0.78mg KOH/g of oil, and 0.39mg of algae oil.

The results obtained from this research suggest the potential of algal oil as a feedstock for biodiesel industry which could be exploited as an alternative source of fuel.

5.2 Recommendations

This research shows the possibility of producing biodiesel from microalgae to use as substitute of petroleum fuel and mitigate environmental pollutions caused by fossil fuel consumption. However, further research and development on selection and isolation of specific species of algal strains from the consortium, identification of the major fatty acid compositions (profile), investigation on the productivity of isolated species, determination of conducive environmental conditions or growth media (i.e. optimal temperature, sun light intensity, and nutrients requirement) for optimal growth of strains with higher fatty acid yield per unit area of land should be carried out.

Besides, further study on fuel property using HPLC or GC analysis, blending conditions, engine performance and emission tests and techno-economic analysis should be carried out in future studies.

Glycerol has relevance in soap industries but in this study the property of glycerol was not determined. Therefore, in addition to the production of biodiesel the property of the byproduct glycerol should be analyzed and purified for further applications.

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