JIMMA UNIVERSITY
JIMMA INSTITUTE OF TECHNOLOGY
SCHOOL OF GRADUATE STUDIES
FACULTY OF CIVIL AND ENVIRONMENTAL ENGINEERING
ENVIRONMENTAL ENGINEERING MASTERS PROGRAM

Biodiesel production from blends of crude castor seed oil and microalgae oil

By
Mohammedsani Abdulkadir (BSc.)

A Thesis submitted to Jimma University, Jimma Institute of Technology, Faculty of Civil and Environmental Engineering, Environmental Engineering Chair in partial fulfillment of the requirements for the Degree of Masters of Science in Environmental Engineering

April, 2019
Jimma, Ethiopia

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Main advisor: Dejene Beyene (PhD)
Co-advisor: Adisu Bafekadu (MSc.)

April, 2019
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DECLARATION

This Thesis, entitled “biodiesel production from blends of crude castor seed oil and microalgae oil” is entirely my original work and has not been presented for a degree in either Jimma University or any other universities.

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ABSTRACT

Global energy crises and environmental concerns arising from oil spillage and exhaust gaseous emissions into the atmosphere are the major problems of fossil fuel use. Due to the environmental problems caused by using fossil fuels, considerable attention has been made to biodiesel production as an alternative to petro-diesel. Biodiesel is an ecofriendly, alternative diesel fuel prepared from renewable resources i.e. vegetable oils and animal fats. It is a renewable source of energy that can be an ideal solution for global energy demands including Ethiopia. The objective of this study was to investigate the production of biodiesel from blends of crude castor oil and microalgae using methanol with KOH (Potassium hydroxide) as a catalyst. Optimum molar ratio of alcohol to oil, volumetric ratio of castor oil to microalgae oil and catalyst concentration was investigated during experimental process. Transesterification process was used to produce biodisiel from oil with methanol in the presence of base catalyst. The oil was extracted by Solvent extraction method, using n-hexane as the solvent from both algae and castor seeds powder separately, and then it was mixed together. Biodiesel was produced through blending of algae and castor oil using methanol in the presence of potassium hydroxide as catalyst at the end of the reaction glycerol and biodiesel were form two layers. After settling, the glycerol layer was at the bottom while the biodiesel layer was at the top of the container. The layers were later separated from each other by draining the glycerol from the bottom of the flask containing the mixture. The biodiesel was washed several time to remove the remaining glycerol and stored properly. Optimum parameters for the production of biodiesel such as molar ratio of methanol to oil, volumetric ratio of castor oil to algae oil and catalyst concentration were investigated. The optimum parameters obtained were molar ratio of methanol to oil, volumetric ratio of castor oil to algae oil and catalyst concentration of 9:1(v/v), 2:1(v/v) and 1.5% (wt/wt) respectively. From optimum parameter the optimum biodiesel yield was 86% (v/v). The yield obtained was nearly similar with the predicted yield by the software (RSM). Finally fuel properties of the biodiesel were tested.

Key words: *Biodiesel, Castor oil, Microalgae, Catalyst, Transesterification, Optimization, Optimum parameters and response surface methodology.*

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ACRONYMS

AEOE	Aqueous enzymatic oil extraction
ANOVA	Analysis of variance
ASTM	American Society for Testing and Materials
AV	Acid value
CCD	Central composite design
DG	Diglycerides
ENS	European Committee for Standardization
EV	Ester value
FAME	Fatty acid methyl esters
FFA	Free fatty acids
FP	Flash point
GHG	Greenhouse gas
GL	Glycerol
HV	Heating value
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
IV	Iodine value
MG	Monoglycerides
N	Normality
NBB	National Biodiesel Bord
NOx	Nitrous oxide
RPM	Revolution per minute
RSM	Response surface method
SG	Specific Gravity
SV	Saponification value
TG	Triacylglycerides
WCO	Waste cooking oil

CHAPTER ONE
INTRODUCTION

1.1 Background

Increasing the cost of petroleum products, global energy problem and environmental issue arising from oil spillage and an exhaust gas emission into the atmosphere are the major problems of conventional fossil energy source (Barnwal and Sharma, 2005). The demand for petroleum is increasing 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 fossil fuel resources is fueling the exacerbating 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 products and increasing threat to the environment, have generated an international interest for searching an alternative, non-petroleum; renewable fuels that have the potential to solve many of the current problems and concerns (Demirbas, 2006).

The essential minimum requirement for biofuels to be more sustainable and alternative for fossil fuels is that they should be produced from renewable raw materials and that their use has a lower negative environmental impact (Knothe *et al.*, 2005)

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 include bioethanol which are obtained by fermentation of starch from wheat, barley, corn, potato or sugars from sugarcane, and sugar beet and biodiesel produced through transesterification of oil crops such as rapeseed, soybeans and sunflower.

Second generation biofuels are fuels produced by conventional technologies from novel starch, oil and sugar crops such as *jatropha*, *cassava* and *lignocellulosic* materials such as straw, wood, and grass. Third generation biofuels are fuels produced from microalgae and seaweeds (Knothe *et al.*, 2005).

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).

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 nonedible components of corn or sugarcane. However, converting the woody biomass into fermentable sugars requires expensive technologies that involve pretreatment with special enzymes, meaning that second generation biofuels cannot yet be produced economically on a large scale (Brennan and Owende, 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). Microalgae are able to produce 15–300 times more oil for biodiesel production than traditional crops on an area basis. Furthermore, compared to the 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).

Industrialized countries consider biofuels as a way of reducing greenhouse gas (GHG) emissions from the transport sector and diversifying energy sources. Developing countries, on the other hand, consider biofuels as a way to stimulate rural developments, create jobs and save foreign exchange. Biodiesel is a more immediate option as a renewable fuel source. Biodiesel contains no sulfur or aromatics, and use of it in a conventional diesel engine results in substantial reduction of unburned hydrocarbons, carbon monoxide and particulate matter emissions (NBB, 2006).

Biodiesel consists of alkyl esters of long chain fatty acids that are derived from lipids (oil and fats) produced by organisms (NBB, 2006). There are a number of animal fat and plant oil 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, peanut, rapeseed and soybean (Fangriu, 1999).The use of this plant can cause food scarcity due to the fact that a huge imbalance in the human nutrition chain versus fuel. This will make biodiesel economically unfeasible. To avoid these situations, non-edible oil seeds need to be used for commercial production of biodiesel. Many researchers have initiated work on the use of low cost nonedible oil source as alternative feedstock for biodiesel production. Among nonedible oil feedstock, seeds of castor and jatropha, and microalgae oil are proved to be one of the highly promising reliable sources having high oil content (Sruthi, 2013). Algae are economical choice for biodiesel production, because of its availability and low cost (Basumatary, 2013).

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 (Fangriu, 1999).

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 countries has increased at a rate of about 7% per year in line with increased economic activity (Mulugetta, 2008).

Ethiopia, imports its entire petroleum diesel requirement and the demand for petroleum diesel is rising rapidly due to a growing economy and expanding infrastructure. With the increase of demand the price of petroleum diesel also increase from time to time, so it has negative impact on economy of the country, on the environment and human health. Thus it is necessary to search for alternative energy source (Yadasa and Jorgi, 2017).

Ethiopia has ample opportunities and potential for the production of biodiesel from varieties of non-edible oilseeds and oil fruits, because it has the favorable agro-ecology (climate and soil). Among the non-edible oil seeds grown in Ethiopia castor and jatropha are the well-known plants (Yadasa and Jorgi, 2017). Like any other vegetable oil, castor oil can be converted to biodiesel which can yield up to 98 % (Chakrabati and Ahmad, 2008).

By identifying the most feasible and reliable oil crops for biodiesel production castor was selected. In this work both castor seed and micro algae oil was used to produce biodiesel, both of them are easily available and have low cost. The availability and sustainability of sufficient supplies of less expensive feedstock will be a crucial determinant delivering a competitive biodiesel to the commercials filling stations. Fortunately, inedible vegetable oils, mostly produced by seed-bearing trees and shrubs can provide an alternative fuel. The comparative advantage of castor is that its growing period is much shorter than that of Jatropha and there is considerably greater experience and awareness among farmers about its cultivation (Hemant *et al*., 2011). It is a fast-growing, suckering perennial shrub which can reach the size of a small tree (around 12 metres) (Momoh *et al*., 2012).

1.2 Statement of the problem

Petroleum fuel consumptions are the major contributor to the increasing concentration of carbon dioxide (CO_2) in the atmosphere which is a key cause of global warming problem, reduces agricultural production and causes other biological and social problems. Petroleum 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). 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). These necessities the search for alternative energy sources such as biodiesel in order to reduce its negative impact on economy, environment and human health. In Ethiopia, there are diverse ecological varieties that have a great potential for biofuel production. Castor oil, *Jatropha*, palm oil, neem, microalgae and others are among some of non-edible naturally occurring feed stocks for biodiesel production in the country.

Since Ethiopia imports the entire petroleum diesel requirement, and the demand for petroleum diesel is rising and also since the country has a huge potential of biomass that can be used as feedstock for biofuel production, search for renewable fuel source which are cheap and have no linkage with food security should be a primary objective. Therefore, it can be possible to overcome the aforementioned problems and the trade imbalance that the country is facing by the use of castor seed and micro algae oil to produce biodiesel which is biodegradable and very low exhaust gas emission as compared to petroleum diesel.

1.3 Objectives of the study

1.3.1 General Objective

The general objective of this study was to investigate biodiesel production from the blends of crude castor seed oil and microalgae and methanol using KOH (potassium hydroxide) as a catalyst.

1.3.2 Specific Objectives

The specific objectives of this study:

i. To extract crude oil from microalgae and castor seed by solvent extraction method
ii. To optimize transesterification process parameters of biodiesel production.
iii. To characterize biodiesel produced from blends of castor seed oil and microalgae

1.4 Research Questions

1. How much oil can be extracted from castor seed and microalgae through solvent extraction method?
2. What are the optimum parameters for biodiesel production?
3. What are the characteristics of the biodiesel produced from blends of castor seed oil and microalgae?

1.5 Justifications

The primary reason for biodiesel production was to reduce environmental pollution by using biodiesel rather than petroleum diesel. Biodiesel is more environmental friendly than petroleum diesel. The Clean Air Act from the Environmental Protection Agency (EPA) concluded that toxic emissions from biodiesel is less than the emissions from petroleum diesel, and the carbon monoxide emissions is also less than emission from petroleum diesel.

The other reason for the biodiesel production is to protect public health and safety. Biodiesel is safer to use and handle than petroleum diesel because it is nontoxic and biodegradable.

Another reason for the biodiesel production was its economic advantages. It can create job opportunities for the farmers. The plantations of castor plant for this purpose protect soil erosion, enhance soil fertility and consume more amount of carbon dioxide (CO_2) from the atmosphere, these helps to increase the amounts of crop product in that area. Therefore, this study has great significance in terms of assuring the production of environmental friendly alternative form of energy from the blends of crude castor seed oil and microalgae which is a nonedible and can be abundantly cultivated and grown on land which is not suitable for other crops.

1.6 Scope of the study

The scope of this study was extraction of oil from castor seed and microalgae using soxhlet extraction method and conversion of blends of the two oils into biodiesel using transesterification reaction method. The transesterification reaction was carried out by varying the parameters such as alcohol to oil molar ratio, castor oil to algae oil ratio and catalyst concentration to identify their effects on the response parameter which is biodiesel yield.

1.7 Limitations of the study

Algae biomass collection was tedious sine it was suspended on the water body so there was quality problem. After collection the moisture present in algae biomass must be removed by means of drying, so it needs large surface area. In order to gets large surface area, sun drying method was selected so there is loss of dried algae biomass by wind. Electric power interruption was their which causes solvent loss during oil extraction. The presence of many species of microalgae’s with different oil contents was the major problem that affect algae oil yield. Caster seed husk removal process was tedious and time consuming. There was no well-arranged equipment for transesterification reaction in the laboratory this also affects the biodiesel yield and its quality.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

The growth of world population would ultimately lead to increase the energy demand in the world. Petroleum is a non-renewable energy source, which means that the resources of this kind of fossil fuel are finite and would be run out upon continuous use. Both of the shortage of resources and increase of petrol price have led to the search for new alternative and renewable energy sources. Apart from these situation, environmental issue are also the driving force for the development of alternative energy source since the burning of fossil fuel would cause various environmental problems including global warming, air pollution, acid precipitation, ozone depletion, forest destruction, and emission of radioactive substances (Dincer, 2000). Depletion of fossil fuel reserves couple with the environmental problems associated with fossil fuel usage have led to the search for alternative fuels which can be obtained from renewable source (Oghenejoboh *et al*., 2010).

Biodiesel is a non-polluting, locally available, accessible, sustainable and reliable fuel obtainable from renewable sources such as vegetable oil or animal fats by transesterification (Adebayo *et al*., 2011). It has an energy content of about 12% less than petroleum based diesel on a mass basis (Oghenejoboh and Umukoro, 2011). It is a biodegradable, renewable fuel that can be produced from a range of organic feedstock including fresh or waste vegetable oils, algae, oilseed and animal fats. Biodiesel has lower emission compared to petro diesel when burnt (either blended or pure). It does not contribute to the rise in carbon dioxide level in the atmosphere and it minimizes the intensity of greenhouse effect (Vicente *et al*., 2004).

2.2 Feed stocks for biodiesel Production

The production and use of biodiesel has increased significantly in many countries around the world using numerous feedstock sources. Different types of fats and oils have been used in different countries as the raw material for biodiesel production, depending on availability, regional climate, geographical location and local soil conditions. Availability of feedstock for producing biodiesel depends on the regional climate, geographical locations, local soil conditions and agricultural practices of any country (Atabani *et al.*, 2012).

These feedstock's include algae, vegetable oils (e.g., soybean, cottonseed, palm peanut, rapeseed/canola, sunflower, safflower, coconut) and animal fats and oil as well as waste oil (Yadasa and Jorge, 2017).

Globally, there are more than 350 oil bearing crops identified as potential sources for biodiesel production (Sajjadi *et al*., 2016). The availability of wide range of biodiesel feedstocks is one of the most significant factors that enables the sustainable production of biodiesel (Shahid and Jamal, 2011). According to Avhad and Marchetti (2015), satisfactory replacement of petroleum diesel with biodiesel depends on two basic requirements: the first is its availability and environmental acceptability and the second is being economically reasonable. Availability of feedstock for producing biodiesel depends on the regional climate, geographical locations, local soil conditions and agricultural practices of any country (Atabani *et al.,* 2012).

From the literature, it has been found that feedstock alone represents about 75% of the overall biodiesel production cost (Atabani *et al.,* 2012).Therefore, minimizing the cost of biodiesel production has been the main agenda for biodiesel producers in order to be competitive with petroleum-derived diesel (Tan *et al.,* 2011). Hence, it is crucial to employ inexpensive feedstocks to replace the expensive refined oils (Bhuiya *et al.,* 2016; Atabani *et al.*, 2012). Using low-cost triglyceride sources such as waste cooking oil and animal fats is also important to minimizing the total cost as these wastes are three times cheaper than refined oils, and are abundantly available (Tan *et al.,* 2011). Feedstocks of biodiesel can be divided into four main categories: edible vegetable oil, non-edible oils, waste or recycled oils, and animal fats (Avhad and marchetti, 2015; Ahmad *et al.,* 2011 and Lim and Teong, 2010).

2.2.1 Edible oil as feed stock for biodiesel production

Edible oils resources such as soybeans, palm oil, sunflower, safflower, rapeseed, coconut and peanut are considered as the first generation biodiesel feedstocks because they were the first crops to be used for biodiesel production (Atabani *et al.,* 2012). Due to higher prices of edible vegetable oils compared to diesel fuel, waste vegetable oils and non-edible crude vegetable oils are now being used as biodiesel sources. The limitations of using edible oil include: competition of crop product between fuel and food, higher viscosity, lower volatility and the reactivity of unsaturated hydrocarbon chains. Currently, more than 95% of the world biodiesel is produced from edible oils such as rapeseed

(84%), sunflower oil (13%), palm oil (1%), soybean oil and others (2%) (Bhuiya *et al.,* 2016 and Atabani *et al.,* 2012). Plantations of these feedstock plants have been also well established in many countries around the world such as Malaysia, USA and Germany (Atabani *et al.,* 2012). However, continuous large-scale usage of edible plant oils for biodiesel production raises many concerns such as food versus fuel crisis and major environmental problems such as deforestation and destruction of vital soil resources, conversion of much available farm lands to oil bearing plants (Atabani *et al.,* 2012 and Balat, 2011).

The prices of vegetable oil have also increased dramatically in the last few decades and this will affect the economic viability of biodiesel industry (Balat, 2011). Furthermore, the use of such edible oils to produce biodiesel is not feasible in the long term due to the growing gap between demand and supply (Atabani *et al.,* 2012). Thus, the current use of edible plant oils as a feedstock for producing biodiesel are considered to be not worthy and stipulates search for relatively less expensive resources (Avhad and Marchetti, 2015). One of the possible solutions to reduce the utilization of the edible oil for biodiesel production is by exploiting non-edible oils. They got great attention as the plants from which these oils obtained are easily available in many parts of the world (Atabani *et al*., 2013 and Bhuiya *et al*., 2016). These plants can grow on wastelands that are not suitable for food crops, eliminate competition for food, reduce deforestation rate, and their oils are very economical compared to edible oils (Atabani *et al.,* 2012).

2.2.2 Non-edible oils as feed stock for biodiesel production

Non-edible oil seeds such as castor, microalgae, *jatropha*, tung, cotton and jojoba’s seeds oil are the well-known. Non edible oils are more suitable to produce biodiesel because they are not competitive with the food material, this will preserve the food sources even though biodiesel from edible oils have properties closer to standard diesel properties. Also, biodiesel from edible oils are not economical compared with non-edible oils.

Non-edible plant oils which are known as the second generation feedstocks can be considered as promising substitutions for traditional edible food crops for the production of biodiesel (Atabani *et al*., 2013). Recently, these oils have gained enormous attention as a new generation feedstock because of their high oil content, easy availability, and having the advantage that it could be grown on lands which are not suitable for agriculture (Atabani *et al*., 2012). Non-edible oil bearing plants could also be grown with less intensive attention; thus, reducing the cost of cultivation (No SY, 2011 and Avhad and

Marchetti, 2015). Therefore, production of biodiesel from non-edible oils is an effective way to overcome the problems associated with edible oils (Atabani *et al*., 2013).

During selection of any feedstock as a biodiesel source, the amount of oil that can be obtained from the seeds or kernel is an important parameter. Estimated oil contents of seed and kernel of some non-edible vegetable oil were shown in the following table.

Table 2.1: Oil contents of non-edible plant (Yadasa and Jorgi, 2017)

Common name	Species name	Oil contents wt/wt	Refrences
Castor	*Ricinus communis L*	45–50	Singh and Singh, 2010
Cotton seed	*Gossypium hirsutum L.*	18–25	Karmark *et al*., 2010 and Quampah *et al*., 2012
Desert date	*Balanites aegyptiaca*	45–50	No SY, 2011
Jatropha	*Jatropha curcas L.*	20–60	No SY, 2011; Singh and Singh, 2010 and Gui *et al*., 2008
Jojoba	*Simmondisa chincnsis*	45–50	Karmark *et al*., 2010
Karanja	*Pongamia pinnata*	30–40	Singh and Singh, 2010
Linseed	*Linum usitatissimum*	35–45	Singh and Singh, 2010
Mahua	*Madhuca indica*	35–40	Singh and Singh, 2010
Neem	*Azadirachta indica*	20–30	Singh and Singh, 2010
Polanga	*Calophyllum inophyllum*	65	No SY, 2011
Rubber seed	*Hevea brasiliensis*	40–60	No SY, 2011
Tobacco	*Nicotiana tabacum L.*	30–43	Karmark *et al*., 2010
Tung	*Vernicia Montana*	16–18	Karmark *et al*., 2010
Ethiopian mustard	*Brassica carinata*	42	No SY, 2011
Croton oil plant	*Croton tiglium*	30–45	Azam *et al*., 2005
Microalgae	-	20–50	Bankovic *et al*., 2012

2.2.3 Animal fats and oils as feed stock for biodiesel production

Animal fats are also an interesting option especially in countries with plenty of livestock resources such as Ethiopia, it may include materials from a variety of domesticated animals, such as cows, chickens, pigs and other animals such as fish and insects. It was normally characterized by a greater percentage of saturated fatty acids in comparison to oils obtained from the plant and algae. Animal fats such as tallow, chicken fat and lard are also considered as feedstocks for biodiesel production (Oner and Altun, 2009; Guru *et al*., 2010 and Lu *et al*., 2007).

According to Adewale *et al*., (2015), animal's fat are low cost, mitigate environmental damage and increase the quality of the resultant biodiesel fuel. However, it has been reported that these may not be plentiful enough to satisfy the global energy demand. Moreover, biodiesel derived from animal fats has a relatively poor performance in cold weather. Furthermore, the transesterification process is difficult for some types of fats due to the presence of a high amount of saturated fatty acids (Bhuiya *et al*., 2016 and Atabani *et al.*, 2012).

2.2.4 Waste oils as feed stock for biodiesel production

The residual obtained after using oil for the cooking purposes is generally discarded with no further application (Avhad and Marchetti, 2015). Over the last few years, waste cooking oil has been considered as a possible feedstock for biodiesel production due its low cost, and as its biofuel was found to fulfill the requirements specified by European standard for biodiesel (EN) and American Society for Testing and Materials (ASTM) standards (Al Hamamre and Yamin , 2014). However, waste oil is highly impure consisting mainly of high free fatty acid (FFA). These oils after the filtration and purification processes could be used for biodiesel production (Avhad and Marchetti, 2015).

2.2.5 Algae as feedstock for biodiesel production

The amounts of oily crops, both edible and non-edible, animal fats and waste cooking oils are limited, so it is unlikely to provide worldwide biodiesel production demand. The search for other renewable sources is needed to provide the required amount of oily feedstocks. In recent years a high interest towards producing biodiesel from microalgae has been developed. The advantages of using microalgae for biodiesel production are: much higher biomass productivities than land plants, some species can accumulate up to

20–50% triacylglycerol, no agricultural land is required to grow the biomass and they required only sunlight and a few simple and cheap nutrients (Bankovic *et al*., 2012).

From various types of biomass, microalgae have the potential of becoming a significant energy source for biofuel production in the coming years. Currently, researches are mainly focusing on optimization of cultivation methods and the conversion of microalgae to biodiesel (lipids for biodiesel production) (Barreiro *et al.*, 2013). Martinez and Gude (2016) has also reported that algal biodiesel production will play a significant role in sustaining future transportation fuel supplies, and a large number of researchers around the world are investigating into making this process sustainable by increasing the energy gains and by optimizing resource-utilization efficiencies. Some of the studies that focus on optimization of biodiesel production from microalgae include the investigations by Misau *et al*.,2016; Gulyurt *et al*. 2016; Barreiro *et al*., 2013 and Rajendran *et al*.2015.

The advantages of using microalgae in energy productions are: algae 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). Interms of their reproduction, the reproduction of microalgae is generally split type breeding, the cell cycle is relatively short, and so it is easy to carry out large scale cultivation. And the other most advantages of microalgae is cultivation land, microalgae can be cultivated in the sea water, alkaline water and even waste water, so it is significant to produce bioenergy in the freshwater shortage areas and barren land areas (Gavrilescu and Chisti, 2005).

2.2.6 Castor oil feedstock for biodiesel production

The castor oil plant, *Ricinus communis L.* is a species of flowering plant in the spurge family, *Euphorbiaceae* (Knothe *et al*., 2005). Castor is indigenous to the southeastern Mediterranean Basin, Eastern Africa, and India (Knothe *et al.*, 2005). Castor plant is originally a tree or shrub and there are different varieties that can be cultivated.

The comparative advantage of castor is that its growing period is much shorter than that of *Jatropha* and *Pongamia* and there is considerably greater experience and awareness among farmers about its cultivation (Hemant *et al*., 2011).

The seed contain ricin and toxin which are also present in lower concentrations throughout the plant. The toxicity of raw castor beans due to the presence of a poisonous substance called ricin. The toxin provides the castor plant with some degree of natural protection from insect pests. Castor seeds usually contain 40 - 55 % oil and the average yield of castor seed in the world is about 1.1 ton per hectare, although it may be possible to obtain a maximum of 4.2 ton per hectare. Therefore, castor is among the plants with the highest oil yield potential (Scholz and Silva, 2008). Castor oil is one of the most often used non-edible oil in biodiesel synthesis (Gui *et al*., 2008).

Table 2.2: Castor oil fatty acid profile and properties (Nuria *et al*., 2013)

Types of fatty acid	Amount in %	Physicochemical Properties	Measured value
C16:0 *Palmitic*	1.3 %	Density at 15 ^oC	961 kg/m^3
C18:0 *Stearic*	1.2%	Viscosity at 40 ^oC	262 cSt
C18:1 *Oleic*	3.6%	Saponification value	179.3 mg KOH/g
C18:2 *linoleic*	4.6%	Iodine Value	80.5 mg I_2 /g
C18:3 *linolenic*	0.4%	Acid Value	1.19 mgKOH/g
C18:1OH *ricinoleic*	88.9%	Molecular weight	926 g/mol

2.3 Mixed feed stock for biodiesel production

Biodiesel production from mixed feedstock was employed to provide biodiesel with improved physical properties in comparison to the individual fuels on their own. It may also arise as a result of economic considerations. For instance, it has an economical advantage to extend the lifetime of a comparatively more expensive feed stock through blending with a less expensive feedstock. The influence of blending various feed stocks on biodiesel properties has been conducted including blends of canola, palm, soybean and sunflower oil methyl esters (Moser, 2008). Many researches were done by mixed feed stock such as; blends of palm, rapeseed and soybean oil methyl esters (Park *et al*., 2008) and blends of jatropha and palm oil methyl esters (Sarin *et al*., 2007).

2.4 Biodiesel production processes

2.4.1 Feedstock preparation

The pre-requisite for biodiesel production is feedstock preparation (Bhuiya *et al*., 2016, Jahirul *et al*., 2013). The preparation of feedstock involves removal of outer layers of the fruit to expose the kernels or seeds, and drying it to reduce moisture content (Jahirul *et al*., 2013). The seeds are separated from fruits, and the fruits that do not dehisce are cracked open manually. The separated seeds or kernels are sieved, cleaned and stored at room temperature (Atabani *et al*., 2012).

According to Atabani *et al.,* (2012) seeds can be either dried in the oven or sun dried to appropriate moisture. The kernels or seeds have to be prepared in such a way that they contain optimum moisture content for high oil extraction. According to the report of Jahirul *et al.,(2013)* seed kernel of beauty leaf (*Calophyllum inophyllum*) prepared to 15% moisture content provided the highest oil yields in both mechanical and solvent extraction methods.

2.4.2 Oil Extraction

One of the important steps in the production of biodiesel is oil extraction, and different methods and techniques of oil extraction are in use (No SY, 2011 and Atabani *et al.,* 2012). After preparation, the raw material is ready for oil extraction. There are three main methods that have been identified for oil extraction: mechanical extraction, enzymatic extraction and chemical or solvent extraction (Bhuiya *et al*., 2016 and Atabani *et al*., 2013). It has been indicated that mechanical pressing and solvent extraction are the most commonly used methods for commercial oil extraction. According to Atabani *et al.,* (2012), the main products during oil extraction are the crude oil, and the important by-products such as seeds or kernel cakes. Seed cakes can be used as fertilizers for soil enrichment, feed for poultry, fish and swine, and some oil cakes have also application in fermentation and biotechnological processes (Ramachandran *et al*., 2007).

2.4.2.1 Mechanical oil extraction method

Mechanical press oil extraction is the most conventional technique. A manual ram press or an engine driven screw press can be used (Bhuiya *et al*., 2016). It has been found that engine driven screw press can extract 68–80% of the available oil while the ram presses only achieved 60–65%. Oil extraction efficiencies calculated from data reported in more recent studies are found to generally correspond to these ranges, although the efficiency range of engine driven screw presses can be broadened to 70–80 % (Bhuiya *et al.,* 2016 and Atabani *et al.,* 2012). The oil extracted by mechanical presses needs further treatment of filtration and degumming in order to produce a more pure raw material (Atabani *et al*., 2013 and Lokanatham, 2013). Another problem associated with conventional mechanical presses is that the design of mechanical extractor is suited for some seeds, and therefore, the oil yield is affected if that mechanical extractor is used for other seeds (Atabani *et al*., 2013 and Lokanatham, 2013). It has been also found that pretreatment of seeds before applying mechanical extractor increases the amount of oil recovery (Atabani *et al*., 2013 and Achten *et al*., 2008). For instance, by cooking jatropha seeds in water for one hour at 70 ^{o}C and using screw pressing, Beerens (2007) obtained oil yield of 89 % after single pass and 91 % after dual pass compared to 79 % and 87 % oil yield recovery of untreated seeds, respectively. Therefore, several other methods have been proposed recently for oil extraction such as solvent extraction, enzymatic extraction and microwave assisted techniques in order to improve the oil extraction yield.

2.4.2.2 Enzymatic oil extraction method

Aqueous enzymatic oil extraction (AEOE) method is a promising technique for extraction of oil from plant materials (Rosenthal *et al*., 2001 and shah *et al*., 2005). In this method, enzymes should be used to extract oil from crushed seeds. AEOE can also be used in combination with other methods of oil extraction. For instance, shah *et al*., (2005) used a combination of ultrasonication and aqueous enzymatic oil extraction (using an alkaline protease at pH = 9.0) method to extract oil from *Jatropha curcas* seeds and obtained 74% of the seed oil which is very large compared to the 17–20% oil extracted by aqueous oil extraction alone. Moreover, using of ultrasonication also resulted in reducing the process time from 18 to 6 hour. The main advantages of using enzymatic oil extraction method are that it is environmental friendly and does not produce volatile organic compounds. However, the long process time is the main limitation associated with this technique.

2.4.2.3 Solvent oil extraction (chemical extraction) method

Solvent extraction is the process in which the oil is removed from a solid by means of a liquid solvent, it is also known as leaching (Bhuiya, *et al*.,2016). The chemical extraction using n-hexane method results in the highest oil yield which makes it the most commonly used solvent (Atabani *et al.,* 2012). According to Jahirul *et al*., (2013) n-hexane has used to extract the oil from Australian native beauty leaf seeds (*Calophyllum in ophyllum*), although the cost of oil extraction technique by mechanical screw press is low it is ineffective due to relatively lower oil yields. On the contrary, the chemical oil extraction method was found to be very effective because of high oil yield and for its consistent performance. It has been observed that there are many factors affecting the rate of solvent extraction such as particle size, the type of solvent used and temperature (Atabani *et al*., 2013). The solvent has to be selected in such a way that it would be a good selective solvent and its viscosity would be sufficiently low to circulate freely. Also Sayyar *et al*., (2009) extracted *Jatropha curcas* oil by n-hexane and petroleum ether and found that the extraction yield with n-hexane to be about 1.3% more than that of petroleum-ether (47.3% and 46.0% respectively) under similar conditions.

The authors recognized n-hexane is a more preferable solvent for extraction of jatropha oil as compared to petroleum ether. In the extraction of olive oil using organic solvents like hexane, ethanol, petroleum ether, isopropyl alcohol and carbon tetrachloride by Soxhlet extractor, Banat *et al*., (2013) also obtained the highest oil yield (12.7%) by n-hexane. However, it has been indicated that this method consumes much more time compared to other methods. Solvent extraction is only economically attractive at a large-scale of production (more than 50 ton biodiesel per day) as reported by Atabani *et al.*, (2012). In addition, n-hexane solvent extraction has a negative environmental impact because of the wastewater it generates, higher energy consumption and emissions of large amount of volatile organic compounds and human health impacts (Atabani *et al*., 2013).

2.5 Characteristics of Oil and its Purification Process

Crude vegetable oils obtained by oil seed processes contain trace amounts of naturally occurring materials such as proteinaceous material, free fatty acids, and phosphatides. It has to be refined to remove undesirable substances. The typical oil refining process includes degumming, chemical or physical refining, bleaching, and deodorization.

Deodorization is an important step in the oil refining process to eliminate free fatty acids, aldehydes, unsaturated hydrocarbons, and ketones, which cause undesirable odors and flavors in the oil (Demirbas and Kara, 2006).

Degummed crude oil is subjected to a further refining process. For this purpose, caustic soda (NaOH) is fed to the oil in the proper quantity to react with the free fatty acids, phosphatides, and the other impurities. Soap stock and other impurities are separated from the oil by centrifuges. Caustic soda is not completely selective in reacting with free fatty acids and phosphatides; therefore, some triglycerides are hydrolyzed and saponified (broken down and converted into soap) (Demirbas and Kara, 2006).

2.5.1 Density of the Oil

Density is the mass per unit volume of any liquid at a given temperature. Specific gravity is the ratio of the density of a liquid to the density of water. According to song (2000), the density values of vegetable oils are between 912 and 921 kg/m^3. This parameter is important in engine performance since fuel injection operates on a volume metering system (Srivastava and Prasad 2000). According to Clark (1988), low relative density is indicator of good ignition properties of fuels.

2.5.2 Kinematic Viscosity of Oil

Viscosity is a measure of the internal fluid friction or resistance of oil to flow, which tends to oppose any dynamic change in the fluid motion (Song, 2000). There are two ways to report or measure a fluid's viscosity. It can either be expressed as dynamic viscosity, or kinematic viscosity. As the temperature of oil increases, its viscosity decreases, and it is therefore able to flow more readily. It is also important for the flow of oil through pipelines, injector nozzles, and orifices (Radovanovic *et al*., 2000). The lower the viscosity of the oil, the easier it is to pump and atomize and achieve finer droplets (Islam *et al*., 2004). The kinematic viscosity values of vegetable oils vary between 39.2 and 65.4 mm2/s at 300 K. Vegetable oils are extremely viscous with viscosities 10 to 20 times greater than that of D2 fuel (Demirbas, 2003).

2.5.3 Acid Value (AV) of Oil

Acid value (acid number) is the mass of potassium hydroxide (KOH) in milligrams that is required to neutralize one gram of chemical substance. The acid number is a measure of the amount of carboxylic acid groups in a chemical compound, such as a fatty acid, or in a mixture of compounds. The acid number is used to quantify the amount of acid present in a sample of oil (Ma and Hanna, 1999).

2.5.4 Free Fatty Acid (FFA) Content of Oil

The free fatty acid value of oil is derived from its acid value by simple mathematical calculation; it is half of Acid value. FFAs easily react with alkaline catalysts to form soap that prohibits the separation of biodiesel and glycerol. The soaps of FFAs also cause foaming in aqueous media. The resulting soaps also cause an increase in viscosity, formation of gels, and foams and make the separation of glycerol difficult (Wright *et al*., 1944; Ma and Hanna, 1999; Demirbas, 2003). In the transesterification process, the vegetable oil should have an acid value of less than 1 and all materials should be substantially anhydrous. If the acid value is greater than1, more KOH is spent to neutralize the free fatty acids (Demirbas, 2003).

2.5.5 Saponification Value of Oil

Saponification value represents the number of milligrams of potassium hydroxide or sodium hydroxide required to saponify 1 gram of fats or oil. It is a measure of the average molecular weight (or chain length) of all the fatty acids present. The long chain fatty acids found in fats have low saponification value because they have a relatively fewer number of carboxylic functional groups per unit mass of the fat as compared to short chain fatty acids. Saponification value (SV) of the oil samples range from 188.71 to 220.78 mgKOH/g of oil. The SV of an oil decreases with increase of its molecular weight. The percentages of C and H in an oil decrease with an increase in molecular weight. The increase in SV results in a decrease in the heat content of an oil (Demirbas, 1998).

2.5.6 Iodine Value (IV) of Oil

Iodine value is the mass of iodine in grams that is consumed by 100 grams of a chemical substance. The iodine values (IVs) of the oil range from 69.82 to 156.74 gI_2/100 of oil. The increase in IV (i.e., carbon-carbon double bond, –C=C–, content of oil) results in a decrease in the heat content of an oil. The iodine value gives a measure of the average degree of unsaturation contained in fatty acids. This unsaturation is in the form of double bonds which react with iodine compounds. The higher the iodine number, the more unsaturated fatty acid are present in a fat (Mittelbach and Remschmidt, 2006).

2.5.7 Moisture Content of Oil

Water content is an important factor in the conventional catalytic transesterification of vegetable oil. The oils used in transesterification should be substantially anhydrous (0.06 % w/w). In transesterification of fats and vegetable oils for biodiesel production, water always produce negative effect since the presence of water causes soap formation, consumes catalyst, and reduces catalyst effectiveness. Thus water has negative effects on the yields of methyl esters (Kusdiana and Saka, 2004).

2.6 Current Technologies for Biodiesel Production

The dominant technologies, which enable us to use oil and fat feedstock types as fuel in diesel engines, are usually described as direct use or blending of oils, micro-emulsion, pyrolysis and transesterification. Transesterification being currently mentioned by various researchers as the most preferable due to better quality of fuel produced (Meher *et al*., 2006 and Leung, *et al*., 2010).

2.6.1 Pyrolysis (Thermal Cracking) Process for Biodiesel Production

Pyrolysis refers to a chemical change caused by the applicationof thermal energy in the absence of air or oxygen, or by the application of heat in the presenceof a catalyst, which results in cleavage of bonds and formation of a variety of small molecules. Pyrolysis is conducted at temperature range of 400–600 ℃. The process produces gases, bio-oil, and a char depending on the rate of pyrolysis (Shemelis and Jorge, 2017)

The pyrolyzate (product of pyrolysis) from any feedstock type has lower viscosity, flash point, and pour point than petroleum diesel fuel and equivalent calorific values. In addition, the cetane number of the pyrolyzate is lower (Mahanta and Shrivastava, 2004). According, to Mahanta and Shrivastava the pyrolyzed vegetable oils contain acceptable amounts of sulfur, water and sediments and give acceptable copper corrosion values but unacceptable quantities of ash, carbon residual and pour point.

The biodiesel fuel produced through a pyrolysis process or known as bio-oil is suitable for diesel engines; however, low-value materials are produced due to the elimination of oxygen during the process (Abbaszaadeh *et al*., 2012). Undesirable properties that sometimes restrict the application of biodiesel produced through this process are low heating value, incomplete volatility and instability (French and Czernik, 2010). In another view, pyrolysis of triglycerides has several advantages including lower processing cost, simplicity, less waste and no pollution (Singh and Singh, 2010).

The limitation of pyrolysis is that it needs distillation equipment for separation of the various fractions. Also the product obtained is similar to gasoline containing sulfur which makes it less environmentally friendly (Ranganathan *et al*., 2008). The equipment for pyrolysis is expensive for modest throughputs. In addition, while the products are chemically similar to petroleum-derived gasoline and diesel fuel, the removal of oxygen during the thermal processing also removes any environmental benefits of using an oxygenated fuel. It produces some low value materials and sometimes, more gasoline than diesel fuel (Ma and Hanna, 1999).

2.6.2 Micro-emulsification Technique

According to International Union of Pure and Applied Chemistry (IUPAC) definition, micro-emulsion is dispersion made of water, oil and surfactants that is an isotropic and thermodynamically stable system with dispersed domain diameter varying approximately from 1 to 100 nm, usually 10 to 50 nm (Slomkowski *et al*., 2011).

The components of a biodiesel micro-emulsion include diesel fuel, vegetable oil, alcohol, and surfactant and cetane improver in suitable proportions. Alcohols such as methanol and ethanol are used as viscosity lowering additives, higher alcohols are used as surfactants and alkyl nitrates are used as cetane improvers (Chiaramonti *et al*., 2003).

Micro-emulsions can improve spray properties by explosive vaporization of the low boiling constituents in the micelles. Micro-emulsion results in reduction of viscosity, increase in cetane number and good spray characters of the biodiesel (Shemelis and Jorge, 2017). Continuous use of micro-emulsified diesel in engines causes problems like injector needle sticking, carbon deposit formation and incomplete combustion (Pairiawi, 2010).

2.6.3 Dilution (Blending) Technique

Direct uses of vegetable oils have generally been considered not satisfactory and impractical for diesel engines. The high viscosity, acid composition, free fatty acid content, as well as gum formation due to oxidation and polymerization during storage and combustion, carbon deposits and lubricating oil thickening are the major problems (Shemelis and Mario, 2017). The oil deterioration and incomplete combustion are the two severe problems associated with the direct use of vegetable oils as fuels (Ma and Hanna, 1999).

2.6.4 Esterification Process

Normally most of the oils are converted into biodiesel esters using the base catalyzed transesterification method. But there are certain exceptional cases where in direct transesterification cannot be performed. Such cases appear in raw vegetable oils (non-edible oil) like olive oil, *jatropha,* castor and cotton seed oil, etc. because these raw vegetable oils possess high free fatty acid (Sattanathan, 2015).

For determining whether the raw vegetable oils can be transesterified directly or not, the acid value is the most important property that must be known. If the acid value is less than three then the raw vegetable oil can be directly trans-esterified. If the acid value is greater than three then there is slight change in the production of biodiesel process. At first the oil undergoes esterification and then followed by transesterification. In the esterification process the excess of the free acid gets reacted. The remaining acid content in the oil undergoes trans-esterification process. So this method is effective for oils that contain high free fatty acid content (Sattanathan, 2015).

2.6.5 Trans-esterification Process

Transesterification is an exchange reaction. The alkaline catalyzed transesterification process is the reaction of a triglyceride (fat/oil) with an alcohol in the presence of alkaline catalysts such as alkaline metal alkoxides and hydroxides as well as sodium or potassium carbonates to form esters (biodiesel) and glycerol. Alkali catalyzed transesterification is much faster than acid catalyzed transesterification and is less corrosive to industrial equipment and therefore is the most often used commercially (French and Czernik, 2010; Ranganathan *et al*., 2008).

Transesterification is the main convenient method to produce biodiesel from oil and fat feedstock types, which chemically resembles petroleum diesel. Through this method, oils and fats (triglycerides) are converted to their alkyl esters with reduced viscosity to near diesel fuel levels. This product is thus a fuel with properties similar to petroleum based diesel fuel, which enable it to be used in existing petroleum diesel engines without modifications (Shemelis and Jorge, 2017). There are a number of ways to produce biodiesel through transesterification among them the two most known transesterification process are homogeneous acid catalyzed transesterification and homogeneous alkaline catalyzed transesterification.

2.7 Biodiesel Production Process by Transesterification Reaction

Transesterification, also called alcoholysis is the action of one alcohol displacing another from an ester (i.e. the transformation of the large, branched, triglyceride molecules of bio-oils and fats into smaller, straight-chain molecules, similar in size to components of diesel fuel). The main factors affecting transesterification are the amount of alcohol and catalyst, reaction temperature, pressure, time, the contents of free fatty acid (FFAs) and water in oils (Demirbas, 2003).

Transesterification is conducted to produce biodiesel with the objective to reduce the viscosity of the parent vegetable oil or animal fat, since it is an order of magnitude greater than that of the corresponding methyl esters (Biodiesel). As Peterson *et al*., (1998) reported the viscosity of canola oil was 37 mm^2/s and reduced to 4.7 mm^2/s after transesterification. 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 nonionic base catalysts but alkali-catalyzed transesterification is faster than that catalyzed by the same amount of an acidic catalyst transesterification (Gerpen, 2004). Potassium hydroxide has added advantage since, at the end of the reaction; the waste mixture can be neutralized with phosphoric acid resulting in potassium phosphate, which can be used as fertilizer (Isigigur *et al*., 1994). Suitable feedstocks for a base-catalyzed transesterification process require FFA < 1% (acid value of oil should be below 2 mgKOH/g) according to ASTM and EN standards (Ghadge and Raheman, 2005). The transesterification reaction is:

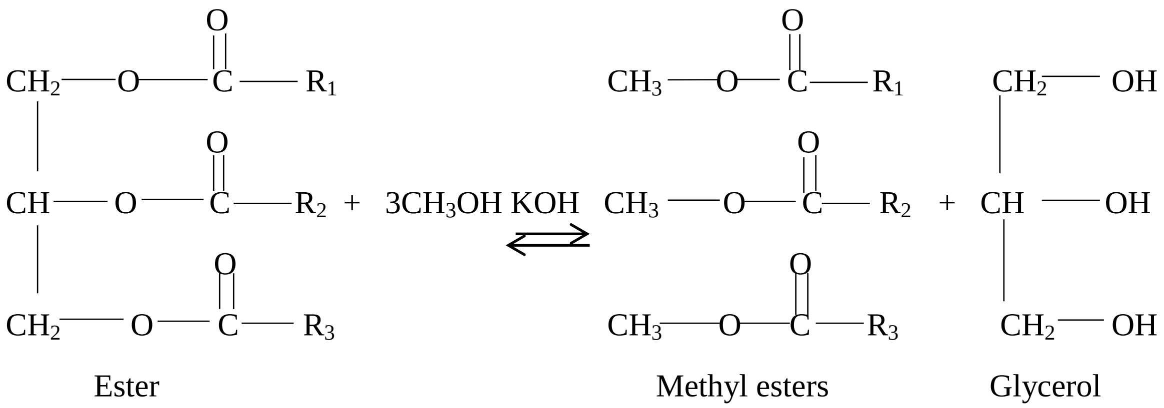


Figure 2.1: The transesterification reaction equation (Freedman *et al.*, 1984)

Where R_1, R_2 and R_3 represents hydrocarbon chains of fatty acid alkyl groups of the triaglyceride

2.7.1 Homogeneous Acid Catalyzed Transesterification Process

Acid catalyzed transesterification process was the first method ever in history to produce biodiesel (ethyl ester) from palm oil using ethanol and sulfuric acid (Gerhard, 2005). The acid catalyzed process is due to the reaction of a triglyceride (fat/oil) with an alcohol in the presence of acid catalyst to form esters (biodiesel) and glycerol. Specially, this method is convenient and economically viable in producing biodiesel from oil or fat resources with high free fatty acid content.

Acid catalyzed transesterifications are usually far slower than alkali catalyzed reactions and require higher temperatures and pressures as well as higher amounts of alcohol but offer the advantage of esterifying free fatty acids contained in the fats and oils. This route is therefore especially suited for the transesterification of highly acidic fatty materials such as waste edible oil. Moreover it enables the production of long and branched chain esters, which poses considerable difficulty in alkaline catalysis (Nimcevic *et al.*, 2000) but the presence of water in the reaction mixture proves absolutely detrimental for acid catalysis (Canacki and Gerpen, 1999).

The transesterification process is usually catalyzed by Bronsted acids, preferably by sulfonic (Anton *et al.*, 2006) and sulfuric acids (Kulkarni and Dalai 2006). These catalysts give very high yields in alkyl esters, but the reactions are slow; typically require temperatures above 100 °C and more than 3 hours to reach complete conversion (Kim *et al.*, 2004). However, the acid catalyzed reaction requires a longer reaction time and a higher temperature than the alkali catalyzed reaction (Zhang, *et al.*, 2003).

Sulphuric acid, sulfonic acid, and hydrochloric acid are the usual acid catalysts but the most commonly used is sulphuric acid. There are also various studies conducted to see the yield effect of using alternative acids. According to Soriano *et al.*, (2009) the $AlCl_3$ could be used to catalyze the esterification of stearic acid suggesting that it is a potential alternative catalyst for biodiesel preparation using cheaper vegetable oil containing high amount of FFA. In their study, optimum conditions to afford 98 % conversion of canola oil to FAME is with the use of methanol to oil molar ratio of 24:1 and reaction time of 18 hours at 100 ℃ in the presence of 5 % $AlCl_3$ as catalyst (Soriano *et al.*, 2009).

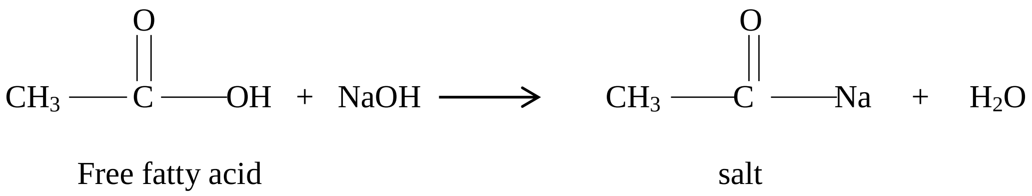
Despite its relatively slow reaction rate, the acid-catalyzed process offers benefits with respect to its independence from free fatty acid content and the consequent absence of a pretreatment step. These advantages favor the use of the acid-catalyzed process when using feedstock types like waste cooking oil as well as most non-edible plant oil, which are usually associated with higher content of fatty acid (Ganesan *et al.*, 2009).

2.7.2 Enzyme catalyzed processes

Enzyme such as lipase catalyzed reactions have advantages over the previously mentioned methods which include: the generation of no by-products, easy product recovery, mild reaction conditions, catalyst recycling, insensitivity to free fatty acid and water content in waste cooking oil (Du *et al.*, 2004 and Ha *et al.*, 2007). Enzyme-based transesterification is carried out at moderate temperatures with high yields, but this method cannot be used in industry today due to high enzyme costs, and the problems related to its deactivation caused by feed impurities. The enzyme can be immobilized on a support to obtain a heterogeneous catalyst however the use will only be possible if the enzyme costs are reduced as in the case of enzymes used in detergents, dairy products, textile, and leather processing. The enzymatic alcoholysis of pure triglycerides with or without solvent has been well-documented in literature (Shah *et al.*, 2004 and Du *et al.*, 2003). Efficient and low energy intensive protocols of the production of biodiesel from waste oils and animal fats combining lipases with alkali catalysts have also been reported (Shah *et al.*, 2004 and Kumari *et al.*, 2007).

2.7.3 Homogeneous alkaline catalyzed transesterification

The alkaline catalyzed transesterification process is the reaction of a triglyceride (fat/oil) with an alcohol in the presence of alkaline catalysts such as alkaline metal alkoxides and hydroxides as well as sodium or potassium carbonates to form esters (biodiesel) and glycerol. Alkali catalyzed transesterification is much faster than acid catalyzed transesterification and is less corrosive to industrial equipment and therefore is the most often used commercially (Ranganathan *et al.*,2008, Marchetti *et al.*, 2007). However, presence of water and high amount of free fatty acid in a feedstock gives rise to saponification of oil and therefore, incomplete reaction during alkaline transesterification process with subsequent formation of emulsion and difficulty in separation of glycerol (Pairiawi, 2010). The saponification reaction is represented by the equation shown below:



$$CH_3-\overset{\overset{O}{||}}{C}-OH + NaOH \longrightarrow CH_3-\overset{\overset{O}{||}}{C}-Na + H_2O$$

Figure 2.2: Saponification reaction

The main drawback resulted due to saponification reaction is the consumption of catalyst and increased difficulty in separation process, which leads to high production cost. In addition to that, formation of water in the product will also inhibit the reaction. In this case, water generated either from vegetable oil or formed during saponification reaction will hydrolyze triglyceride to form more free fatty acid as shown in the equation below.

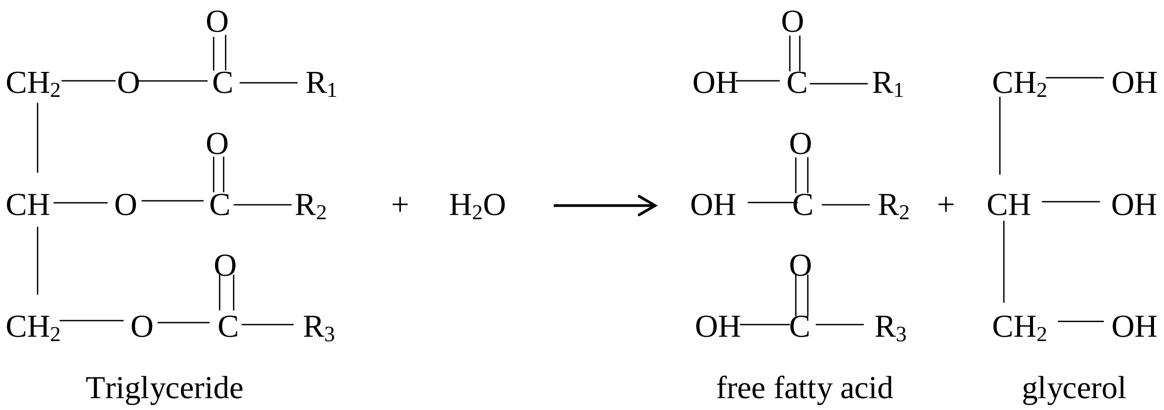


Figure 2.3: hydrolysis of triglyceride

R_1, R_2 and R_3 represents hydrocarbon chains of FA alkyl groups of the triaglyceride.

Generally, base catalyst manifest much higher catalytic activity than acid catalysts in the transesterification reaction, but are selectively suitable for deriving biodiesel only from refined oils having low content of free fatty acids (FFA) usually less than 0.5% (Demirbas, 2009). Esterification as additional step to decrease the free fatty acid content

of feedstock with greater than 0.5% FFA, this will enable us to choose among different feedstock types with higher FFA content.
The efficient production of biodiesel using base catalyzed transesterification process is not only dependent on the quality of feedstock, it is also dependent on the crucial reaction operation variables such as: alcohol to oil molar ratio, reaction temperature, rate of mixing, reaction time, type and concentration of catalyst and also on the type of alcohol used (Fadhil and Ali, 2013; Leung *et al*., 2010 and Singh and Sing, 2010).

Sodium hydroxide, potassium hydroxide and sodium methoxide are catalysts usually used in base catalyzed transesterification process. Sodium hydroxide is mostly preferable owing to its intermediate catalytic activity and a much lower cost (Atadashi *et al*., 2013). Leung and Gua, (2006) evaluated the effect of catalyst on transesterification process by comparing the maximum ester content and yield percentage attained using three base catalysts while other determinant variables were kept the same for all conditions during the base catalyzed transesterification processes.

2.8 Factors that Influence the Transesterification Reaction

Transesterification of oil or fats to produce a high yield biodiesel is typically investigated by optimizing reaction variables such as: alcohol to oil molar ratio, catalyst concentration, reaction temperature and reaction time involved in the process (Betiku and Adepoju, 2013).

2.8.1 Effect Reaction Temperature and Time on Transesterification Process

Temperature plays an important role during biodiesel production; this is because the rate of reaction is strongly influenced by the reaction temperature (Devanesan *et al*., 2007). Studies indicate that alkaline transesterification are conducted close to the boiling point of the alcohol used and that temperature higher than this burns the alcohol resulting into lower yield. According to Patil and Deng, (2009) report, alkaline transesterification at temperature above 60^{o}c cause excessive methanol loss due to evaporation and cause a significant reduction of the overall biodiesel yield.

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 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, (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^{o}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^{o}c; yielding a methylester content of 98.6% w/w. similar result is also reported by other researchers (Freedman, 1984 and Matthew, 2008).

2.8.2 Effect of Molar Ratio of Alcohol to Oil and Types of Alcohol

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).

2.8.3 Effect of Catalysts on Transesterification Process

The concentration of the catalyst is an important parameter of the transesterification reaction and strongly influence on the yield of the isolated methylesters. Sodium hydroxide (NaOH) and potassium hydroxide (KOH) are the common catalyst used in the reaction process (Ojiego *et al.*, 2014). High concentrations of alkaline catalyst form soaps in the presence of large residues of fatty acids resulting in emulsion formation between soaps and water molecules, thus leading to low yields of methyl esters (Babajide, 2009). Catalyst type and concentration can affect the yield of biodiesel product. As mentioned earlier, 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 oil a small amount of water will be produced, which will affect the product yield due to 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.

The methyl ester content increased as concentration of basic catalyst increase from 0.5% to 1% (w/w) but slight decrease from 1% to 1.5% (w/w). In general, as the catalyst concentration increased, the conversion of triglyceride also increased (Leung *et al*., 2010). Because of an insufficient catalyst concentration an incomplete conversion of triglyceride into methyl ester happen, due participation of oil in the production soap and so there is reduction in production of ester (Eevera *et al*., 2009).

2.8.4 Effect of Mixing Intensity on Transesterification Process

Most studies indicate that during the transesterification reaction, the reactants initially form a two-phase liquid system. The mixing effect has been found to play a significant role in the slow rate of the reaction. As phase separation ceases, mixing becomes insignificant. The effect of mixing intensity on the kinetics of the transesterification process forms the basis for process scale-up and design (Shereena and Thangaraj, 2009)

2.8.5 Effect of Purity of Reactants on Transesterification Process

Impurities in the oil affect the conversion level considerably. It is reported that about 65–84 % conversion into esters using crude vegetable oils has been obtained as compared to 94–97 % yields refined oil under the same reaction conditions. The free fatty acids in the crude oils have been found to interfere with the catalyst. This problem can be solved if the reaction is carried out under high temperature and pressure conditions (Shereena and Thangaraj, 2009).

2.9 Separation and Purification of Biodiesel Product

The separation process is one of the most crucial parts of biodiesel production. The products of transesterification process are composed of FAME, glycerine, alcohol, catalyst and unreacted glycerides (free glycerin). Due to their different chemical affinities, most of the catalyst and excess alcohol will concentrate in the lower phase glycerin, while most of the mono-, di - and triglycerides will concentrate in the upper phase FAME. Once the interphase is clearly and completely defined, the two phases may be physically separated. Removing glycerin from biodiesel is important since it significantly affect the quality of biodiesel. Severe consequences may result due to the high content of free and total glycerin, such as buildup in fuel tanks, clogged fuel systems, injector fouling and valve deposits (Hayyan *et al.*, 2010). The by-product glycerin is separated from FAME using a simple gravitational settling method and left for a certain period of time. The separation process using the settling or centrifugal tank was considered to be cost effective (Gomes *et al.*, 2010). After the separation of glycerin, the FAME mixture contains impurities such as remnants of alcohol, catalyst and mono-, di -, triglycerides and other by-products. These impurities confer undesirable characteristics to FAME such as increased cloud point and pour point, lower flash point. Therefore, biodiesel must be purified before being used as a fuel in order to fulfill standard specifications; otherwise the methyl esters formed cannot be classified as biodiesel. Biodiesel purification methods can be classified into two categories: namely wet washing and dry washing technologies. Water becomes the most popular reagent in wet washing technologies since the by-products i.e. glycerin and methanol are highly soluble in water (Javidialesaadi *et al.*, 2013). Water washing techniques have been improved by the addition of acid in water solution. The presence of acid in aqueous solution could reduce the purification steps as it simultaneously neutralizes the basic constituent in the product, thus producing a better quality of biodiesel (Canakci and Van Gerpen, 2001). Dry washing process, in this water-less purification method, a synthetic adsorbent or an ion exchange resin is employed to bind and remove the ionic salts, traces of catalyst, soaps, glycerin and water from biodiesel. Products that are being promoted as an eco-friendly alternative to the water washing process includes magnesol, which is a synthetic magnesium silicate adsorbent and ion exchange resin. (Alemayehu and Amanu, 2014).

2.10 Properties of Biodiesel Fuels

Since biodiesel is produced from vegetable oils of varying origin and quality, the pure biodiesel must meet standard specification before being used as a pure fuel or being blended with conventional diesel fuels. The various parameters which define the quality of biodiesel are discussed below.

2.10.1 Viscosity

The viscosity of a liquid is a measure of its resistance to flow due to internal friction; this is a very important property of a diesel fuel because it affects the engine fuel injection system predominantly at low temperatures. Biodiesel is slightly viscous but their viscosities are still close to that of the petroleum diesel. This is an advantage of biodiesel over its source oils (Alnuami *et al*., 2014). The standard specification of biodiesel viscosity is 1.9 – 6.0 mm^2/s and 3.5 – 5.0 mm^2/s for ASTMD6751 and EN14214, respectively.

2.10.2 Density

Density is a key fuel property, which affects the engine performance characteristic. It affects the mass of fuel injected into the combustion chamber and thus, the air-fuel ratio. This is because fuel injection pumps meter fuel by volume not by mass and a denser fuel contains a greater mass in the same volume. Thus, the changes in the fuel density will influence engine output power due to a different mass of fuel injected (Alemayehu *et al*., 2014). The standard density for biodiesel is 870–900 kg/m^3 for ASTM D6751. It is known that biodiesel density mainly depends on its esters content and the remained quantity of alcohol; hence this property is influenced primarily by the choice of vegetable oil (Encinar *et al*., 2010).

2.10.3 Flash point

Flash point of a fuel indicates the minimum temperature at which the fuel will ignite (flash) on application of an ignition source under specified conditions. It is noted that the biodiesel component must meet a flash point criteria, prior to blending, for the purpose of assuring that the biodiesel component does not contain methanol (Alemayehu *et al*., 2014). The standard flash point for biodiesel is a minimum of 120^oc and 130^oc for EN14214 and ASTM D6751, respectively. The flash point of biodiesel is higher than the petro diesel, which is safe for transport purpose. High values of flash point decreases the risk of fire (Sanjay, 2013; Vuppaladadiyam *et al*., 2013).

2.10.4 The Cetane number

The cetane number of a fuel is a measure of the ignition quality of the fuel, the higher the cetane number the better the ignition quality. The standard cetane numbers for biodiesel is a minimum of 47 for ASTM D623. On the basis of ignition quality, biodiesel can be said to be better than the petroleum diesel because they have cetane numbers higher than that of the petroleum diesel, this high cetane number is due to higher oxygen contents. This means that they will burn smoothly and with less noise in a diesel engine than petroleum diesel (Alnuami *et al*., 2014 and Bello and Agge, 2012).

2.10.5 Acid Value

Acid number is a measure of acids in the fuel. The standard acid numbers for biodiesel is a maximum of 0.5 mgKOH/g and 0.8 mgKOH/g as the specification of EN14214 and ASTM D 6751 respectively. These acids emanate from two sources: acids utilized in the production of the biodiesel that are not completely removed in the production process; and degradation by oxidation (Sanjay, 2013). According to the report of Bello and Agge, (2012), the acid values of ground nut oils and its biodiesel are 15.37 mgKOH/gm and 3.37 mgKOH/gm respectively.

2.10.6 Saponification Value

Production of biodiesel using an alkali catalyst always produces some amount of soap. After the transesterification reaction is complete, the left over catalyst and soap tends to concentrate in the glycerol phase; however some soap and a small amount of catalyst may be left in the biodiesel phase. During the process development, it can be useful to know the amount of soap formed, and how effective the washing process was in removing this soap (Ma and Hanna, 1999).

2.10.7 Iodine number

Iodine number is a measure of total unsaturation within a mixture of fatty material. Its value only depends on the origin of the vegetable oil, the biodiesel obtained from the same oil should have similar iodine values (Encinar *et al*., 2010). It is related to the chemical structure of the fuel. Higher iodine value indicates higher unsaturation in fats and oils. The standard iodine value for biodiesel is a maximum of 120 I_2g/100g for EN 14214 specification. This requirement is limited by the standard limits of linolenic acid methyl ester composition for biodiesel. The limitation of unsaturated fatty acids is necessary due to the fact that heating higher unsaturated fatty acids results in polymerization of glycerides. This can lead to the formation of deposits or deterioration of the lubricating property.

2.10.8 Ash content

Ash content is a measure of the mineral ash residue when a fuel is burned. The standard ash content value for biodiesel is a maximum of 0.02 and 0.03 % w/w for EN14214 ASTM D6751 respectively. It is an important test for biodiesel because it is an indicator of the quantity of residue metals in the fuel that came from the catalyst used in the transesterification process (Alnuami *et al*., 2014).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

The samples of caster seed and algae biomass were collected from Jimma Zone, Dedo Werada and Jimma town, Boye stream. Dedo Werada and Boye stream were found at about 360 and 380 km south west of Addis Ababa (the capital city of Ethiopia), respectively. Both areas are locally categorized as woyna-dega in climatic nature.

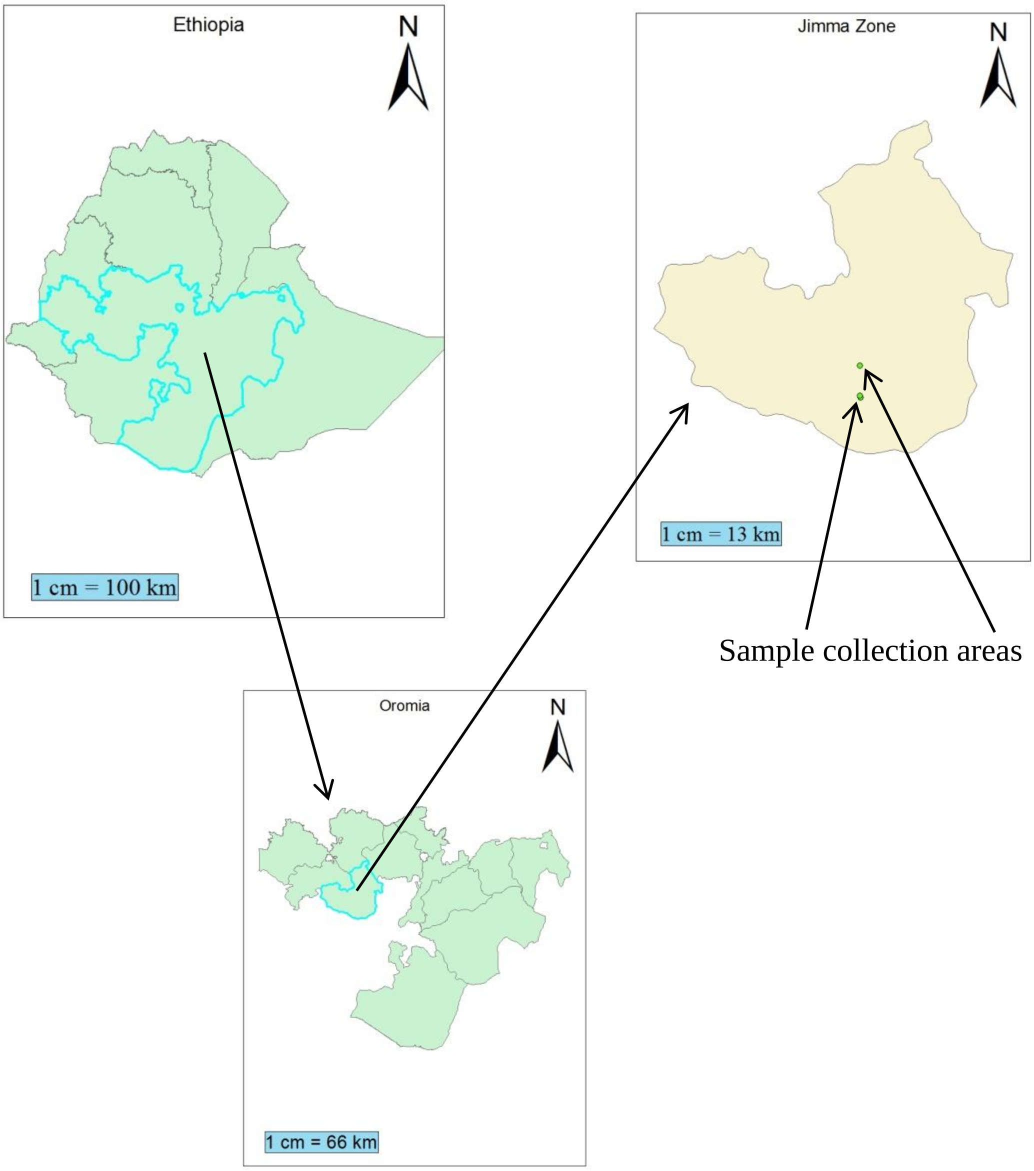


Figure 3.1: Location map of sample collected area.

3.2 The raw material

The major raw materials used for biodiesel productions were; microalgae, castor seed, methanol and potassium hydroxide. The chemicals used in this study are listed in table: 3.1. The major equipment's used were oven, furnace, mortar and pestle, soxhlet extractor, rotary evaporator, vibro-viscometer, condenser, hotplate with magnetic stirrer and electronic balance.

Table 3.1: List of Chemicals, solvents and Acid used in the study

Chemicals/solvents	Grade/purity
N-hexane	99 % w/w
Methanol	99 % w/w
Ethanol	98 % w/w
potassium hydroxide	analytical grade
sodium hydroxide	analytical grade
phosphoric acid	98 % w/w
hydrochloric acid	98 % w/w
potassium iodide	Analytical grade
Chlorophorm	99 % w/w
Phenolphthalein	Analytical grade

3.3 Experimental Design

All experimental works were conducted at Jimma University department of Chemistry, Organic and Inorganic Chemistry research laboratory.

The general experimental setup is shown in figure 3.2. The Castor seed was collected from Dedo Werada, Jimma Zone. The seeds were selected according to their condition where poor quality seeds were discarded and seeds in good condition were cleaned, crushed to small size and oven dried. The algae biomass was collected from Boye stream by filtering using a piece cloth as filter media and dried in a sun. The oil was extracted from both the dried and grinded castor seed and microalgae separatly using soxhlet extraction method with a solvent n-hexane. After the oil obtained was refined the two oils were blended and analyzed for its physicochemical properties, it was subjected to a chemical reaction transesterification with methoxide (a mixed solution of methanol and potassium hydroxide). After the transesterification reaction was completed the crude biodiesel was separated from the glycerol using a separator funnel and the methanol was distilled off. Then washing process with hot water was used to remove the impurities from the biodiesel and it 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.

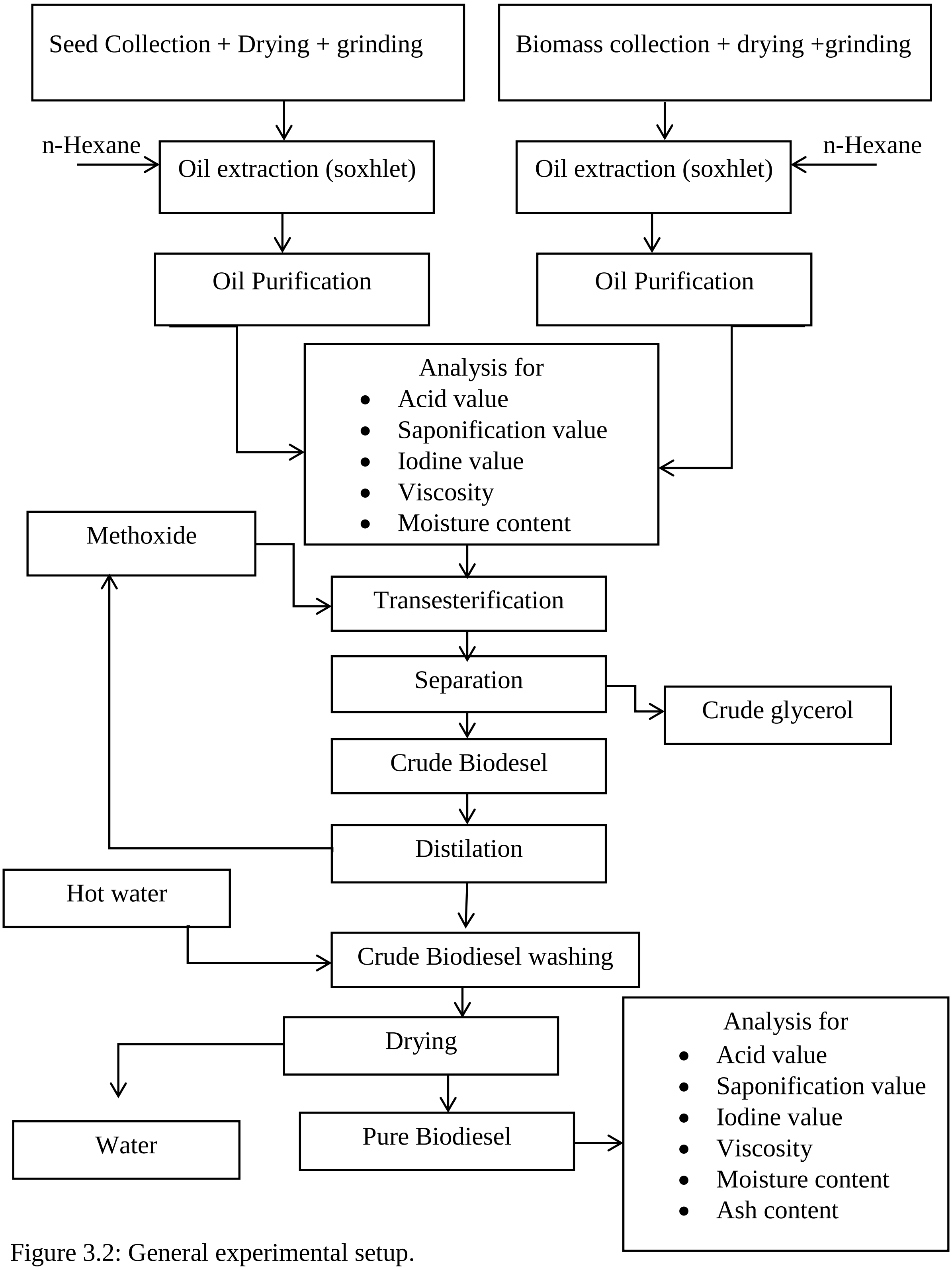


Figure 3.2: General experimental setup.

3.4 Sample collection

The algae biomass and caster seed samples were collected from Dedo Werada, Jimma Zone and Jimma town, Boye stream. 3 kg of castor seed was collected manually in four rounds with two days intervals from two rural villages called Sito and Coma. About 0.75 kg of caster seed was collected in each round. The seeds were selected according to their condition where poor quality seeds were discarded and seeds in good condition were used. 8 kg of algae biomass was collected manually using a piece of cloth and sieve as a filter media in six rounds with one day interval from Boye stream about 1.3 kg of wet algae biomass was collected in each round.

Figure 3.3: Castor seed and Algae Paste.

3.5 Sample preparation

For the caster seed hand cleaning was performed to remove foreign materials such as sand, sticks, stems and leaves. The cleaned beans were dried in the sun for seven hours until the casting splits and shed the seeds. The shell was separated from nibs (cotyledon) by hand. The seed were crushed and dried in the oven at a temperature of 105 ^{o}C for 2hr to remove moisture in the seed. It was further crushed to a sieve size of about 1.2 mm using mortar and pestle for easy oil extraction.

For the microalgae as it was collected from water body by filtration no more foreign materials present it simply needs moisture removal, since algae have high moisture content and it can be easily decomposed because of this problem it was sun dried using large dish or container to increase surface area and to put algae slurry with small thickness. The dried algae biomass was collected immediately to minimize loss due to wind and crushed to a sieve size of about 1.2 mm using mortar and pestle for easy oil extraction.

Figure 3.4: Sample preparation.

3.6 Determination of Seeds Moisture Content

100 g of cleaned bean sample was dried in an oven at 105 °C, weighed at 2 hour intervals, until it reaches a constant weight up to 6 hours. The moisture content was calculated using the equation:

$$\% \, \mathrm{MC} = \frac{\mathrm{W1} - \mathrm{W2}}{\mathrm{W2}} * 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots .3.1$$

Where: $W_{1=}$ weight of samples before drying

$W_{2=}$ weight of samples after drying

MC= moisture content

3.7 Oil extraction

Both castor and algae oil were extracted by using a soxhlet extractor. About 50 g of ground samples were weighed and packed in a filter paper, placed in the thimble and 250 ml of hexane was measured and poured in a round bottom flask. The long glass containing thimble inside of it was connected with a condenser and fixed with a round bottom flask. The fitted apparatus was then heated in a heating mantle to boil the solvent. When the solvent was boiled, the vapour rose through the vertical tube into condenser to the top and the vapour condensed, dripped into the thimble in the centre. The extractor seeped through the pores of the thimble and filled siphon tube where it flowed back down into the round bottom flask (Akpan *et al* .2006). The extraction prolonged to six hours after which the resulting mixture in the round bottom flask was concentrated in rotary evaporator to recover the solvent from the extracted oil. The weight of the extracted oil was recorded.

Figure 3.5: Soxlet extractor and rotary evaporator.

Castor Oil Yield

The % yield of castor seed oil was calculated using the following equatiom

$$\%\, yield = \frac{\mathrm{W1}}{\mathrm{W2}} * 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots .. 3.2$$

Where: W1= weight of extracted oil
W2= weight of caster seed used

3.8 Purification of crude oil

The crude oil was refined by degumming and neutralization processes. In degumming process, the crude oil was treated with hot water and phosphoric acid to remove gums, hydrated phosphate and other impurities, and then it was neutralized with 0.1N NaOH to remove FFA and soap.

3.8.1 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.

3.8.2 Neutralization

After determining the free fatty acid (FFA) of oil, the free fatty acid was neutralized by 0.5 N of NaOH and 0.86 gram of NaOH was added per 100 ml of oil to neutralize and coagulate the FFA (Antony *et al.*, 2011). Neutralization was done by heating the oil at 70 ^{o}C and adding NaOH. The mixture of oil and NaOH were stirred at 200 rpm at a temperature of 70 ^{o}C for 1 hour. The coagulated free fatty acid (soap) was separated using a centrifuge at a speed of 800 rpm for 20 minutes. Later the mixture was washed with a distilled water to remove a trace NaOH. Finally, trace water was removed in an oven through drying at a temperature of 105 ^{o}C for 1 hour. This process brought the free fatty acid content to below 2 % and is perfect source for biodiesel production.

3.9 Physicochemical Properties of Extracted Oil

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

3.9.1 Determination of Specific Gravity of Extracted Oil

The specific gravity or relative density of the oil can be determined by taking the ratio of the mass of the 50 ml oil at 20 ^{o}C (room temperature) to the mass of an equal volume of water at 20 ^{o}C (room temperature).

$$\mathrm{Sg} = \frac{\mathrm{Mo\ at\ 20\ ^{o}c}}{\mathrm{Mw\ at\ 20\ ^{o}c}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots .3.3$$

Where: Sg = specific gravity

Mo = mass of 50ml of oil at 20 ^{o}c

Mw = mass of 50ml of water at 20 ^{o}c

Density of oil = (Specific gravity of oil) X (Density of water) ……..……………….….. 3.4

3.9.2 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.

$$V = \frac{\eta}{\rho} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots 3.5$$

Where: η = dynamic viscosity of the oil

ρ = density of the oil

ν = kinematic viscosity of the oil

3.9.3 Acid Value (AV) of Extracted oil

The acid value is the number that expresses, the quantity of potassium hydroxide required to neutralize the free acids present in 1 g of the substance. To determine the acid value of oil a titration solution of 0.1N of KOH in distilled water was prepared. 2 g of oil was added to a beaker, Then 25 ml of anhydrous ethanol (99.5%w/w), and 3 drops of phenolphthalein were added into the titration beaker that contains sample (oil).Then sample was mixed thoroughly with a mixture of 20ml of ethanol, and 3 drops of phenolphthalein and heated at 70 ^{o}C for 10 minutes. Finally, titration solution, 0.1N of KOH was added 1 drop at a time until the first color (pink) 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;

$$AV = \frac{MxNxV}{W} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots 3.6$$

Where: AV= Acid Value

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

3.9.4 Free Fatty Acid (FFA) Determination

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

$$\%\ FFA = \frac{AV}{2} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots 3.7$$

Where: % FFA = percentage of free fatty acid,

AV = Acid Value of the oil.

3.9.5 Saponification Value (SV) of Oil

The saponification value is the number of mg of potassium hydroxide required to neutralize the free acids and to saponify the esters in 1g of the substance. Saponification value was determined using indicator method according International Organization for

Standardization, (ISO) 3657, (1988). 2 g of the sample was weighed and added to a conical flask then 25 ml of 0.1N ethanolic KOH solution also added. A reflux condenser was connected to the flask containing the mixture and the sample was constantly stirred and allowed to boil at 70 ^{o}C for 60min. After that,3 to 5 drops of phenolphthalein indicator was add to the warm solution and then titrate with 0.5M HCl (volume Va was record) to the end point until the pink color of the indicator was disappeared. Then a blank was prepared following the same procedure and (volume Vb was record). Then the SV was calculated using the equation below:

$$SV = \frac{\mathrm{Mx\ Nx(Vb - Vs)}}{\mathrm{W}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.8$$

Where: SV= saponification value

M= molecular weight of KOH

N = normality of HCL solution

Vb= volume of HCl solution used in blank

Vs= volume of HCl solution used in the sample

W = weight of oil used

3.9.6 Iodine Value (IV) of Extracted Oil

The iodine value gives a measure of the average degree of unsaturation of a lipid: the higher the iodine value, the greater the number of C=C double bonds. By definition the iodine value is expressed as the grams of iodine absorbed per 100 gram of lipid**.**

The method specified by ISO 3961, (1989) was used to determine iodine value of oils. Approximately 0.25 g of the oil was put into a 250 mL conical flask and 10 ml of chloroform and 30 ml of hanus solution was added, then the flasks was closed completely by Para film and leave the solution for 30 minutes with shaking continuously. Next 10 ml of 15 % potassium iodide solution and 100 ml of distilled water were added and the mixture was shacked. Finally the solution was titrated against 0.1 N sodium thiosulfate solution till yellow color formed, then 2-3 drops of starch solution was added then the blue solution was formed and then the titration was continued till the blue color was disappeared, the volume (ml) of $Na_2S_2O_3$ at end point represents S. For blank the same procedure was conducted without sample and the volume (ml) of $Na_2S_2O_3$ at end point represents B. Then the iodine value (IV) was calculated as follows:

$$\mathrm{IV} = \frac{\mathrm{(B - S)x\ Nx12.69}}{\mathrm{W}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.9$$

Where: B = V ml of $Na_2S_2O_3$ for blank

S = V ml of $Na_2S_2O_3$ for sample

N = Normality of $Na_2S_2O_3$

W = weight of sample (oil)

3.9.7 Moisture Content of Extracted Oil

A dish was weighed with and without oil. The dish with oil was dried in an oven at 105°C for 6 hours, weighing each 2 hours till constant weight was obtained and finally the weight was taken and compared with the initially recorded weight. The moisture content of the oil was calculated using the following formula;

$$\%\ \mathrm{MC} = \frac{\mathrm{W1} - \mathrm{W2}}{\mathrm{W1}} \mathrm{x}100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots..3.10$$

Where: MC= Moisture content

W1= Original weight of the sample

W2= Weight of the sample after drying

3.9.8 Ash Content (AC) of Extracted Oil

Furnace was used to determine the ash content of the oil. A burning cup containing 20 g 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 as follows.

$$\%\ \mathrm{AC} = \frac{\mathrm{M2}}{\mathrm{M1}} \mathrm{x}\ 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots..3.11$$

Where: AC= Ash content

M1= Mass of oil before burning

M2= Mass of oil after burning

3.10 Biodiesel production process

Biodiesel was made using three main components: oil, alcohol, and a base catalyst by transesterification method by using the three necked bottle reactor. The reactor was put in the water bath which was on the magnetic stirrer with heating mantle that provide the mixing requirement in a temperature controlled hot plate as shown in the figure: 3.6, in addition, the condenser fixed to the middle neck of the bottle to provide cooling system for the experiment to control the leakage of methanol by supplying cooled water through the inlet and the hot water was rejected through the outlet part.

The oil was prepared from the blends of two oils such that, crude algae oil was mixed with crude castor oil at three different ratio. Since the acid value of the blended oil was 20.56 g KOH/g oil which contains high free fatty acid (FFA) then there was slight change in the production of biodiesel process. At first the oil undergoes esterification and then followed by transesterification.

Esterification reaction was done by using 5 % H_2SO_4 (v/v) and 30 % CH_3OH (v/v) of oil mixed together and it was added to the oil which was preheated to 60 ^oC in the three necked bottle reactor then the mixture was heated for one hour in the water bath to 65 ^oC. In the esterification process the excess of the free acid gets reacted. The remaining acid content in the oil undergoes trans-esterification process. So this method is effective for oils that contain high free fatty acid (FFA) content.

The transesterification reaction was done by the following methods and procedures. The base catalyst (KOH) and alcohol (methanol) was mixed in a catalysts preparation reactor to prepare potassium methoxide and then it was added to the pre heated oil in the reactor. The ratio of oil to alcohol that was used in this experiment was 1:6, 1:9 and 1:12. The oil was prepared from the blends of two oils with the volume ratio of 1:1, 1:2, and 1:3 to search the best biodiesel yield by using three different catalyst concentrations such that 1 %, 1.5 % and 2 % weight by weight of oil's used.

Before starting the biodiesel production process the following technique was done, keeping the reaction temperature, stirring rate and reaction time constant at 65 ^oC, 400 rpm and 2 hour respectively, three different biodiesel production conditions were varied. Firstly, transesterification was done keeping the molar ratio of oil to methanol at 1:6, the volume ratio of algae oil to castor seed oil at 1:1 and varying catalyst concentration. Secondly, keeping the methanol to oil molar ratio at 1:6, the catalyst load at 1 % weight by weight and varying the volume ratio of algae oil to castor seed oil, another set of biodiesel was produced. Thirdly, the methanol to oil molar ratio was varied while keeping the volume ratio of algae oil to castor seed oil at 1:1 and the catalyst concentration at 1 %. After shaking the solution from each experiment it was transferred to separator funnel and was kept for 24 hr to settle then the biodiesel and sediment layers clearly observed, the biodiesel was separated from sediment by draining the bottom layer (sediment) carefully. The biodiesel was washed with hot distilled water until the washing water become clear, then the biodiesel was dried in an oven to get a pure biodiesel, the biodiesel yield was calculated separately from each experiment to know the best yield.

By knowing the best yield, the optimum parameter was known then the last experiment was conducted with the optimum parameters obtained from the privies conducted experiments to obtain the optimum biodiesel yield. Finally, the biodiesel was checked for its physicochemical properties and compared with the international standards. The yield was calculated for each individual experimental result as follows.

$$\% \text{ Yield} = \frac{\text{Vb}}{\text{Vo}} \text{ x } 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.\ldots 3.12$$

Where: Vb= Volume of biodiesel

Vo= Volume of oil

Figure 3.6: Tranisterification and separation process.

3.10.1 Feed Material Requirement for the transesterification process

For each experimental run of the transesterification process, 30 ml 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 6:1

$$\frac{\text{mole of methanol}}{\text{mole of oil}} = 6 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.13$$

$$\frac{\text{given mass of methanol} \big/ \text{molecular mass of methanol}}{\text{given mass of oil} \big/ \text{molecular mass of oil}} = 6$$

$$\frac{\text{Density of methanol x Volume of methanol} \Big/ \text{molecular mass of methanol}}{\text{Density of oils x volume of oils} \; /\text{molecular mass of oil}} = 6$$

$$\frac{0.791\ \text{g/ml} * \text{Volume of methanol} \Big/ 32.04\ \text{g/mol}}{\frac{\frac{0.920\ \text{g}}{\text{ml}} \text{x30 ml}}{926\ \text{g/mol}}} = 6$$

Volume of methanol = 7.24 ml

Similarly, Volume of methanol required when the molar ratio of methanol to oil is 9:1 and 12:1 was calculated and obtained as 10.85 ml and 14.5 ml respectively.

Amount of Catalyst required

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

$$\frac{\text{mass of catalyst (KOH)}}{\text{mass of oil}} \text{x } 100 = 1\ \% \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots .3.14$$

$$\frac{\text{mass of catalyst (KOH)}}{\text{Density of oil x volume of oil}} \text{x}100 = 1\%$$

$$\frac{\text{mass of catalyst(KOH)}}{0.920\ \text{g}\big/_{\text{ml}}\ \text{x30 ml}} \text{x}100 = 0.01$$

Mass of Catalyst = 2.76 mg

Similarly, the amount of catalyst required when catalyst weight to oil ratio is 1.5 % and 2 % was obtained as 4 mg and 5.5 mg respectively.

3.11 Design of Experiment for Response Surface Methodology (RSM)

A three variable central composite design for response surface methodology was used to study the combined effects of methanol to oil molar ratio, castor oil to microalgae oil volumetric ratio and catalyst concentration on the amount of biodiesel yield over three levels. The process variables were: molar ratio of methanol to oil, volumetric ratio of castor oil to microalgae oil and catalyst percent in weight. The reaction time, temprature and mixing intensity were fixed at 2 hours, 65 ^{o}C and 400 rpm respectively for all experimental runs. The design requires experimental number of runs according to the following equation.

$$N = 2^{K} + 2K + Cp \dots\dots\dots 3.15$$

Where: K is the factor number which is three in this case and Cp is the number of replications at the center point which is six there for; the number of runs are twenty.

3.12 Physicochemical Properties of Biodiesel

The physicochemical properties of biodiesel, such as, density, kinematic viscosity, acid value, saponification value and iodine value was determined with the same procedures and methods as the physicochemical properties of oil but the rest physicochemical properties including, flashpoint, heating value and cetane number was determined as follows.

3.12.1 Determination of Flash point (FP)

The Flash point of the biodiesel was determined using empirical formula suggested by Ayhan Demirbas (2008). The equation between flash point (FP) and heating value (HV) for biodiesel was:

$$HV = 0.12FP + 32.12 \dots\dots\dots 3.16$$

$$FP = \frac{HV - 32.12}{0.12} \dots\dots\dots 3.17$$

Where: HV= Heating value of biodiesel

FP = Flash point of biodiesel

3.12.2 Determination of cetane number (CN)

The Cetane number of biodiesel was determined using the empirical formula suggested by (Kalayasiri *et al.*, 1996), using the result of SN and IV of the biodiesel.

$$CN = 46.3 + \frac{54.58}{SN} - 0.225 * IV \dots\dots\dots 3.18$$

Where: CN = Cetane number of biodiesel

SN= saponification value of biodiesel

IV = Iodine Value of biodiesel

3.12.3 Determination of Heating Value (HV)

The HV of biodiesel was determined using the empirical formula suggested by Demirbas (1998).

$$HV = 49.43 - [0.041(SN) + 0.015(IV)] \dots\dots\dots 3.19$$

Where: HV = Heating value of biodiesel

SN = Saponification number of biodiesel

IV = Iodine Value of biodiesel

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Oil Extraction and Characterization

4.1.1 Castor seed preparation, Oil extraction and refining

3 kg of seed was brought from Dedo werada, Jimma zone. During seed preparation first it was cleaned, oven dried at 105°c for 6 hours to moisture content of 14.43%. During the moisture determination three samples with 25 g were prepared and the average moisture content was determined table (4.1). From 3 kg of raw castor seed 2.28 kg of cleaned kernel was obtained and crushed to about 1.2 mm using mortar and pestle to increase extraction efficiency. Then oil was extracted by Soxhlet extraction method using the solvent n- hexane. Based on equation 3.2 the yield of extracted oil from 100 g of powdered caster seed was 42 g (42%). From 8 kg of wet algal biomass 625 g dried algal biomass was obtained and based on equation 3.2 the yield of extracted oil from 100 g of powdered algae was 25.5 g (25.5%). After the oil was extracted and separated from the solvent n-hexane, using rotary evaporator 288 ml and 212 ml of castor seed oil and microalgae oil were obtained, respectively.

Table 4.1: Moisture content determinations of caster seed

Sample	Drying time in hours				
	0	2	4	6	Moisture cont.
Sample 1	25 g	23.46	22.25	21.35	14.6%
Sample 2	25 g	23.45	22.35	21.46	14.16%
Sample 3	25 g	23.35	22.38	21.37	14.52%
Average					14.43%

The moisture content of caster seed for the three samples were 14.6%, 14.16% and 14.52%, respectively and the average moisture content was found to be 14.43% w/w. the same procedure was used to determine the moisture content of algae biomass and the result found to be 10.5% w/w.

4.2 Characterization of Oils (Castor and Algae)

4.2.1 Density of the Oil

The specific gravity was determined using the method stated under section 3.9.1. The result was found to be 0.920 g/ml. From the simple empirical formula explained in equation 3.4, the density of the oil was obtained to be 920 kg/m^3 which fell with the ranges reported in literature song (2000).

4.2.2 Kinematic Viscosity of the Oil

The dynamic viscosity of the oil was measured using at 40^{o}c .The device detects the dynamic viscosity, which is the resistance to flow with vibration. The observed kinematic viscosity was 44.6 mm^2/sec that was in agreement with the literature data (Ayalew, 2014). The result shows that the oil obtained is highly viscous & needs transesterification so as to reduce its viscosity.

4.2.3 Acid Value of the Oil

The acid value of the oil was determined using the method stated under section 3.9.3. The results were recorded in table 4.2. From this table the titration volume at which the first color change observed on average was 7.33 ml. The acid value of the oil was calculated using equation 3.6 and the result was found to be 20.56 mg KOH/g oil. Since this value is very high the oil needs treatment, in this case the treatment technique used was esterification (Sattanathan, 2015) thus the acid value after esterification was reduced to 1.98 mg KOH/g of oil. The Acid value is higher in crude oil, while it is less after esterification. The result agree with the literature (Molla and Nigus, 2014).

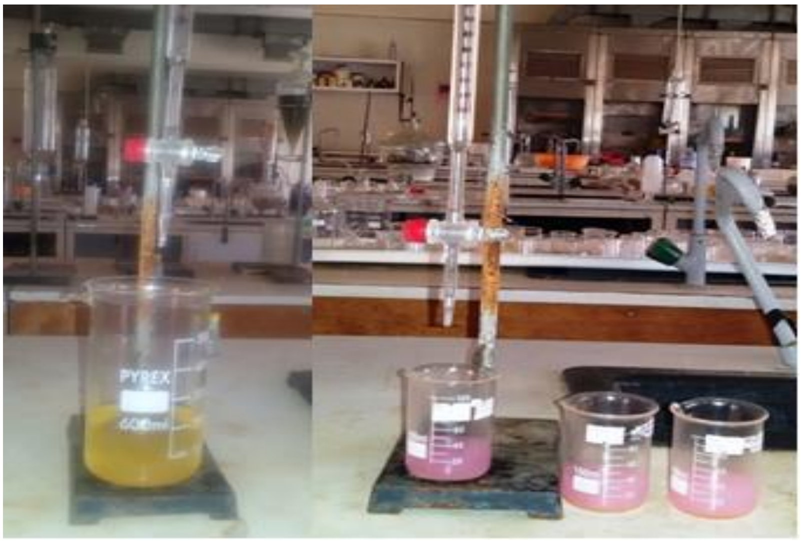


Figure 4.1: Acid value determinations by titration method.

Table 4.2: Acid value test result

Run No.	Mass of oil	Volume of KOH consumed	Color change	Acid Value mg KOH/g oil
1	2 g	7.2	Yellow to pink	20.196
2	2 g	7.5	Yellow to pink	21.037
3	2 g	7.3	Yellow to pink	20.477
Average		7.33		20.56

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. Equation 3.7 was used to calculate the free fatty acid of crude oil and found to be 10.28% and 0.98% for crude and esterified oil, respectively. The results indicated that the free fatty acid values of the oils decreased significantly after esterification. It was somewhat similar with the report of literature data (Demirbas, 2003).

4.2.5 Saponification Value of the Oil

The procedure used to determine saponification value of oil was stated in section 3.9.5. The titration results for saponification test are illustrated in Table 4.3. The titration volume at which the first color change observed was the amount of hydrochloric acid consumed. The amount of hydrochloric acid consumed by blank solution and solution with sample on average were 16.4 ml and 3.5 ml, respectively.

The saponification value was then calculated using equation 3.8 the observed value was taken for three trials and the average value obtained was 180.9 mgKOH/g of oil, which is in agreement with the result obtained in many literature (Molla and Nigus, 2014, Ayalew, 2014).

Table 4.3: Saponification value test result

Run No.	Mass of oil	Vb of HCl consumed	Vs of HCl consumed	Vb –Vs of HCl	Saponificatio value mgKOH/g oil
1	2 g	16.4	3.5	12.9	180.9
2	2 g	16.3	3.6	12.7	178.12
3	2 g	16.5	3.4	13.1	183.72
Average		16.4	3.5	12.9	180.9

Figure 4.2: Saponification value determinations by titration method

4.2.6 Iodine Value of Oil

It is the measure of the degree of unsaturation of a particular oil or fat. It was determined using titration. The procedure used to determine Iodine value of oil was stated in section 3.9.6. The titration results for Iodine value test were illustrated in Table 4.4. The titration volume at which the first color change observed was the amount of $Na_2S_2O_3$ consumed by blank solution (Vb) and sample (Vs). The amount of $Na_2S_2O_3$ consumed by blank solution and sample on average were 25.93 ml and 9.43 ml respectively. The Iodine value was then calculated using equation 3.9 and the average Iodine value was 83.82 I_2/100g of oil, which was in the range of (82-88 I_2/100g) reported in literature (Demirbas, 2008).

Figure 4.3: Iodine value determinations by titration method

Table 4.4: Iodine value test result

Run No.	Mass of oil	Vb of $Na_2S_2O_3$ Consumed	Vs of $Na_2S_2O_3$ consumed	Vb –Vs of $Na_2S_2O_3$	Iodine value gI_2 /100g oil
1	0.25 g	25.6	9.2	16.4	83.31
2	0.25 g	26.3	9.7	16.6	84.33
3	0.25 g	25.9	9.4	16.5	83.82
Average		25.93	9.43	16.5	83.82

4.2.7 Moisture content of the oil

Moisture content of the oil was determined using equation 3.10 and it was found to be 0.06% which was beyond the data reported in the literature, Kusdiana and Saka, (2004). The result shows that further removing of the moisture is required to avoid soap formation in transesterification reaction since moisture content greater than 0.05% lead to increase acid value of oils and then soap formation will happen.

4.2.8 Ash content of the oil

Using equation 3.11 the ash content of the oil was determined to be 0.045%. Since high ash content of the oil can cause combustion deposits and injection system wear but the result shows that the oil has low ash content so it was possible to use the oil for biodiesel production. The result obtained was somewhat similar to the work done by Bekele, (2018).

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 3.7. The actual results obtained from the 20 runs were recorded in table 4.4 and used to analyze the effect of each variable on the yield (biodiesel) production.

4.3.2 Statistical analysis

The design expert software was used for the analysis of variance (ANOVA). Response surface method of Central Composite 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 were obtained and from those values the yield was calculated and recorded in table 4.4. The response of the transesterification process which is biodiesel yield was calculated using equation 3.12.

Table 4.5: Actual and predicted values of biodiesel yield

Run No.	**Factors**			Yield %	
	Alcohol to oil ratio	Castor oil to algae oil ratio	Catalyst concentration	Actual	Predicted
1	14.05	2.00	1.50	54.00	53.99
2	12.00	1.00	1.00	66.00	66.59
3	6.00	3.00	1.00	68.00	68.41
4	6.00	1	2.00	74.00	74.77
5	9.00	0.32	1.50	80.00	78.85
6	9.00	2	1.50	86.60	86.74
7	3.95	2.00	1.50	60.00	59.06
8	9.00	3.68	1.50	80.00	80.20
9	9.00	2.00	1.50	86.50	86.74
10	9.00	2.00	0.66	75.00	74.28
11	12.00	1.00	2.00	67.00	67.26
12	9.00	2.00	1.50	86.80	86.74
13	6.00	1.00	1.00	68.50	69.35
14	9.00	2.00	2.34	79.00	78.77
15	12.00	3.00	2.00	70.00	69.81
16	6.00	3.00	2.00	73.00	73.08
17	9.00	2.00	1.50	86.80	86.74
18	9.00	2.00	1.50	86.80	86.74
19	12.00	3.00	1.00	70.00	69.89
20	9.00	2.00	1.50	87.00	86.74

The values in this report table are used to produce the diagnostics graphs. From the twenty runs twenty responses (yields) were calculated based on the laboratory result which we call Actual Value. After the actual yield data filled to the software (RSM) the predicted value was obtained**.** This value was predicted from the model, generated using the prediction equation. The table above shows that the actual and predicted value of the biodiesel yield was somewhat nearer.

The model equations that correlates the response (yield of biodiesel) of the transesterification process variables in terms of coded and actual value after excluding the insignificant terms was given below.

Final Equation in Terms of Coded Factors

$$\begin{aligned}\text{Yield} = {} & +86.93 - 0.0732 * \text{A} + 4.56 * \text{B} + 0.0732 * \text{C} - 0.3750 * \text{AB} + 1.12 * \text{AC} \\ & + 1.87 * \text{BC} - 11.11 \ * \text{A}^2 - 6.87 * \ \text{B}^2 - 2.09 * \ \text{C}^2 \dots\dots\dots\dots \quad \dots\dots..4.1\end{aligned}$$

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

Final Equation in Terms of Actual Factors

$$\begin{aligned}\text{Yield} = {} & -57.84388 \ + \ 21.34299 \text{ methanol to oil ratio} \\ & + \ 7.98361 \text{ castor oil to Algae oil ratio} \\ & + \ 53.88987 \text{ catalyst concentration } + \ 0.354167 \text{ methanol to oil ratio} \\ & * \ \text{castor oil to Algae oil ratio } \ - 0.791667 \text{ methanol to oil ratio} \\ & * \ \text{catalyst concentration } \ - 0.375000 \text{ castor oil to Algae oil ratio} \\ & * \ \text{catalyst concentration } \ - \ 1.18702 \text{ methanol to oil ratio}^2 \\ & - \ 2.55147 \text{ castor oil to Algae oil ratio}^2 \\ & - 14.44852 \text{ catalyst concentration}^2 \dots\dots\dots\dots..4.2\end{aligned}$$

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space.

The quality of the model developed was evaluated based on the correlation coefficient value, R square (R^2). The R^2 value for Equation (4.1) was 0.9998. This indicated that 99.98% of the total variation in the biodiesel yield was attributed to the experimental variables studied. The closer the R^2 value to unity, the better the model will be, as it will give predicted values, which are closer to the actual values for the response.

Table 4.6: Analysis of variance (ANOVA) for response surface quadratic model

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	2587.95	9	287.55	6328.34	< 0.0001	Significant
A	0.0732	1	0.0732	1.61	0.2330	
B	283.53	1	283.53	6239.96	< 0.0001	
C	0.0732	1	0.0732	1.61	0.2330	
AB	1.13	1	1.13	24.76	0.0006	
AC	10.13	1	10.13	222.83	< 0.0001	
BC	28.13	1	28.13	618.97	< 0.0001	
A^2	1778.30	1	1778.30	39136.56	< 0.0001	
B^2	679.33	1	679.33	14950.54	< 0.0001	
C^2	63.12	1	63.12	1389.09	< 0.0001	
Residual	0.4544	10	0.0454			
Lack of Fit	0.3211	5	0.0642	2.41	0.1785	Not significant
Pure Error	0.1333	5	0.0267			
Cor Total	2588.40	19				

As table 4.7 shows, The Model F-value of 6328.34 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise. P-values less than 0.0500 indicate model terms are significant. In this case B, AB, AC, BC, A^2, B^2, C^2 are significant model terms.

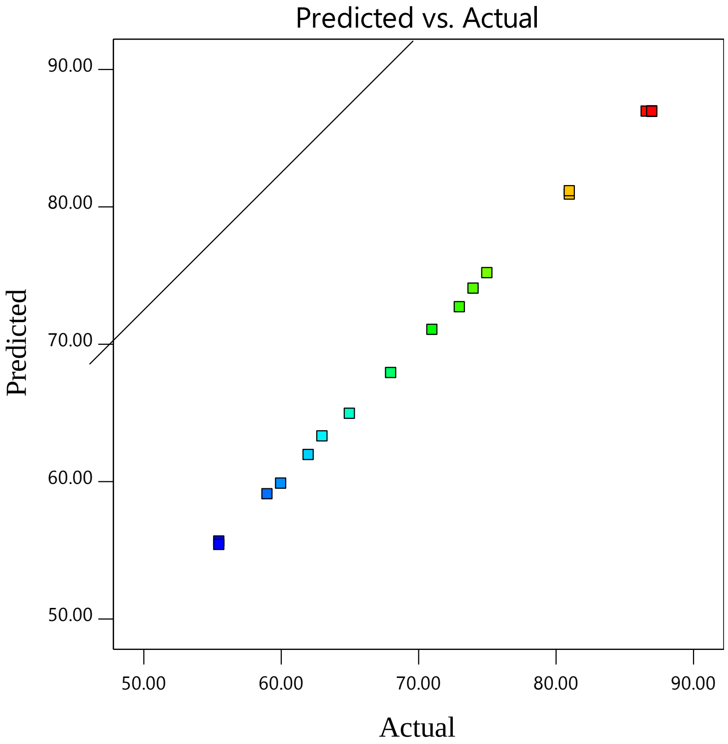


Figure 4.4: Graph of actual versus predicted value of biodiesel

As figure 4.4 shows the plot contains a line of unit slope which is the line of perfect fit, with the points corresponding to zero error between predicted values and actual values. This plot therefore clarifies the performance of the correlation in an evident way. Hence, the 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.

4.4 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.

4.4.1 Effect of methanol to oil molar ratio

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 result obtained in this study was in agreement with Dennis. As shown in figure 4.5, 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 inhance the solubility of glycerol and shift the equilibrium to the left.

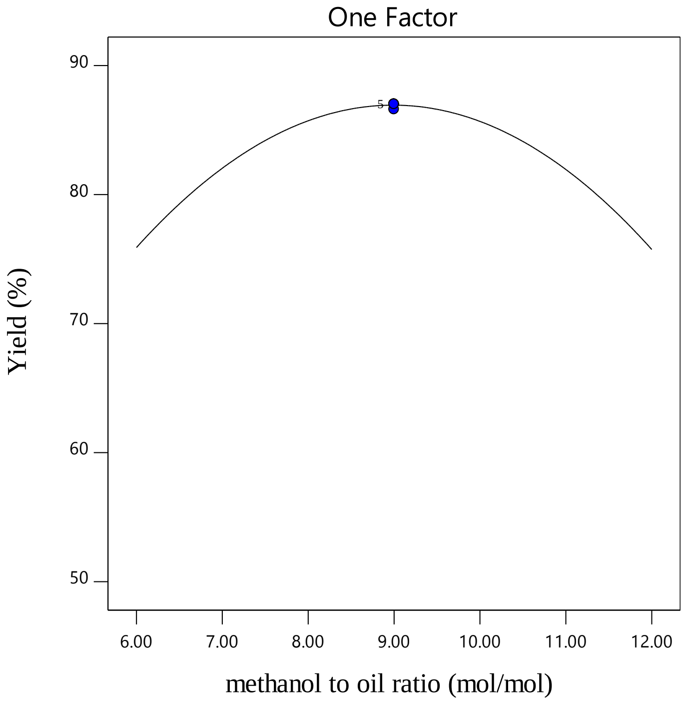


Figure 4.5: Effect of methanol to oil molar ratio on biodiesel yield

4.4.2 Effect of castor oil to algae oil ratio

The castor oil to algae oil ratio clearly influence the biodiesel yield in positive manner. As the castor oil to algae oil ratio increases gives high yield of biodiesel. But as it reaches the maximum point the yield of biodiesel start to decreases as it was observed from the figure 4.6. The decrease in the yield was may be due to the misibility problem of oil and methanol resulted from an increase in viscosity due to the high castor oil. Higher ratio of castor oil used could increases the viscosity of oil, which reduces the contact rate of triglyceride molecules with the catalyst’s active sites and decrease the catalyst activity.

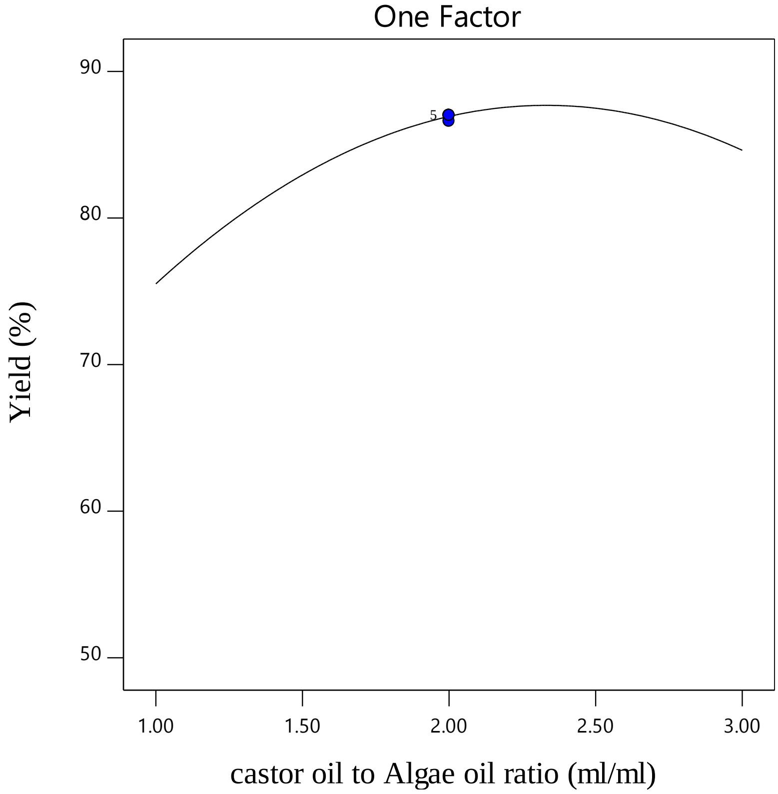


Figure 4.6: Effect of castor oil to algae oil ratio on biodiesel yield

4.4.3 Effect of Catalyst Concentration

The transesterification reaction was strongly dependent upon the amount of catalyst applied. Without the addition of catalyst, the transesterification reaction did not occur. The presence of the catalyst increased the reaction rate. From the figure 4.7 it can be seen that the biodiesel yield was increased as the catalyst concentration increased up to a certain level. This might be due to the reason that when the catalyst amount was increased the active site of the catalyst also increased thus the transesterification reaction was accelerated and biodiesel yield was increased. But as it reaches the maximum point the yield of biodiesel start to decreases which may be as a result of the formation of an emulsion which led to the formation of gels possibly owing to an increase in soap content this factor hindered the glycerol separation.

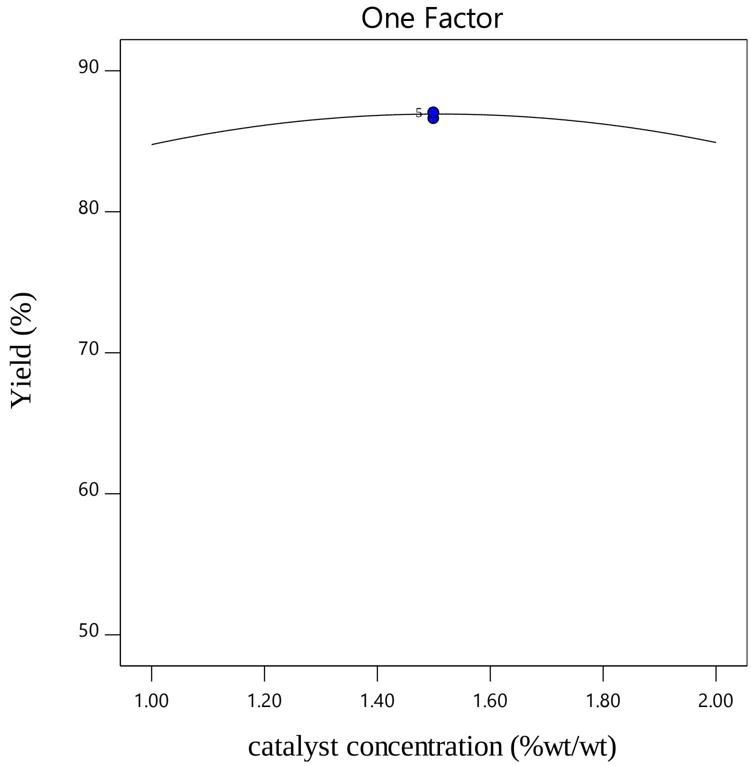


Figure 4.7: Effect of Catalyst concentrations on biodiesel yield

4.5 Effects of interaction between process variables on biodiesel yield

An interaction occurs when the response is different depending on the settings of two factors. They will appear with two non-parallel lines, indicating that the effect of one factor depends on the level of the other.

4.5.1 Effect of Methanol to Oil Molar Ratio and Catalyst Concentration

The molar ratio of methanol to oil is a key parameter in the reaction. Biodiesel production could be improved by excess amounts of methanol because it shift the equilibrium to the right-hand side. However, the presence of high amounts of alcohol complicates the separation between glycerol and biodiesel it also increase the cost of the process. From the graph beolw it was seen that the biodiesel yield increase when the methanol to oil ratio increase. But after an optimal concentration level, the yield starts to decrease. The decrease of methyl ester content with higher methanol to oil ratios could be caused by the higher solubility of glycerol in biodiesel and the higher difficulty to wash out the glycerol from the mixture. Similarly when the catalyst concentration increased at first, the yield also increased. However after the catalyst concentration 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 of the catalyst species methoxide radical (CH3O^{-}), which react with triglyceride are obtained from the reaction of KOH and CH$_3$OH, Because potassium hydroxide is a strong base, its dissociation constant is very large: therefore the amount of methoxide ions depends only on the catalyst concentration; as a result, the higher the amount of catalyst, the faster the reaction proceeds (Thanh *et al.*, 2010). However for further increase of catalyst the yield started to decline this could be due to the formation of soap.

Methanol to oil ratio and catalyst concentration have strong interaction on biodiesel yield, as it was shown on the Figure 4.8 the two non-parallel lines, indicate that the effect of methanol to oil ratio factor depends on the level of catalyst concentration.

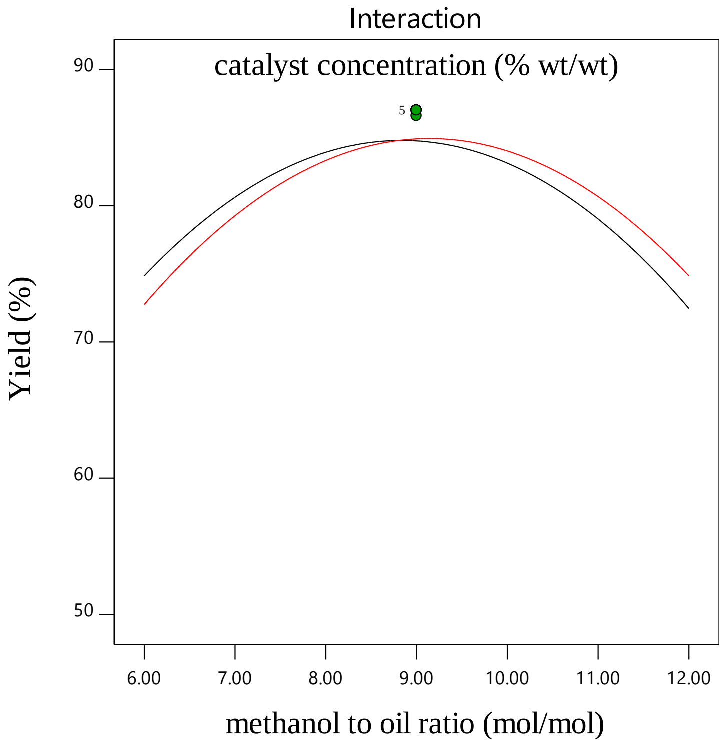


Figure 4.8: Effect of Methanol to oil ratio and catalyst concentration on biodiesel yield

4.5.2 Effect of castor oil to algae oil ratio and catalyst concentration

As it was seen from the graph bellow the biodiesel yield increases when the castor oil to algae oil ratio increase. But after an optimal concentration level, the yield starts to decreases. The increase of yield to an optimal level as the castor oil to algae oil ratio increases could be due to the increase on the catalyst activity which facilitates the transesterfication reaction. However for further increase of castor oil to algae oil ratio the yield started to decline this could be due to the effect of increase in volume of castor oil which affect the catalyst activity beacause of its high dencity and viscosity than algae oil. Similarly when the catalyst concentration increased at first, the yield also increased. However after the catalyst concentration reached an optimal level the yield started to decline. The increase of yield to an optimal level as the catalyst increases could be due due to the increase of the catalyst species methoxide radical ($CH3O^{-}$) 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. As it was shown on the Figure 4.9 the two non-parallel lines, indicate that the two factors have strong interaction on the biodiesel yield.

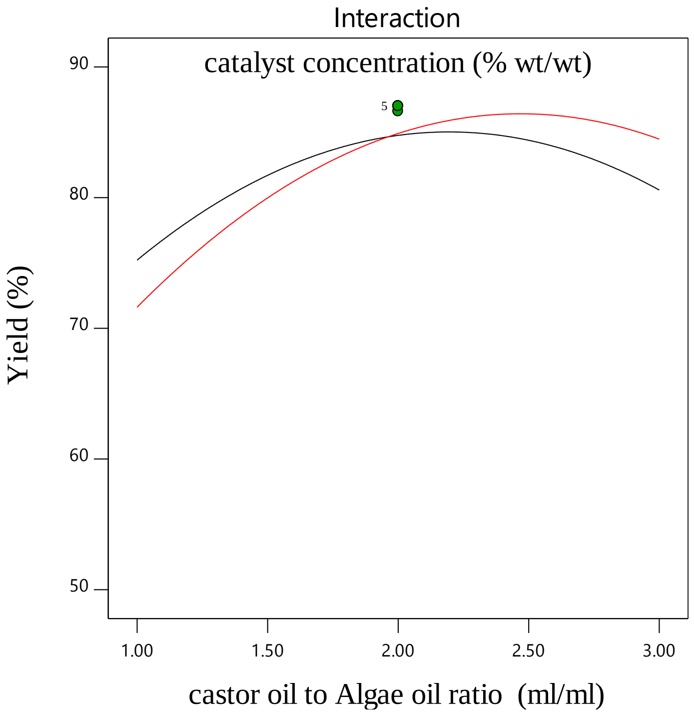


Figure 4.9: Effect of castor oil to algae oil ratio and Catalyst concentration on biodiesel yield

4.5.3 Castor oil to algae oil ratio and Methanol to oil ratio

As it was seen from the graph bellow the biodiesel yield increases when the castor oil to algae oil ratio increase. But after an optimal concentration level, the yield starts to decreases. The increase of yield to an optimal level as the castor oil to algae oil ratio increases could be due to the increase on miscibility of castor oil in the methanol which facilitates the transesterfication reaction. However for further increase of castor oil to algae oil ratio the yield started to decline this could be due to the effect of increase in volume of castor oil which affect miscibility in the methanol beacause of its high dencity and viscosity than algae oil. similarly the biodiesel yield increase when the methanol to oil ratio increase. But after an optimal concentration level, the yield starts to decrease. The increase of yield to an optimal level as the methanol to oil ratio increases could be due to the increase on miscibility of methanol and triglyceride in the oil which facilitates the transesterfication reaction. The decrease of methyl ester content with higher methanol to oil ratios could be caused by the higher solubility of glycerol in biodiesel and the higher difficulty to wash out the glycerol from the mixture. Methanol to oil ratio and castor to algae oil ratio have no interaction on biodiesel yield, as it was shown on the Figure 4.10 the two parallel lines, indicate that the effect of methanol to oil ratio factor does not depends on the level of catalyst concentration.

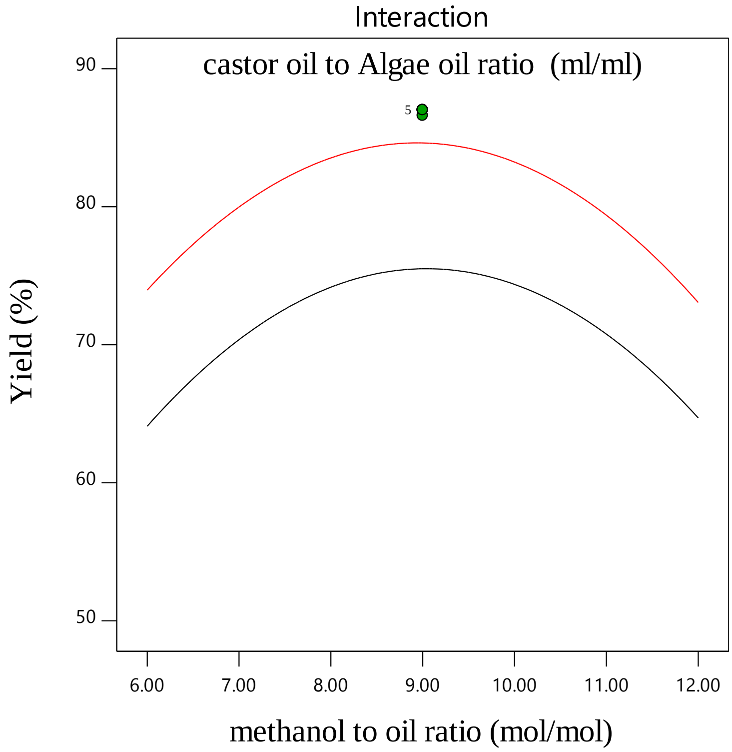
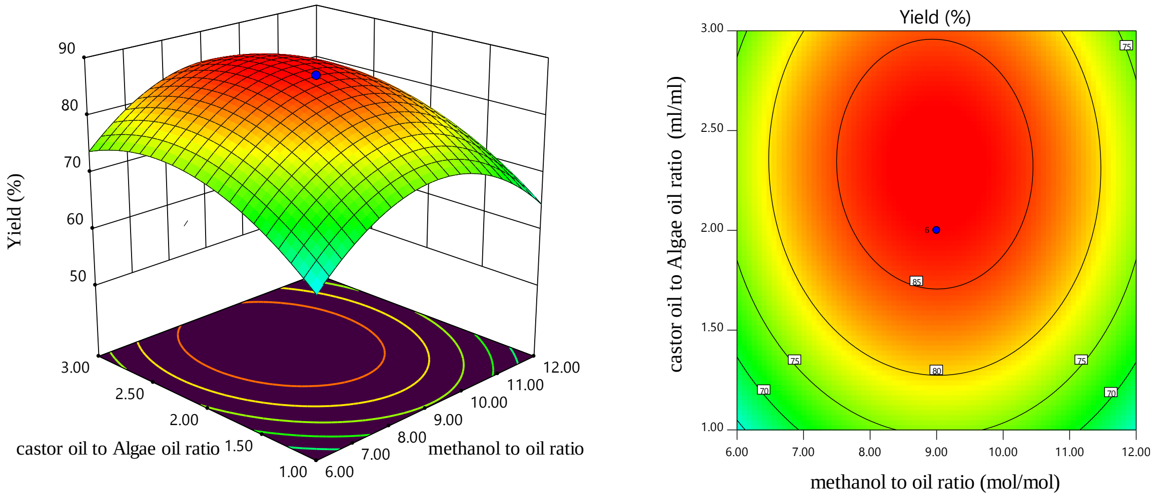


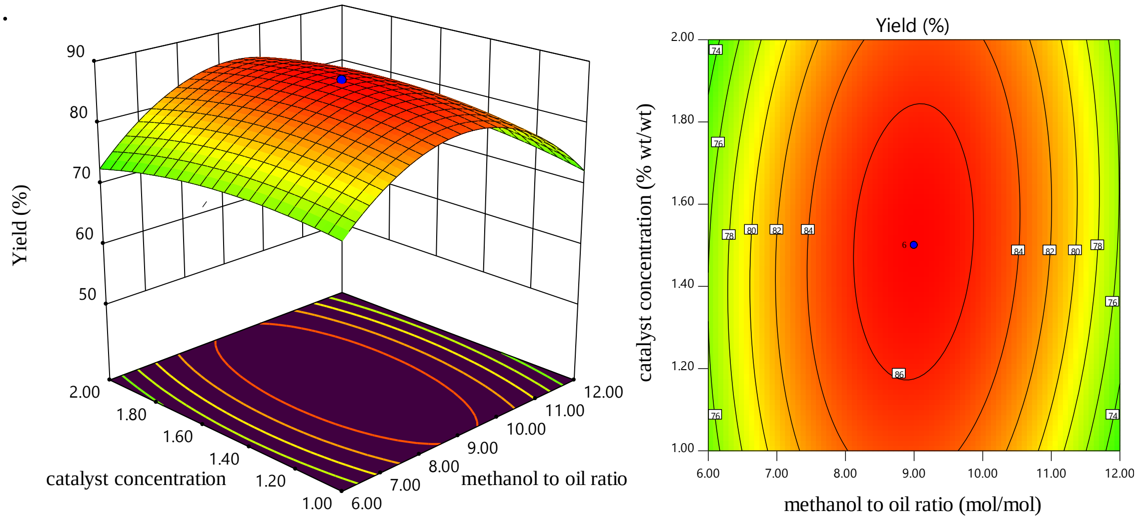
Figure 4.10: Effect of castor oil to algae oil ratio and Methanol to oil ratio on biodiesel yield

Response surface and Contour plot of Biodiesel yield

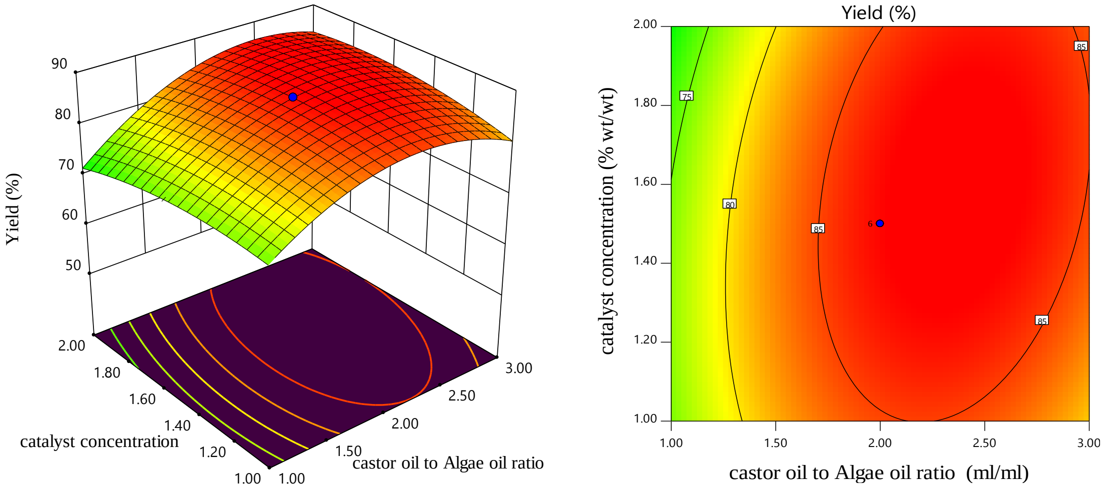
The 3D surface and Contour plots were drawn to show the interaction effect of the process variables or independent variables on the biodiesel yield. Each contour curve presented the effect of two variables on the methyl ester yield, holding the third variable at constant level (at zero level).



A) Methanol to oil ratio verses castor oil to algae oil ratio when catalyst concentration at 1% wt/wt.



B) Methanol to oil ratio verses catalyst concentration when the castor oil to algae oil ratio at 1:1.



C) Castor oil to algae oil ratio verses Catalyst concentration when the methanol to oil ratio at 6:1.

Figure 4.11: Response surface and Contour plot of Biodiesel yield (%) in terms of the factors (A, B & C)

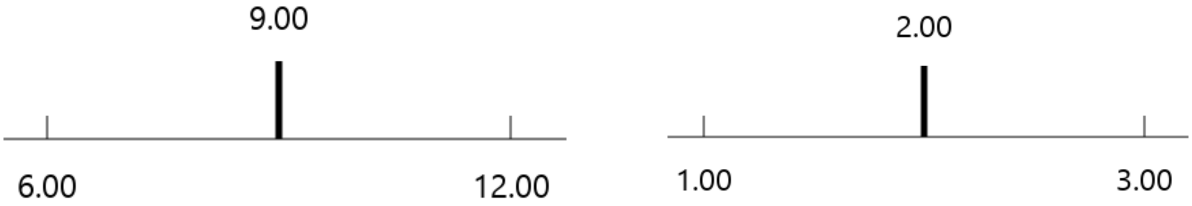
4.6 Optimization of process variables

The optimization function in Design Expert 11.1.0.1 software was employed for the optimization of process variables. The goal of the three variables methanol to oil molar ratio, Castor oil to Algae oil ratio and catalyst concentration was set in range and the goal of the response was set to maximize. Accordingly, the optimum yield predicted was 23 ml at the process variable of methanol to oil molar ratio of 9:1, Castor oil to Algae oil volume ratio of 2:1 and catalyst concentration of 1.5% (wt/wt).

Table 4.7: optimization of process variables.

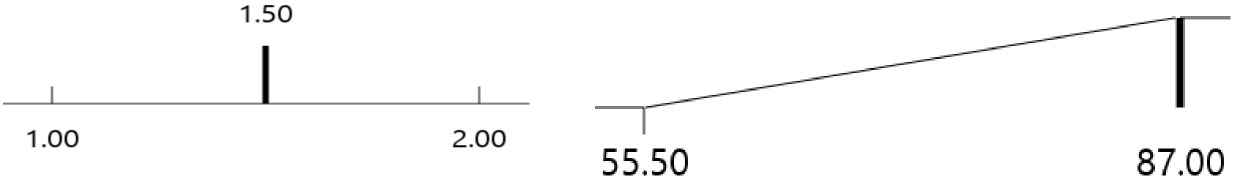
Constraints Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper weight	Importance
Methanol to oil ratio	in range	6	12	1	1	3
Castor oil to Algae oil	in range	1	3	1	1	3
Catalyst	in range	1	2	1	1	3
Yield	Maximize	55.5	87	1	1	3
Solution						
Solution number	methanol to oil ratio	Castor oil to Algae oil ratio	catalyst concentration	Yield	Desirability	
1	9.00	2.00	1.50	86.933	0.998	Selected

The possible optimum solution in transesterification reaction to produce biodiesel was presented in table 4.7 above. The predicted optimum yield of biodiesel was 86.945%, it was obtained at 9 methanol to oil molar ratio, 1.50%(wt/wt) catalyst concentration and 2.00 castor oil to algae oil volume ratio.



Methanol to oil molar ratio = 9:1

Castor oil to algae oil volume ratio = 2:1



Catalyst concentration = 1.5% (wt/wt)

Yield = 87% (vol/vol)

Figure 4.12: Optimization of process variables.

In order to check this prediction, experiments were conducted at the specified parameters. The yield obtained through experiment at methanol to oil molar ratio 9:1, catalyst concentration 1.5% and Castor oil to algae oil ratio 2:1 was 86.6%. Which is near to the value obtained using the design expert software. Therefore, this study shows that KOH is a potential catalyst for the production of biodiesel from castor bean oil via homogeneous transesterification process.

4.7 Physicochemical Properties of Biodiesel

The physicochemical properties of the biodiesel were determined by using titration method and empirical formulas.

4.7.1 Density

The density of biodiesel was measured with the same procedure as the density of oil. Thus it was found to be 880 kg/m^3. The density of the oil changed from 920 kg/m^3 to 880 kg/m^3, the change in the density shows that the transesterification reaction was done well. The ASTM D6751 standard for biodiesel states that the fuel should have a density between 870 and 900 kg/m^3 the result shows that the value was with in the acceptable limit. This property is important mainly in airless combustion systems because it influences the efficiency of atomization of the fuel (Ryan *et al*., 1984).

4.7.2 Viscosity

Viscosity of biodiesel was determined by the same procedure as viscosity of oil determination that was explained in Sections 4.2.2. Kinematic viscosity of biodiesel was found to be 5.8 mm^2/s. It is worth mentioning that the extent of the reaction, as well as the experimental conditions used in the biodiesel production processes, greatly influenced the fuel properties and especially its viscosity. Transesterification reaction is responsible for minimizing the viscosity of vegetable oil in order to apply it as a fuel for engines. Thus, it reduced the viscosity of vegetable oil from 44.6 mm^2/s to 5.8 mm^2/s. The result shows that the value was in agreement with standard specification limit of ASTM D6751 with maximum limit of 1.9-6.0 mm^2/s.

4.7.3 Acid Value

The acid value measures the content of free fatty acids in biodiesel, the presence of free fatty acids influences fuel aging. The same procedure was done to determine the acid value of biodiesel as it was done for oil. The titration was done three times and the observation of the result was taken as the average. The average acid value of the biodiesel was calculated using the equation 3.5 and the result was found to be 0.74 mg KOH/g oil, which was in agreement with standard specification limit of ASTM D6751 with maximum limit of 0.8 mg KOH/g oil.

4.7.4 Saponification Value

The same procedure was done to determine the saponification value of biodiesel as it was conducted for oil. The titration was done three times and the average observation of the result was taken. The average saponification value of the biodiesel was calculated using the equation 3.7 and resulted 1.8 mg KOH/g oil. The value was in agreement with in the value reported in the literature (Ma and Hanna, 1999).

4.7.5 Iodine Value

The iodine value is an important measure that allows determination of the unsaturation degree of the fuel. This property greatly influences fuel oxidation and the type of aging products and deposits formed in diesel engines injectors. According to EN 14214 (2003), methyl esters used as diesel fuel must have an iodine value below 120 gI_2 per 100 g of sample. Similar procedure as that of oil iodine value determination was employed to determine the iodine value of biodiesel. The value was calculated using the equation 3.8 and the result was found to be 82.22 $gI_2/100g$, which was in agreement with standard specification limit of EN14214 with a maximum limit of 120 g $I_2/100g$.

4.7.6 Heating Value

The heating value of biodiesel was determined by empirical formula depending on the titration result of Saponification Value and Iodine Value of Biodiesel. The calculated result of heating value was 48.12 MJ/kg. The heating value result reported by Graboski *et al*. (1999) was 40.39 and 40.37 MJ/Kg for methyl rape and methyl soy, respectively. The result of this study was beyond the results of this author but it was in agreement with the standard specification limit of EN14214 >35MJ/kg.

4.7.7 Flash point

Flash point was determined by empirical formula depending on the calculated result of heating value and result was found to be 133.33^{o}c, which was in agreement within the lower limit of standard specification of EN14214 >120^{o}c and ASTM D6751 >130^{o}c. Flash point is used in safety regulations to define “flammable” and “combustible” materials, this higher value indicate that the biodiesel is less likely to ignite accidentally.

4.7.8 Cetane number

Better ignition quality of the fuel is always associated with higher cetane number value (CN). This is one of the important parameter, which is considered during the selection of methyl esters for use as biodiesel. Generally, methyl esters with higher CN are favored for use as biodiesel; however, an increase in CN will lead to the solidification of methyl esters at higher temperature. To avoid this situation, the upper limit of CN (65) has been specified in US biodiesel standards. The cetane number was determined by empirical formula depending on the titration result of saponification value and iodine value of biodiesel and the result was found to be 58.12 and the result was in agreement with the standard specifications of both ASTM D6751 and EN14214 which stated that a minimum amount of 47 and 51, respectively.

4.7.9 Moisture content

Determination of moisture contents of biodiesel was conducted in the same procedures and methods with that of oil moisture content determination as discussed in Sections 3.6.8. Moisture content of the biodiesel was determined using equation 3.10 and the result was found to be 0.027%. Fuel contaminated with water can cause engine corrosion or react with the glycerides to produce soaps and glycerol. Therefore, EN14214 (2003) imposes a maximum content of 0.05% of water in fuels. The result was in agreement within the limit of standard specifications of ASTM D6751 and EN14214 which stated that a maximum content of 0.03% and 0.05% of water in fuels, respectively.

4.7.10 Ash content

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 0.028%. The result was in agreement within the limit of standard specifications of ASTMD6751 and EN14214 which stated that a maximum content of 0.03% and 0.02%, respectively.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

Castor seeds and microalgae are more promising feed stocks to their wide spread availability and higher oil yields. Using castor seed and microalgae to produce biodiesel does not compromise production of food, fodder and other products derived from crops. In this study the oil extraction method used was soxlet extraction using the solvent n-hexane and the yield obtained from the castor seed and microalgae was about 42 % and 25.5 % of its dry weight, respectively.

Physicochemical properties of extracted oil such as density, viscosity, Acid value, free fatty acid, saponification value, iodine value, moisture content and ash content were studied. The result obtained were 920 kg/m^3, 44.6 mm^2/sec, 20.56 mg KOH/g oil, 10.28 %, 180.9 mg KOH/g oil, 83.82 g I_2/100g, 0.006% and 0.045%, respectively.

The blends of extracted crude castor seed oil and microalgae oil were 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 11.1.1 software. From the experiment performed it was obtained that an optimal biodiesel yield of 86.6% (26 ml) at methanol to oil ratio of 9:1, castor oil to algae oil ratio of 2:1 and amount of catalyst 1.5% with constant temperature of 65^{o}c and agitation speed of 400 rpm. The physicochemical properties of biodiesel were tested. The result showed that the fuel properties are within the ASTM and EN standards. The density, kinematic viscosity, acid value, saponification value, iodine value, heating value, flash point, catene number, moisture content, ash content were studied 880 kg/m^3, 5.8 mm^2/s, 0.74 mg KOH/g of oil, 1.8 mg KOH/g oil, 82.22 I_2/100g oil, 48.12 MJ/kg,133.33^{o}c, 58.12, 0.027% and 0.028% respectively. The result of this study satisfies the standard of biodiesel and suggests that blend oil from crude caster seed and microalgae can be used as an alternative source of biodiesel.

5.2 RECOMMENDATIONS

The result of this study shows that the possibility of producing biodiesel from blends of crude castor seed oil and crude microalgae oil to use as substitute of petroleum fuel and mitigate environmental pollutions caused by fossil fuel consumption. However, further study and development on selection and isolation of specific species of castor plant and microalgae, identification of the major fatty acid compositions (profile), investigation on the productivity of isolated species, determination of environmental conditions or growth media for optimal growth with their high fatty acid yield per unit area of land should be carried out.

Species improvement through organized selection and breeding programs, improving oil extraction technology, maximization of biodiesel production efficiency, raising the quality of by-products and developing the techniques of their conversion into useful products are the major issues requiring further investigations.

Using the availability of various seed oils with distinct composition and physicochemical characteristics as advantage, it is recommended to plan on the implementation of the blends of biodiesels from different types of vegetable oils in variable ratios; in order to achieve the final desired properties of the fuel.

Further study on fuel property analysis, blending conditions, engine performance and emission tests and economic analysis should be carried out in the future. Product quality is the major factor that determines the widespread use of biodiesel fuel, especially for local production and use. Therefore, since the concept and principles of biodiesel production is novel to the country, it is essential to establish the product quality standardization, pertinent to the country, which agrees with the internationally accepted standard specifications as ASTM and EN.

Byproduct properties such as, husk of castor seed, extract residue, glycerol and waste water were not tasted in this study and it must be tasted for the future. Since those byproduct has a relevance importance to use as a fertilizer, in soap industries, to save water consumption. Therefore, in addition to the production of biodiesel the property of the byproduct should be analyzed for further applications.

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APPENDICES

Figure: algae biomass collection

Figure: algae biomass drying

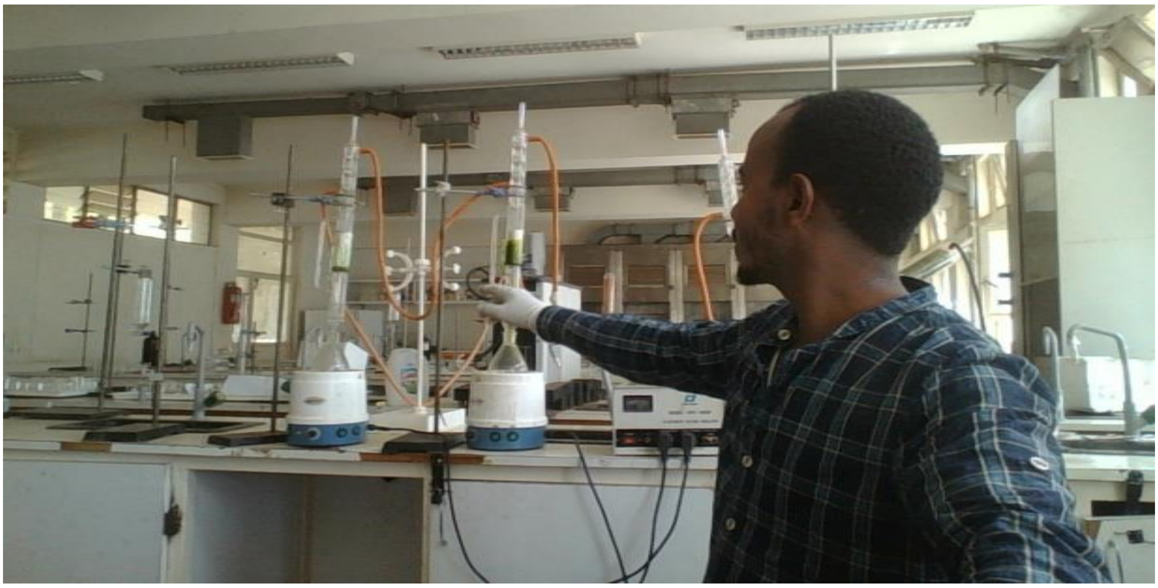

Figure: soxhlet extracter

Figure: sample preparation

Figure: Extraction process

Figure: Transesterification

Figure : Separation process of biodiesel and glycerol

Figure: Iodine value test by titration method.

Calculation of Heating Value, Catane Number and Flash Point of biodiesel based on saponification number and Iodine value.

Heating Value

$$\mathrm{HV} = 49.43 - [0.041(\mathrm{SN}) + 0.015(\mathrm{IV}) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots..\ldots\ldots\ldots\ldots\ldots\ldots..3.19$$

$$\mathrm{HV} = 49.43 - [0.041(1.8) + 0.015(82.22)]$$

$$\mathrm{HV} = 49.43 - [0.0738 + 1.233]$$

$$\mathrm{HV} = 49.43 - 1.31$$

$$\mathrm{HV} = 48.12 \text{ MJ/kg}$$

Flash Point

$$\mathrm{FP} = \frac{\mathrm{HV} - 32.12}{0.12} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots.\ldots..3.17$$

$$\mathrm{FP} = \frac{48.12 - 32.12}{0.12}$$

$$\mathrm{FP} = \frac{16}{0.12}$$

$$\mathrm{FP} = 133.33\ {}^{\mathrm{O}}\mathrm{C}$$

Catane Number

$$\mathrm{CN} = 46.3 + \frac{54.58}{\mathrm{SN}} - 0.225 * \mathrm{IV} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots...3.18$$

$$\mathrm{CN} = 46.3 + \frac{54.58}{1.8} - 0.225 * 82.22$$

$$\mathrm{CN} = 46.3 + 30.32 - 18.5$$

$$\mathrm{CN} = 58.12$$

ASTM D6751 and EN14214 standard of Biodiesel

Properties	ASTM D6751	EN14214
Density (kg/m^3)	870–890	860–900
KinematicViscosity at 40^{o}c (mm^2/sec)	1.9-6.0	3.5-5.0
Flash point (^{o}C)	130 Minimum	120 Minimum
Heating value MjKg^{-1}		>35
Cetane number	> 47	
Acid Values(mg KOH/g)	≤ 0.8	≤ 0.5
Iodine values (I2g100g-1)	Maximum of 115	120
Moisture Content (%w/w)	<0.03	
Ash Content(% w/w)	<0.03	<0.02

Experimental results obtained based on the experimental design

Run No.	**Factors**			Yield %
	Alcohol to oil ratio	Castor oil to algae oil ratio	Catalyst concentratio	
1	14.05	2.00	1.50	54.00
2	12.00	1.00	1.00	66.00
3	6.00	3.00	1.00	68.00
4	6.00	1	2.00	74.00
5	9.00	0.32	1.50	80.00
6	9.00	2	1.50	86.60
7	3.95	2.00	1.50	60.00
8	9.00	3.68	1.50	80.00
9	9.00	2.00	1.50	86.50
10	9.00	2.00	0.66	75.00
11	12.00	1.00	2.00	67.00
12	9.00	2.00	1.50	86.80
13	6.00	1.00	1.00	68.50
14	9.00	2.00	2.34	79.00
15	12.00	3.00	2.00	70.00
16	6.00	3.00	2.00	73.00
17	9.00	2.00	1.50	86.80
18	9.00	2.00	1.50	86.80
19	12.00	3.00	1.00	70.00
20	9.00	2.00	1.50	87.00