ADDIS ABABA UNIVERSITY

ADDIS ABABA INSTITUTE OF TECHNOLOGY

SCHOOL OF CHEMICAL AND BIO ENGINEERING

UTILIZATION OF WASTE ANIMAL BONE AS A HETEROGENEOUS SOLID BASE CATALYST FOR TRANSESTERIFICATION OF JATROPHA OIL

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A Thesis Submitted To

The School of Chemical and Bio Engineering

Presented in Partial Fulfillment of the Requirement for the Degree of Master of Science (Process Engineering)

Addis Ababa University

Addis Ababa, Ethiopia

June 2018

Addis Ababa University

Addis Ababa Institute of Technology

School of Chemical and Bio Engineering

This is to certify that the thesis prepared by Kaleab Bizuneh, entitled: *Utilization of waste animal bone as a heterogeneous solid base catalyst for transesterification of Jatropha oil* and submitted in partial fulfilment of the requirement for the degree of Master of Science (Process Engineering) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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DECLARATION

I declare that this thesis entitled "*Utilization of waste animal bone as a heterogeneous solid base catalyst for transesterification of Jatropha oil* " has not been submitted in any form for another degree, diploma or an award at any university or other institution of the tertiary education. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due reference to the literature and discussions. Information taken from published and unpublished work of others has been acknowledged in the text and a list of references is given. The work was under the guidance of Professor Belay Woldeyes, instructor in Addis Ababa University, School of Chemical and Bio Engineering.

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ABSTRACT

Heterogeneous catalyst plays a vital role in transesterification process, especially in view of cost and reusability. In this study, heterogeneous solid base catalyst was prepared by impregnating the waste animal bone with different weight percent (wt%) KOH solution followed by drying and calcination at 900 °C for 3 hr. Having screened the catalytic performance and basicity of animal bone loaded with different K-compounds, 12 wt% KOH loaded on animal bone was chosen. The physico-chemical properties of animal bone, support and the KOH-impregnated calcined animal bone catalyst were characterized by means of titration (for basicity of catalyst), Fourier Transform Infra-Red (FT-IR) spectroscopy and X-Ray Diffraction (XRD) spectroscopy measurements. Thermogravimetric Analysis (TGA) was also performed for the selected catalyst. Catalytic performance of the catalyst was evaluated by transesterificating Jatropha oil with methanol in batch reactions. The Box-Behnken Design (BBD) of the Response Surface Methodology (RSM) was employed to investigate the effects of reaction temperature, catalyst loading and methanol to oil molar ratio on Fatty Acid Methyl Ester (FAME) yield. Maximum yield of 96.74% was obtained at 11.56:1 methanol to oil molar ratio, 5.08 wt% catalyst loading, 64.05 ^{o}C reaction temperature and 500 rpm stirring speed in 3 hr reaction time. Reusability studies indicated that the prepared catalyst could be applied to at least three consecutive batches of the transesterification reaction without any significant loss of activity. The effects of temperature (313, 321.3, 329.6, and 338 K) and time (1, 2, 3, and 4 hr) at optimum conditions on the conversion of the oil into FAME were also investigated. The results obtained indicated a pseudo-first order kinetics for the transesterification reaction using the prepared catalyst. The activation energy obtained was 38.55 KJ.mol^{-1}. The physical, chemical and fuel properties of the produced FAME met the standard specifications.

Keywords: *Animal bone, Heterogeneous catalyst, Potassium hydroxide, Jatropha oil and Transesterification*

ACKNOWLEDGMENTS

First and foremost, I am grateful to the almighty GOD for giving the strength and wisdom to successfully complete this thesis work. Then, there are many people to whom I owe a dept of ingratitude for their help towards this thesis. Firstly, my sincere thanks go to my advisor Professor Belay Woldeyes for his sustainable and appreciable guidance up to the successful completion of this thesis.

Thanks to all Chemical Engineering laboratory staff members, in particular Mr. Hintsasilasie Seifu and Mr. Aklilu G/Hawaria for their technical assistance and for fixing everything that went wrong. I am also grateful to my classmates for their help and friendliness.

Finally, I also would like to thank my family for their incredible support over the past two years and always wish the best for me.

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ACRONYMS

AETDP	Alternative Energy Technology Development and Promotion Directorate
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
ASTM	American Society for Testing and Material
AV	Acid Value
BBD	Box -Behnken Design
FAME	Fatty Acid Methyl Ester
FFA	Free Fatty Acid
FP	Flash Point
FT-IR	Fourier Transform Infrared Spectroscopy
GC-MS	Gas Chromatography Mass Spectroscopy
HHV	Higher Heating Value
IV	Iodine Value
JCPDS	Joint Committee on Powder Diffraction Standards
KOH	Potassium Hydroxide
LIDI	Leather Industry Development Institute
rpm	revolution per minute
RSM	Response Surface Methodology
SV	Saponification Value
TGA	Thermogravimetric Analysis
XRD	X-ray Diffraction

1. INTRODUCTION

1.1 Background

Today the greatest attention in the world is devoted to energy resources because their use is usually irreversible and very vital in the socio-economic development of any country. According to the current energy scenario, because of the ever-increasing world population and high speed of economic development energy consumption is increasing day by day. This leads the world towards a global energy crisis in the near future. As a result, petroleum reserves are being depleted that result an increase in crude oil prices. Moreover, continued and increasing use of petroleum will intensify local air pollution and magnify the global warming problems caused by carbon dioxide emission. This is why over the last decades attention is focused on renewable energy resources and ways to increase energy efficiency (Verma et al., 2016).

Nowadays, there has been increased interest in alternative fuels (such as biodiesel, methanol, ethanol, biogas, hydrogen, etc.). Biodiesel also known as FAME is believed to be the most feasible alternative energy source for replacing petro-diesel due to its better performance, biodegradability, non-toxic, carbon neutral, low pollutant, environment-friendly and carries efficient combustion due to higher oxygen content and higher flash point (Gupta et al., 2016). Biodiesel is produce by reacting vegetable oils or animal fats with monohydric alcohol in presence of a catalyst (Gupta et al., 2016).

Edible and non-edible vegetable oils, waste or recycled oil and animal fats are commonly used feedstocks for biodiesel production. More recently, microalgae have become the newly emerged feedstock. Currently, biodiesel is produced from different feedstocks by transesterification reaction, but non-edible oil resources such as Jatropha are gaining worldwide attention because they are easily available in many parts of the world, eliminate competition for food, more efficient, more environmentally friendly, produce useful by-products and they are very economical (Atabani et al., 2012; Verma et al., 2016).

Among various oil-bearing seeds Jatropha becomes a worldwide issue for its multi-dimensional advantage including drought-resistant, easy to establish, grows almost everywhere even on gravelly, sandy and saline soils. It produces seeds for 50 years with a high oil content of about 37% or more (Koh et al., 2011). Moreover, the seed contains 35-40% oil content and 50-60% in

the kernel. The oil comprises of about 79% unsaturated fatty acids and 21% saturated fatty acids. There are some toxic components in the oils which is poisonous and render the oil not appropriate for human consumption. At present, Jatropha trees, as a potential alternative biodiesel crop, are widely cultivated in semi-cultivated tropical or subtropical areas in Central and South America, India, Africa and South China (Amalia et al., 2013).

Catalysts used for biodiesel production are categorized in to three; homogeneous, heterogeneous and enzymes. Homogeneous and heterogeneous catalysts were then categorized into alkali and acid catalysts. As compare to enzyme catalysts, alkali and acid catalysts are more commonly used in biodiesel production (Talha & Sulaiman, 2016).

Currently, homogeneous alkaline catalysts, such as NaOH and KOH, are most commonly used in industrial transesterification processes for biodiesel production. They are very effective because of their ability to efficiently promote the reaction at relatively low reaction temperature and atmospheric pressure, high conversion in shorter time, and economically available (Sivasamy et al., 2009; Talha & Sulaiman, 2016). However, they bear several process problems because of their sensitivity to water and free fatty acids contents in the feedstock, which results in soap formation and ester saponification and creates difficulties during purification and generate a large amount of waste water during washing. On the other hand, homogeneous acid catalysts such as HCl and H_2SO_4 has been largely ignored, mainly because of their relatively slower reaction rate and increase corrosiveness (Sivasamy et al., 2009).

Heterogeneous catalysts are being employed to overcome the problems caused by homogeneous catalyst in biodiesel production. In contrast, heterogeneous catalysts have the general advantage of being reusable, environmentally friendly, lack of toxicity, long catalyst life times and easy to separate from the reaction products (Talha & Sulaiman, 2016). However, in case of heterogeneous transesterification existence of three phases with alcohol and oil leads to diffusion limitations, thus lowering the rate of reaction. One of the ways to overcome this mass transfer problem is to use structure promoters or catalyst supports which can provide more specific surface area and pores for active species (Zabeti et al., 2009). Different researchers used different types of supports for heterogeneous transesterification. It has been reported that aluminium oxide (Zabeti et al., 2009), zinc oxide (Xie & Huang, 2006) and palm shell activated carbon (Baroutian et al., 2010) showed favorable support properties for alkali-catalyzed biodiesel production.

Recently, researchers synthesized and used different kinds of natural source derived heterogeneous catalyst (such as mollusk shells, eggshells, calcined fish scale, sheep bone, etc.) as a new trend in order to produce environmental friendly and cost-effective catalyst for biodiesel production (Farooq & Ramli, 2015; Obadiah et al., 2012; Talha & Sulaiman, 2016). Among these natural sources, animal bone is one that is obtained as a waste in huge quantities particularly in developing countries like Ethiopia. Calcium phosphate is the main component of bone and can be transformed to hydroxyapatite by calcination, which has relatively high catalytic activity, good thermal and chemical stability (Ghanei et al., 2016). However, these catalysts are required in high amount, high methanol/oil molar ratio with longer time for the reaction to occur. Hence this research focuses on the development of solid base catalyst, KOH-impregnated calcined waste animal bone for transesterification reaction.

1.2 Statement of the Problem

The increase in the emission of pollutants into the environment, depletion of world petroleum reserves, instability of world political condition, and economic considerations are the main reasons that stimulate the search for alternative renewable energy sources that are capable of fulfilling an increasing energy demand (Caban et al., 2013). Among the different renewable energy sources, biodiesel is one that would probably reduce the dependency on petroleum.

Biodiesel is produce mainly by transesterification, reacting vegetable oils or animal fats with monohydric alcohol in presence of a catalyst. The transesterification reaction is affected by various parameters. One of this parameter is catalyst type. Generally, catalysts used in transesterification reaction can be divided into three categories: homogenous, heterogeneous and enzymes. Industrially biodiesel production is being carried out homogeneously (Acids and Bases). However, using homogenous catalyst not qualified by researchers because of issues with catalyst deactivation, catalyst recovery, large wastewater production, and corrosion to operation facilities, all of which increase the production cost of biodiesel. Recently, to overcome the problems encountered with homogeneous catalysts a lot of research is being conducted on developing heterogeneous catalysts having high catalytic activity and stability.

Heterogeneous catalysts offer numerous advantages over homogeneous catalysts as it can easily be separated from reaction mixture and can be reused, possess high thermal stability, have limited corrosion effect, and easily applied in continuous processes. Preparation of heterogeneous base catalysts derived from commercial chemicals sometimes consists of multiple complicated steps. As a result, those catalysts might become uneconomical, especially on a commercial scale. One possible way to address the problem is to synthesize catalysts from natural sources, lowering the production cost and alleviating environmental pressure simultaneously.

Among the different kinds of natural sources (such as mollusk shells, eggshells, calcined fish scale, sheep bone, etc.), animal bones tend to be a suitable choice due to their high abundancy and non-toxic features. It has been shown recently, by simple thermal conversion animal bone can effectively catalyzed transesterification reaction (Farooq & Ramli, 2015; Jazie et al., 2013; Obadiah et al., 2012). However, the reaction required high catalyst amount, high methanol to oil molar ratio with longer reaction time. All these disadvantages make waste bone derived catalysts practically and economically unsuitable. A modification step, like wet impregnation with other

chemicals, could be combined with thermal conversion to improve the performance of bone-derived catalysts (Ghanei et al., 2016).

1.3 Objective of the Research

1.3.1 General Objective

The main objective of this research is to convert animal bones in to heterogeneous solid base catalyst having high catalytic activity for production of biodiesel (FAME) from the non- edible Jatropha oil, via transesterification reaction.

1.3.2 Specific Objectives

The specific objectives of this research are: -

- To prepare and characterize several KOH-impregnated calcined animal bones.
- To investigate the catalytic performance/efficiency of the prepared catalyst by producing biodiesel (FAME) from Jatropha oil in batch process under varying reaction conditions (methanol/oil molar ratio, catalyst loading and reaction temperature).
- To study the main and interaction effect of transesterification reaction parameters using RSM.
- To develop model equation and determine the optimal reaction condition aiming to obtain maximum FAME yield.
- To determine the kinetics of the base-catalyzed transesterification reaction.
- To characterize the physico-chemical properties of produced biodiesel (FAME).

1.4 Significance of the Study

Currently Ethiopia is one of the countries where their economies are booming. This fast-growing economy together with the increasing population of the country increases the energy demand day by day. Fossil fuels take the major portion in fulfilling the energy demand of the country. The Ethiopian government spends over 87% of the foreign earning annually on these fossil fuel (Beemnet et al., 2016).

In recent years, the country gives attention to alternative fuels (such as biodiesel, methanol, ethanol, biogas, hydrogen, etc.). Biodiesel has gained attention as a source of alternative fuel due to its environmental benefits and renewability.

This research produces biodiesel using wastes of animal bone as raw material for catalyst synthesis. This could eliminate the wastes and simultaneously produced the heterogeneous catalysts with high cost effectiveness. And production of biodiesel from non-edible Jatropha oil by using this cost effective heterogeneous catalyst develop cheap, efficient and environmentally friendly biodiesel technology for sustainable energy production.

1.5 Scope of the Study

The thesis work generally covers solid base catalyst preparation and characterization, Jatropha oil extraction, refining and characterization, production of biodiesel using the prepared catalyst in a two-step acid-base catalyzed transesterification reaction and characterization of the produced biodiesel using standard procedures and test methods, and determination of reaction kinetics for the base-catalyzed transesterification reaction.

2. LITERATURE REVIEW

2.1 Renewable Energy

The depletion of fossil fuel, the resulting high price, and its adverse effects on the environment such as greenhouse effects and acid rain due to excessive sulfur emissions is compelling researchers to investigate other renewable energy sources. Thus, there is an urgent need for alternative and renewable fuels.

Currently, a number of renewable energy sources are under consideration. For example, solar, wind, tidal, wave and so forth are all green and renewable energy sources and have been playing a more and more significant role in today's energy market (Dincer, 2000). Nevertheless, several existing problems are limiting their potential to be used extensively. Firstly, many of those including, solar and wind energy, largely depend on the location and weather, which prevents them from being applied nationwide and worldwide. Secondly, the efficiency of the machines that are used for energy conversion and export is low. Hence, at this time, those energy sources are less economical to substitute fossil fuels.

Among all the renewable energy sources, energy generated from biomass shows a series of advantages over the others. Biomass refers to all the biological material derived from living, or recently living organisms, like wood, algae, and waste manure. In contrast to fossil fuels, biomass is generated from various bio-resources in a short-term cycle. Furthermore, since biomass uses sunlight and atmospheric CO_2, it is more environmentally friendly than fossil fuels. Currently, biomass is converted into other usable forms of energy, such as biogas, bioethanol, and biodiesel. Those energy forms could be stored and transported easily. Although the current conversion method of biomass into alternative energy form is expensive and inefficient which make it in competitive with fossil fuels.

2.2 Biodiesel

Biodiesel refers to vegetable oil or animal fat-derived diesel fuel consisting of long-chain alkyl (methyl, ethyl, or propyl) esters. Biodiesel has excellent lubricating properties and cetane ratings compared with diesel fuels. Despite possessing lower heating value than diesel fuels, its better lubricity and a complete combustion could compensate for its lower energy density. Biodiesel can

be used alone or mixed with petroleum diesel at any concentration ratio. The fuel efficiency depends on the mix ratio and the type of combustion engine (Agarwal, 2007).

2.2.1 Feedstocks for Biodiesel Production

The high cost of biodiesel is a major obstacle to its commercialization, which is 1.5–3 times higher than petroleum derived diesel. The feedstocks, accounts for about 75% of the total production costs, contribute to a major portion of the overall biodiesel production cost as shown in figure 2-1 (Atabani et al., 2012). Therefore, it is critically important to select a feedstock, which is cheap, domestically available, and not compete with the food materials, to ensure low production cost of biodiesel.

In general, biodiesel feedstocks can be divided into four main categories as edible vegetable oil, non-edible vegetable oil, waste or recycled oil and animal fats. The main feedstocks of biodiesel are shown in Appendix A.

Oils from rapeseed, soybean, palm, sunflower, coconut and peanut fall under the edible vegetable oil category and considered as the first generation of biodiesel feedstocks because they were the first oil crops to be used for biodiesel production. Their plants were cultivated in many countries around the world such as Malaysia, USA, Indonesia, Canada, and Germany. 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%)(Atabani et al., 2012). However, their use for biodiesel production competes with their primary use as food sources, giving rise to “food vs fuel” debate and environmental concerns such as destruction of vital soil resources, deforestation and usage of much of the available arable land.

One of the possible solutions to reduce the dependence of biodiesel production on edible oils is by exploiting non-edible oils. Non-edible oils, such as oil from Pongamia pinnata (Karanja or Honge), Jatropha curcas, Cotton seed, rubber seed tree, Moringa, Croton megalocarpus, Salmon oil and so on, are gaining worldwide attention because they are easily available in many parts of the world especially wastelands that are not suitable for food crops, eliminate competition for food, reduce deforestation rate, more efficient, more environmentally friendly, produce useful by-products and they are very economical comparable to edible oils (Atabani et al., 2012). Non-edible oils are regarded as the second generation of biodiesel feedstocks.

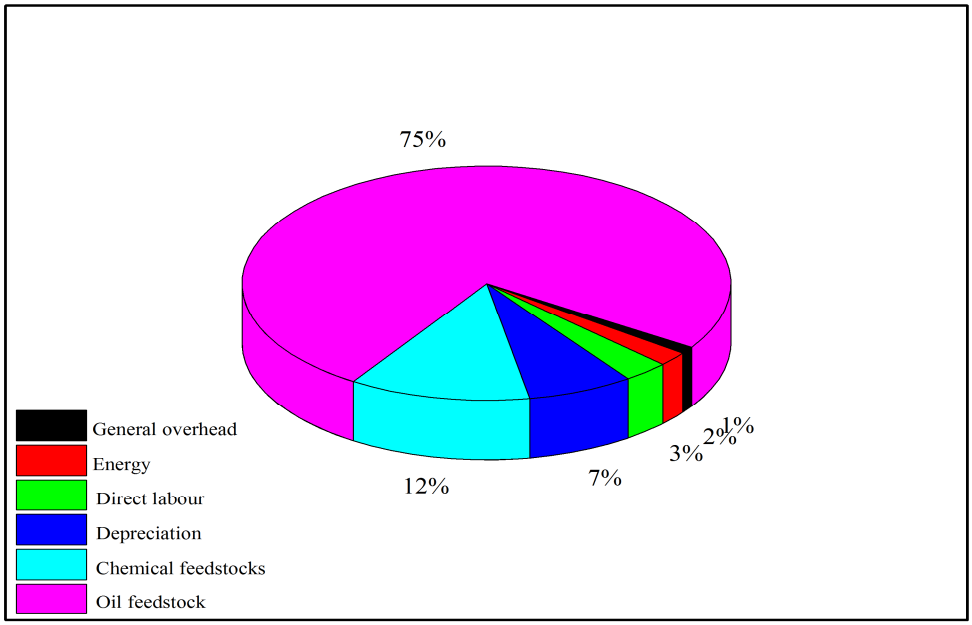


Figure 2-1: General cost breakdown for production of biodiesel (Atabani et al., 2012)

Animal fats such as beef tallow, poultry fat, pork lard and by-products from fish oil, waste oils and grease are also considered second generation feedstocks. The use of these types of feedstock as a raw material for biodiesel production eliminates several disposal problems and contamination to water and land resources. However, the drawback of these feedstocks is the presence of high free fatty acids (FFA) and water content. Excess FFA and moisture will affect the transesterification reaction through saponification reaction, and, eventually, decrease the yield and complicate the product separation and in the case of waste cooking oil, infrastructure and logistics needed for collection could be hurdle as the sources are generally scattered (Atabani et al., 2012).

More recently, algal lipids derived from microalgae are gaining interest all over the world and have emerged to be the third generation of biodiesel feedstock. Microalgae are photosynthetic microorganisms that convert CO_2 into sugar and proteins in the presence of sunlight. Higher photosynthetic efficiency, higher growth rates and productivity, high oil content comparing to edible and non-edible feedstocks and easy of cultivation (either in in farm or bioreactor) makes microalgae a promising feedstock for biodiesel production in the near future. However, its high production cost from requiring high-oil yielding algae strains and effective large-scale bioreactors are the main obstacles for its commercialization (Atabani et al., 2012; Ramli et al.,2016).

2.2.1.1 Jatropha Curcas L. Seed Oil

Jatropha curcas L. is a perennial small tree or large shrub that belongs to Euphorbiaceae family, known for its toxicity. Its name is derived from two Latin words jatros (doctor) and trophe (food). The genus consists of over 170 species of succulent shrubs and trees. It reaches a height of three to five meters but can reach up to eight to ten meters when grown under favorable conditions. It has an average life span of 50 years with a high oil content of about 37% or more and reaches maturity within four to five years. Jatropha curcas is native to Mexico, Central America, Africa, India, Brazil, Argentina and Paraguay. The Jatropha curcas plant is drought-resistant and has the capability to semi-arid and arid conditions, can grow almost on any type of soil whether gravelly, sandy or saline and thrives even on the poorest stony soils and crevices. The potential areas of Jatropha curcas were distributed around the world is presented in Figure 2-2 (Silitonga et al., 2013). The oil from the Jatropha curcas L. seeds has valuable properties such as a low acidity, good stability as compared to soybean oil, low viscosity as compared to castor oil and better cold properties as compared to palm oil. Besides, Jatropha oils consist of mainly oleic and linoleic acids which are unsaturated fatty acids, the biodiesel produced has desirable good low temperature properties. Although Jatropha oil cannot be used for human consumption and the cake as well cannot be used as animal feed which makes it a good alternative feedstock for biodiesel production (Koh et al., 2011). However, most non-edible oils contain a high level of free fatty acids (FFA) which makes transesterification reaction difficult and lowers the yield of biodiesel. This is because a high FFA (>1%w/w) will promote more soap formation and the separation of products will be difficult during alkali-catalyzed transesterification. Therefore, pre-treatments of non-edible oils for lowering the FFA in feedstock for alkali transesterification are inevitable. Table 2-1 tabulates the physical and chemical properties of Jatropha curcas oil (Koh et al., 2011).

Apart from being potential feedstock in the production of biodiesel as a diesel substitute, Jatropha oil has other uses such as producing soap, and biocides (insecticide, molluscicide, fungicide and nematicide). Further, as the stability of biodiesel is highly critical to meet storage requirements, usually biodiesel requires the addition of an antioxidant. Appropriate blends of Jatropha and palm biodiesel have been established in order to minimize the dosage of antioxidant needed as Jatropha biodiesel has good low temperature properties but a poor oxidation stability, whereas palm biodiesel has a good oxidative stability with poor low temperature properties. The blending and combinations of Jatropha and palm give an additive effect on these critical properties of biodiesel.

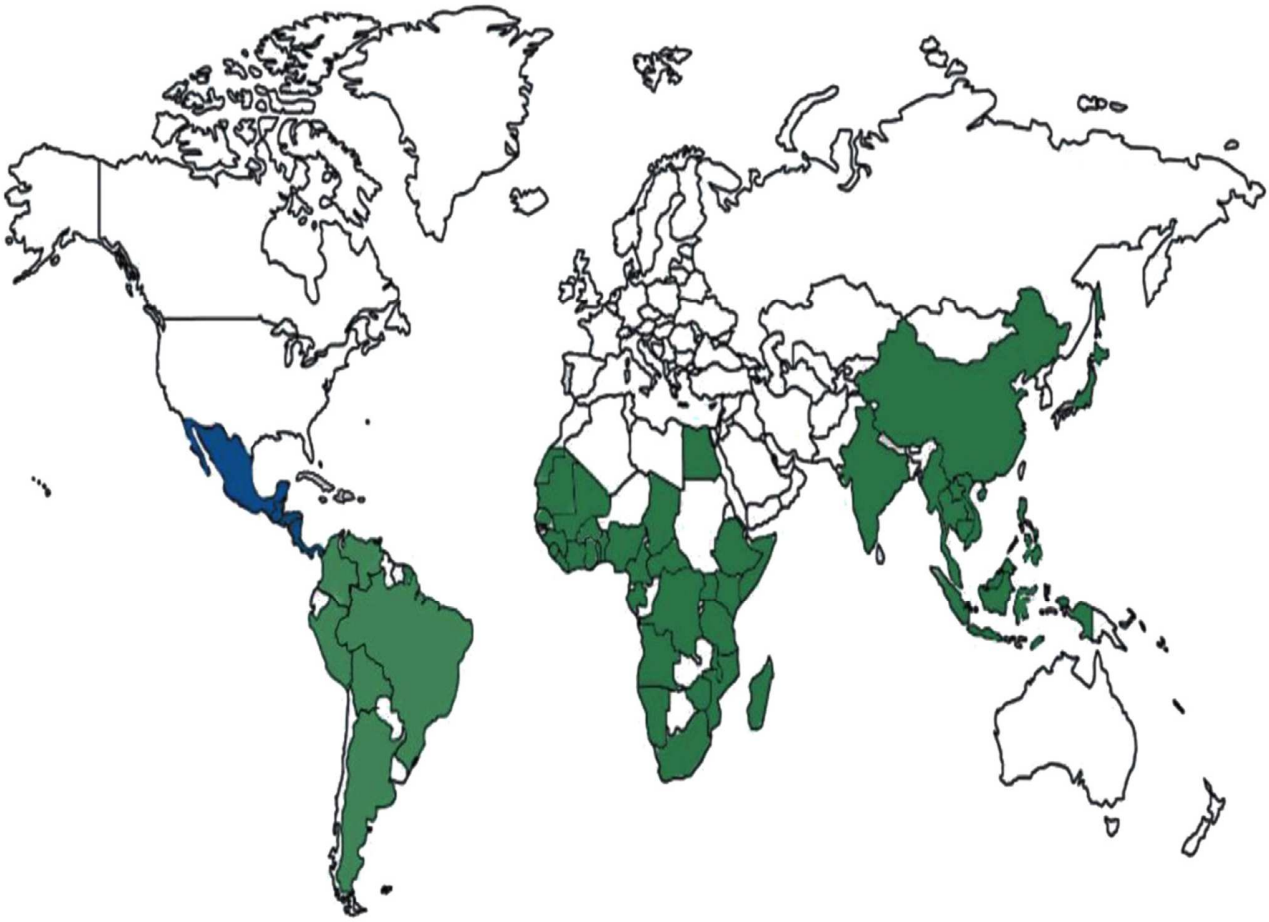
Moreover, direct use of Jatropha oil without any modifications can be used in older engines and motors equipped with current technologies such as pumps and generators running at a constant speed (Koh et al., 2011). Although the seeds can be used for many purposes such as lighting, lubricant and biomass elements (Silitonga et al., 2013).

Table 2-1: Physical and chemical properties of Jatropha curcas oil

property of Jatropha curcas oil	**Range**
Specific gravity, gcm^{-3}	0.860–0.933
Calorific value, MJ kg^{-1}	37.83–42.05
Pour point, ˚C	−3
Cloud point, ˚C	2
Flash point, ˚C	210–240
Cetane value	38.0–51.0
Saponification number, mg KOH g^{-1}	102.9–209.0
Viscosity at 30 ˚C, cSt	37.0–54.8
Free fatty acids %, kg kg^{-1} ×100	0.18–3.40
Iodine number, mg I g^{-1}	92.0–112.0
Acid number, mg KOH g^{-1}	0.92–6.16

Source:(Koh et al., 2011)

Jatropha grows in many parts of Ethiopia, as a hedge around homesteads and farmlands, such as in Wolayita, Metekel, Southern Wollo, Northern and Eastern Shoa, Tigrai, Gamo Gofa zones and Gambella region. Beemnet et al. conducted a study in five regions namely Southern Nation Nationalities Peoples Regional State (SNNPRS), Amhara region, Tigrai region, Oromia region and Benishangul Gumuze Regions, where Jatropha is found abundantly. Based on their report, the oil content of Ethiopian Jatropha populations ranged from 29.88 to 34.34% with up to 42.8-51.4%, 21.73-25.43%, 10.9-19.3% and 10.62-15.91% oleic, linoleic, stearic and palmitic acids respectively (Beemnet et al, 2016). In another study the oil yield of Jatropha curcas seed cultivated in Tigray Ethiopia was found to be 42.19% (wt/wt) (Amabye & Bezabh, 2015). Fatty acid composition of Ethiopian Jatropha oil compared with different Jatropha oils obtained from different countries are shown in Appendix A.



Native : Belize, Costa Rica, El Salvador, Guatemala, Honduras, Mexico, Nicaragua, Panama

Exotic : Angola, Antigua and Barbuda, Argentina, Bahamas, Barbados, Benin, Bolivia, Brazil, Burkina Faso, Cambodia, Cameroon, Cape Verde, Central African Republic, Chad, China, Colombia, Cote d'Ivoire, Cuba, Democratic Republic of Congo, Dominica, Dominican Republic, Ecuador, Egypt, Eritrea, Ethiopia, French Guiana, Gabon, Gambia, Ghana, Grenada, Guadeloupe, Guinea, Guinea-Bissau, Haiti, India, Indonesia, Jamaica, Japan, Kenya, Laos, Liberia, Madagascar, Malawi, Malaysia, Mali, Martinique, Mauritania, Montserrat, Mozambique, Myanmar, Namibia, Nepal, Netherlands Antilles, Nigeria, Peru, Philippines, Portugal, Puerto Rico, Sao Tome et Principe, Senegal, Sierra Leone, Somalia, South Africa, Sri Lanka, St Kitts and Nevis, St Lucia, St Vincent and the Grenadines, Tanzania, Thailand, Togo, Trinidad and Tobago, Uganda, US, Venezuela, Vietnam, Virgin Islands (US), Zanzibar, Zimbabwe

Figure 2-2: The distribution of Jatropha curcas L. plants around the world (Silitonga et al., 2013)

2.2.2 Conventional Biodiesel Production Methods

Vegetable oils are the most widely used raw materials for biodiesel production. The fact that vegetable oils are renewable and have an energetic content close to diesel fuels make them an attractive raw material for biodiesel (Rudolph & He, 2004). However, there are still some problems that hinder the use of vegetable oil for substitute of petrodiesel. The viscosity of vegetable oil is several times higher than that of mineral diesel due to large molecular weight and complex chemical structure; hence they have to be modified to bring their combustion-related properties

closer to those of mineral diesel. This fuel modification is mainly aimed at reducing the viscosity to get rid of flow and combustion-related problems. A number of methods are currently used for biodiesel production from different feedstocks to overcome the high viscosity of the vegetable oils as a fuel. However, there are four primary methods employed for biodiesel production: dilution, micro-emulsification, pyrolysis and transesterification. In present, transesterification is the most efficient method to produce biodiesel with high quality.

2.2.2.1 Dilution

Vegetable oil can be used directly or blended with diesel fuel without any change to the engine. Vegetable oil was proposed to be used as an alternative to petroleum in the 1980 by Caterpillar (Brazil). It used pre-combustion chamber engines with a mixture of 10% vegetable oil to maintain total power without any alterations or adjustments to the engine. At that point, it was not practical to substitute 100% vegetable oil for diesel fuel, but a blend of 20% vegetable oil and 80% mineral diesel was successful (Agarwal, 2007).

Advantage of vegetable oil as diesel fuel includes: its high heat content, renewability, readily availability and portability. Conversely, the major problems arise from the high viscosity, acid composition, free fatty acid content, as well as gum formation due to oxidation and polymerization during storage and combustion. In long term engine tests injector coking, higher carbon deposits, sticking of piston rings, and lubricating oil thickening and gelling are obvious problems(Agarwal, 2007).

2.2.2.2 Micro-emulsification

A micro-emulsion can be defined as a clear and thermodynamically stable dispersion of optically isotropic fluid microstructures with dimension generally in the 1–150 nm range, formed spontaneously, without mechanical action, from two normally immiscible liquids. Vegetable oils with an ester or a dispersant, or a vegetable oil, alcohol and a surfactant could form a micro-emulsion.

In this method low viscosity is obtained for the fuel, but the fuel has lower heating value than that of diesel, a low cetane number and lower energy and consequently a lower power output and the combustion would be done incompletely and much carbon deposits will come out (Gorji et al.,

2014). One of the major problems reported was the difficulty in starting the engine even at room temperature (Caban et al., 2013).

2.2.2.3 Pyrolysis

Pyrolysis or thermal cracking is the conversion of long chains of carbon-, hydrogen and oxygen-containing substance into smaller molecules by means of heat in the absence of oxygen. Pyrolysis studies were carried for more than 100 years, especially in those areas of the world that lack deposits of petroleum. The pyrolyzed raw materials can be vegetable oils, animal fats, and natural fatty acids or methyl esters of fatty acids (Gorji & Ghanei, 2014).

The liquid fraction obtained from the decomposition of vegetable oil leads to the creation of several groups of material such as alkanes and alkenes, alkadyns, the aromatics and carboxylic acid. The resulting fuel from this method has similar chemical properties to that of diesel fuels. This method has some technological shortcomings like poor thermal stability, presence of solids in the oils, corrosive nature of oil, dissolved char in oil and the production of pyrolytic water, inappropriate amounts of ash, carbon residue and cloudy spot as a by-product (Gorji & Ghanei, 2014).

2.2.2.4 Transesterification

In organic chemistry, transesterification is the process of exchanging the alkoxy group of an ester compound by another alcohol. Transesterification is the most common methods used to reduce oil viscosity in the biodiesel industry. The transesterification process is the reaction of a triglyceride (fat/oil) with an alcohol to form esters and glycerol. A catalyst, often an acid or base, is usually used to improve the reaction rate and yield.

In this method, the resulting biodiesel has high cetane number, low emission of pollutants, and high combustion efficiency and besides, this method leads to conserve oxygen atoms in biodiesel molecule. However, the method requires higher cost of downstream processing and produce large amount of wastewater. The details of the process of transesterification are discussed as follows.

2.2.3 Fuel Properties and Qualities of Biodiesel

The advancements of biodiesel quality are being developed globally to maintain the quality of the end product and to ensure better criteria of biodiesel storage and feedstock for consumers' confidence and successful commercialization of biodiesel. Since biodiesel is produced from wide variety of feedstocks of having different qualities, it is necessary to install a standardization of fuel

quality to guarantee an engine performance without any difficulties. Currently, the properties and qualities of biodiesel must adhere with the international biodiesel standard specifications. These specifications include the American Standards for Testing Materials (ASTM D6751) or the European Union (EN 14214) Standards for biodiesel fuel. However, there are some other standards available globally such as in Germany (DIN 51606), Austria (ON) and Czech Republic (CSN) (Atabani et al., 2012).

2.2.3.1 Cetane Number (CN)

One of the most important parameters, which is considered during the selection procedure of methyl esters for using as biodiesel is the dimensionless cetane number (CN). It is the indication of ignition characteristics or ability of fuel to auto-ignite quickly after being injected. Higher CN value is an indication of better ignition quality of the fuel, which is shorter time between the ignition and the initiation of fuel injection into the combustion chamber. Conversely, lower cetane number indicates long ignition delay (long time between fuel injection and start of combustion). Cetane number increases with increasing chain length of fatty acids and increasing saturation (Giakoumis, 2013). Biodiesel has usually higher cetane number than conventional diesel fuel, which results in higher combustion efficiency. The CN of biodiesel fuel, specified by ASTM D613 and EN ISO 5165 is at least 47 and 51 min respectively (Atabani et al., 2012).

2.2.3.2 Kinematic Viscosity

Viscosity is an important property of any fuel as it indicates resistance to flow of a fluid under gravity. It therefore affects the operation of fuel injection equipment and spray atomization, particularly at low temperatures when the increase in viscosity affects the fluidity of the fuel. Higher viscosity leads to a higher drag in the injection pump and thus causes higher pressures and injection volumes, especially at low engine operating temperatures. On the contrary, significant reduction in viscosity can result: loss of lubricity which causes excessive wear, higher mechanical friction which causes high heat generation and/or high energy consumption (Isioma et al., 2013).

Viscosity is closely related to the fatty acid composition of a given biodiesel sample. Larger proportions of saturated fatty acids with longer carbon chains cause kinematics viscosity to increase. The kinematic viscosity of biodiesel is 10–15 times greater than that of diesel fossil fuels. This is because of its large molecular mass and large chemical structure. The maximum allowable

limit according to ASTM D445 ranges are (1.9–6.0 mm^2/s) and (3.5–5.0 mm^2/s) in EN ISO 3104 (Atabani et al., 2012).

2.2.3.3 Specific Gravity

Specific gravity and therefore density is another important parameter of biodiesel quality. Specific gravity of a substance is the ratio of density of the substance to that of density of water, which is unit less. Fuel injection equipment operates on a volume metering system, hence a higher density for biodiesel results in the delivery of a slightly greater mass of fuel into combustion chamber it will affect the stochiometric ratio of air to fuel which in turn negatively affect the combustion process. The specific gravity of biodiesel ranges between 0.86-0.9 at 15 ^oC. The standard analytical procedure for the determination of density of biodiesel fuel involves the use of a standardized glass hydrometer or an oscillating U-tube at the prescribed temperature.

2.2.3.4 Flash Point (FP)

Flash point of a fuel is the minimum temperature at which it will ignite when exposed to a flame or a spark. It is a measure of the flammability of fuels and thus, an important parameter for assessing hazards during fuel transport and storage. Flash point varies inversely with the fuel's volatility. The flash point of biodiesel (>150 ^oC) is higher than the prescribed limit of diesel fossil fuel (55-66 ^oC), which is safe for transport, handling and storage purpose. The limit of flash point ranges in ASTM D93 is ≥130 ^oC and in EN ISO 3679 is ≥120 ^oC (Atabani et al., 2012).

2.2.3.5 Acid Value

Acid value or neutralization number is a measure of mineral acids and free fatty acids contained in a fuel sample. Free fatty acids (FFAs) are the saturated or unsaturated monocarboxylic acids that occur naturally in fats, oils or greases but are not attached to glycerol backbones. Higher amount of free fatty acids leads to higher acid value. Biodiesel with high acid value causes increase in fueling system deposit and increases the likelihood of corrosion, consequently, it will damage fuel pumps and fuel filters. Acid value is expressed in milligrams of KOH required to neutralize 1 g of fatty acid methyl esters. It measures the amount of unreacted acids, which remained in the fuel. European specification (EN 14104) and US standard (ASTM D664) sets to a maximum value of 0.5 and 0.8 mg KOH/g respectively (Atabani et al., 2012).

2.2.3.6 Iodine Number

The iodine number (IN, or iodine value IV) is a parameter used to indicate the degree of unsaturation of the fuel. The higher the iodine value, the greater the number of double bonds, which indicates greater potential to polymerize resulting in formation of engine deposits and problems in storing the fuel. There is no specification in the US for the IN, whereas European specifications (EN 14111) require that biodiesels used in compression ignition engines have a (rather low) maximum value of IN of the order of 120 (Giakoumis, 2013).

2.2.3.7 Cloud Point (CP), Pour Point (PP) and Cold Filter Plugging Point (CFPP)

Low temperature flow properties of biodiesel are an important quality criterion. This is because partial or full solidification of the fuel may cause blockage of the fuel lines , pumps and filters, leading to fuel starvation, problems of starting, driving and engine damage due to inadequate lubrication (Atabani et al., 2012; Giakoumis, 2013).

The cloud point is the temperature at which wax crystals first becomes visible when the fuel is cooled down. Pour point is the lowest temperature at which the fuel becomes semi solid and loses its flow characteristics being no longer pumpable; hence it is a measure of the fuel gelling point. Cloud and pour points are measured using ASTM D2500 and D97 procedures respectively. Generally, biodiesel has higher CP and PP compared to conventional diesel (Isioma et al., 2013). Cold filter plugging point (CFPP) refers to the temperature at which the test filter starts to plug due to fuel components that have started to gel or crystallize. CFPP defines the fuels limit of filterability, having a better correlation than cloud point for biodiesel as well as diesel. CFPP is measured using ASTM D6371. There is no European or US specifications for low temperature properties (each country is free to determine its own limits according to local weather conditions) (Atabani et al., 2012).

2.3 Transesterification Reaction

Transesterification, also called alcoholysis, is the reaction of an oil or fat with an alcohol in presence of appropriate catalyst (alkali, acid, enzyme) to produce alkyl ester and glycerin as a valuable by-product. It is the most common way to produce biodiesel. The general scheme of the transesterification reaction is presented in Figure 2-3.

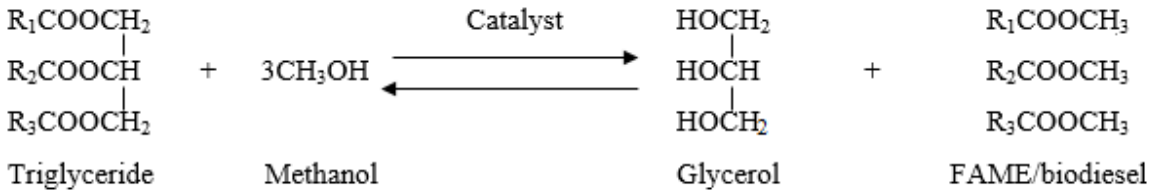


Figure 2-3: Transesterification reaction scheme: Overall reaction

The stoichiometry of the reaction suggests that the molar amount of methanol needed is three times as much as triglyceride. It is also worth noting that the reaction is reversible, and consequently, the triglyceride is only partially converted into FAMEs. To increase the yield of FAMEs, more than the stoichiometric amount of methanol is added into the reaction system to shift the chemical equilibrium towards the product. Generally, the methanol to oil ratio will largely exceed 3:1. The transesterification reaction between triglyceride and methanol consists of three consecutive reactions, wherein diglyceride and monoglyceride are formed as intermediates (figure 2-4). Since the three consecutive reactions are all reversible, triglyceride, diglyceride, and monoglyceride all exist in the reaction mixture. The excessive methanol not only increases the conversion of triglyceride but also decreases the amount of those intermediates throughout the reaction period (Romero et al.).

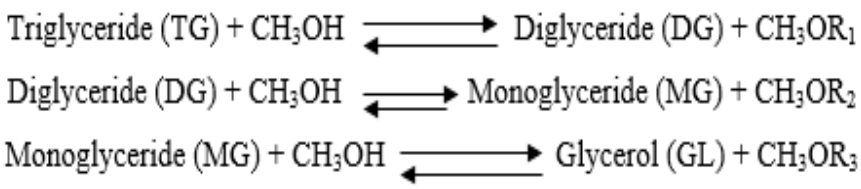


Figure 2-4: Transesterification reaction scheme: Stepwise, consecutive and reversible reaction

2.3.1 Types of Transesterification Reactions

As with any chemical reaction, the catalyst is crucial to the transesterification reaction because it determines the reaction rate and affects the downstream processing of FAMEs. Generally, non-catalytic transesterification requires a higher temperature, pressure, and methanol to oil ratio to achieve a considerable reaction rate and oil conversion, and consequently, the process requires high energy input and expensive equipment that are temperature, pressure and corrosion resistant. The high production cost suggests that the non-catalytic transesterification is not favored by biodiesel industry. The catalytic transesterification reaction proceeds rapidly under mild conditions with the aid of catalyst that substantially lowers the activation energy (Thanh et al., 2012).

In general, the catalysts that can be used for producing biodiesel are divided into three groups: alkaline, acidic and enzymatic. As compare to enzymatic catalysts, alkaline and acidic catalysts are more commonly used in biodiesel production. Enzymatic catalysts have become more attractive since it can avoid soap formation and the purification process is simple to accomplish. However, they are not commercially used because of the longer reaction times and higher cost. Alkaline and acidic catalysts are also classified into two groups: Heterogeneous and homogeneous catalysts (Talha & Sulaiman, 2016).

2.3.1.1 Homogeneous (Alkali) Base-Catalyzed Transesterification

The most common basic catalysts are potassium hydroxide (KOH), potassium methoxide ($KOCH_3$), sodium hydroxide (NaOH), sodium methoxide ($NaOCH_3$), and sodium ethoxide ($NaOCH_2CH_3$) (Talha & Sulaiman, 2016). These catalysts are commonly used because of several advantages such as able to catalyze reaction at mild condition, high conversion in shorter time, and economically available. NaOH and KOH are the most common homogeneous base catalyst in biodiesel production.

One of the biggest drawbacks of the alkaline catalysts are their sensitivity to the purity of the reactant. They give good performance when raw materials with high quality (FFA < 1 wt.% and moisture < 0.5 wt.%) are used. These catalysts bear process problem through soap formation. The formation of soap decreases the activity of the catalyst and inhibits the separation of glycerol from the reaction mixture and the purification of FAME with water. Removal of these saponified catalysts is technically difficult and it adds extra cost to the production of biodiesel. Furthermore, since homogeneous base catalysts mainly dissolve in the glycerol and alcohol phase after the reaction is completed, they cannot be recycled for the following batches, and the crude biodiesel must be purified by a washing process with water this generate a large amount of waste water (Thanh et al., 2012).

Homogeneous alkaline-catalyst transesterification is much faster than the acid-catalyst transesterification and commonly used in commercial biodiesel production from vegetable oils or fats containing low FFA and water.

2.3.1.2 Homogeneous Acid-Catalyzed Transesterification

The liquid acid-catalyzed transesterification process is not much popular as the base-catalyzed process. Homogeneous acid catalyzed reaction is about 4000 times slower than the homogeneous

base-catalyzed reaction (Kombe et al., 2007). However, the performance of the acid catalyst is not strongly affected by the presence of FFAs in the feedstock. With starting raw materials containing a high amount of FFA such as waste cooking, Jatropha curcas, rubber, tobacco oils, etc., an acid-catalyst, usually a strong acid such as sulfuric, hydrochloric or phosphoric acid, is more favorable than base-catalyst because the reaction does not form soap.

A great advantage with acid catalysts is that they can directly produce bio-diesel from materials containing a high amount of FFA. However, acid-catalysts require higher temperature and longer reaction time, in addition to causing undesired corrosion of the equipment. Moreover, to increase the conversion of triglyceride, a large excess amount of methanol, e.g., molar ratio of methanol to oil of higher than 12:1, should be used. In practice, therefore, to reduce the reaction time, the process with an acid-catalyst is adapted as a pretreatment step only when it is necessary to convert FFA to esters, and is followed by a base-catalyst addition for the transesterification step to transform triglyceride to esters (Thanh et al, 2012).

2.3.1.3 Heterogeneous Acid and Base-Catalyzed Transesterification

Research is now being directed to the application of heterogeneous catalysts to overcome the problems encountered with homogeneous catalysts for biodiesel forming reactions. It is believed that heterogeneous acid catalysts have the potential as alternative to homogeneous acid catalysts. Some of advantages of heterogeneous acid catalyst are insensitive to FFA content, can simultaneously conduct esterification and transesterification, easily separated from the reaction mixture, non-formation of soap that caused to less washing and regenerating and reusing the catalyst is possible and also reduce the corrosion problems (Sivasamy et al., 2009; Talha & Sulaiman, 2016).

A major shortcoming of a heterogeneous catalyst, compared with a homogeneous catalyst, is its relatively low catalytic activity. Unlike the homogeneous catalytic system where the reaction occurs ubiquitously, the reaction in a heterogeneous catalytic system only occurs on the surface of the solid catalyst. Thus, the reaction rate is substantially limited by the mass transfer rate of reactants to the catalyst surface. One of the strategy to minimize mass transfer limitation could be employing vigorous agitation and adding co-solvent, however, the agitation might affect the surface structure of the catalyst and the addition of co-solvent complicates the composition of the reaction system. Another strategy to enhance the reaction rates is to increase the catalytic activity

of the solid base catalyst. Structure promoters or catalyst supports are to be used which can provide pores for active species and more surface area for transesterification reaction. Different types of supports have been used in heterogeneous transesterification. It has been stated that aluminium oxide, zinc oxide, mordenite, bentonite and palm shell activated carbon showed favorable support properties for alkali catalysts in biodiesel production (Xie & Huang, 2006; Zabeti et al., 2009)(Intarapong et al., 2013; Soetaredjo et al, 2011). Similar to homogeneous catalyst, solid base-catalysts are more active than solid acid-catalysts (Thanh et al., 2012).

2.3.1.4 Enzyme-Catalyzed Transesterification

Enzymatic transesterification using lipase has drawn researcher's attention for reasons of easy product separation, minimal wastewater generation, easy glycerol recovery, mild reaction condition and insensitive to high FFA oil. However, enzyme catalyzed biodiesel production has some limitations for implementing in industrial scale such as prolongation of reaction time, high cost and enzyme deactivation.

2.3.2 Process Variables Affecting Transesterification Reaction

Production of biodiesel in the process of transesterification is affected by several process parameters/variables. The most important variables include reaction temperature, molar ratio of alcohol to oil, catalyst concentration and type, reaction time, alcohol type, stirring and presence of moisture and free fatty acids (FFA).

2.3.2.1 Effect of Reaction Temperature

The rate of transesterification reaction is strongly influenced by the reaction temperature. Depending on the type of oil used transesterification can proceed at different temperature. At lower temperature the conversion efficiency is very low, as the temperature increases the viscosities of oils can decrease and result in an increase in reaction rate as more energy is being supplied for the reaction to occur. Thus, the yield of the biodiesel product is improved. However, the reaction temperature must be less than the boiling point of alcohol to ensure the alcohol will not be lost through vaporization. Generally, alcoholysis of vegetable oils is normally conducted near the boiling point of the alcohol at atmospheric pressure (Koh et al., 2011).

2.3.2.2 Effect of Molar Ratio of Alcohol to Oil

One of the most important variables affecting the yield of ester is the molar ratio of alcohol to vegetable oil employed. The stoichiometry of this reaction requires 3 mol alcohol per mol triglyceride to yield 3 mol fatty ester and 1 mol glycerol. In practice, it is necessary to use excessive alcohol because the transesterification reaction is a reversible reaction in its nature. As a result, higher amount is important to drive the reaction to forward side and for complete conversion of the reactant at higher rate. When 100% excess alcohol is used, the reaction rate is at its highest. Higher molar ratio of alcohol to oil increase the conversion rate but leads to difficulties in the separation of the glycerol and added useless expense to the separation. Lower molar ratios require a longer time to complete the reaction. A molar ratio of 6:1 is normally used in industrial processes to obtain methyl ester yields higher than 98% by weight (Agarwal, 2007; Shikha & Rita, 2012).

2.3.2.3 Effect of Catalyst Concentration and Type

A catalyst is needed to improve the transesterification reaction and yield. Catalysts are classified as alkali, acid, or enzymes. Alkali catalysts are the most effective for transesterification compared to others because of the higher reactivity and the lower process temperature required. However, if a vegetable oil has high free fatty acid and water content, acid-catalyzed transesterification reaction is suitable. The alkalis include NaOH, KOH, carbonates and corresponding sodium and potassium alkoxides such as sodium methoxide, sodium ethoxide, sodium propoxide and sodium butoxide. Sodium methoxide was found to be more effective than NaOH because of the reduced amount of water produced upon mixing sodium hydroxide with methanol. Due to its low cost, NaOH, has attracted its wide use in large scale transesterification. The alkaline catalyst concentrations in the range of 0.5–1% by weight yield 94–99% conversion of vegetable oils into esters. Further increase in catalyst concentration does not increase the yield, but it adds to the cost and makes the separation process more complicated because it is necessary to remove it from the reaction medium at the end. Sulfuric acid, sulfonic acids and hydrochloric acid are usually used as acid catalysts (Agarwal, 2007; Koh et al., 2011).

2.3.2.4 Effect of Reaction Time

Among others parameters, reaction time is one of the important parameter that has a profound effect on the FAME yield in transesterification reaction. Generally, the conversion rate increases with reaction time. Jazie and Sinha studied the effect of reaction time from 0.5 to 5hr on

transesterification of peanut and rapeseed oils using waste of animal bone as catalyst. The FAME content increased on increasing the reaction time from 0.5 to 5hr. However, the maximum yield 96% for rapeseed oil and 94% for peanut oil after 4 hr (Jazie et al., 2013). Obadiah et al. produced biodiesel from Palm oil using calcined waste animal bone as catalyst and achieved methyl ester conversion of 96.78% in 4 hr (Obadiah et al., 2012). Ghanei et al. also reported a reaction time of 5 hr to produce biodiesel from food grade canola oil using CaO-impregnated calcined animal bone catalyst and achieved maximum conversion of 95.18 % (Ghanei et al., 2016).

2.3.2.5 Effect of Moisture and FFA

Free fatty acids and moisture negatively affect the biodiesel yield. In the case of alkali-catalyzed transesterification, the starting materials should meet certain specifications. The glyceride should have a total FFA of <1% and substantially anhydrous because water makes the reaction partially change to saponification, which produces soap (water content <0.5%) (Thanh et al, 2012). Moisture content from the vegetable oil is removed by heating in oven for 1 hr at 383 K. The presence of high amount of FFA in the glyceride requires more alkali to neutralize it, which consumes the catalyst and reduce the effectiveness of the catalyst. Therefore, FFA and water content should be minimal otherwise saponification shall occur and the separation of products shall be exceedingly difficult, and as a result, the yield of biodiesel product would be low (Azhari et al., 2008).

Feedstock that contains higher amount of FFA, pre-treatment method (such as esterification) is required to bring to acceptable level before transesterification was carried out (Kombe et al., 2007).

2.3.2.6 Effect of Alcohol Type

Appropriate alcohols in transesterification method for producing biodiesel can be primary or secondary monohydric aliphatic alcohols having from 1 to 8 carbon atoms. Among the alcohols that have been used to produce biodiesel are methanol, ethanol, propanol, isopropanol, butanol, pentanol and amyl alcohol (Gorji & Ghanei, 2014; Romero et al.). Among which methanol is the most commonly used alcohol in biodiesel production, mainly because of its quick reactivity with triglycerides, availability and a relatively inexpensive alcohol. For this reason biodiesel is also called fatty acid methyl esters or "FAME" (Gorji & Ghanei, 2014).

The use of ethanol as solvent is becoming more popular since this alcohol is a renewable resource and does not raise the same toxicity concerns than methanol. However, the same reaction using

ethanol has a drawback that the produced ethyl esters are less stable and a carbon residue is observed after reaction. In addition, methanol is considerably easier to produce and recover than ethanol. Ethanol forms an azeotrope with water so it is expensive to purify the ethanol during recovery. Even though methanol is more toxic and sustainable methods of its production are currently not economically viable and the majority of it is formed from syngas, which is extracted from natural gas (a non-renewable source), it is the preferred alcohol for producing biodiesel (Gorji & Ghanei, 2014).

2.3.2.7 Effect of Stirring

Stirring can play an important role in the yield of biodiesel production. Transesterification reaction was carried out with 180, 360 and 600 revolutions per minute (rpm) and reported incomplete reaction with 180 rpm. The yield of methyl ester was same with 360 and 600 rpm (Shikha & Rita, 2012).

2.4 Solid Base Catalyst

The most commonly used solid base catalysts are derived from alkaline earth metal oxides. Alkaline earth metal commonly refers to beryllium (Be), magnesium (Mg), calcium (Ca), strontium (Sr), barium (Ba), and radium (Ra). Their oxides typically have a strong basic strength. From Lewis theory of acids and bases, the basic strength of alkaline metal oxides is in the order of BeO < MgO < CaO < SrO < BaO < RaO (Kouzu & Hidaka, 2012). Among all the alkaline earth metal oxides, MgO, CaO, and SrO have been extensively tested for the transesterification reaction because of their high activity for using in the typical process which at low temperature and under atmospheric pressure condition , high accessibility (high abundancy) and relatively low production cost (Thanh et al., 2012)(Dagmawi, 2015).

Some researchers have been focused on mesoporous material supported base catalysts, to improve the catalytic performance and increasing the yield of biodiesel. Alumina and Zeolite have been extensively used as a support for dispersing catalytic material (Venkateswarulu et al., 2014).

2.4.1 Natural Source-derived Base Catalyst

In the past decade, researchers have focused on deriving catalysts from natural sources other than commercial chemicals in order to lower production costs. Among all the natural sources Ca containing sources has special place to biodiesel production because of its cheapness, availability, possibility to reusing and having various renewable resources, such as eggshell, oyster shell,

mollusk shell, and bone. (Farooq & Ramli, 2015). Among these sources, animal bones tend to be the most feasible ones because of their high abundancy. There are many advantages of using animal bones as the precursor of solid base catalysts. Firstly, animal bones are produced in huge quantities daily due to the large food consumption, thus making the catalyst supply potentially abundant. Secondly, the utilization of those bones tackles the disposal of wastes, alleviating the environmental pressure. It is, therefore, both economically and environmentally friendly to derive solid base catalysts from waste natural bones.

Calcium phosphate ($CaPO_4$) is the main component of bone and can be transformed to hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$), $Ca(OH)_2$ and CaO by calcination. Hydroxyapatite is highly porous and also has a large surface area which allows catalyst to disperse over it largely and effectively. Besides, it has relatively high catalytic activity, good thermal and chemical stability (Nisar et al., 2017; Obadiah et al., 2012). So far, animal bones have been used to derive CaO to catalyze the transesterification reaction. However, the reaction required high catalyst amount, high methanol to oil molar ratio with longer reaction time. All these disadvantages make animal bone derived catalysts practically and economically unsuitable. Table 2-2 summarizes animal bone-derived catalysts used for biodiesel production.

Recently, many efforts have been made to improve the catalytic performance of animal bone derived catalysts. Ghanei et al. produce biodiesel from canola oil using CaO-impregnated calcined animal bone. First, they calcined sheep bone in electrical furnace at 600 ^oC for 8 hr and then impregnated with CaO, followed by recalcination at 600 ^oC for 4 hr. A FAME yield of 95.18 % was obtained at reaction temperature of 60 ^oC, methanol/oil molar ratio of 12:1, catalyst loading of 5 wt% and reaction time of 5 hr. Their catalyst also exhibited a slight improvement, increased surface area from 4.5808 m^2/g of the calcined animal bone to 5.2499 m^2/g of the final catalyst (Ghanei et al., 2016). This study indicated that the impregnation method followed by thermal conversion could increase the specific area of the catalyst, which improved the catalytic activity of calcined animal bone. In another study, Nisar et al. produce biodiesel from Jatropha oil using a catalyst derived from animal bone. First, they calcined animal bone at 900 ^oC for 5 hr followed by KOH impregnation on calcined animal bone. A FAME yield of 96.1% obtained at the optimal parametric conditions of methanol/oil molar ratio, 9:1, catalyst concentration, 6 wt%, reaction temperature, 70 ± 3 ^oC and reaction time, 3 hr (Nisar et al., 2017).

Table 2-2: Summary of recently reported natural bone-derived catalysts for biodiesel production

Entry	Source material	Calcination temperature (°C)	Calcination time (hr)	Oil type	Catalyst loadings (wt%)	Molar ratio	Reaction temperature (°C)	Reaction time (hr)	FAME yield (%)	Reference
1	Chicken bones	900	4	waste cooking oil	5	15:1	65	4	89.33	(Farooq & Ramli, 2015)
2	Waste animal bones	800	N.A.	Palm oil	20	18:1	65	4	96.78	(Obadiah et al, 2012)
3	Goat bones	900	2	Peanut oil Rapeseed oil	18	20:1	60	4	94 96	(Jazie et al., 2013)
N.A. - not available										

3. MATERIAL AND METHODS

3.1 Material and Reagents

The cattle bones used in this study were collected as wastes from Burau area. Dry Jatropha curcas L. seed was obtained from Awash Melkasa Agricultural Research Center. Methanol, potassium hydroxide, sulfuric acid, phenolphthalein indicator, hydrochloric acid, ethanol, diethyl ether and filter paper. All the chemicals were analytical reagent grade and obtained from different chemical stores in Addis Ababa.

3.2 Equipment

Equipment used during the experimental works are electrical furnace, Bielenberg ram press, water bath, micropipette, three necks round bottom flask, condenser, overhead stirrer, desiccators, crucibles, mortar and pestle, crusher, separating funnel, balance, oven, orbital shaker, different size conical and Erlenmeyer flasks, beakers, measuring cylinders, hot plate magnetic stirrer, rotary evaporator, density meter, vibro viscometer, bomb calorimeter, GC-MS (Agilent 7890A/5975C), FT-IR (FTIR-65, Perkin-Elmer), XRD (Miniflex 300/600) and TGA (DSC-TGA instrument, model: SDT Q600 V20.9 Build 20).

Mechanical extraction of Jatropha oil using Bielenberg ram press was done at Alternative Energy Technology Development and Promotion Directorate workshop, Ministry of Water, Irrigation and Electricity. Jatropha oil refining and characterization, animal bone preparation, calcination, impregnation of calcined animal bone, characterization of the solid base catalyst such as basicity and performance evaluation of the catalyst was done at School of Chemical and Bio Engineering Laboratory, AAIT. The other characterization of the catalyst, FT-IR and XRD analysis was done at faculty of natural science department of chemistry, Arat Killo. TGA analysis of the catalyst and GC-MS of biodiesel was done at Leather industry development institute (LIDI). The overall framework of the experiment is shown in Figure 3-1.

3.3 Preparation of KOH-impregnated Calcined Animal Bone Catalyst

Firstly, the discarded cattle bones collected from Burau area were broken up into small particles. To remove impurity and undesirable material such as dust, meat and cartilages, the prepared bone particles were washed several times with boiled water and then dried in a laboratory electric oven

at 105 ^{0}C for 12 hr. After cooling them at room temperature the bone particles were then crushed to finely to 100–500 µm particle size powder in a hammer mill.

The prepared bone powder was then placed in electrical furnace using crucibles and calcined at 600 °C for 2 hr to remove the remained organic substances from the mineral substances. The prepared white powder was then used as support for impregnation of active sites.

In order to stabilize the active site, wet impregnation method was employed. The white powder was divided in 5 g portions and placed in separate Erlenmeyer flasks and 50 ml of potassium containing solutions of different concentrations (8, 10, 12, 14 and 16 wt%) was prepared by dissolving KOH in deionized water. The prepared white powder was then soaked with potassium hydroxide solutions. The mixture was then agitated in an orbital shaker at 180 rpm at constant temperature of 25 °C for 24 hr and then heated gradually on hot plate magnetic stirrer. The obtained precipitate dried overnight at 105 ^{o}C to remove water through evaporation. The dried cake was then milled into powder and calcined at 900 ^{o}C for 3 hr. The prepared catalyst was placed in a desiccator and kept in the close vessel to avoid the reaction with CO_2 and humidity in air before used. In this way KOH-impregnated calcined animal bone catalysts with loading percent of 8, 10, 12, 14 and 16 wt% were prepared. Also, corresponding assignments were BI-8, BI-10, BI-12, BI-14, and BI-16, respectively. Moreover, the support (calcined animal bone powder) without impregnation and pure animal bone were specified as B and N respectively.

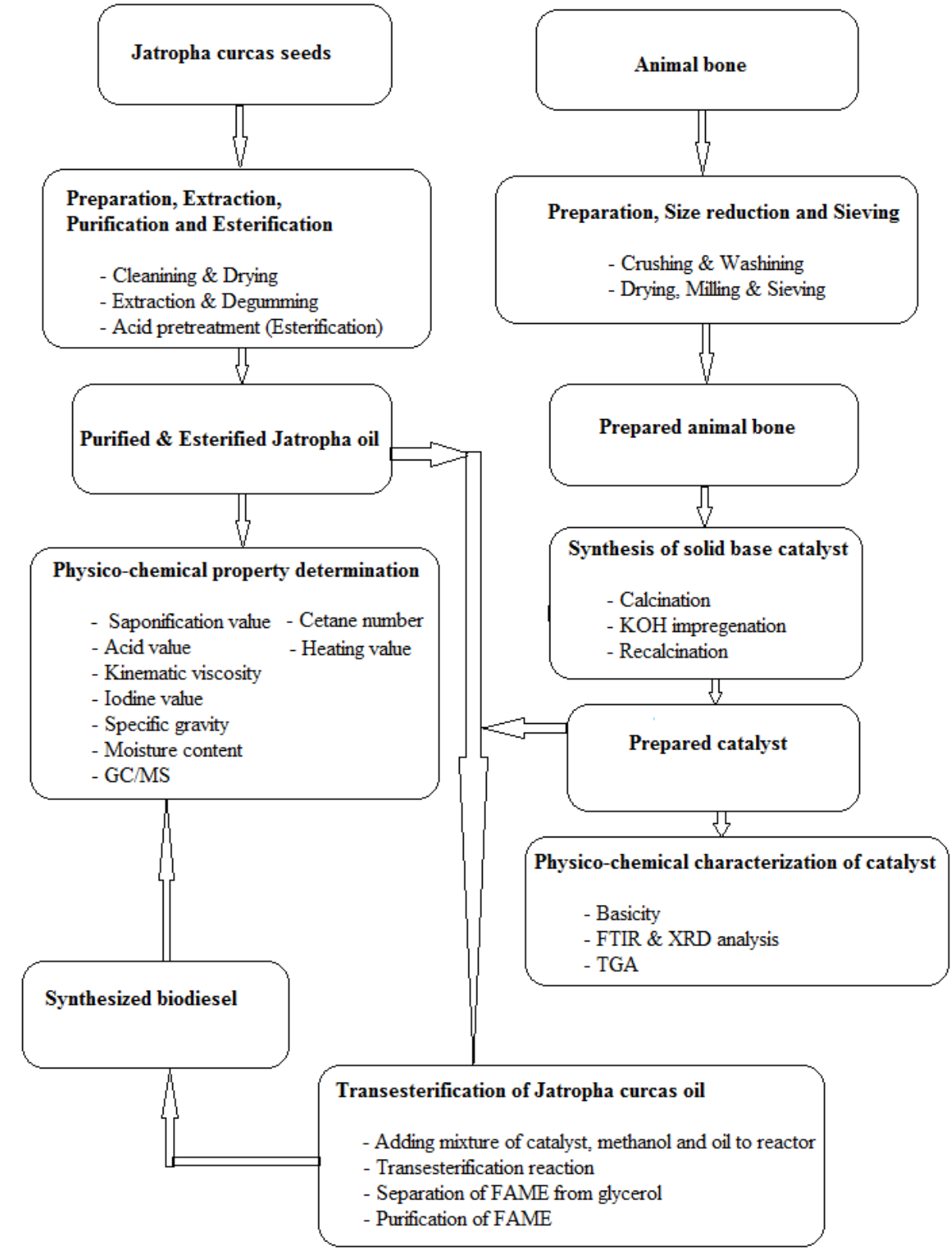


Figure 3-1: Frame work of the experiment

3.4 Screening of Catalyst

Screening of the catalytic activity of KOH-impregnated calcined animal bone catalyst was carried out to obtain catalyst with better loading. In order to compare the performance of the catalysts, the same reaction conditions were used for each catalyst in all the transesterification processes. Acid treated oil (50 ml), methanol to oil molar ratio of 11, catalyst loading of 8.5wt%, reaction temperature of 52.5 ^{o}C, and 3 hr reaction time. Catalyst with high basicity and FAME yield was selected for further investigation and its properties were studied.

3.5 Characterization of Catalyst

3.5.1 Determination of Basicity of the Catalyst

The basicity (total basic sites) of the catalyst was measured by titration method following the procedure reported in the literature (Intarapong et al., 2013). For each catalyst, 20 mg of sample was put in a beaker containing 5 mL of 0.02 M aqueous HCL, and the mixture was stirred for 1 hr at room temperature. The remaining acid was then titrated in the presence of three drop of phenolphthalein as indicator using 0.02 M aqueous KOH as the titrant. The titration was continued until the end point (colorless to pink color) is recognized. Basicity in mmol/g was calculated as follows:

$$Basicity = \frac{Volume\ of\ HCL * Normality\ of\ HCL - Volume\ of\ KOH * Normality\ of\ KOH}{Mass\ of\ catalyst} \ldots\ldots 3.1$$

3.5.2 Fourier Transform Infrared (FT-IR) Spectroscopy

The functional groups associated with the catalyst was identified using FT-IR spectroscopy (FTIR-65, Perkin-Elmer) equipped with KBr beam splitter. The solid samples were ground into fine powder and mixed with potassium bromide (KBr) and pressed. The infrared spectrum was recorded by passing a beam of infrared light through the sample. The spectra were obtained at a resolution of 4 cm^{-1} in the range of 4000 cm^{-1} to 400 cm^{-1}. The functional groups were determined by comparing the spectra obtained with the standard spectrum group frequencies.

3.5.3 X-ray Diffraction (XRD) Spectroscopy

Crystalline phases of the catalyst were identified by conducting XRD measurements. Miniflex 300/600 powder X-ray diffractometer with the Cu Kα radiation using an acceleration voltage of 40 kV and a current of 15 mA, over a 2θ range of 10–80^{o} at a scanning speed of 10^{o}/min were used

to record the XRD patterns of the catalyst. The resultant intensity data was processed by using in-built diffraction software Match! 3 to monitor the peak position.

3.5.4 Thermogravimetric Analysis (TGA)

Thermal stability characteristics of the KOH-impregnated calcined animal bone catalyst was determined using Thermogravimetric analyzer (DSC-TGA instrument, model: SDT Q600 V20.9 Build 20). The programme heating range was from room temperature to 800 ^{o}C at a heating rate of 10 ^{o}C/min under a nitrogen atmosphere with a flow rate of 100 ml/min to remove all corrosive gases and avoid thermoxidative degradation. The measurement was done for 77.313 mg sample. The thermal degradation onset temperature and the thermal degradation weight loss of composites were recorded and analyzed.

3.6 Extraction and Purification of Jatropha Oil

3.6.1 Extraction of Jatropha Oil

Jatropha oil was extracted using mechanical press (Bielenberg ram press) (Appendix E) found in AETDP workshop, Ministry of Water, Irrigation and Electricity. Prior to oil extraction, seeds were first cleaned from dust, sand, small stones and heated in full sunlight on a black plastic sheet for several hours. A black color mixture was obtained from the extraction and it is kept in closed vessel to settle for a week. The crude oil was then collected after it is separated from the sediment by simple decantation.

3.6.2 Degumming

Water degumming was done to remove hydrated phosphatides or gums to avoid rancidity (increase in free fatty acid) and gum deposits on storage of oil for long time. Initially a sample of oil was taken and heated under stirring to 70 ^{o}C. Then 3 % distilled water (which first was heated to approximately 90 ^{o}C) was added to the heated sample and kept in stirring (350 rpm) for 1 hr at 70^{o}C and then allowed to cool to room temp. The white-formed precipitate was separated and the degummed oil was dried at 100 ^{o}C for 30 minutes. The purified oil was then stored for further characterization.

3.7 Two-step Acid-base Catalyzed Transesterification of Jatropha Oil

A two-stage process was used for the transesterification of jatropha oil, acid-catalyzed esterification and base-catalyzed transesterification reaction. The first step will be used to reduce

the high free fatty acid (FFA) content of Jatropha oil below 1% by converting it into esters and in the second step transesterified Jatropha oil (biodiesel) was produced in the presence of alcohol and base catalyst.

3.7.1 Acid-catalyzed Esterification

The purified Jatropha oil was poured into a 500 ml three-necked round bottom flask equipped with agitator and water-cooled condenser (Appendix E) and heated to a temperature of 60 ^{o}C. A mixture of concentrated H_2SO_4 (1 wt%) with methanol (13 wt%) (based on weight of oil) was heated separately at 50 ^{o}C and then added to the preheated oil in the flask. The mixture was stirred constantly at 600 rpm for 1:30 hr. After completion of the reaction, it was transferred to separator funnel and allowed to settle for 2 hr. the methanol–water mixture separated at the top layer was removed and the oil with lower content of FFA was settle at the bottom layer. The esterified oil at the bottom layer was collected and washed with hot distilled water (about 90 ^{o}C) again and again until the color of the washing water clears. Finally, the treated oil was dried in oven at 100 ^{o}C for 6 hr and titrated to check the acid value. The esterification reaction was continued until the FFA content of the oil reduced below the limit (1%).

3.7.2 Base-catalyzed Transesterification of Jatropha Oil

For transesterification reaction, the same experimental set up was used as employed for acid pretreatment, a 500 ml three-necked round-bottom flask fitted with a water-cooled condenser and agitator (Appendix E). A known amount of catalyst was first activated by dispersing it in a known volume of methanol at 40 ^{0}C with constant stirring (500 rpm) for 40 min. After the catalyst activation, 50 ml of acid treated oil (heated to reaction temperature) was added into the mixture under vigorous stirring. The transesterification reaction was carried for 3 hr under atmospheric pressure. After completion of the transesterification, the reaction mixture was cooled and the catalyst was removed through simple filtration. The mixture was then allowed to settle for 24 hours in a separating funnel. FAME was obtained at the top layer while glycerol, excess methanol, catalyst and any soap formed during the reaction was obtained at the bottom. The FAME was then poured into a separate beaker and enter in to a rotary evaporator (40 to 45 ^{o}C) to remove the remaining methanol. The FAME was then stored for further characterization. For catalyst stability/reusability test, the filtered catalyst was dried fully for reutilization.

The yield of the transesterification reaction, which is used to evaluate catalytic performance of the prepared heterogeneous solid base catalyst, was determined by comparing weight of FAME produced to weight of esterified oil used.

$$Yield\ (\%) = \frac{weight\ of\ fatty\ acid\ methyl\ ester}{weight\ of\ oil\ used} * 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.2$$

3.8 Characterization of Purified Jatropha Oil and Biodiesel

3.8.1 Determination of Moisture Content

The sample was weighed and put into crucible. It was then placed in an oven at 105 ^{o}C. The sample was taken out in every 2 hours from the oven and placed in the desiccator for 30 minutes to cool and re-weighed till constant weight is obtained. The percentage moisture content in the sample was then calculated using the formula below:

$$Moisture\ content, \% = \frac{W1 - W2}{W1} * 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.3$$

Where, W1 is the weight of the sample before drying

W2 is the weight of the sample after drying

3.8.2 Determination of Kinematic Viscosity

Viscosity of the sample was measured using Vibro viscometer according to the test method recommended by ASTM (D445-04) (ASTM D445-04, 2004) . The sample was kept in the water bath heated by thermostat until it reaches 60 ^{o}C. After that the sample was poured in to the holder and then tip of the vibro viscometer was inserted in to the sample and the viscosity (mpa.sec) reading was recorded from the controller when the temperature of the sample reached 40 ^{o}C. The Kinematic viscosity was calculated using the formula below:

$$\upsilon = \frac{\mu}{\varrho} * 10^3 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.4$$

Where, μ is dynamic viscosity, mpa.sec

υ is kinematic viscosity, mm^2/s

ϱ is density, kg/m^3

3.8.3 Determination of Specific Gravity and Density

Density meter was used to measure the density of the sample according to the test method recommended by ASTM (D4052-96) (ASTM D4052-96, 2002). The sample was injected using syringe to the density meter and density of the sample was recorded from density meter screen at 15 ^{o}C.

3.8.4 Determination of Saponification Value

A weighted quantity of sample (5 g) was added to 250 ml conical flask and 25 ml of 0.5M ethanolic potassium hydroxide solution, which is prepared by dissolving KOH pellet in 95% ethanol, was added to the oil. A reflux condenser was connected to the flask and allowed to boil gently for one hour while stirring.

Five drops of phenolphthalein indicator, was added to the warm solution and then titrated with 0.5M HCl until the pink color disappeared. The same procedure was followed for the blank solution (all chemicals except oil). The saponification value (SV) mgKOH/g was calculated by the following formula:

$$Saponification\ value = \frac{56.1 * N * (Vb - Vs)}{w} \quad \ldots\ldots 3.5$$

Where, N is concentration of Hydrochloric acid

Vb is volume of HCL solution used in blank (ml)

Vs is volume of HCL solution used in sample (ml)

w is weight in gram of sample taken

3.8.5 Determination of Acid Value or Acid Number

Standard alcoholic potassium hydroxide solution (0.1 N) was prepared by dissolving KOH (pellet) with 95% v/v ethanol. The solution was filtered and stored in brown bottle for five days. Furthermore, a mixture of 95% ethanol and diethyl ether in a ratio of 1 to 1 by v/v was prepared by mixing 250 ml diethyl ether and 250 ml of ethanol.

A weighted quantity of sample (2 g) was dissolved in 25 ml of 1 to 1 mixture of ethanol and diethyl ether in 250 mL conical flask, 4 drops of phenolphthalein indicator was then added and titrated against 0.1 N ethanolic KOH solution. The content was constantly stirred until a pink color which persisted for 15 seconds was obtained. The volume of 0.1 N ethanolic KOH (V) for the sample titration was noted. The acid value in mgKOH/g was calculated by using the following equation:

$$Acid\ value\ (AV) = \frac{V * N * 56.1}{M} \quad \ldots\ldots 3.6$$

Where, V is the volume of 0.1 N ethanolic KOH solution expressed in milliliters

N is concentration of ethanolic KOH

m is the mass of a sample in grams

3.8.6 Determination of Ash Content

A weighted quantity of sample (20 g) was added into weight measured crucible then, placed in the furnace at 500 ^{0}C for an hour. After all organic substance burned out, placed in the desiccators till it attain room temperature. The weight of residual inorganic substance was measured. Ash content was calculated using the following formula:

$$Ash, \% = \frac{W1}{W2} * 100 \quad \ldots 3.7$$

Where, W1 is the weight of the sample after drying

W2 is the weight of the sample before drying

3.8.7 Determination of Iodine Value

The iodine value of the sample was determined by Wiji's method (AOAC 993.20) in JIJE analytical testing service laboratory. 0.4gm of the sample was weighed into a conical flask and 15 ml cyclohexane and glacial acetic acid mixture was added to the test sample and swirl to dissolve completely. Then 25 ml Wiji's solution was added and swirl to mix. The flask was then stored for 1 hour at ambient temperature in the dark. After completion of the reaction, the flask was removed from the dark and 20 ml of 10% KI solution and 150 ml water was added and gradually titrated with 0.1N standard sodium thiosulphate ($Na_2S_2O_3$) solution with constant and vigorous shaking until yellow color has just disappeared. A few drops of starch indicator (1-2 ml) was added and continued titrating until blue color has just disappeared. The volume of 0.1N sodium thiosulphate was recorded. The same procedure was followed for the blank solution (all chemicals except oil). The iodine value (IV) g I_2/100g was calculated by the following formula:

$$Iodine\ value\ (IV) = \frac{[(Vb - Vs) * N * 12.69}{w} \quad \ldots 3.8$$

Where, Vb is volume of $Na_2S_2O_3$ used for blank (ml)

Vs is volume of $Na_2S_2O_3$ used for sample (ml)

N is normality of $Na_2S_2O_3$ solution

w is weight of sample (g)

3.8.8 Determination of Higher Heating Value

The higher heating value (energy content per unit quantity) of the sample was determined using oxygen bomb calorimeter in Geological Survey of Ethiopia analytical testing laboratory.

3.8.9 Determination of Flash Point

A flash point of the biodiesel was determined using an open cup method according to the test method recommended by ASTM (D93-02) (ASTM D93-02, 2002). A cup was filled with the biodiesel up to a mark (about 75 ml) and was heated by a Bunsen burner. A hand thermometer was inserted into the cup to read the temperature. A small open flame was maintained from an external supply of natural gas. Periodically, the flame was passed over the surface of the oil. When the flash temperature was reached, the surface of the biodiesel caught with fire. The temperature at this moment was noted and reported as flash point temperature.

3.8.10 Determination of Fatty Acid Composition

The fatty acid composition of sample was determined using Gas Chromatography-Mass Spectrometer (GC-MS) in LIDI. The analysis was performed with Agilent 7890A/5975C GC-MS system. Samples were injected by a sampler injector. The data, obtained using MS-Agilent Technologies EMS detector and processed using Chemstation software, were used to obtain fatty acid composition of sample.

3.9 Experimental Design for Base-catalyzed Transesterification Reaction

To design the experiment and to obtain suitable predictive model and optimum reaction conditions of transesterification reaction using KOH-impregnated calcined animal bone catalyst, Design Expert software, Version 7.0.0 was used. The design of experiment selected was response surface method coupled with Box-Behnken Design (BBD). This design consisted of seventeen randomized experiments with five replicates at the center point to minimize error.

The yield of fatty acid methyl esters was tested against three input variables, reaction temperature, molar ratio of methanol to oil and catalyst loading. The reaction time of 3 hr and rotational speed of 500 rpm was chosen to obtain maximum FAME yield at atmospheric pressure based on literature on heterogeneous alkali transesterification. Table 3-1 shows the coded levels of the process parameters used in the experimental design for base-catalyzed transesterification reaction.

Selection of levels for each factor was based on the literature reports. The lower level of temperature was 40 °C since below that the reaction rate is relatively slow. The upper level of temperature, 65 °C, was limited by boiling point of methanol. Above this temperature the mass transfer on the three phases interface is limited by the formation of the methanol bubbles. Levels

of methanol to oil molar ratio between 8:1 and 14:1 and 5-12 wt% for the catalyst loading were selected.

Table 3-1: Coded levels of the process parameters used in the experimental design

Parameters (Factors)	Factor Coding	Unit	Coded levels		
			-1	0	+1
Methanol to Oil ratio	A	-	8	11	14
Catalyst loading	B	Wt%	5	8.5	12
Reaction temperature	C	^{0}C	40	52.5	65

Aided by design expert software, model was fitted to the experimental results. Adequacy of the model was determined by evaluating the lack of fit, coefficient of determination (R^2) and the Fisher test value (F-value) using design expert software analysis of variance (ANOVA) and significance of the model and variables were determined at 5% probability level ($P<0.05$).

3.10 Reaction Kinetics for Base-catalyzed Transesterification Reaction

To determine the kinetics of the reaction, the effects of temperature and time were investigated. Since the amounts of methanol used were in sufficient amount to convert the oil into FAME, thus, the reaction equilibrium shift towards the formation of fatty acid methyl esters. Thus, the reverse reaction could be ignored and change in concentration of the catalyst during the course of reaction can be assumed to be negligible. Assuming that the transesterification reaction is carried out in one step, transesterification reaction rate can be calculated through the following equation (Birla et al., 2012).

$$-r_{TG} = \frac{-d[TG]}{dt} = k'.[TG].[KOH]^3 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.9$$

Where, [TG] is the concentration of triglycerides

[ROH] is the concentration of methanol

k' is the equilibrium rate constant

This overall reaction follows a second order reaction rate law. However, because of the high methanol to oil molar ratio, the change in methanol concentration can be considered as constant during reaction and can be considered as a pseudo-first order reaction. Therefore, the reaction rate could be expressed as follows:

$$-r_{TG} = \frac{-d[TG]}{dt} = k.[TG] \ldots 3.10$$

Where, k is the modified reaction constant which is equal to k'. $[ROH]^3$

I assumed that the initial triglyceride concentration was $[TG_0]$ at time t = 0, and at time t it falls down $[TG_t]$. The integration of above equation for t = 0, [TG] = $[TG_0]$ and at t = t, [TG] = $[TG_t]$ gives the following equation.

$$\ln[TGo] - \ln[TGt] = k.t \ldots 3.11$$

From mass balance,

$$X_{ME} = 1 - \frac{[TG_O]}{[TG]} \ldots 3.12$$

On integration and rearrangement gives,

$$-\ln(1 - X_{ME}) = k.t \ldots 3.13$$

Where, X_{ME} is methyl ester conversion

The methyl ester conversion (X_{ME}) and FAME yield were assumed to be each other as:

$$[TG] = [TG_O](1 - X_{ME}) \ldots 3.14$$

$$Yield = \frac{[FAME]}{[TG_0]} \ldots 3.15$$

$$[TG] = [TG_O] - [FAME] \ldots 3.16$$

Substituting 3.15 in to 3.16 gives,

$$[TG] = [TG_O](1 - Yield) \ldots 3.17$$

According to 3.14 and 3.17 X_{ME} is equal to Yield.

To determine the activation energy Arrhenius equation will be used. The equation establishes a relation between reaction rate constant (K), temperature (T) and activation energy (E_a) as follows.

$$k = k_o \exp\left(\frac{-E_a}{RT}\right) \ldots 3.18$$

Where k_0 and R are frequency factor and universal gas constant, respectively. This equation could be rewritten as follows.

$$\ln(k) = -\frac{E_a}{RT} + \ln(k_o) \ldots 3.19$$

By plotting the diagram of ln (k) vs. 1/T, the slope is equal to $-E_a/R$ and the intercept will be ln (k_0).

4. RESULT AND DISCUSSIONS

4.1 Influence of KOH Loading on the Catalytic Activity

KOH-impregnated calcined animal bone catalysts were prepared by impregnation of KOH varying from 8 to 16 wt% on animal bones calcined at 600 ^{o}C followed by drying and calcination at 900 ^{o}C and were used to catalyze the transesterification reaction. The reaction was carried out over the solid base catalyst (8.5 wt%) with 11:1 methanol to oil molar ratio at reaction temperature of 52.5 ^{o}C and 500 rpm stirrer speed for 3 hr, and the FAME yield is listed in Table 4-1, in which are also included the basicities of the catalysts measured by the approximated acid titration method.

Table 4-1: Basicity and methyl ester yield for all catalyst samples

Catalyst name	Basicity (mmol/g)	FAME yield (%)
N	0.21	38.6
B	1.42	62.3
BI-8	2.58	70.0
BI-10	3.01	76.8
BI-12	4.21	84.2
BI-14	3.26	81.8
BI-16	3.14	78.6

The results shown in Table 4-1 revealed that the support showed less catalytic activity for the reaction as compared to KOH-impregnated calcined animal bone catalysts where their FAME yield exceeded 70%. In regard to basicity, when KOH was loaded on the surface of calcined animal bone, the basicity of the catalyst increased significantly, which indicated that catalytic activity was strongly dependent on the basicity of the catalyst.

Moreover, as illustrated in Table 4-1, the FAME yield was increased with increasing the amount of KOH loaded on calcined animal bone, accompanied with the increase in the basicity of the catalysts. A maximum conversion of 84.2% and the highest basicity of 4.21 mmol/g were obtained at 12 wt% KOH loading. However, further increase of the loading amount of KOH beyond 12 wt% led to the decrease in the resulting basicity of the catalyst and thus caused a decrease in the FAME yield. This was possibly due to agglomeration of the active KOH phase during calcination and/or

the covering of the basic sites by the excess KOH, and hence lower the surface area of the active components, which leads to the reduction in catalytic activity. The optimum loading amount of 12 wt% KOH was therefore, chosen for further investigation.

4.2 Catalyst Characterizations

4.2.1 Fourier Transform Infrared (FT-IR) Spectroscopy

Analysis on presence of functional groups on the surface of the catalyst was conducted using Fourier Transform Infrared (FT-IR) spectroscopy as shown in Figure 4-1.

The FTIR spectrum of ground animal bones showed absorption bands at 3442 cm^{-1} and 2927 cm^{-1}, which are attributed to the stretching vibrational bands of the O–H group associated with the physically absorbed water (Jazie et al., 2013). In addition, the absorption bands at 470-670 cm^{-1} and 1035 cm^{-1} are corresponding to the O-P-O and P-O stretching vibrations, respectively. Besides, the characteristic absorption of C=O, the bands between 1400 cm^{-1} and 2000 cm^{-1} indicates the presence of carbonate (Obadiah et al., 2012; Tang et al., 2013). For the support (Figure 4-1 (B)), many absorption peaks are transformed due to calcination. The O-H stretching vibration at 3446 cm^{-1} is reduced and that at 2927 cm^{-1} is disappeared completely. In addition, the broad peaks at 1035 cm^{-1} and 470-670 cm^{-1} of the pure animal bone is converted in to a high intensity absorption peak at 1043 cm^{-1} and 575 cm^{-1} for the support, respectively. For the solid base catalyst (Figure 4-1 (BI-12)), the addition of KOH affected the structure of the support. The O-H stretching vibration at 3446 cm^{-1} is converted in to broad peak between 3000 cm^{-1} and 3500 cm^{-1}. New peak at 872 cm^{-1} arises, which indicated the presence of a new functional group in the catalyst (K–O–P group or compound) (Nisar et al., 2017).

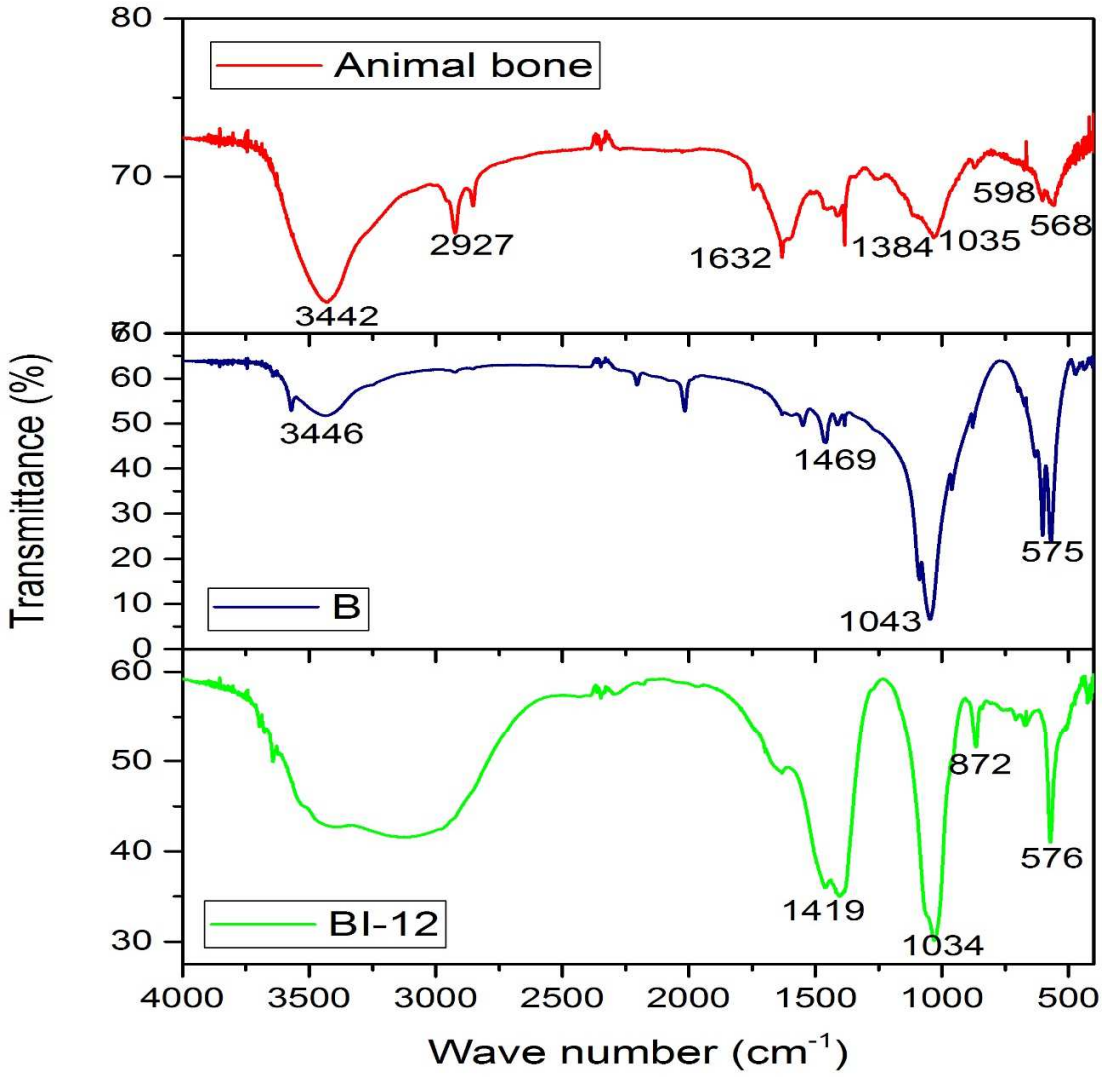


Figure 4-1: FTIR spectra of pure animal bone, support and KOH-impregnated calcined animal bone catalyst

4.2.2 X-ray Diffraction (XRD) Spectroscopy

The powdered X-ray diffraction patterns of animal bone, support (B) and solid base catalyst (BI-12) are shown in Figure 4-2. The XRD pattern of the animal bone exhibit peaks at 24.14, 29.18^{o} and broad peak at 2θ^{o} = 30-35^{o}, the patterns indicate the presence of calcium on those bones in two different phases of $CaCO_3$, known as aragonite and calcite. Similar observations were reported by

(Lesbani et al., 2015; Nisar et al., 2017). Diffraction 2θ from JCPDS for calcium oxide being used is at 32.2, 37.3, 53.8, 64.1, and 67.3° (Lesbani et al., 2015). The XRD pattern of the support or animal bone calcined at 600 °C shows a present of calcium oxide at 32.24 and 53.68°. Additionally, the peaks at 26.24, 40.12, 47.04, and 49.8° are attributed to the presence of hydroxyapatite (Farooq & Ramli, 2015). Impregnation of KOH and subsequent calcination showed a few additional peaks on the XRD pattern of the KOH-impregnated calcined animal bone catalyst than those observed with the support.

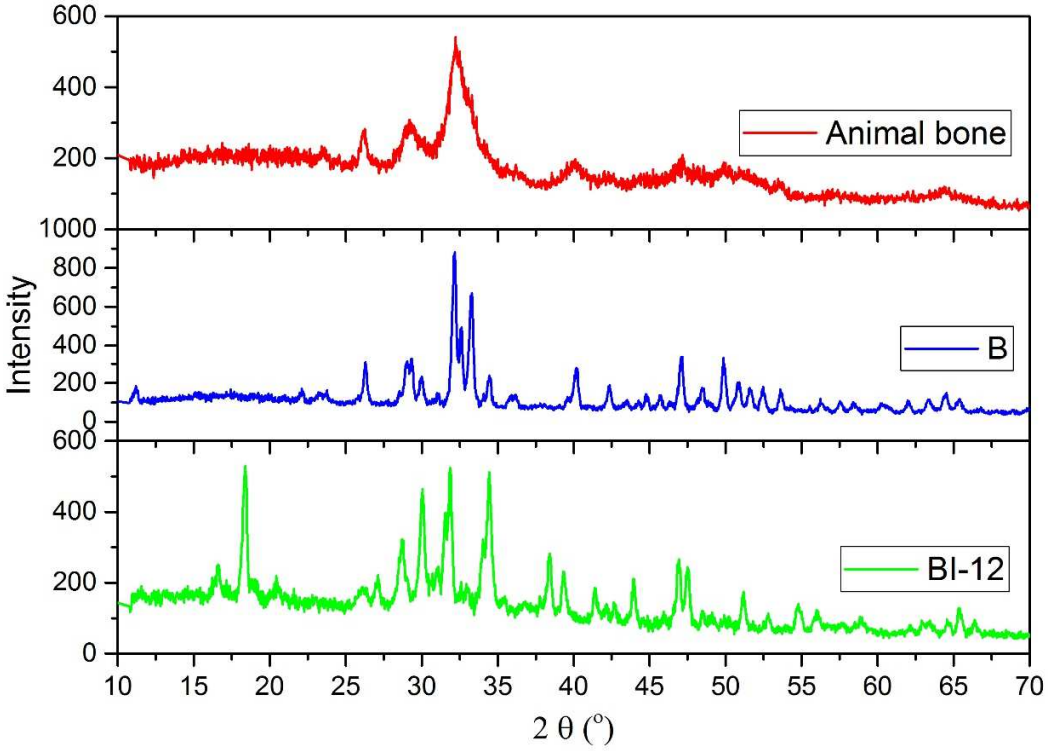


Figure 4-2: XRD patterns of pure animal bone, support and KOH-impregnated calcined animal bone catalyst

4.2.3 Thermogravimetric Analysis (TGA)

The thermal behavior of KOH-impregnated calcined animal bone catalyst is shown in Figure 4-3. The first weight loss at lower temperature (<150 °C) corresponds to the loss of water in the form of evaporation of adsorbed water from internal and external surfaces of the catalyst. The second weight loss in the temperature range of 200–400 °C, was due to the decomposition of KOH. An

additional very small and steady weight loss is also observed in the temperature range 500-600 ºC. This may be attributed to the decomposition of the residual hydroxyl groups bonded to the oxide lattice (Takase et al., 2014). The curve further shows that weight loss above 600 ºC is almost constant, thus confirms the completion of decomposition process.

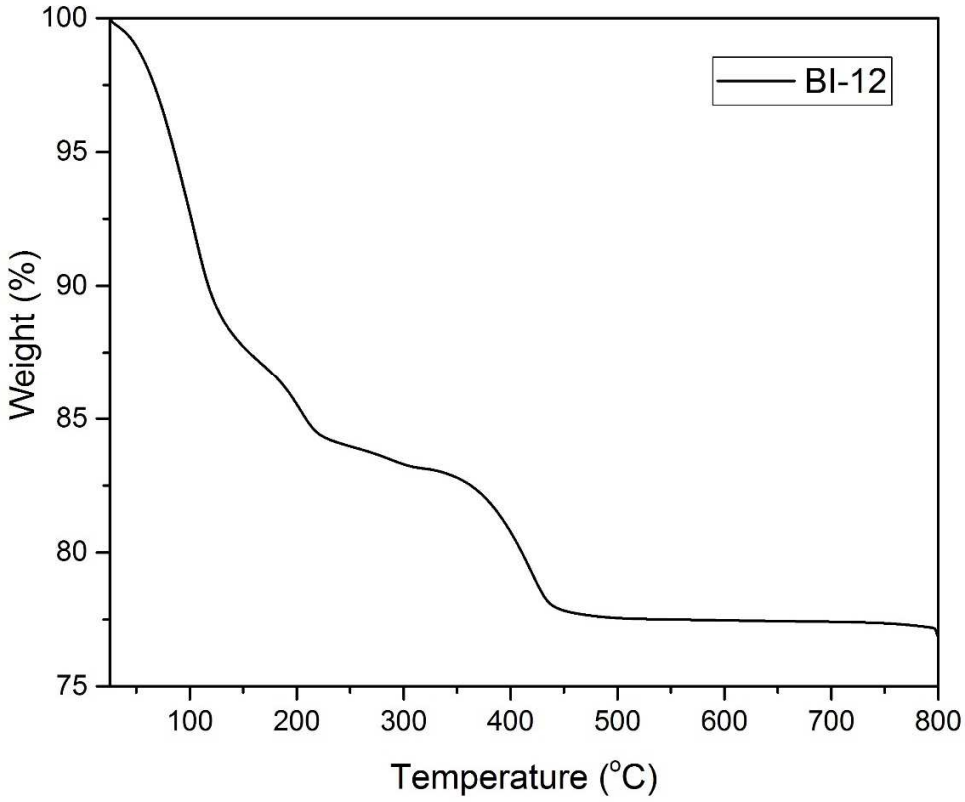


Figure 4-3: Thermal behavior of KOH-impregnated calcined animal bone catalyst

4.3 Extraction and Purification of Jatropha Oil

About 12 kg of dried Jatropha curcas L. seeds were used to extract oil using mechanical press (Bielenberg ram press) found in AETDP workshop. Four liter of oil mixture (black in color) was obtained from the extraction. After kept a week 2.4 liter of crude oil was obtained by decantation. The yield of oil was 18.352%. The crude oil was further purified using degumming to remove hydrated phosphatides or gums.

4.4 Activity Test of BI-12 Catalyst in A Two-step Acid-base Catalyzed Transesterification

Free fatty acid and moisture content are key parameters for determining the viability of the vegetable oil transesterification process. For a successful transesterification using the alkaline catalyst, the FFA value of the oil should be less than 1%, which corresponded to the acid value of 2 mg KOH/g oil (as of oleic acid). This avoid soap formation, which has a potential of reducing catalyst efficiency and consequently reducing biodiesel yield. To reduce the acid value of Jatropha oil from 3.46 mg KOH/g oil to less than 2 mg KOH/g oil, acid-catalyzed esterification was employed as shown below.

$$\underset{\text{(FFA)}}{RCOOH} + \underset{\text{(Methanol)}}{CH_3OH} \xrightleftharpoons{H_2SO_4} \underset{\text{(Methyl ester)}}{RCOOCH_3} + H_2O$$

The reaction was conducted at 60 ^oC for one and half an hour with methanol to oil molar ratio of 13:1 and H_2SO_4 catalyst concentration of 1%w/w of oil. It was found that by the first acid-catalyzed esterification the acid value of the oil was reduced to 1.6 mg KOH/g oil, which is suitable for carrying the second step, base-catalyzed transesterification.

The base-catalyzed transesterification was carried out using a 500 ml round-bottom flask equipped with a reflux condenser and overhead stirrer. KOH- impregnated calcined animal bone (BI-12) was used as a catalyst. In order to investigate the activity of solid base catalyst (BI-12), a series of experiments was conducted. The stirrer speed and reaction time was adjusted to the desired conditions.

4.4.1 RSM and Model Fitting for Base-catalyzed Transesterification

In this work, the relationship between one response (biodiesel production yield) and three independent parameters (reaction temperature, catalyst loading and methanol to oil molar ratio) was studied. The results from the 17-run design including the actual and predicted values of yield of production as well as experimental points and the ANOVA are shown in Table 4-2 and Table 4-3 respectively. Aided by the Design Expert software (Version 7.0.0), the best fitting model was fitted to the experimental results and tested for significance.

Table 4-2: Box-Behnken experimental design matrix, experimental and estimated data for three-level-three-factors response surface analysis

Run	Methanol to Oil ratio	Catalyst loading, wt%	Reaction temperature, ^{o}C	Yield (%)		Residuals
				Experimental	Predicted	
1	11.00	8.50	52.50	81.90	83.22	-1.32
2	8.00	5.00	52.50	82.40	81.51	0.89
3	11.00	5.00	40.00	83.50	83.59	-0.087
4	11.00	12.00	65.00	83.00	82.91	0.088
5	14.00	5.00	52.50	88.80	88.24	0.56
6	11.00	8.50	52.50	83.20	83.22	-0.020
7	11.00	8.50	52.50	82.70	83.22	-0.52
8	14.00	12.00	52.50	76.80	77.69	-0.89
9	14.00	8.50	65.00	92.80	92.00	0.80
10	8.00	8.50	40.00	69.20	70.00	-0.80
11	11.00	8.50	52.50	84.40	83.22	1.18
12	11.00	12.00	40.00	73.00	71.64	1.36
13	14.00	8.50	40.00	77.10	77.57	-0.47
14	8.00	12.00	52.50	65.00	65.56	-0.56
15	11.00	8.50	52.50	83.90	83.22	0.68
16	8.00	8.50	65.00	81.20	80.73	0.47
17	11.00	5.00	65.00	96.10	97.46	-1.36

4.4.1.1 Development of Regression Model Equation

The regression model equation that correlates the response variable, yield of FAME, with the transesterification process variables, in terms of actual values after excluding the insignificant terms was given below. Diagnostics of the residuals and the difference between actual and predicted yield, indicated that no transformation is required to improve the yield model. The quadratic model to predict the biodiesel production yield in terms of coded factors is given in equation (4.1).

$$FAME\ yield\ (\%) = 83.22 + 4.71A - 6.62B + 6.29C + 1.35AB + 0.93AC - 0.65BC - 4.40A^2 - 0.57B^2 + 1.25C^2 \quad \ldots\ldots 4.1$$

Where, A= Methanol to oil molar ratio

B= Catalyst loading, wt%

C= Reaction temperature, ^oC

4.4.1.2 Model Adequacy Check

The regression model was found to be highly significant with the correlation coefficients of determination of R-Squared, adjusted R-Squared and predicted R-Squared having a value of 0.9879, 0.9725 and 0.8655 respectively.

Figure 4-4 shows the experimental values versus the predicted values for FAME yield using the model equation developed. This figure indicate that the model was successful in capturing the correlation between the reaction parameters to the response (yield) with correlation coefficients R^2=0.9879. It implies that 98.79% of the total variation in FAME yield is attributed to the experimental variables studied.

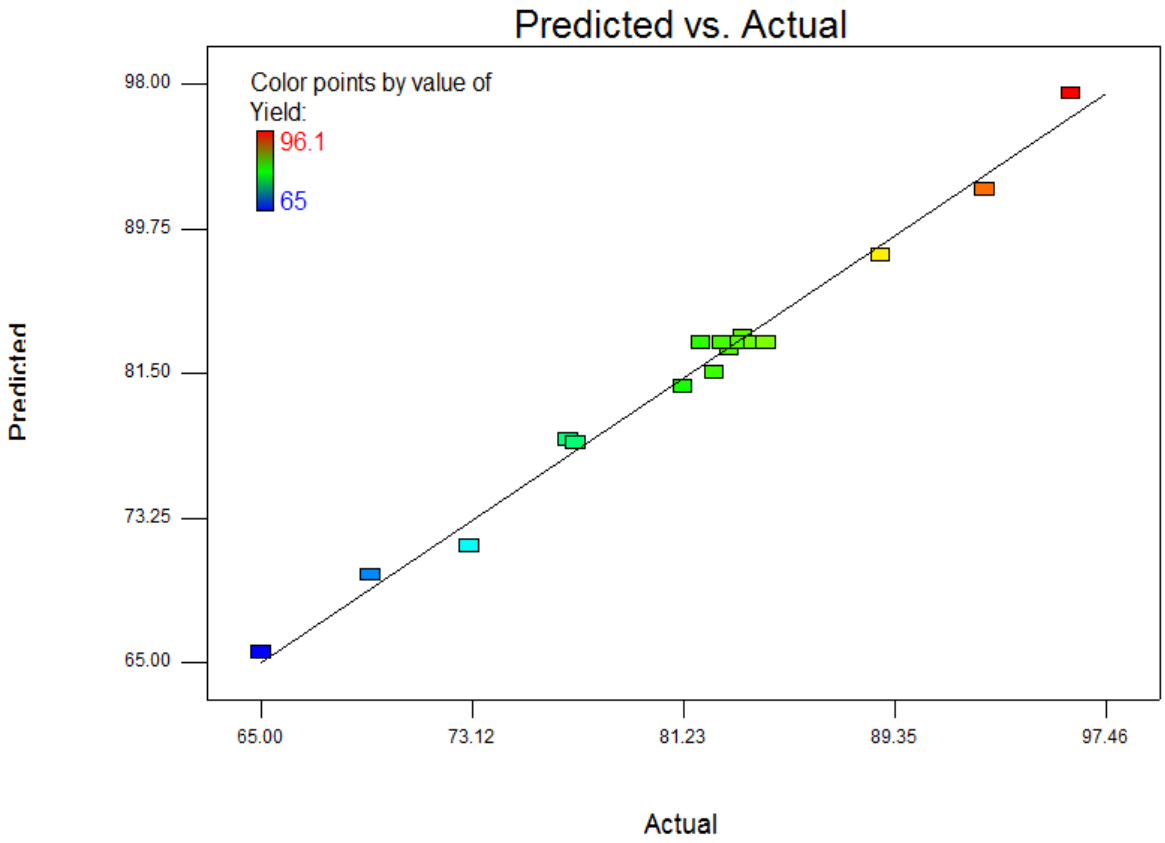


Figure 4-4: Predicted from model versus measured FAME yield

Fitness and significance of the model was further investigated with Design Expert software analysis of variance (ANOVA) as shown in Table 4-3. It can also present the effects of individual parameters and interaction of variables on the response. As can be seen the statistical analysis of variance revealed overall model p-value (probability of error value) less than 0.0001 which are very significant. On the other hand, lack of fit testing produced p-value of 0.1855 that indicates the models well fitted to all data. The ANOVA table also shows that the three parameters of methanol to oil molar ratio (A), catalyst loading (B) and reaction temperature (C) were highly significant and also quadratic term of methanol to oil ratio (A^2) is significant to the FAME yield based on the p-value less than 0.05. Values greater than 0.05 indicate the model terms are not significant.

Table 4-3: ANOVA for response surface model

Source	Sum of Squares	Df	Mean Square	F – value	P – value Prob > F	Remarks
Model	945.64	9	105.07	63.76	< 0.0001	Significant
A- Methanol to Oil molar ratio	177.66	1	177.66	107.81	< 0.0001	Significant
B- Catalyst loading	351.12	1	351.12	213.07	< 0.0001	Significant
C- Reaction temperature	316.26	1	316.26	191.91	< 0.0001	Significant
AB	7.29	1	7.29	4.42	0.0735	Not significant
AC	3.42	1	3.42	2.08	0.1927	Not significant
BC	1.69	1	1.69	1.03	0.3449	Not significant
A^2	81.42	1	81.42	49.41	0.0002	Significant
B^2	1.38	1	1.38	0.84	0.3906	Not significant
C^2	6.61	1	6.61	4.01	0.0854	Not significant
Residual	11.54	7	1.65			
Lack of fit	7.67	3	2.56	2.64	0.1855	Not significant
Pure Error	3.87	4	0.97			
Cor Total	957.18	16				

4.4.2 Effect of Process Variables on Transesterification Reaction

4.4.2.1 Effect of Individual Factors on FAME Yield

A. Effect of molar ratio of methanol to oil

Transesterification process consists of three consecutive reversible reactions where triglyceride is successively transformed into diglyceride, monoglyceride, and to glycerine and FAME. The molar ratio of methanol to oil is one of the important parameter that affects the conversion of triglycerides to esters. Stoichiometrically, the molar ratio of alcohol to triglyceride for the transesterification reaction is 3:1. Since the transesterification reaction is reversible, excess methanol can generally be used to improve the conversion of oil to esters. The transesterification of Jatropha oil is

equilibrium-limited reaction. In order to overcome the equilibrium limitation, the reaction was conducted in excess methanol in order to favor the forward reaction, where the un-reacted methanol could be easily separated and recycled through evaporation. In the present work, the effect of different molar ratios of methanol to oil was studied and the dependence of FAME yield on the molar ratio of methanol to oil (8:1-14:1) at constant catalyst loading of 8.5 wt% and 52.5 ^{o}C reaction temperature is shown in Figure 4-5.

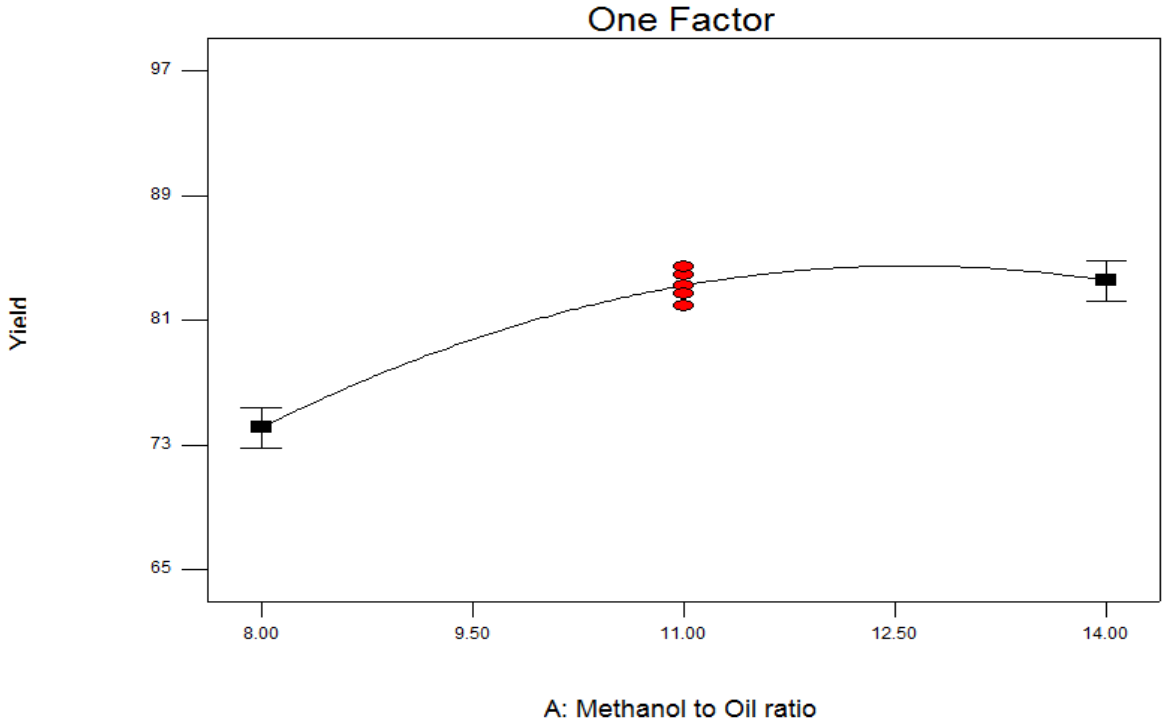


Figure 4-5: Effect of molar ratio of methanol to oil on FAME yield

As shown in Figure 4-5, with increasing molar ratio of methanol to oil, resulting in an increase in the reaction rate of transesterification of Jatropha oil with methanol, a high yield of FAME was observed. As the molar ratio is high enough, the methanol adsorption is probably not the rate determining step. Therefore, further increase in molar ratio had decrease the FAME yield possibly due to the dilution of the oil with the excess alcohol, which cover active site of the catalyst (catalyst deactivation). Hence excessive use of methanol decreased the conversion by shifting the equilibrium in the reverse direction. Therefore, use of lower molar ratio of methanol makes the process more economic and environmentally benign.

B. Effect of catalyst loading

Catalyst plays an important role in transesterification reaction. In this study, catalyst amount in the range of 5-12 wt% (relative to weight of Jatropha oil) on FAME yield was investigated and the results at constant methanol to oil molar ratio of 11:1 and 52.5 ^{o}C reaction temperature is shown in Figure 4-6. Increasing the amount of catalyst used in the transesterification beyond 5 wt% has no significant positive effect on FAME yield. Which indicate that high concentrations of alkaline catalyst form soap in the presence of high free fatty acids which results from emulsion formation between the soap and water molecules that leads to low FAME yields.

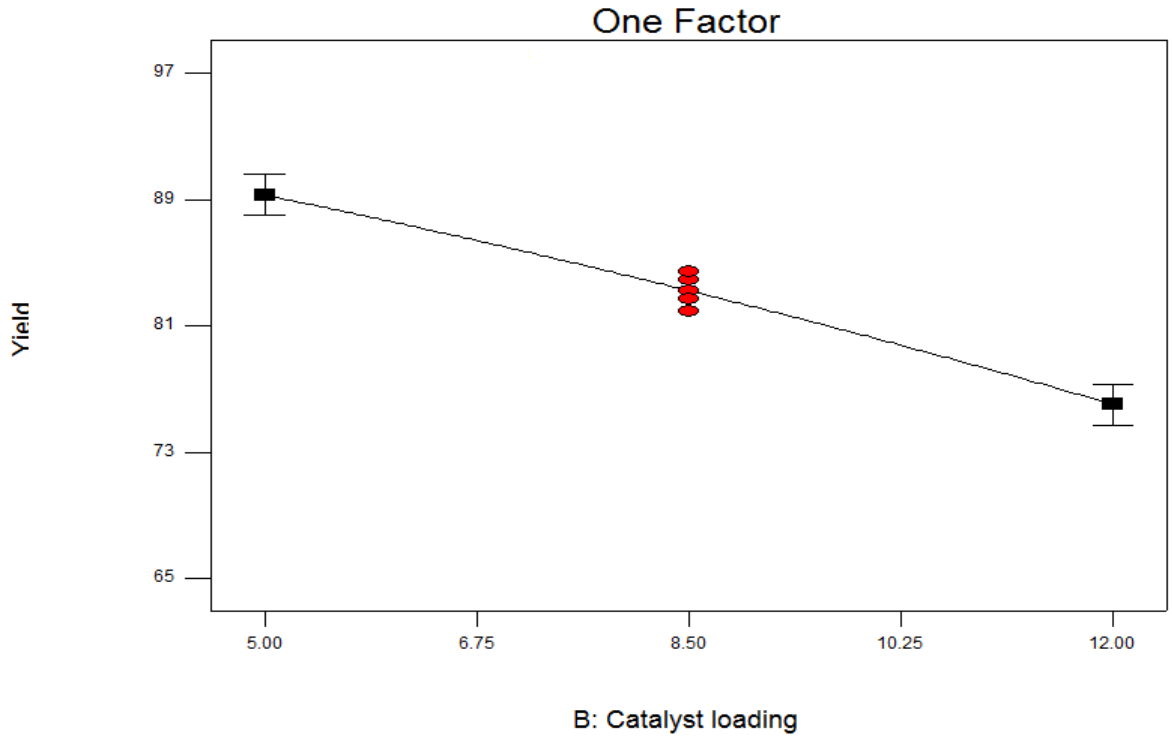


Figure 4-6: Effect of catalyst loading on FAME yield

C. Effect of reaction temperature

Figure 4-7 shows the effect of reaction temperature on the yield of the transesterification reaction at constant methanol to oil molar ratio of 11:1 and 8.5 wt% catalyst loading. It can be seen that with increasing reaction temperature the yields of fatty acid methyl ester (FAME) were increase significantly. The low rate of reaction at lower reaction temperature could possibly be due to the high viscosity of the oil at the lower temperatures, resulting in poor mixing between the phases of oil–methanol–catalyst.

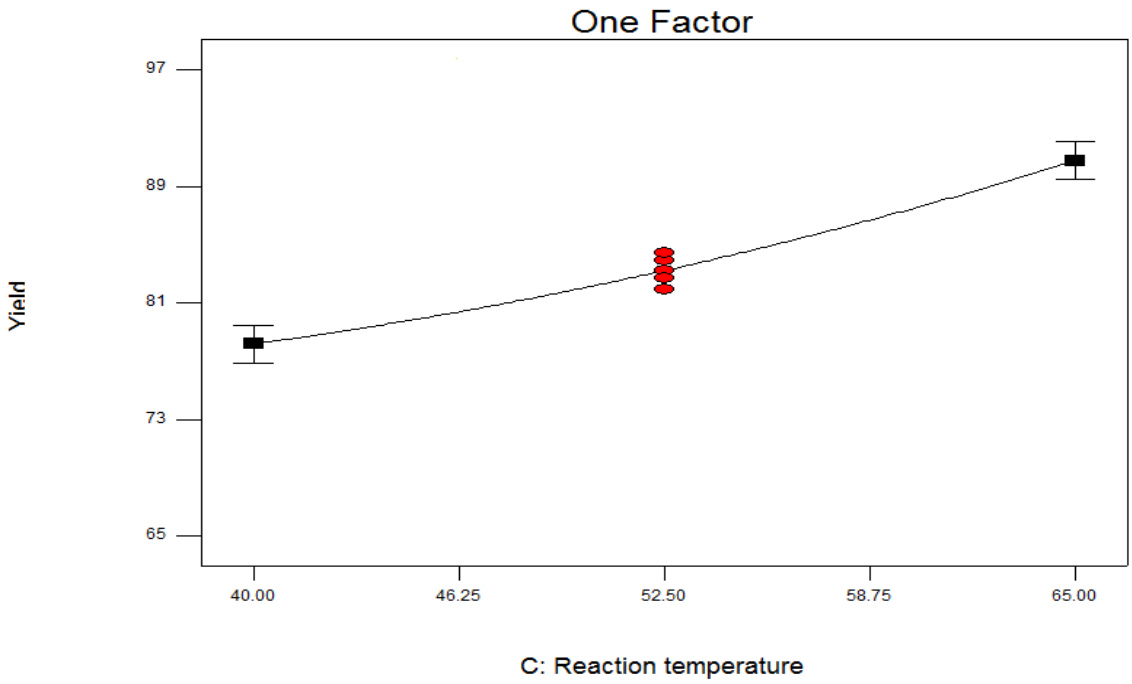


Figure 4-7: Effect of reaction temperature on FAME yield

4.4.2.2 Interaction Effect of Process Variables on FAME Yield

The relationship between the parameters were demonstrated clearly by plotting a-three-dimensional graph of the predicted FAME yield with two independent variables as shown in Figure 4-8 and Figure 4-9.

Figure 4-8 and Figure 4-9 shows the interaction between catalyst loading and molar ratio of methanol to oil, and catalyst loading and methanol to oil molar ratio on FAME yield respectively. As can be seen from the figures, conversion of oil to FAME initially increases by an increase in the methanol to oil molar ratio and reaction temperature at lower concentration of catalyst. However, at higher catalyst loading a reduction can be seen in the yield of biodiesel production. The decrease in conversion of oil to biodiesel at higher concentration of the catalyst can be attributed to the soap formation during the transesterification.

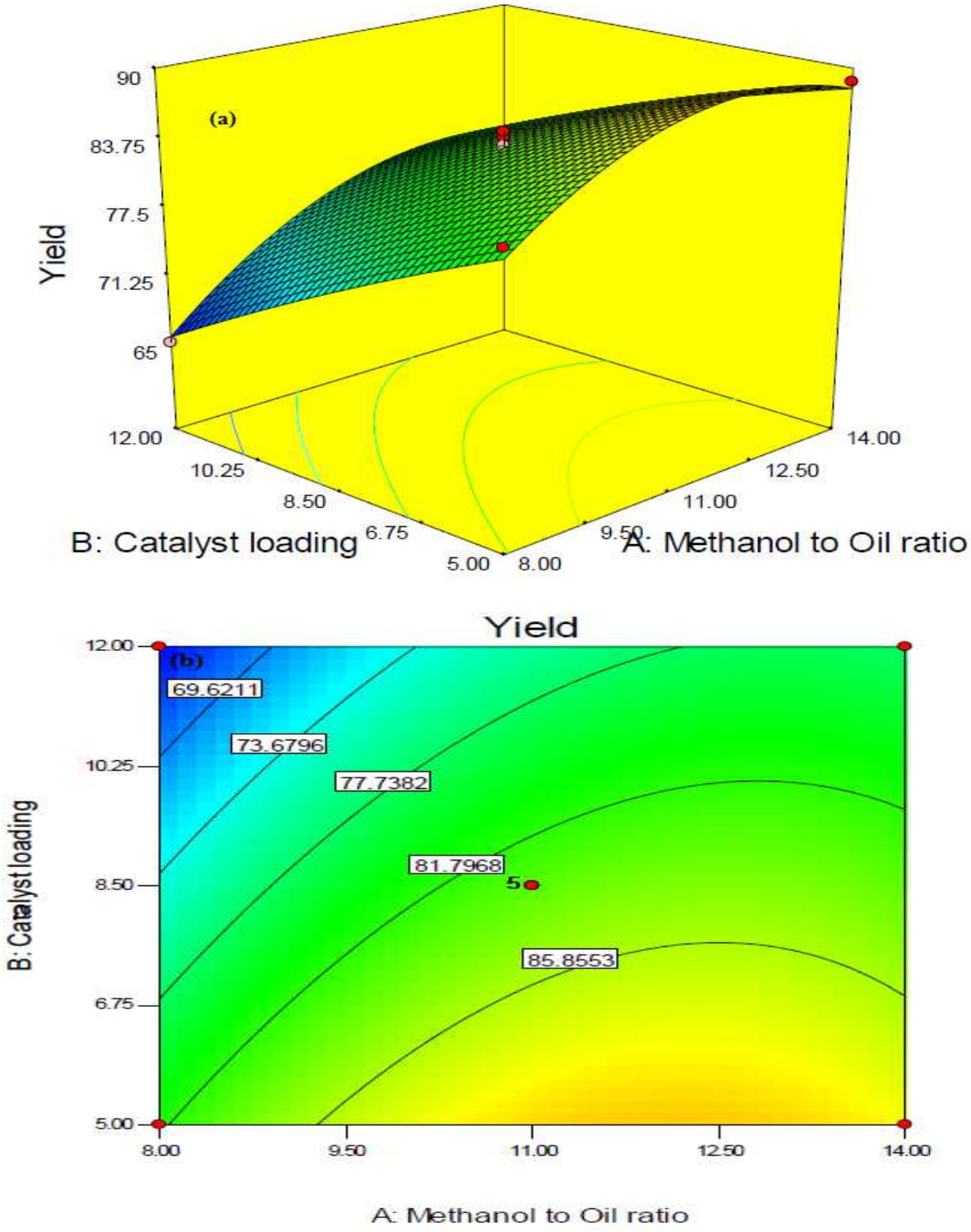


Figure 4-8: Interaction effect of catalyst loading and methanol to oil molar ratio at reaction temperature of 52.5 ^{o}C, a) 3D response surface plot and b) Contour plot

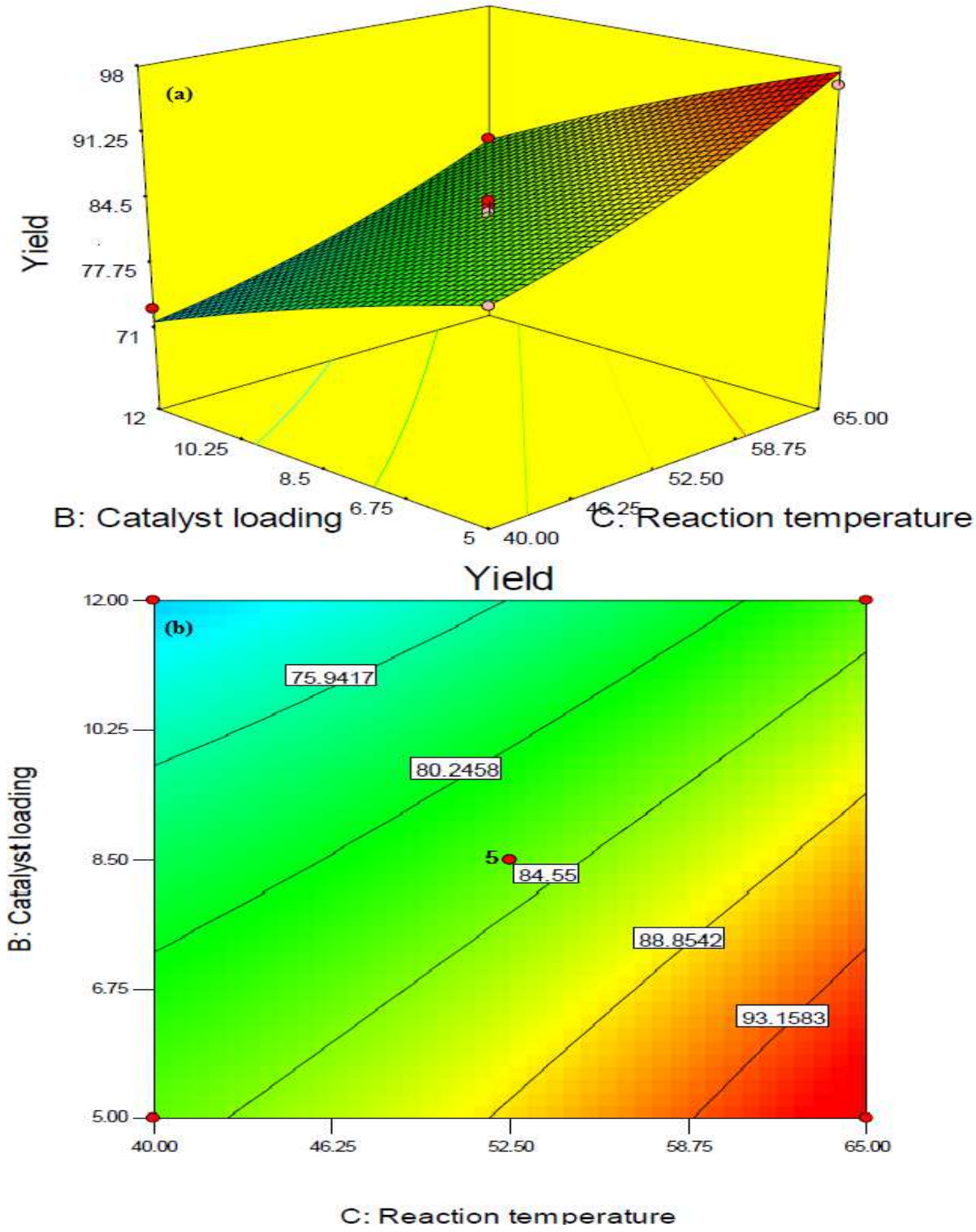


Figure 4-9: Interaction effect of catalyst loading and reaction temperature at methanol to oil molar ratio of 11:1, a) 3D response surface plot and b) Contour plot

4.4.3 Optimization of Process Variables

One of the main objectives of this study is to maximize the yield of biodiesel production from Jatropha oil using KOH-impregnated calcined animal bone catalyst. The optimum conditions for the three factors, i.e. molar ratio of methanol to oil (A), catalyst loading (B) and reaction temperature (C) were determined using the numerical optimization feature of the Design Expert software. The software generates combination of parameters that satisfy the requirement for the response and each of the factors. All the parameters and response with respectively high and low limits to satisfy the creations defined for the optimum condition are listed in Table 4-4. The optimum conditions obtained were then evaluated by the composite desirability, which has a value from 0 to 1, to determine the degree of satisfactory of the optimum conditions for the ultimate goal of response. Based on the predicted combination of parameters as shown in Table 4-5, triplicate experiments were conducted to validate the optimum conditions and FAME yield of 96.74% was obtained and the results are closely related with the data obtained from optimization analysis using desirability functions.

Table 4-4: Constrains for the factors and response in numerical optimization

Parameters	Ultimate goal	Experimental region	
		Lower limit	Upper limit
Methanol to oil molar ratio	In range	8	14
Catalyst loading (wt%)	In range	5	12
Reaction temperature (^oC)	In range	40	65
FAME yield (%)	Maximize	65	96.1

The produced biodiesel was analyzed directly after separating from glycerol by-product without passing through a washing step by water as it is the case when using homogeneous catalysts. Based on our results KOH-impregnated calcined animal bone catalyst has the potential to serve as a solid base catalyst for transesterification of oils into FAMEs.

Table 4-5: Results of optimization and model evaluation

Methanol to oil molar ratio	Catalyst loading (wt%)	Reaction temperature (^oC)	Yield (%)		Desirability
			Predicted	Measured	
11.56	5.08	64.05	97.26	96.74	1.0

4.4.4 Catalyst Reusability

Reusability/stability of the KOH-impregnated calcined animal bone catalyst in the transesterification of Jatropha oil with methanol, under the optimum reaction conditions, was studied. The catalyst was repeatedly used for four cycles. In each cycle the used catalyst was taken out from the reactor and dried fully for reutilization. The results are shown in Figure 4-10. Results indicated that no significant differences in FAME yields were observed between three runs. Which is around 90%, suggesting that the catalyst was durable and possessed activity to catalyze transesterification for at least three times. This decay could be due to the leaching of active sites to the reaction media. Leaching of active sites are due to the breakage of bond with the creation of CH_3O^- and K^+ ions; this is in agreement with the results of Baroutian et al. who investigated KOH supported on palm shell activated carbon catalyst for transesterification of palm oil (Baroutian et al., 2010).

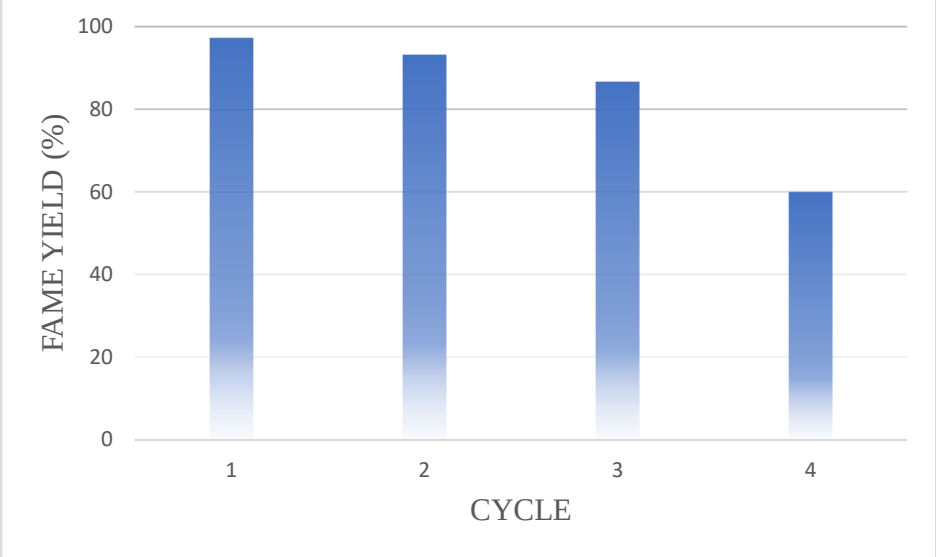


Figure 4-10: Reusability of catalyst

4.5 Physico-chemical and Fuel Properties of Purified Jatropha Oil and FAME

Table 4-6 presents the physical, chemical and fuel property of purified oil and FAME along with ASTM D6751 and EN 14214 Standards.

It was observed that the purified Jatropha oil has golden yellow color, pleasant smell and liquid physical state at room temperature. The chemical properties of the oil analyzed were presented

on table 4-6. The acid value 3.46 mgKOH/g was obtained which signifies substantial reduction of FFA was needed for base-catalyzed transesterification reaction. The saponification value was found to be 192.3 mgKOH/g. Oil with a saponification value of 200 mgKOH/g and above is regarded as high molecular weight fatty acid oil and is used in making of soaps.

Table 4-6: Physico-chemical characteristics of purified Jatropha oil and FAME along with American and European Standards

Property	Test method	Purified Jatropha oil	FAME	FAME standards	
				ASTM D6751	EN 14214
Moisture content, %w/w	-	0.036	0.021	-	-
Specific gravity	-	0.9176	0.8813	-	-
Density at 15ºC, Kg/m^3	ASTM D4052	917.6	881.3	-	860-900
Kinematic Viscosity, mm^2/s	ASTM D445	39.8	3.87	1.9-6.0	3.5-5.0
Acid value, mg KOH/g oil	ASTM D664	3.46	0.378	≤ 0.8	≤ 0.5
Saponification value, mgKOH/g	-	192.3	-	-	-
Free Fatty Acid	-	1.73	0.19	-	-
Iodine Value, gI_2/ 100g	AOAC 993.20	104.67	97.58	-	≤ 120
Higher heating value, MJ/Kg	-	37.48	39.52	-	-
Ash content, %	-	0.016	0.018	-	-
Flash point, ºC	ASTM D93	-	175	≥130	>101

Table 4-7 shows the GC-MS identification of major composition for FAME produced from Jatropha oil with KOH-impregnated calcined animal bone as catalyst. The produced biodiesel mainly consists of four fatty acid methyl esters: methyl isopalmitate (C16:0) 15.73%, methyl linoleate (C18:2) 36.20%, methyl oleate (C18:2) 38.73% and methyl stearate (C18:0) 9.33%. Its properties are shown in Table 4-6, which are within the described standards. The alkali-catalyzed transesterification clearly reduced the viscosity of the crude Jatropha Curcas L. seeds oil from 39.8 mm^2/s to 3.87 mm^2/s, and this value was closer to that of diesel (3.068 mm^2/s). The flash point

temperature of biodiesel relates to the ignition of the fuel, and the minimum value of this property is required for proper safety and handling of the fuel. Since the flash point of the biodiesel from Jatropha oil with KOH-impregnated calcined animal bone as catalyst was 175 ^{o}C. which is higher than both the American and European standards. Thus, the fuel is safe for handling and storage for some period of time. The Iodine value of biodiesel is a measure of the average amount of unsaturation of the biodiesel component. The result obtained in the present study indicates that the iodine value reduced by 6.77%, but still it was higher than diesel (93 gI_2/ 100g). The Acid value of the biodiesel produced was found to be 0.378 mgKOH/g. The result of which indicates that acid value of the biodiesel decreased significantly after the two-step acid-base catalyzed transesterification reaction. The test also indicated 5.16% energy content increment was obtained by transesterifying Jatropha oil. This is probably due to the presence of relatively higher number of hydrogen in fatty acid methyl ester than crude Jatropha oil.

Table 4-7: GC-MS identification of major components of the produced FAME

	Systematic Name	Synonyms	Residence Time (min.)	Area (%)	Molecular Formula
1	Pentadecanoic acid, 14-methyl ester	Methyl palmitate	46.353	15.73	$C_{17}H_{34}O_2$
2	9,12-Octadecadienoic acid, methyl ester	Methyl linoleate	48.066	36.20	$C_{19}H_{34}O_2$
3	9-Octadecenoic acid, methyl ester	Methyl oleate	48.126	38.73	$C_{19}H_{36}O_2$
4	Methyl stearate		48.324	9.33	$C_{19}H_{38}O_2$

4.6 Determination of Reaction Kinetics for Base-catalyzed Transesterification Reaction

To determine the kinetics of base-catalyzed transesterification reaction of Jatropha oil with methanol (11.56:1 methanol to oil molar ratio) in the presence of KOH-impregnated calcined animal bone catalyst (5.08 wt%) at the temperatures of 313, 321.3, 329.6, and 338 K under the stirring speed of 500 rpm at reaction times of 1, 2, 3, and 4 hr were investigated. Table 4-8 shows

yield/conversion (%) of oil into FAME at different times intervals and temperatures using KOH-impregnated calcined animal bone catalyst. By fitting the data between ln(1-X_{ME}) and t, a linear relationship was obtained which supports the hypothesis that the reaction is of pseudo-first order (Birla et al., 2012).

Table 4-8: Conversion (%) of oil into FAME at different times and temperatures

Temp. (K) / Time (h)	313	321.3	329.6	338
1	55.00	60.18	66.68	72.48
2	61.57	70.48	82.21	87.37
3	75.45	81.27	92.78	95.23
4	78.27	85.68	93.85	96.82

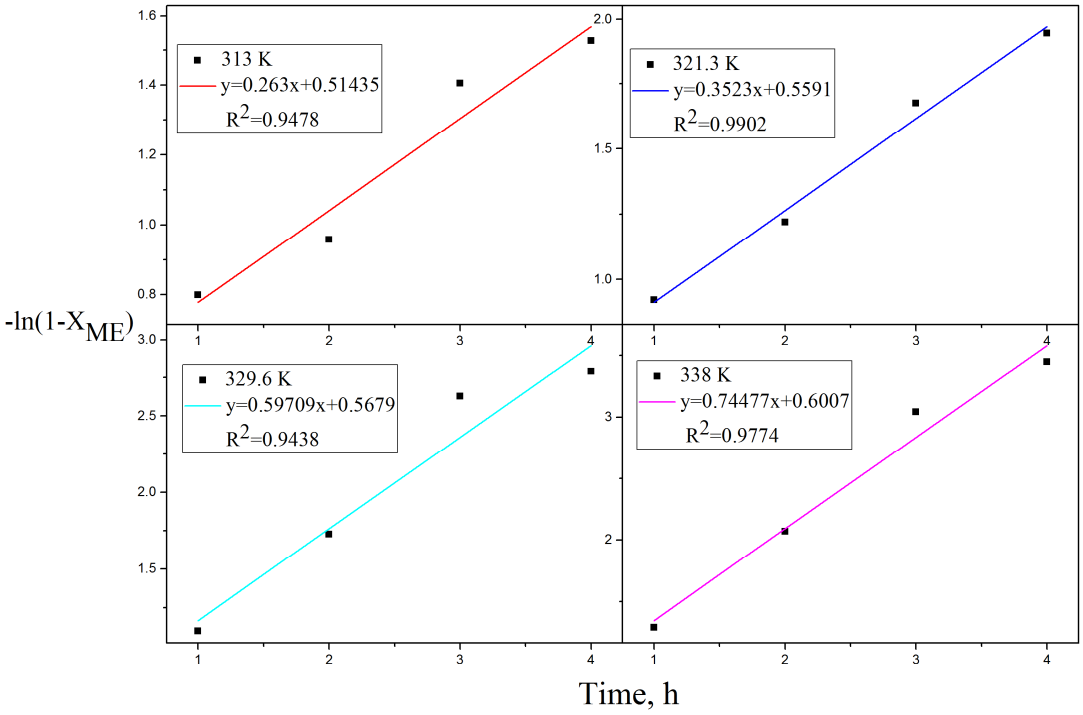


Figure 4-11: Kinetics plot of -ln (1-X_{ME}) via time (t) at different temperatures (methanol to oil molar ratio = 11.56:1, catalyst loading = 5.08 wt.% relative to oil)

The K and R^2 values for each temperature are presented in Table 4-9. As seen in Table 4-9, the kinetic rate constant increased with increasing the temperature.

Table 4-9: Transesterification reaction constant rate at different temperatures

T(K)	K, h^{-1}	R^2
313	0.263	0.9287
321.3	0.3523	0.9902
329.6	0.59709	0.9366
338	0.74477	0.9363

The activation energy of transesterification reaction was calculated using the Arrhenius equation (Eq. 3.20). The slope and intercept of the graph between ln k and $1/T*10^3$ (Figure 4-12) give the values of activation energy and frequency factor. The activation energy (Ea) determined from graph was 38.55 kJ/mol and the frequency factor (ko) was $7.03*10^6$ h^{-1}. And the rate constant for base-catalyzed transesterification reaction in the presence of KOH-impregnated calcined animal bone catalyst was $k = 7.03 * 10^6 \exp(-\frac{4637}{T})$.

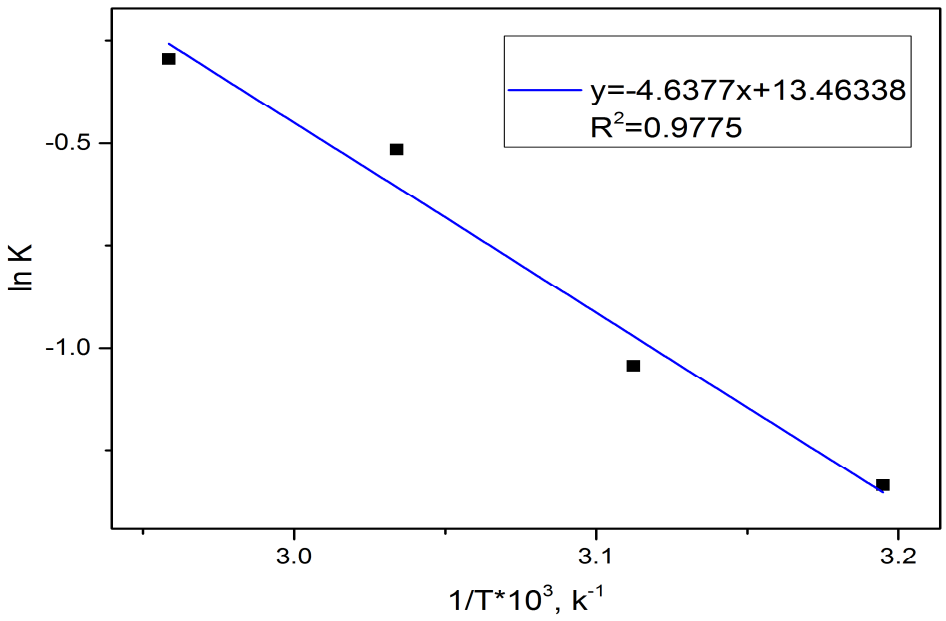


Figure 4-12: Arrhenius plot ln k versus $1/T*10^3$ for transesterification of Jatropha oil by KOH-impregnated calcined animal bone catalyst

5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

A highly active solid base catalysts was synthesized by wet impregnation of KOH on animal bone, followed by calcination and used to transesterificate purified Jatropha oil; a non-edible oil with methanol to biodiesel. The FFA of Jatropha oil was 1.73%. which is higher than the limit (FFA of ≤1%) for alkali-catalyzed transesterification. To improve process efficiency and yield as well, acid-catalyzed esterification reaction was conducted using sulfuric acid as catalyst to reduce FFA content to 0.8%.

The main objective of this research was to synthesize solid base catalyst with porous structures and superior catalytic activity for the transesterification reaction. Impregnation of KOH varying from 8 to 16 wt% on calcined animal bones followed by drying and calcination produced five different catalysts. The basicity and FAME yield of all the synthesized catalyst was determined. Based on the result, catalyst with 12 wt% loading (BI-12) was selected for further characterization using FT-IR, XRD and TGA, and for testing the activity of the catalyst.

The prepared catalyst exhibited good catalytic activity in the transesterification of Jatropha oil and the maximum yield (96.74%) was obtained under optimal reaction conditions of methanol to oil molar ratio of 11.56:1, catalyst loading of 5.08wt%, reaction temperature of 64.05 ^{o}C in 3 hr. Under these optimal conditions the catalyst can be used for at least three runs without any significant decrease of FAME yield. The produced FAME under the optimal reaction conditions was characterized and its physio-chemical and fuel properties were comparable to the American (ASTM D6751) and European (EN 14214) Standards.

The reaction kinetics of BI-12 catalyzed transesterification of esterified Jatropha oil was also carried out in order to determine the reaction rate constant (k) and activation energy (Ea). Since the variations in the methyl ester conversion were exponential at all the studied temperature values, therefore, a pseudo-first order kinetics for the transesterification reaction was considered. The kinetic data can be used to optimize the process of biodiesel synthesis.

In summary, the study indicates that KOH-impregnated calcined animal bone catalyst (BI-12) shows high catalytic activity. Therefore, it could be utilized as potential catalyst for producing biodiesel from Jatropha oil.

5.2 Recommendations for Future Works

KOH-impregnated calcined animal bone catalyst (BI-12) had proved to be effective in transesterifying esterified Jatropha oil with methanol into FAMEs. Some of the most important future research works are listed below.

- By further studying the textural properties of the catalyst such as surface area, pore volume and pore diameter, and the surface morphology, the KOH-impregnated calcined animal bone catalyst could be tested on reactions catalyzed by solid bases, such as Michael addition, alkene and alkyne isomerization, Knoevenagel condensation and so on.
- The issue of catalyst leaching has been found to be the main problem in most of the heterogeneous catalysts studied so far. Therefore, studies on leaching of the catalyst still need to be carried out for its commercialization.
- The reusability of the catalyst was moderate and future studies need to be conducted to enhance the reusability of the catalyst after being treated with solvents.
- The performance of the synthesized biodiesel in compression ignition engines also needs to tested.

Further study on improvement of the transesterification process parameters reaction time, molar ratio of methanol to oil and speed of stirring on percentage of FAME yield is also suggested.

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APPENDICES

Appendix A: Composition of Jatropha Oil

Table A-1: Fatty acid profile of Ethiopian Jatropha oil compared with different Jatropha oils obtained from different countries

Fatty acid	Beemnet et al (2016) in SNNPRS, Oromia, Tigray, Amhara and Benishangul Gumuze regions, Ethiopia	Mesfin (2008) in Bati wereda, Ethiopia	Akbar et al (2009) in Malaysia	Adebowale and Adedire (2006) in Nigeria	Nzikou et al (2009) in Kongo Brazzaville	Berchmans and Hirata (2008) in Indonesia
Palmitic acid	10.62-15.91%	11.23±0.46%	14.2%	11.3%	16.07 ± 1.53%	14.1-15.3%
Stearic acid	10.9-19.3%	17±0.12%	7%	17%	6.03 ± 0.18%	3.7-9.8%
Oleic acid	42.8-51.4%	12.3±0.66%	44.7%	12.8%	41.39 ± 0.38%	34.3-45.8%
Linoleic acid	21.73-25.43%	47.1±0.58 %	32.8%	47.3%	35.61 ± 0.12%	29.0-44.2%

Source: (Beemnet et al., 2016)

Table A-2: Main feedstocks of biodiesel

Edible oils	Non-edible oils	Animal fats	Other sources
Soybeans (Glycine max)	Jatropha curcas	Pork lard	Bacteria
Rapeseed (Brassica napus L.)	Mahua (Madhuca indica)	Beef tallow	Algae (Cyanobacteria)
Safflower	Pongamia (Pongamia pinnata)	Poultry fat	Microalgae (Chlorellavulgaris
Rice bran oil (Oryza sativum)	Camelina (Camelina Sativa)	Fish oil	Poplar
Barley	Cotton seed (Gossypium hirsutum)	Chicken fat	Tarpenes
Sesame (Sesamum indicum L.)	Karanja or honge (Pongamia pinnata)		Switchgrass
Groundnut	Cumaru		Miscanthus
Sorghum	Cynara cardunculus		Latexes
Wheat	Abutilon muticum		Fungi
Corn	Neem (Azadirachta indica)		
Coconut	Jojoba (Simmondsia chinensis)		
Canola	Passion seed (Passiflora edulis)		
Peanut	Moringa (Moringa oleifera)		
Palm and palm kernel (Elaeis guineensis)	Tobacco seed		
Sunflower (Helianthus annuus)	Rubber seed tree (Hevca brasiliensis)		
	Salmon oil		
	Tall (Carnegiea gigantean)		
	Coffee ground (Coffea arabica)		
	Nagchampa (Calophyllum inophyllum)		

Source:(Atabani et al., 2012)

Appendix B: Standard Specifications of Biodiesel

Table B-1: American biodiesel specifications (ASTM D6751-02)

Property	Test Method	Limits	Units
Flash point (closed cup)	D 93	130.0 min	°C
Water and sediment	D 2709	0.050 max	% volume
Kinematic viscosity, 40°C	D 445	1.9–6.0	mm^2/s
Sulfated ash	D 874	0.020 max	% mass
Sulfur	D 5453	0.05 max	% mass
Copper strip corrosion	D 130	No. 3 max	
Cetane number	D 613	47 min	
Cloud point	D 2500	Report	°C
Carbon residue	D 4530	0.050 max	% mass
Acid number	D 664	0.80 max	mg KOH/g
Free glycerin	D 6584	0.020	% mass
Total glycerin	D 6584	0.240	% mass
Phosphorus content max	D 4951	0.001	% mass
Distillation temperature, Atmospheric equivalent temperature, 90 % recovered	D 1160	360 max	°C

Source:(ASTM D 6751-02)

Table B-2: European biodiesel specifications (EN 14214:2008)

Property	Test Method	Limits	Units
Density @ 15 °C	EN 12185	860-900	kg/m^3
Viscosity @ 40 °C	EN 3104	3.5-5.0	mm^2/s
Flash point	EN 3679	>101	°C
Cold filter plugging point	EN 116	Country specific	°C
Cloud point		Country specific	°C
Sulfur content	EN 20884	<10	mg/kg

Carbon residue	EN 10370	<0.30 (10% dist. residue)	% w/w
Sulfated ash	EN 3987	<0.02	% w/w
Water	EN 12937	<500	mg/kg
Contamination	EN 12662	<24	(mg/kg)
Copper strip corrosion (3 h @ 50 ^{o}C)	EN 2160	Class 1	
Oxidation stability (h @110 ^{o}C)	EN 14112	>6	
Cetane number	EN 5165	>51	
Acid value	EN 14104	<0.5	mg KOH/g
Methanol	EN 14110	<0.20	% w/w
Ester content	EN 14103	>96.5	% w/w
Monoglyceride	EN 14105	<0.80	% w/w
Diglyceride	EN 14105	<0.20	% w/w
Triglyceride	EN 14105	<0.20	% w/w
Free glycerol	EN 14105	<0.02	% w/w
Total glycerol	EN 14105	<0.25	% w/w
Iodine value	EN 14111	<120	
Linolenic acid ME	EN 14103	<12	% w/w
Poly-unsaturated acid MEs	EN 14103	<1	% w/w
Phosphorus	EN 14107	<4	mg/kg
Gp I metals (Na, K)	EN 14538	<5	mg/kg
Gp II metals (Ca, Mg)	EN 14538	<5	mg/kg
PAHs	-		% w/w
Lubricity/wear (mm @ 60 ^{o}C)	-		

Source: (Giakoumis, 2013)

Appendix C: Experimental Result

Table C-1: Moisture content of purified Jatropha oil

Run	Sample weight (g)			Moisture Content (%)	Average Moisture Content (%)
	W1	W2	(W1 – W2)		
1	1.00	0.99958	0.00042	0.042	0.036
2	1.00	0.9969	0.0031	0.031	
3	1.00	0.9965	0.0035	0.035	

Table C-2: Ash content of purified Jatropha oil

Run	Sample weight (g)		Ash Content (%)	Average ash Content (%)
	W1	W2		
1	0.0052	20.00	0.026	0.016
2	0.0022	20.00	0.011	
3	0.0022	20.00	0.011	

Table C-3: Acid Value of purified Jatropha oil and FAME

Type of Sample	1st Result	2nd Result	Average	Color change
Purified Jatropha oil	3.84	3.08	3.46	Gray to Pink
FAME	0.41	0.346	0.378	Gray to Pink

Table C-4: Catalyst screening

No	Catalyst name	Catalyst weight (g)	Measurement of basicity				FAME yield (%)
			Initial volume of titrant (ml)	Final volume of titrant (ml)	Volume difference (ml)	Basicity (mmol/g)	
1	N	0.02	250	245.21	4.79	0.21	38.6
2	B	0.02	250	246.42	3.58	1.42	62.3
3	BI-8	0.02	250	247.58	2.42	2.58	70.0
4	BI-10	0.02	250	248.01	1.99	3.01	76.8
5	BI-12	0.02	250	249.21	0.79	4.21	84.2
6	BI-14	0.02	250	248.26	1.74	3.26	81.8
7	BI-16	0.02	250	248.14	1.86	3.14	78.6

Table C-5: Experimental Processes Conditions for Transesterification process

Factors	**Run #1**		**Run #2**		**Run #3**	
Methanol to Oil ratio	11	23.01ml	8	16.74ml	11	23.01ml
Catalyst loading	8.5	3.89g	5	2.29g	5	2.29g
Reaction temperature	52.50	52.50^{o}c	52.50	52.50^{o}c	40	40^{o}c
	Run #4		**Run #5**		**Run #6**	
Methanol to Oil ratio	11	23.01ml	14	29.29ml	11	23.01ml
Catalyst loading	12	5.50g	5	2.29g	8.5	3.89g
Reaction temperature	65	65^{o}c	52.50	52.50^{o}c	52.50	52.50^{o}c
	Run #7		**Run #8**		**Run #9**	
Methanol to Oil ratio	11	23.01ml	14	29.29ml	14	29.29ml
Catalyst loading	8.5	3.89g	12	5.50g	8.5	3.89g
Reaction temperature	52.50	52.50^{o}c	52.50	52.50^{o}c	65	65^{o}c
	Run #10		**Run #11**		**Run #12**	
Methanol to Oil ratio	8	16.74ml	11	23.01ml	11	23.01ml
Catalyst loading	8.5	3.89g	8.5	3.89g	12	5.50g

Reaction temperature	40	40°c	52.50	52.50°c	40	40°c
	Run #13		**Run #14**		**Run #15**	
Methanol to Oil ratio	14	29.29ml	8	16.74ml	11	23.01ml
Catalyst loading	8.5	3.89g	12	5.50g	8.5	3.89g
Reaction temperature	40	40°c	52.50	52.50°c	52.50	52.50°c
	Run #16		**Run #17**		**Optimum condition**	
Methanol to Oil ratio	8	16.74ml	11	23.01ml	11.56	24.19ml
Catalyst loading	8.5	3.89g	5	2.29g	5.08	2.33g
Reaction temperature	65	65°c	65	65°c	64.05	64.05°C

Appendix D: Experimental Method Calculations

D-1: Feed material requirement

Methanol to oil molar ratio and catalyst to oil weight ratio need to be specified in equivalent term as of the volume of oil.

- Calculation of Molecular weight of Oil

Molecular weight of Jatropha oil was determined from its acid value and saponification value using the following formula:

$$M = \frac{56.1 * 1000 * 3}{S.V - A.V}$$

Where, S.V - Saponification Value

A.V- Acid value

$Molecular\ weight\ of\ purified\ Jatropha\ Oil = \frac{56.1*1000*3}{192.3-3.46} = 891.2$g/mol

- Methanol to oil molar ratio calculation

The following basic formula works for all cases:

$$Methanol\ to\ oil\ molar\ ratio = \frac{number\ of\ mole\ of\ methanol}{number\ of\ mole\ of\ oil}$$

$$Number\ of\ mole = \frac{Given\ mass}{Molecular\ mass}$$

$$Density = \frac{Mass}{Volume}$$

Substitute Number of mole and Density in to methanol to oil molar ratio equation:

$$Methanol\ to\ oil\ molar\ ratio = \frac{\left(\frac{Density * Volume}{Molecular\ mass}\right) of\ methanol}{\left(\frac{Density * Volume}{Molecular\ mass}\right) of\ oil}$$

Then, solving for Volume of methanol:

$$Volume\ of\ methaol = (methanol\ oil\ ratio) * \frac{\left(\frac{Density * Volume}{Molecular\ mass}\right) of\ methanol}{\left(\frac{Density * Volume}{Molecular\ mass}\right) of\ oil}$$

Where, Density of methanol=0.79g/ml

Molecular mass of methanol=32.04g/mol

Density of oil=0.9176g/ml

Volume of oil=50ml

Molecular mass of oil=891.2g/mol

- ❖ Catalyst to oil weight ratio calculation

As it was cited before, from Density and Volume of oil, mass of Oil can easily be calculated.

Mass of catalyst was calculated as follow:

$$Catalyst\ weight = \frac{Mass\ of\ catalyst}{Mass\ of\ oil}$$

Then,

$$Mass\ of\ catalyst = Catalyst\ weight * mass\ of\ oil$$

D-2: Purification of Crude Jatropha Oil (Degumming)

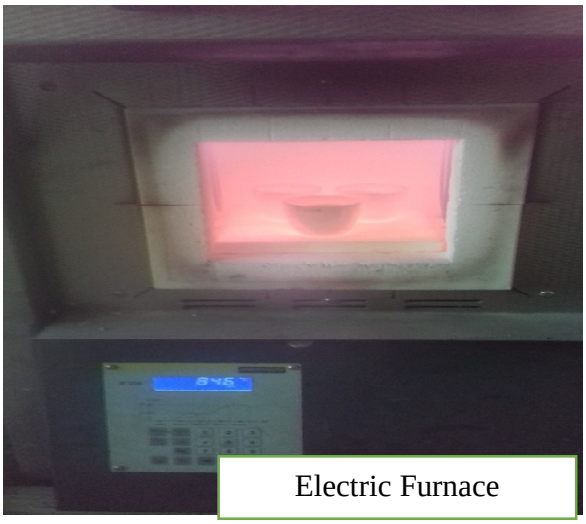
It is the removal of phosphatides, gums and other complex compounds from crude Jatropha oil. Hence, based on the method discussed in previous chapter 3 wt% of distilled water is required for degumming process of crude oil.

$$Amount\ of\ distilled\ water\ required\ = amount\ of\ Jatropha\ oil \times 3\%$$
$$= 2.4 liter * 0.03$$
$$= 0.072\ liter = 72\ ml$$

Appendix D: Fourier Transform Infrared Spectroscopy (FT-IR) Correlation Table

	Type of Vibration causing IR absorption	Absorption Ranges, cm^{-1}	Intensity
C-H	Alkanes (stretch)	Alkanes (stretch)	Alkanes(stretch)
	-CH3 (bend)	-CH3 (bend)	-CH3 (bend)
	-CH2- (bend)	-CH2- (bend)	-CH2- (bend)
	Alkenes (stretch)	Alkenes (stretch)	Alkenes (stretch)
	(out-of-plane bend)	1000-650	Strong
	Aromatics (stretch)	3150-3050	Strong
	(out-of-plane bend)	900-690	Strong
	Alkyne (stretch)	~3300	Strong
	Aldehyde	2900-2800	weak
		2800-2700	weak
C-C	Alkane not interpretatively useful		
C=C	Alkene	1680-1600	medium
	Aromatic	1600 and 1475	Weak
C≡C	Alkyne	2250-2100	medium
C=O	Aldehyde	1740-1720	Strong
	Ketone	1725-1705	Strong
	Carboxylic Acid	1725-1700	Strong
	Ester	1750-1730	Strong
	Amide	1670-1640	Strong
	Anhydride	1810 and 1760	Strong
	Acid Chloride	1800	Strong
C-O	Alcohols, Ethers, Esters, Carboxylic Acids, Anhydrides	1300-1000	Strong
O-H	Alcohols, Phenols		
	Free	3650-3600	Medium
	H-bonded	3500-3200	Medium
	Carboxylic Acids	3400-2400	Medium
N-H	Primary and Secondary Amines and Amides		
	(stretch)	3500-3100	Medium
	(bend)	1640-1550	Strong
C-N	Amines	1350-1000	Medium
C=N	Imines and Oximes	1690-1640	Weak
C≡N	Nitriles	2260-2240	Medium
X=C=Y	Allenes, Ketenes, Isocyanates, Isothiocyanates	2270-1950	Strong
N=O	Nitro (R-NO_2)	1550 and 1350	Strong
S-H	Mercaptans	2550	Weak
S=O	Sulfoxides	1050	Strong
	Sulfones, Sulfonyl Chlorides, Sulfates, Sulfonamides	1375-1300 and 1200-1140	Strong

Appendix E: Laboratory Equipment and Samples Photos



Electric Furnace

FT-IR Instrument

Density meter

Vibro viscometer

Bielenberg Ram Press

Size reducing equipment

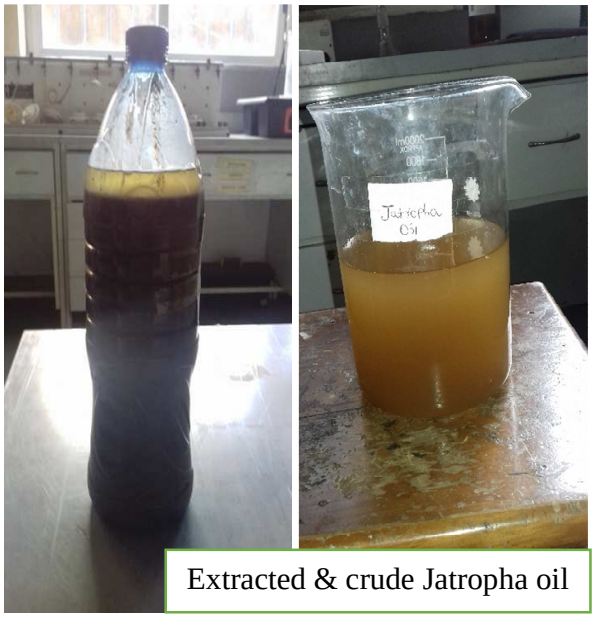
Animal bone

Bone powder

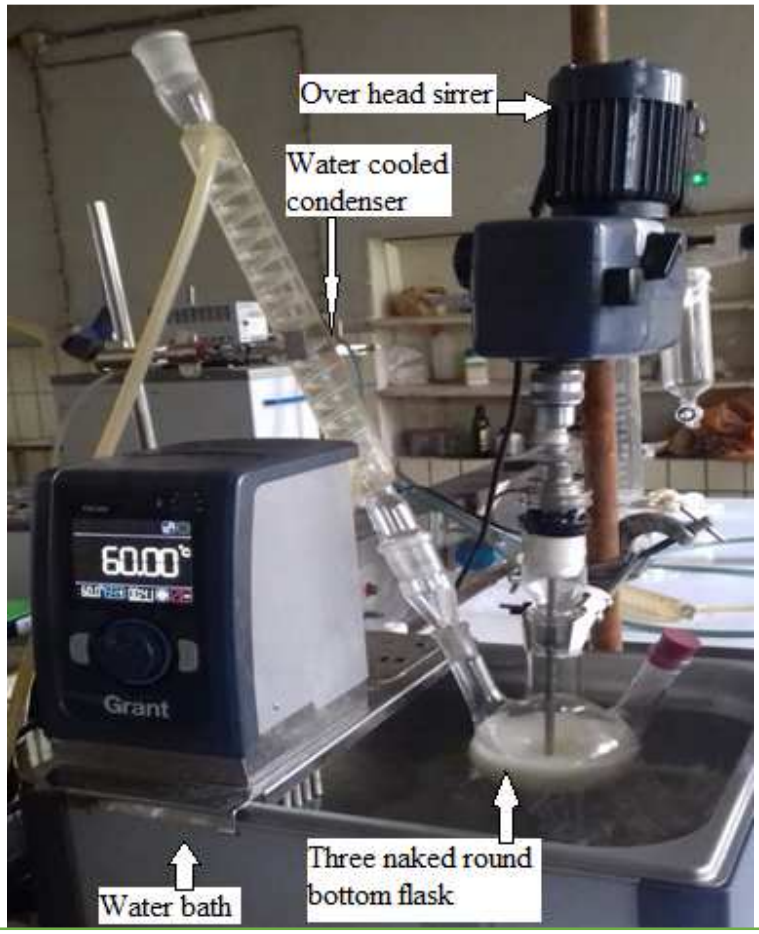
Support (white powder)

Wet KOH impregnation

KOH-impregnated calcined bone catalyst



Extracted & crude Jatropha oil



Experimental setup for a two-step acid-base catalyzed transesterification reaction

Result of acid-catalyzed Esterification

Result of base-catalyzed Transesterification