ADDIS ABABA UNIVERSITY
ADDIS ABABA INSITUTE OF TECHNOLOGY
SCHOOL OF CHEMICAL & BIO-ENGINEERING

Preparation and Characterization of KOH Impregnated Calcined Fish bone Catalyst for Biodiesel Production from WCO

A Thesis Submitted to School of Chemical and Bio Engineering, Addis Ababa Institute of Technology in Partial Fulfillment of the Requirements for the Degree of Masters of Science in Process Engineering

BY: KIDIST GETACHEW

Addis Ababa University
Addis Ababa, Ethiopia
June 2019

ADDIS ABABA UNIVERSITY
ADDIS ABABA INSTITUTE OF TECHNOLOGY
SCHOOL OF CHEMICAL AND BIO ENGINEERING

This is to certify that the thesis is prepared by Kidist Getachew, entitled: ***Preparation and characterization of KOH impregnated calcined fishbone catalyst for biodiesel production from waste cooking oil*** *and submitted in partial fulfillment of the requirements for the Degree of Master of Science in Process Engineering complies with the regulations of the University and meets the accepted standards with respect to originality and quality.*

Signed by examining committee:

Prof. Belay W.	_____	_____
Advisor	Signature	Date
Dr. Beteley T.	_____	_____
External Examiner	Signature	Date
Dr. Abubeker Y.	_____	_____
Internal Examiner	Signature	Date

School or center chairperson: _____

DECLARATION

I declare that this thesis for the M.Sc. degree at Addis Ababa University, hereby submitted by me, is my original work and has not previously been submitted for degree at this or any other university, and that all resources of materials used in this thesis have been duly acknowledged.

Name: Kidist Getachew

Signature: ----------

Date of submission: ----------

This thesis has been submitted for examination with my approval as university advisor.

Name: Prof. Belay W.

Signature: ----------

Date of submission: ----------

ACKNOWLEDGEMENT

First and foremost, I am grateful to the almighty GOD for giving the strength and wisdom to successfully complete this thesis work. I wish to express my sincere gratitude and earnest appreciation to my research advisor, Professor Belay Weldeyes for their guidance, advice, continues follow up, crucial remarks, priceless suggestion and comments throughout the thesis.

My appreciation goes to Chemical Engineering Laboratory Staff; Mr. Hintsa-Selassie for his unreserved support to my laboratory work.

I extend my appreciation to to all Debre Berhan university of Chemical Engineering department staff members and Special thanks to Kitaw, Ayalew, Aman, Getahun, Menilik and Damenech for their invaluable support and making my laboratory work easier.

It is my pleasure to express my sincere appreciation to my family Ato Getachew Adere, Belete, Alemdar, Takelu; I am sure that I would not have been able to achieve without their support, help and prayer. I would like to acknowledge my friends at Addis Ababa institute of technology who have contributed to my wor

ABSTRACT

KOH/Calcined fishbone catalyst was prepared through wet impregnation process by impregnating 5, 10 & 15 wt% of KOH catalyst with calcined fishbone. The catalyst prepared by impregnating 10 wt% of potassium hydroxide in calcined fishbone as a support was found to show the best catalytic activity among the prepared catalysts. The characterization of KOH/Calcined fishbone catalyst, supported catalyst and raw fishbone was conducted by XRD and FTIR analysis. The effects of various reaction variables on the yield of biodiesel were investigated. The highest yield of biodiesel over KOH/Calcined fishbone catalyst was 97.46%. It was obtained at the optimum condition of methanol to oil ratio of 10.56, catalyst dosage of 3.96 wt%, reaction time of 2.7 h and the reaction temperature at 65^0C. The physiochemical and fuel properties of biodiesel were studied and it is within the biodiesel standard specification. The produced biodiesel under the optimal reaction condition was characterized by using FTIR and GC-MS analysis. The selected catalyst has been reused successfully for three catalytic cycles.

***Key factor**: Solid base catalyst, fishbone, impregnation, potassium hydroxide, waste cooking oil and Transterification*

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

ACRONOMY

ANOVA	Analysis of Variance
ASTM D6751	American Standards for Testing Materials
AV	Acid Value
BBD	Box-Behnken Design
CaO	Calcium oxide
EIA	Energy Information Administration
EN 14214	European Union Standard
FAME	Fatty Acid Methyl Esters
FFA	Free Fatty Acid
FT-IR	Fourier Transform Infrared Spectroscopy
GC-MS	Gas Chromatograph Mass Spectroscopy
HCl	Hydro Chloric acid
H_2SO_4	Sulphuric acid
IV	Iodine Value
JCPDS	Joint Committee on Powder Diffraction Standards
KBr	Potassium Bromide
KOH	Potassium Hydroxide
PM	Particulate Matter
RSM	Response Surface Methodology
rpm	Revolution per minute
SV	Saponification Value
TG	Triglyceride
TGA	Thermogravimetric analysis
USA	United State of America
XRD	X-Ray Diffraction
WCO	Waste Cooking

1. INTRODUCTION

1.1. Background

Energy is one of the most important resources for humankind and its sustainable development. Today, the energy crisis becomes one of the global issues confronting us. Fuels are of great importance because they can be burned to produce significant amounts of energy. Many aspects of everyday life rely on fuels, in particular the transport of goods and people. Main energy resources come from fossil fuels such as petrol oil, coal and natural gas. Fossil fuel contributes 80% of the world's energy needs. Most industries use diesel machines for the production process. In the transportation sector, private vehicles, buses, trucks, and ships also consume significant amounts of diesel and gasoline. This situation leads to a strong dependence of everyday life on fossil fuels. However, the growth of the population is not covered by domestic crude oil production (Mutreja et al., 2011).

Ethiopia imports its entire petroleum fuel requirement by spending over 80% of the foreign earning annually. The transport sector in general and road transport which accounts for about 52% of the country in particular is one of the most key sector which consume the majority of the imported petroleum and contributed more CO_2 to the environment. Since the nation's economy is increasing, it is expected that the demand for petroleum fuel will increase. Hence, in order to ensure the country's sustainable development and fuel security, it is critical to look for locally available alternative fuels such as biodiesel (Birhanu & Ayalew, 2017).

Biodiesel was produced from any fat or oil through a process called transesterification. In the production of biodiesel, the triglycerides present in various sources viz. edible oil, non-edible oil, animal fats, waste oil and oil from algae are trans-esterified with methanol/ethanol in presence of a catalyst (acid, base or biocatalyst) to give fatty acid alkyl esters (FAAE) and glycerol as a byproduct. Most of biodiesel was produced from vegetable oil and methanol alcohol. Since the discovery of biodiesel, there is a problem associated with cost of production that make biodiesel uncompetitive with petro diesel. The vegetable oil price increased as the world food price increased so producing biodiesel from such kind of feed stocks are not commercially feasible. There are efforts to produce biodiesel from cheap feedstock's like waste cooking oil, yellow grease and waste fats. Moreover, it has been reported that nearly 70-95 % of the total production cost is related to the cost of raw materials. This issue can be overcome by the use of Waste Cooking Oil (WCO) as

raw material, which can effectively reduce the feedstock cost to 60-70%. Waste cooking oil is easy to collect from other industries such as domestic usage and restaurant and cheaper than other oils (refine oils). These oils need to be treating before dispose to the environment to prevent pollution. Due to the high cost of disposal, many individuals dispose waste cooking oils directly to the environment especially in rural area. So that, the use of waste cooking oils is an effective way to reduce the cost of biodiesel production (Gashaw & Teshita, 2014).

The transesterification reaction for biodiesel production can be carried out using both homogeneous (acid or basic) and heterogeneous (acid, basic or enzymatic) catalysts. The base-catalyzed transesterification of oils proceeds faster and give very high yields in short reaction times than that catalyzed by the same amount of an acidic catalyst. Enzyme catalyzed transesterification is carried out at moderate temperatures with high yields, but this cannot be used for biodiesel production due to high enzyme costs (Raut et al., 2016).

Different researchers synthesized and used different kinds of solid wastes based catalysts (such as mollusk shells, eggshells, fish scale, sheep bone, etc.) in order to produce cost effective catalysts and biodiesel. Among these solid wastes, fishbone is one of the best solid wastes that are easily and abundantly available in Ethiopia. Fishbone is constituted by the remaining meat after removal of the fillet, bones and cartilages. Fish bone consists of 60% to 70% of inorganic substances and is mainly comprised of calcium phosphate and hydroxyapatite. Edible fish bone contains a high amount of calcium, phosphorous elements (Sulaiman et al., 2014).

Although, the waste bone derived catalysts have shown a reasonable performance and constancy in the reaction, however these catalysts are required in high amount, high alcohol/oil molar ratio with longer time for the reaction to occur. To overcome this drawback wet impregnation method was suggested by researchers. Wet impregnation method is the process of impregnating calcined fishbone in an aqueous solution of alkali and alkaline earth compounds to make fishbone derived catalyst more active and to boost the surface chemical properties. Calcined fish bones have proved to be highly effective as a catalyst support. The properties of calcined bone make it advantageous for use as catalyst support in transesterification reaction. Calcined fishbone contains hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] and B- TCP, that is highly porous and also has a large surface area which allows catalyst to disperse over it largely and effectively (Nisar et al., 2017).

1.2. Statement of the problem

Biodiesel was produce by transesterification reaction by reacting vegetable oils or animal fats with monohydric alcohol in presence of a catalyst. Commercially biodiesel was produced mainly from edible oils that compete with human food consumption and made the biodiesel to be expensive. Utilization of waste materials such as waste cooking oil as a raw material was the preferable way for reducing the production cost and simultaneously it minimizes environmental impact like water and soil pollution, human health problem and disturbance to the aquatic ecosystem.

The transesterification reaction was 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. Biodiesel producer countries were used homogeneous acid and base catalysts in esterification and transterification processes respectively. However, homogenous catalysts were not recommended by researchers nowadays due to a problem with large wastewater production, difficulty in catalyst recovery, soap formation and corrosion to operating equipment 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 had many advantages over homogeneous catalysts as it can easily be separated from reaction mixture and can be reused, does not form soap, have limited corrosion effect and can be applied for continuous processes. Heterogeneous catalysts derived from waste solid resources were preferable than commercial chemicals since it was renewable and eco-friendly to the environment; it reduces the present high production cost of biodiesel and making it competitive with petro-diesel fuels. Therefore, heterogeneous base catalyst derived from fishbone was lowering the production cost of biodiesel and simultaneously protecting the environment.

Research was not conducted on biodiesel production from waste cooking oil using potassium hydroxide impregnated calcined fishbone catalysts. The impregnated catalyst was improved the problem of slow rate of reaction caused by heterogeneous (calcined fishbone) catalyst due to mass transfer limitation between the substrate and the active site of the catalyst.

1.3. Objective

1.3.1. General objective

Preparation and characterization of potassium hydroxide impregnated calcined fishbone catalyst for biodiesel production from waste cooking oil, via transterification reaction.

1.3.2. Specific objective

- To synthesize and characterize KOH impregnated calcined fishbone catalyst at different calcination temperature and weight ratio of KOH solution.
- To investigate the catalytic performance of the prepared catalyst via transterification reaction under varying reaction parameters of methanol to oil molar ratio, catalyst dosage and reaction time.
- To study the main and interaction effect of the transterification reaction using Response Surface Methodology.
- To develop model equation and determine the optimum operating parameters that results a maximum biodiesel/FAME yield.
- To characterize the physico-chemical properties of the produced biodiesel.

1.4. Significance of the study

Currently in Ethiopia, the demand for modern energy sources such as petroleum fuels is increasing with increase in population and economic growth. The annual Ethiopian petroleum consumption, which grows ten percent yearly, has reached 3.8 million metric ton in 2018. The government spends more than 2.8 billion dollars on fuel imports yearly, draining 80 percent of the country's foreign currency earnings (Mahiber, n.d.). The government of Ethiopia gives attention to environmental friendly renewable energies such as biodiesel since importing of petroleum add a lot of cost to the country besides to higher environmental pollution through emitting of CO_2 and NOX gases. Biodiesel developed from waste cooking oil using KOH/Calcined fishbone as a catalyst simultaneously reduces cost for raw materials, minimize environmental pollution like greenhouse gas emission and has higher combustion efficiency because it is non-toxic, renewable, and biodegradable. This study have a significant advantage for the country by installing heterogeneous base catalyst plants as a means of foreign income generation; create job opportunity and reduce environmental pollution.

1.5. Scope of the research

The thesis work started from preparation of necessary equipment's, raw materials and reagents for the experiment. Then based on the procedures different experiments were performed such as preparation of heterogeneous solid base catalyst by wet impregnation method, characterization (density, acid value, saponification value) of waste cooking oil, production of biodiesel by esterification/acid-catalyzed and Transterification/base-catalyzed process using the prepared catalyst and finally characterization of a catalyst and a biodiesel by different test methods.

2. LITERATURE REVIEW

2.1. Solid base catalyst

Solid base catalysts, such as alkaline earth metal oxides, are practiced to assist transesterification reaction due to their moderate basic strength. The basic strength of group II oxides and hydroxides is promoted with the increase in atomic number, i.e., from Magnesium (Mg) to Barium (Ba), which significantly affect their catalytic capability to transesterify lipid feedstock. The origin of basic sites in the alkaline earth metal oxides could be attributed to the presence of the M^{2+}–O^{2-} ion pair and surface hydroxyl groups in different co-ordination environments. The amount of the surface oxygen could be affected by the surface compositions of the solid catalyst and the calcination temperature. Besides, the specific surface area, basicity, degree of leaching in the reaction medium, and selectivity toward the transesterification reaction products must also be considered as contributing factors for the selection of appropriate catalysts for biodiesel production. 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 (Avhad & Marchetti, 2016).

Alkali metals (Li, Na, K) and alkaline earth metals (Mg, Ca, Ba) are the most common sources of super basicity, and selected as the active species of supported catalysts for biodiesel synthesis. They are frequently used in the metallic form or as various ionic forms of hydroxide, halide, carbonate and nitrate, such as K^+, Li^+ $La3^+$, KOH, NaOH, KF, K_2CO_3, KNO_3. Alumina, silica, zinc oxide, zirconium oxide and zeolite were used as supports for these catalysts. Surface basicity is the primary determinant of catalyst activity, then the specific surface area and pore volume (Guo & Fang, 2012).

As different researchers studied so far, CaO is one of a solid base catalyst capable of transesterification of various oils to fatty acid methyl esters (FAME). However, its activity was still found to be lower than that of homogeneous base catalysts. Hence, to increase the catalytic activity of CaO, wet impregnation methods have been employed to modify the surface of CaO to generate more active sites. Recently, naturally waste derived raw materials have attracted attention, since they could serve as the economical precursors for CaO.

2.1.1. Waste-derived heterogeneous solid base catalysts

The use of heterogeneous catalyst is promising to reduce the present high production cost of biodiesel making it competitive with petro-diesel fuels. Searching and developing economically viable, ecofriendly, and renewable heterogeneous catalysts for biodiesel production is therefore very attractive. Recently, researchers have focused on several heterogeneous catalysts derived from renewable resources 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).

There are many advantages of using bone-derived resources as the precursor of solid base catalysts. Firstly, fish 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 (Ca_3PO_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).

Different researches has been done on biodiesel production using waste naturally derived heterogeneous solid base catalysts. Recently, many efforts have been made to improve the catalytic performance of bone derived catalysts.

The waste chicken bone was used as a catalyst for the production of biodiesel from waste cooking oil. This catalyst consists mainly of $CaCO_3$, which is converted to CaO after calcination at 800°C for 5 h. The maximum yield of the produced biodiesel was 96.31 ± 0.72% under the optimum condition of 3.0 wt% catalyst, 3:1 methanol to oil ratio, and reaction temperature 80 ± 5°C for 3 h. The reusability of the catalyst was decreased after four times of use, resulting in activity loss in the fourth repetition (Suwannasom et al., 2016).

Similar work has been reported using sheep bone that was calcined at 800 °C, transformed calcium phosphate of the bone into hydroxyapatite with an increase in surface area. A methyl ester content of 96.78% was obtained under the reaction conditions of methanol to oil 18:1 (molar ratio),

reaction time of 4 hr. and catalyst dosage of 20% at reaction temperature of 65 °C. The catalyst was stable for five cycles at 83.7% conversion (Lesbani et al., 2015).

Ghanei et al. produce biodiesel from canola oil using CaO-impregnated calcined animal bone. First, they calcined sheep bone in electrical furnace at 600^0C for 8 hr and then impregnated with CaO, followed by re-calcination at 600^0C for 4 hr. A FAME yield of 95.18 % was obtained at reaction temperature of 60^0C, 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 m2/g of the calcined animal bone to 5.2499 m^2/g of the final catalyst (Khalili & Salehi, 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. The catalyst (KOH/CAB) is synthesized at an optimum variables of calcination temperature 900^0C, impregnated amount of 10 wt% KOH and calcination time of 3hr. The effect of reaction variables such as methanol to oil ratio (3:1 to 13:1), catalyst dosage (2 to 6 wt%), reaction temperature (60 to 70^0C) and reaction time (0.5 to 4 hr) were varied and evaluated experimentally. The experimental results revealed the optimal parametric conditions viz. methanol/oil molar ratio of 9:1, calcination temperature of 900^0C and catalyst concentration of 6 wt % of oil corresponding to a maximum fatty acid methyl esters (FAME) yield of 96.1% at temperature of 70 ± 3^0C in reaction time of 3 h. Reusability results of the prepared catalyst confirmed that it could be reutilized up to 4 times without losing much activity (Nisar et al., 2017). Bone from waste Rohu fish (*Labeo rohita*) has been reported as a low-cost heterogeneous catalyst for the synthesis of biodiesel from soybean oil. The analysis of TGA and XRD revealed that a significant portion of the main component of fish scale, hydroxyapatite, could be transformed into β-tri-calcium phosphate when calcined above 900 °C for 2 h, with optimal conditions of MeOH/oil molar ratio, 6.27:1, catalyst at 1.01 wt.% for 5 h. This compound was able to yield 97.73% of methyl ester. The reusability of the catalyst shows that it could be re-employed up to six times (Chakraborty et al., 2011).

Similarly synthesis of biodiesel from waste cooking oil using fish bone as a catalyst yield 86% of methyl ester at the optimum conditions of catalyst dosage 6 wt%, mixing rate of 300 rpm, methanol to oil ratio of 18:1 and reaction temperature of 65 °C for 120 min (Sulaiman & Amin, 2016).

It is obvious from the above literature review that there is no report on biodiesel production from waste cooking oil, using potassium hydroxide impregnated calcined fish bone catalyst. The activity of the KOH/calcined fishbone catalyst directly depends on the preparation conditions, especially precursor loading and calcination temperature. In other words, right amounts of precursor when calcined at right temperature will maximize the formation of active sites on the surface that can significantly enhance transesterification reactions. However, to our knowledge, there is limited information on how the activity of KOH/calcined fishbone catalyst is influenced by preparation conditions. Knowing of how calcination temperature and impregnation conditions affect the catalyst activity will not only improve catalyst synthesis processes but also allow us to predict the performance of the catalyst. It is therefore the goal of this research is preparation and characterization of potassium hydroxide impregnated calcined fish bone catalyst for biodiesel production from waste cooking oil. Experiments under different reaction conditions, such as methanol to oil molar ratio, catalyst loading and reaction time was performed in order to optimize biodiesel yield.

2.2. Biodiesel

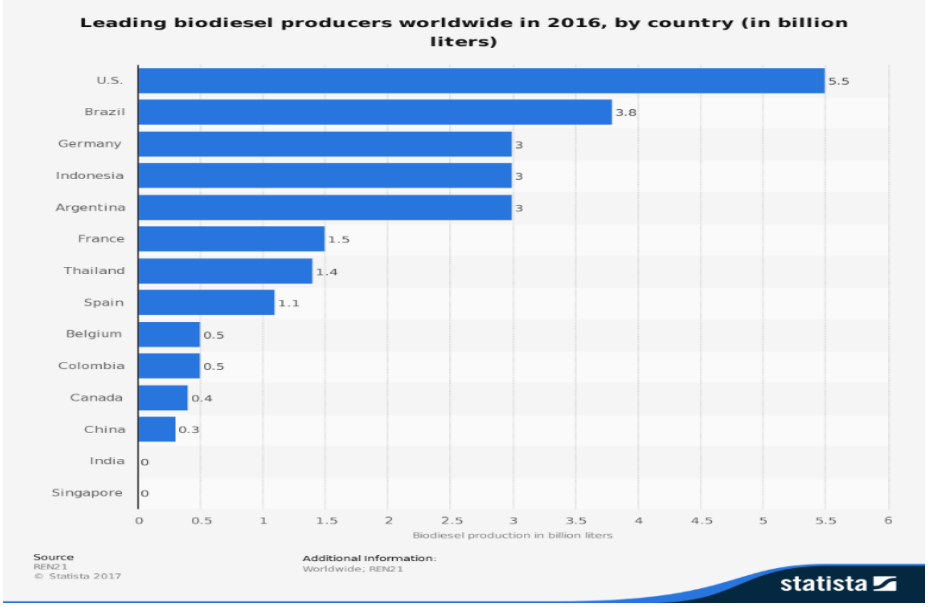
‘‘Biodiesel’’ has been defined by the American Society for Testing and Materials (ASTM) as a mono alkyl ester of fatty acids or fatty acid methyl ester derived from renewable feed- stocks, such as vegetable oils, animal fats…etc. The term “bio” indicates the biological source of biodiesel in contrast with conventional diesel. Biodiesel is a clear liquid with a light-to dark-yellow color. It has a boiling point of over 200 ^{0}C, a flash point between 145–175 ^{0}C, a distillation range of 195–325 ^{0}C, and a vapor pressure (at 22 ^{0}C) less than 5 mmHg. It is also insoluble in water, has alight soapy odor, is biodegradable, non-toxic and has stable reactivity (Yaakob et al., 2013).

2.3. Current Status of Biodiesel Production

2.3.1. Biodiesel production in the world

Biodiesel production in the United States in 2015 reached a volume of 1.263 billion gallons, declining 1.2% from the 2014 level (1.278 billion gallons). Production from January to September

2016 was up 19.7% to 1.128 billion gallons compared with the same period the previous year (0.943 billion gallons). Production estimates for the entire 2016 year by the U.S. Energy Information Administration, published in the January 2017 edition of the Short Term Energy Outlook report, indicated biodiesel production in 2016 averaged 1.518 billion gallons/year (99,000 barrels/day), increasing 20.1% compared with 2015. The report also indicated 2017 U.S. biodiesel production is expected to increase 5.1% to 1.594 billion gallons/year (104,000 barrels/day) relative to 2016. EIA projects 2018 biodiesel production at 1.701 billion gallons (111,000 barrels/day) up 12.1% from the 2016 estimate. The United States and Brazil were among the largest biodiesel producers in the world, totaling some six and 4.3 billion liters, respectively, in 2017. The United States is projected to reach production levels of over 1 billion gallons of biodiesel by 2025. After the implementation of the Energy Policy Act of 2005, which provided tax incentives types of energy, biodiesel production in the U.S. began to increase. In 2010, the U.S. exported about 85 million gallons of their biodiesel products. Comparatively, Argentina accounted for over half of the world's total exports. The United States has one of the highest bioenergy capacities in the world, totaling 13,151 megawatts in 2017 (States et al., 2018).



Source: (States et al., 2018)

Figure 2.1: Leading biodiesel producers worldwide in 2016

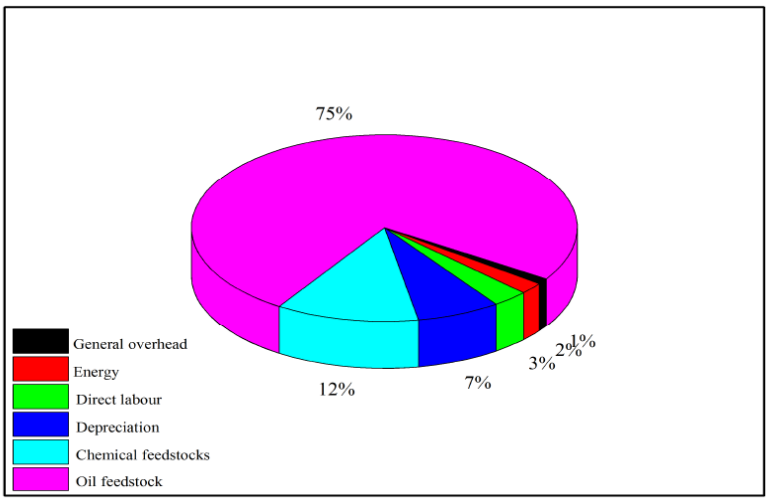
2.3.2. The Potential and Status of Biodiesel Production in Ethiopia

The Government of Ethiopia considers biofuel as an opportunity for enhancing food security and meeting the growing energy demand in the country, thereby saving scarce foreign exchange. Moreover, the government has already started blending gasoline and currently ethanol to gasoline ratio 10:90 and according to the strategy, this ratio will increase to 25% by 2015. Due to the favorable air condition and suitable soil type for bio-diesel development, different types of plant species, which can be used for producing bio-diesel, grow in the country. Jatropha, which is very important feedstock for biodiesel grows in many parts of the country and used as a hedge and medicinal plant. Another plant, which grows in many parts of the country and important feedstock for producing bio-diesel, is castor oil. Imported Palm tree seedlings are planted in 100ha area in the Gambella Regional State for producing oil and soap around Tepi Area. Ethiopia is endowed with natural resource suitable for bio-diesel development and at national level; an estimated area of 25 million hectare suitable land is available for development of biodiesel. (Birhanu & Ayalew, 2017).

2.4. Feedstock for Biodiesel Production

Globally, there are more than 350 oil-bearing crops identified as potential sources for biodiesel production. The wide range of available feedstocks for biodiesel production represents one of the most significant factors of producing biodiesel. As much as possible the feedstock should fulfill two main requirements: low production costs and large production scale. The availability of feedstock for producing biodiesel depends on the regional climate, geographical locations, local soil conditions and agricultural practices of any country (Atabani et al., 2012) .

The high cost of biodiesel is a major obstacle to its commercialization, which is 1.5–3 times higher than petroleum derived diesel. The feedstock's, accounts for about 75% of the total production costs, contribute to a major portion of the overall biodiesel production cost as shown in figure 2-2 (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 feedstock's can be divided into four main categories as edible vegetable oil, non-edible vegetable oil, waste or recycled oil and animal fats. The main feedstock's of biodiesel are shown in Appendix A.



Source : (Atabani et al., 2012)

Figure 2.2: General cost breakdown for production of biodiesel

Edible oils resources such as soybean, palm oil, sunflower, safflower, rapeseed, coconut and peanut are considered as the first generation of biodiesel feedstock because they were the first crops to be used for biodiesel production. Their plantations have been well established in many countries around the world such as Malaysia, USA 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%). However, their use raises many concerns such as food versus fuel crisis and major environmental problems such as serious destruction of vital soil resources, deforestation and usage of much of the available arable land. Moreover, in the last 10 years the prices of vegetable oil plants have increased dramatically which will affect the economical viability of biodiesel industry. Furthermore, the use of such edible oils to produce biodiesel is not feasible in the long-term because of the growing gap between demand and supply of such oils in many countries. For instance, dedicating all US soybean to biodiesel production would meet only 6% of diesel demands.

One of the possible solutions to reduce the utilization of the edible oil for biodiesel production is by exploiting non-edible oils. Non-edible oils resources 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. The main sources for biodiesel production from non-edible oils are jatropha (Jatropha curcas), karanja, rubber seed tree…etc. and it regarded as the second generation of biodiesel feedstocks.

Animal fats such as beef tallow, poultry fat and pork lard waste oils and grease are also considered second-generation feedstocks. The use of these types of feedstock eliminates the need to dispose them. However, it has been reported that second generation feedstocks may not be plentiful enough to satisfy the global energy demand. Moreover, biodiesel derived from vegetable oils and animal fats has a relatively poor performance in cold weather. Furthermore, for many types of animal fats the transesterification process is difficult because they contain high amount of saturated fatty acids. More recently, microalgae have emerged to be the third generation of biodiesel feedstock. Microalgae are photosynthetic microorganisms that convert sunlight, water and CO_2 to algal biomass but they do it more efficiently than conventional crop plants. It represents a very promising feedstock because of its high photosynthetic efficiency to produce biomass, higher growth rates and productivity and high oil content compared 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).

2.4.1. Waste vegetable oil

Waste cooking oil refers to the used vegetable oil obtained from cooking food. Repeated frying for preparation of food makes the edible vegetable oil no longer suitable for consumption due to high free fatty acid (FFA) content. Used vegetable oil (UVO) is a by-product from hotels, fast food, restaurants and shops selling fritter and by-product of an operating vegetable oil refinery. For serving better quality food, they usually throw this waste cooking without any treatment. WCO collected can also be used to prepare soaps and additive for lubricating oil. Many researchers have successfully converted used vegetable oil into biodiesel. In fact, using waste vegetable oil reduces the need for biodiesel-producing crops and the competition with food. UCOs have different properties from those of refined and crude vegetable oils (Raqeeb & Bhargavi, 2015). Many developed countries have set policies that penalize the disposal of waste cooking oil into waste

drainage. The Energy Information Administration (EIA) in the United States (USA) estimated that around 100 million gallons of waste cooking oil is produced per day in USA, where about 9 pounds of waste cooking oil are generated per person per year. The estimated amount of waste cooking oil collected in Europe is about 0.49 - 0.7 million gallons (Patil et al., 2012).

The chemical and physical properties of WCO are different from those of fresh oil since some changes due to chemical reactions - such as hydrolysis, oxidation, polymerization, and material transfer between food and vegetable oil occur during the frying process. The typical chemical and physical characteristics of WCO are shown in Table 2.1:

Table 2.1: Physical properties of waste cooking oil

Property	**Units**	**Value**
Density	g/cm^3	0.91 - 0.924
Kinematic viscosity (40^0C)	mm^2/s	36.4 – 42
Acid value	mgKOH/g	1.32 – 4.6
Saponification value	mgKOH/g	188.2 – 207
Iodine number	$gI_2/100g$	83 - 141.5

Source: (Raqeeb & Bhargavi, 2015)

The properties of WCO can change depending on the frying conditions, such as temperature and cooking time. Indeed a vegetable oil subjected to thermal stress such as during frying can completely vary its chemical and physical original characteristics. The cooking process causes the vegetable oil, Triglyceride to break-down to form, Di-glycerides, Mono-glycerides, and free fatty acids (FFAs).The amount of heat and water in the frying increases the hydrolysis of triglycerides, therefore it causes a growth of the Free Fatty Acids (FFAs) in the WCO. Moreover, because of oxidation and polymerization reactions, there is an increase in the viscosity and the saponification number of the WCO when compared with the original oil .Furthermore the transport of matter and heat between the frying food and the vegetable oil occurs and causes a higher content of water in the WCO. During the transesterification reaction, the presence of water in the WCO samples often lead to hydrolysis, whereas high FFA content and high saponification number can lead to

saponification reactions. Both hydrolysis and saponification reactions cause low biodiesel yield and high catalyst consumption.

2.5. Biodiesel Production Technologies

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. The direct use of vegetable oils and its blends as fuel in diesel engines had been considered both unsatisfactory and impractical, primarily due to high viscosity, acid composition and free fatty acid content of such oils, as well as gum formation due to oxidation and polymerization during storage and combustion. Carbon deposits and lubricating oil thickening are two of the more obvious problems. Because of these problems, efforts have been undertaken to convert these vegetable oils to suitable and viable biodiesel fuels (Aransiola et al., 2013).

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. The dominant technologies, which enable us to use oil and fat feedstock types as fuel in diesel engines, are usually described as direct use or blending of oils, micro-emulsion, pyrolysis and transesterification. Transesterification being currently mentioned by various researchers as the most preferable due to better quality of fuel produced (Gebremariam & Marchetti, 2017).

2.5.1. Direct use/blending

Direct uses of vegetable oils have generally been considered not satisfactory and impractical for both direct and indirect diesel engines. The high viscosity, acid composition, free fatty acid content, as well as gum formation due to oxidation and polymerization during storage and combustion, carbon deposits and lubricating oil thickening are obvious problems. In addition to that, oil deterioration and incomplete combustion are the two severe problems associated with the direct use of vegetable oils as fuels (Gebremariam & Marchetti, 2017).

2.5.2. Micro-emulsification process

The problem of the high viscosity of vegetable oil was solved by micro-emulsions with solvents such as methanol, ethanol, and 1-butanol. Micro-emulsion is defined as a colloidal equilibrium dispersion of optically isotropic fluid microstructures with dimensions generally in the 1-150 nm

range formed spontaneously from two normally immiscible liquids and one or more ionic or non-ionic amphiphilic. The components of a biodiesel micro-emulsion include diesel fuel, vegetable oil, alcohol, and surfactant and Cetane improver in suitable proportions. Alcohols such as methanol and ethanol are used as viscosity lowering additives, higher alcohols are used as surfactants and alkyl nitrates are used as Cetane improvers. Micro-emulsions can improve spray properties by explosive vaporization of the low boiling constituents in the micelles. Micro-emulsion results in reduction in viscosity increase in Cetane number and good spray characters in the biodiesel. However, continuous use of micro-emulsified diesel in engines causes problems like injector needle sticking, carbon deposit formation and incomplete combustion (Gashaw & Teshita, 2014).

2.5.3. Pyrolysis/Thermal cracking

Pyrolysis refers to a chemical change caused by the application of thermal energy in the absence of air or oxygen, or by the application of heat in the presence of a catalyst, which results in cleavage of bonds and formation of a variety of small molecules. Pyrolysis is conducted at temperature range of 400–600 ℃. The process produces gases, bio-oil, and a char depending on the rate of pyrolysis. Pyrolytic chemistry is difficult to characterize because of the variety of reaction paths and the variety of reaction products that may be obtained from the reactions that occur. The pyrolyzed material can be vegetable oils, animal fats, natural fatty acids and methyl esters of fatty acids. In another study it was mentioned that pyrolyzate (product of pyrolysis) from any feedstock type has lower viscosity, flash point, and pour point than petroleum diesel fuel and equivalent calorific values. In addition, the cetane number of the pyrolyzate is lower. According to them, the pyrolyzed vegetable oils contain acceptable amounts of sulfur, water and sediments and give acceptable copper corrosion values but unacceptable quantities of ash, carbon residual and pour point. Thermal pyrolysis of triglycerides has several advantages such as lower processing cost, simplicity, less waste, and no pollution. Another disadvantage of pyrolysis is the need for distillation equipment for separation of the various fractions. In addition, the product obtained is similar to gasoline containing Sulphur, which makes it less ecofriendly (Gebremariam & Marchetti, 2017).

2.5.4. Transesterification (Alcoholysis)

Transesterification, also known as alcoholysis is a multistep reversible reaction in which triglycerides are converted to di-glycerides and then to mono-glycerides which finally is converted to biodiesel and glycerol (by-product). This is a chemical process in which carboxylic acid ester is converted into different carboxylic acid esters. During the process, exchange of 'R' group of an ester with 'R' of an alcohol takes place in presence of a catalyst resulting in an ester with larger alkoxy group (starting from methyl/ ethyl esters).

Transesterification is the most widely employed process for commercial production of biodiesel. It involves heating the oil to a designated temperature with alcohol and a catalyst, thereby restructuring its chemical structure. This conversion reduces the high viscosity of the oils and fats. For the transesterification of triglyceride (TG) molecule, three consecutive reactions are needed. In these reactions, FFA is neutralized by the TG from the alcohol. One mole of glycerol and three moles of alkyl esters are produced (for each mole of TG converted) at the completion of the net reaction. These separate into three layers, with glycerol at the bottom, a middle layer of soapy substance, and biodiesel on top. Transesterification is a reversible reaction. To obtain reasonable conversion rates therefore, it requires a catalyst. The reaction conditions, feedstock compositional limits and post-separation requirements are predetermined by the nature of the catalyst (Sani et al., 2013).

2.6. Types of Transesterification Reaction

2.6.1. Homogeneous Acid - Base Catalyzed Transesterification

This method involves the use of catalyst in liquid form, mainly acid and alkali catalysts. The basic factor in the acid catalysis is the protonation of the carbonyl group in triglycerides and the alcohol attacking the protonated carbon to create a tetrahedral intermediate. However, in a homogeneous-base catalyzed reaction, the important factor is to create nucleophilic alkoxide from the alcohol to attack the electrophilic part of the carbonyl group of the triglycerides.

Acid catalyzed transesterification (esterification process) method is more suitable for feedstock's with high FFAs, which may be of low grade and are less expensive. The acid catalysts used include sulphuric, hydrochloric, sulfonic and phosphoric acids. This method has the advantage of

producing tailored biofuel, as the biodiesel properties can be customized based on the fatty acids present in the feed and consequently the fatty esters obtained in the product. However, this method is sensitive to the presence of water. The reaction is a slow one, usually performed at high oil to alcohol molar ratios, low to moderate temperatures and pressures, and high acid catalyst concentrations. The separation and purification of the downstream process is tedious and capital intensive.

The homogeneous alkali-catalyzed transesterification has been the commonest method used at laboratory, pilot and industrial scale levels. This process is catalyzed by alkaline metal hydroxides and alkoxides as well as sodium or potassium carbonates. The production cost of these catalysts is low and they show a very high performance when feedstock's (vegetable oils) with low free fatty acid are used. In addition, this reaction leads to high conversion of triglycerides to their corresponding methyl esters in short reaction times. However, this method also has its own shortcomings, as it is energy intensive, recovery of glycerol is difficult, the catalyst has to be removed from the product, alkaline wastewater requires treatment, free fatty acid and water interfere with the reaction, and low selectivity leads to undesirable side reactions (Aransiola et al., 2013).

2.6.2. Heterogeneous Acid - Base Catalyzed Transesterification

The high production cost of biodiesel is due to both the cost of raw materials and processing costs. The application of heterogeneous (solid) catalysts in biodiesel production alleviates the problems associated with homogeneous catalysis. Heterogeneous catalysts can be recycled and re-used several times with better separation of the final product, minimizing material and processing cost. The process is environmentally benign and can be applied in either batch or continuous mode without the need for further purification steps (Aransiola et al., 2013).

Heterogeneous acid catalyst has the ability to catalyze both transesterification and esterification reactions simultaneously, which becomes quite important when using low quality feedstock's. This catalyst is less corrosive, less toxic, and generates fewer environmental problems (Sani et al., 2013). Industrially, heterogeneous acid catalysts have been determined to be useful because they contain a variety of acid sites with different strengths of Bronsted or Lewis acidity as compared to the homogeneous acid catalysts. Solid acid catalysts such as Nafion-NR50, sulphated zirconia and

tungstated zirconia, were chosen to catalyze biodiesel-forming transesterification due to the presence of sufficient acid site strength.

The use of heterogeneous alkali transesterification has been interesting in biodiesel production as a result of its simplification of production and purification processes, the decrease in the amount of basic waste water, the downsizing of process equipment, and the reduction of environmental impact and process costs. Apart from ease of catalyst recovery, it has been shown that activity of a heterogeneous alkali catalyst may resemble a homogeneous counterpart at the same operating condition (Aransiola et al., 2013).

2.6.3. Nano Catalyzed Transesterification

There a number of recent developments in catalytic conversion of oils and fats to biodiesel. Among them biodiesel production using Nano catalyst and Ionic liquid catalysts are more promising in terms of few advantages over the conventional acid/base catalysts. Nano catalysis involves the use of nanomaterials as catalysts for a variety of homogeneous and heterogeneous catalysis applications. Nanoscale catalysts have high specific surface area and surface energy resulting in high catalytic activity. Generally, Nano catalysts improve the selectivity of the reactions by allowing reaction at a lower temperature, reducing the occurrence of side reactions, higher recycling rates and recovery of energy consumption. The catalytic activity of Nano catalysts are usually affected by calcination temperature during catalyst preparation with calcination. This is because in the preparation process of the catalyst, calcination treatment of catalyst at high temperature is favorable for the interaction between support and active ingredient, which generates new active sites for the catalyst (Gebremariam & Marchetti, 2017).

The synthesis of Nano-CaO catalysts is usually conducted in two main steps: preparation and activation of nanoparticles. The first step includes different methods such as thermal decomposition, impregnation, mixing and precipitation, while the second step is most frequently the calcination of the produced nanoparticles in order to achieve the specific catalytic activity (Banković-Ilić et al., 2017).

2.6.4. Supercritical Transesterification

One of the approaches to overcome problems associated with poor immiscibility between the reactants and at the same time, technical problems caused by catalysts is to use supercritical

method. Supercritical alcohol transesterification reaction takes place under extremely high temperature and pressure. When a gas or liquid is under high pressure and temperature beyond its critical point, unusual phenomena are exhibited on its properties. In this case, liquid and vapor phase are no longer confined under these conditions and single supercritical fluid phase is generated. In the supercritical transesterification method, methanol and oil, which are immiscible liquids at room temperature, will form a homogenous fluid. This is due to the sharp drop in the solubility of methanol and reduction in dielectric constant, which makes methanol a non-polar substance. In this case, the reaction will be accelerated, as there is no mass transfer limitation under such conditions (Gebremariam & Marchetti, 2017).

2.7. Process variables affecting biodiesel production

The process of transesterification brings about drastic change in viscosity of the vegetable oil. The high viscosity component, glycerol, is removed and hence the product has low viscosity like the fossil fuels. The biodiesel produced is totally miscible with mineral diesel in any proportion. Flash point of the biodiesel is lowered after transesterification and the Cetane number is improved. The yield of biodiesel in the process of transesterification is affected by several process parameters which include; presence of moisture and free fatty acids (FFA), reaction time, reaction temperature, catalyst, molar ratio of alcohol to oil and mixing intensity (Gashaw & Teshita, 2014).

2.7.1. Reaction temperature

Temperature has significant influence on transesterification reaction. If the reaction temperature is increased, then the rate of reaction and yield of product will also tend to increase. However, the increase in reaction temperature beyond the optimal level leads to decrease of biodiesel yield, because higher reaction temperature accelerates the saponification of triglycerides and causes methanol to vaporize resulting in decreased yield. However, if the reaction temperature is maintained below 50^0C, then the viscosity of biodiesel will increase. Usually the transesterification reaction temperature should be below the boiling point of alcohol in order to prevent the alcohol evaporation. The range of optimal reaction temperature may vary from 50°C to 60°C for methanol depends upon the oils or fats used. Therefore, the reaction temperature near the boiling point of the alcohol is recommended for faster conversion by various literatures (Gnanaprakasam et al., 2013).

2.7.2. Reaction time

Among others parameters, reaction time is one of the important parameter, that has a profound effect on the FAME yield in the transesterification reaction. The increase in fatty acid esters conversion observed when there is an increase in reaction time. The reaction is slow at the beginning due to mixing and dispersion of alcohol and oil. After that, the reaction proceeds very fast. Nisar et al. studied the effect of reaction time from 0.5 to 4 hr. on the Transterification of Jatropha oil using KOH impregnated calcined animal bone as a catalyst. The FAME content increased on increasing the reaction time from 0.5 to 4 hr. However, the maximum yield of 96.10% was attained after 3 h and on further increasing the reaction time, the yield of biodiesel started to decline (Nisar et al., 2017). Jazie and Sinha studied the effect of reaction time from 0.5 to 5 hr. 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 & Sinha, 2013).

2.7.3. Water content and Free fatty acid

Water content in vegetable oil will accelerate the hydrolysis reaction and simultaneously reduce the amount of ester formation. Water content should not always exceed 0.5% to obtain 90% yield of biodiesel and it is more critical for an acid-catalyzed reaction than base catalyzed reaction. When acid catalysts are used for esterification of free fatty acid to form ester water is obtained as by-product. Water will naturally inhibit acid-catalyzed reaction and its presence in the product will decrease the efficiency of engines. Similarly, if free fatty acid content exceeds 3%, transesterification reaction will not proceed even with homogenous base catalyst. Hence, this problem could be solved by using heterogeneous catalyst and also on pretreatment with acid homogenous catalyst or heterogeneous catalyst to esterify the free fatty acid to form free fatty acid ester (Gnanaprakasam et al., 2013).

2.7.4. Alcohol to oil molar ratio

To produce three moles of alkyl esters, three moles of alcohol and one mole of triglyceride are required. Alcohol to oil ratio always has positive, effect on biodiesel conversion. According to LeChateliers principle, the rate of formation of product increases as reactant concentration is increased. Therefore, if the concentration of alcohol is increased automatically, the rate of product

formation will be accelerated. Further increase in the molar ratio of alcohol to oil will increase the product formation. The recovery of glycerol and unreacted methanol becomes tedious and increases the cost of product biodiesel by increasing its post treatment cost. In most cases, methanol is used for the production of biodiesel, because recovery of methanol from the final product is much easier. Yield of biodiesel obtained from vegetable oil using methanol is higher than other alcohols (ethanol, butanol) and viscosity of biodiesel obtained using methanol is lesser than that of biodiesel obtained from other alcohols. The cost of methanol is lesser than that of other alcohols, but ethanol is less toxic than methanol. Ethanol can be obtained from renewable source. When ethanol or isopropanol is used it will form azeotrope with water, which in turn makes difficulty in separation of water from alcohol during distillation process (Gnanaprakasam et al., 2013).

2.7.5. Type and amount of catalyst

In recent years, various catalysts (homogenous, heterogeneous, and enzyme catalyst) had been tested for the production of alkyl esters. Some of the researchers used concentrated sulfuric acid as acid catalyst but it requires high reaction time and high reaction condition. Even 1% (mole) can give up to 99% conversion. In the case of raw material having higher free fatty acid content, acid catalyst (homogenous/heterogeneous) are used for esterification reaction. Acid catalyst can be used simultaneously for both esterification and transesterification reactions. One of the main disadvantages in using homogenous catalyst is the recovery of catalyst from the final product and formation of soap. To overcome this issue most of researchers used heterogeneous catalyst because heterogeneous catalyst is not affected by free fatty acid and moisture present in the raw material. When catalyst concentration is increased, the yield of the product will also increase. This is due to enhancement in rate of reaction. However, the conversion decreases with excess catalyst concentration, which may be due to increased viscosity of the reaction mixture. Many researchers studied by varying the catalyst concentration and optimized the concentration of catalyst based on product yield and catalyst recovery (Gnanaprakasam et al., 2013).

2.7.6. Mixing Intensity

The mixing of reactants is very important to achieve completion of transesterification reaction and it increases the yield of product. Agitation increases the collision between the particles and

diffusion of one reactant into another, thorough mixing of catalyst with reactants and rate of reaction. Increase in stirrer speed will shorten the reaction time and increase the conversion. Beyond certain speed of stirrer, there would not be significant rise in the yield. Therefore, optimization of stirrer speed is necessary for different raw materials based on the different physical properties (Gnanaprakasam et al., 2013).

2.8. Biodiesel properties and specification

For commercial fuel, the finished biodiesel must be analyzed using sophisticated analytical equipment to ensure it meets international standards. Some properties in the standard, such as the Cetane number or density, can reflect the properties of the chemical compounds that make up the biodiesel, and other properties provide an indication of the quality of the production process. Generally, biodiesel standards identify the parameters that pure biodiesel must meet before being used as a pure fuel or being blended with distillate fuels. These specifications include the American Standards for Testing Materials (ASTM D6751) or the European Union (EN 14214) Standards for biodiesel fuel and it is shown in appendix B.

2.8.1. Kinematic viscosity

Kinematic viscosity is the primary reason why biodiesel is used as an alternative fuel instead of neat vegetable oils or animal fats. The high kinematic viscosities of vegetable oils and animal fats ultimately lead to operational problems such as engine deposits when used directly as fuels (Moser, 2009). The viscosity of an engine fuel is one of the most critical fuel features. It plays a dominant role in the fuel spray, mixture formation and combustion process. The high viscosity interferes with the injection process and leads to insufficient fuel atomization. Moreover, the mean diameter of the fuel droplets from the injector and their penetration increases with increasing fuel viscosity. The insufficient mixing of fuel with air contributes to incomplete combustion in the engine.

Viscosity of any fuel is related to its chemical structure. Viscosity increases with the increase in the chain length and decreases with the increase in the number of double bonds (unsaturation level). In addition, viscosity and heat content of the feedstocks and biodiesel fuels tend to increase together. Viscosities of biodiesels from fats and greases are higher compared to those from vegetable oils since their saturation level are higher. However, these differences in the viscosity values of the biodiesel fuels from oils and low cost feedstocks are mostly within the specification

given in the standards and there is no problem in terms of this fuel property. The maximum allowable limit according to ASTM D445 ranges are (1.9–6.0 mm2/s) and (3.5–5.0 mm2/s) in EN ISO 3104 (Sanli, 2008).

2.8.2. Density

The density of fuel has some effect on the breakup of the fuel injected into the cylinder. In addition, more fuel is injected by mass as the fuel density increases. All biodiesel fuels regardless of produced from vegetable oils or from fats are denser and less compressible than the diesel fuel. Like viscosity, the density and compressibility have very important influences on the engine fuel injection system. The injected fuel amount, injection timing and injection spray pattern are directly affected by these parameters. With increasing density, the diameter of the fuel droplets increases. Since the inertia of the big droplets is big, their penetrations in the combustion chamber will be higher, as well. When a fuel with lower density and viscosity is injected, improved atomization and better mixture formation can be attained. Like viscosity, the heating content of a fuel is a function of its density. Fuel density also affects the exhaust emissions. The density can be correlated with particulate matter (PM) and NOx emission. The fuel which has high density generally causes an increase in PM and NOx emission in diesel engines. The chain length and saturation level of the fuel raise the density. Thus, biodiesels produced from feedstocks such as fats or greases have more saturated fatty acids than the biodiesels produced from vegetable oils. However, this rise in the density is not a problem in terms of the required standard value (Sanli, 2008).

2.8.3. Flash point

The flash point is the minimum temperature at which the fuel will start to burn when it comes to contact with fire. It is an important temperature from the safety point of view during storage and transportation. This temperature is correlated with its volatility, which is an important fuel feature for an engine's starting and warming. The combination of high viscosity and low volatility of a fuel causes bad cold engine start up, misfire and ignition delay. A fuel with high flash point may cause carbon deposits in the combustion chamber. Thus, measuring the biodiesel flash point helps indicate the presence of methanol or ethanol. For example, the presence of 0.5% methanol in biodiesel reduces biodiesel flash point from 170 °C to 50 °C. The limit of flash point ranges in ASTM D93 is ≥130 ^{0}C and in EN ISO 3679 is ≥120 ^{0}C (Sanli, 2008).

2.8.4. Acid value

The acid value (AV), also called neutralization number or acid number is the mass of potassium hydroxide (KOH) in milligrams that is required to neutralize the acidic constituents in one gram of sample. The acid value determination is used to quantify the presence of acid moieties in a biodiesel sample. In a typical procedure, a known amount of sample dissolved in organic solvent is titrated with a solution of potassium hydroxide with known concentration and with phenolphthalein as a color indicator.

The acidic compounds that could possibly be found in biodiesel are: 1) residual mineral acids from the production process, 2) residual free fatty acid from the hydrolysis process or the post-hydrolysis process of the esters and 3) oxidation byproducts in the form of other organic acids.

This parameter is a direct measure of the content of free fatty acids, thus the corrosiveness of the fuel, of filter clogging and the presence of water in the biodiesel. A too high amount of free glycerin can cause functioning problems at reduced temperatures and fuel filter clogging. This parameter can also be used to measure the freshness of the biodiesel. Fuel that has oxidized after long-term storage will probably have a higher acid value. European specification (EN 14104) and US standard (ASTM D664) sets to a maximum value of 0.5 and 0.8 mg KOH/g respectively (Barabás & Todoru, n.d.)..

2.8.5. Iodine value

The iodine value (IV) or iodine number was introduced in biodiesel quality standards for evaluating their stability to oxidation. The IV is a measurement of total unsaturation of fatty acids measured in g iodine/100 g of biodiesel sample, when formally adding iodine to the double bonds. Biodiesel with high IV is easily oxidized in contact with air. The iodine value highly depends on the nature and ester composition of the feedstocks used in biodiesel production. Biodiesel with high IV tends to polymerize and form deposits on injector nozzles, piston rings and piston ring grooves. The tendency of polymerization increases with the degree of unsaturation of the fatty acids (Barabás & Todoru, n.d.).

2.8.6. Water content and sediment

Water and sediment contamination are basically housekeeping issues for biodiesel. Water can present in two forms, either as dissolved water or as suspended water droplets. While biodiesel is generally considered to be insoluble in water, it actually takes up considerably more water than

diesel fuel. Biodiesel can contain as much as 1500 ppm of dissolved water while diesel fuel usually only takes up about 50 ppm. Sediment may consist of suspended rust and dirt particles or it may originate from the fuel as insoluble compounds formed during fuel oxidation. Water and sediment testing is done using 100 mL of biodiesel and centrifuging it at 1870 rpm for 11 min. If the water and sediment level is below 0.005 vol%, the result is reported as <0.005 vol%. Water in the fuel generally causes two problems. First, it can cause corrosion of engine fuel system components. The most direct form of corrosion is rust, but water can become acidic with time and the resulting acid corrosion can attack fuel storage tanks. Water contamination can contribute to microbial growth. The species of yeast, fungi, and bacteria can grow at the interface between the fuel and water at the bottom of a storage tank. The organisms produce sludges and slimes that can cause filter plugging. Moreover, high water contents can also contribute to hydrolysis reaction that is responsible for converting biodiesel to free fatty acids which is also linked to fuel filter blockage. The standard of water content and sediment for biodiesel in ASTM D2709 and EN ISO 12937 specifications is 0.05 (vol%) max (Atabani et al., 2012).

3. MATERIAL AND METHODS

3.1. Materials

3.1.1. Raw material and Reagents

The raw materials required for this study were collected from different places. The fishbone was obtained from Addis Ababa fish restaurant around Lideta. The waste cooking oil was collected from different restaurants, fast foods and shops selling fritter of Addis Ababa.

The chemicals required for this research were methanol, ethanol, potassium hydroxide, Sulphuric acid, hydrochloric acid, iso-propyl alcohol, phenophitaline, di-ethyl ester, potassium iodide, carbon tetrachloride, sodium tiosulphate, acetone, chloroform, glacical acetic acid, iodine, bromine, potassium iodide, starch, distilled and deionized water.

3.1.2. Equipment

The equipment used for the experimental works were oven, grinder, sieve, digital balance, spatula, maffle furnace, crucible, tong, desiccator, three neck round bottom flask, condenser, oil bath, hot plate with magnetic stirrer, magnetic bar, centrifuge, separating funnel, different sizes of conical and Erlenmeyer flask, beakers, graduate cylinder, pipette, titration instrument, density meter, vibrio viscometer, GC-MS, FT-IR and XRD.

3.2. Methods

The methodology that used to generate data for the research was a laboratory experiment.

The experimental procedure had four phases that were performed.

- Synthesize of heterogeneous base catalyst.
- Pretreatment and characterization of the waste cooking oil.
- Production of biodiesel using the synthesized catalyst.
- Characterization of products (catalyst and biodiesel).

The experimental activity such as pretreatment of waste cooking oil, preparation of catalyst, production of biodiesel and characterization of catalyst such as basicity and performance evaluation was performed in School of Chemical and Bio Engineering Laboratory, AAIT and Debre Berhan University Laboratory. The other characterization of the catalyst, FT-IR and XRD

analysis was done at faculty of natural science department of chemistry, Arat Killo. GC-MS analysis of biodiesel was done at JIJE private limited company.

3.2.1. Preparation of KOH-Impregnated Calcined Fishbone Catalyst

The fishbone waste was collected from fishery restaurants of Addis Ababa around Lideta. First, the fishbone was separated from the meat manually and then it was soaked in boiled water for several hours in order to remove dust, meat, and flesh associated with bones. Then fishbone was soaked with 1% of sodium hydroxide and acetone for 24 hours to remove fillets and gelatinous matter and rinsed with distilled water.

The fishbone was dried in a laboratory electric oven at 105°C for 24 hour. After reached to room temperature the fishbone was milled with grinder in the form of powder and sifted to a particle size of 180-355µm. The fishbone powder was put in to a crucible and calcined in a muffle furnace at different temperatures of 800^0C, 900^0C & 1000^0C for a calcination time of 3 hr. As the calcination process completed it was cooled in the desiccator over silica gel for 24r in order to avoid reaction with air.

The next step was impregnation of different concentrations of KOH in calcined fishbone. The prepared white powder fishbone was used as a support for impregnation of active sites. In order to stabilize the active site, wet impregnation method was employed. In all cases, 10 g of calcined fishbone powder was suspended in 40 ml of deionized water and placed in different Erlenmeyer flasks and to this 10 ml aqueous solutions of different potassium hydroxide concentrations of (5, 10 & 15 wt.%) was added (Nisar et al., 2017). The prepared white powder was then soaked with potassium hydroxide solutions. The mixture was then shake in a shaker for 20 hour at 200 rpm and then heated gradually on hot plate. The obtained precipitate dried in the oven at 120^0C to remove water through evaporation. The dried cake was milled into powder and re- calcined at 600^0C for 2 hr. in order to activate the catalyst. The prepared catalyst was placed in a desiccator over silica gel and kept in the closed vessel to avoid the reaction with humidity in air before used.

In this way, KOH-impregnated calcined fishbone catalysts were prepared at different calcination temperature (800, 900 & 1000^0C) of fishbone with loading percent of 5, 10, and 15-wt%. KOH and the corresponding assignments were $F_{800}I_5$, $F_{800}I_{10}$, $F_{800}I_{15}$, $F_{900}I_5$, $F_{900}I_{10}$, $F_{900}I_{15}$, $F_{1000}I_5$ $F_{1000}I_{10}$ and $F_{1000}I_{15}$ respectively. Moreover, the support (calcined fishbone powder) without Impregnation and pure fishbone that is not calcined were specified as F and N respectively, was investigated.

All above mentioned catalyst samples were characterized and tested in transesterification reaction in similar condition according to ‘‘Materials and Methods’’ section.

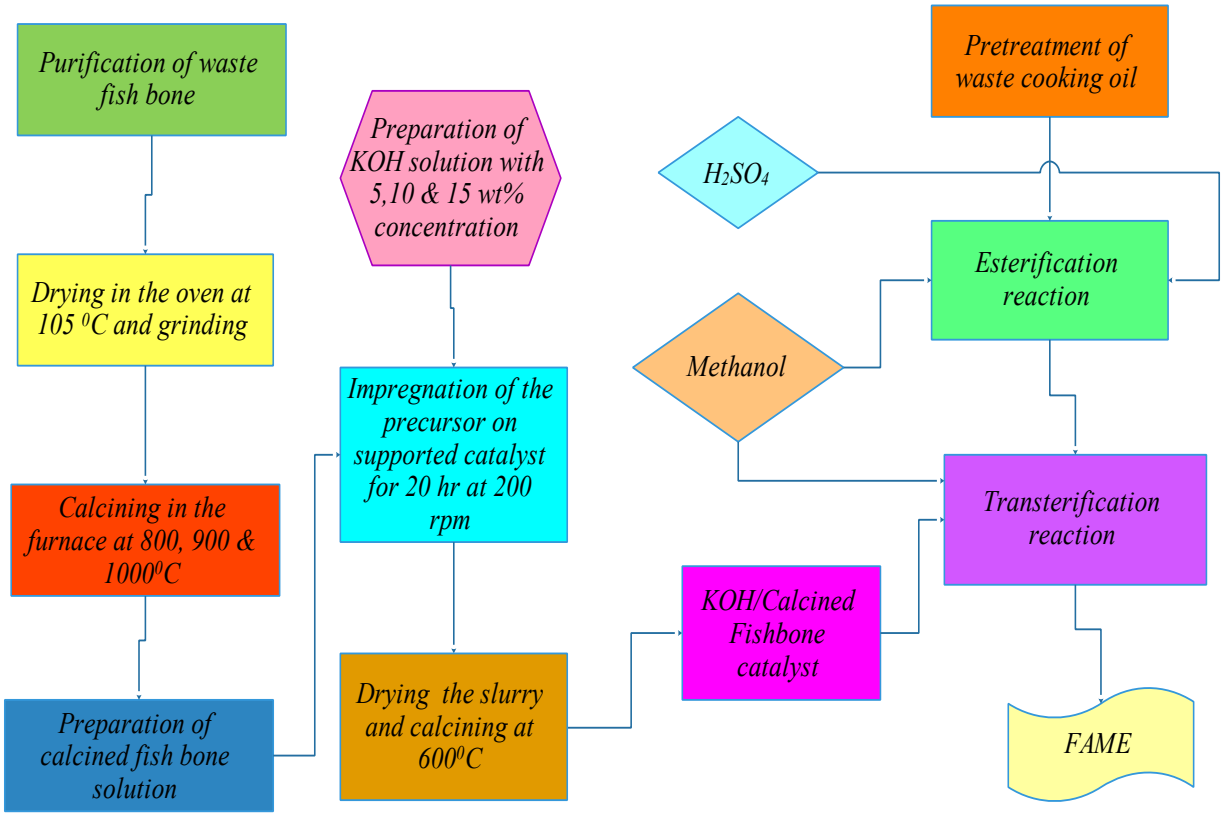


Figure 3.1: Process flow diagram of the experiment

3.3. Catalyst screening

Selection of an efficient solid base catalyst was carried out in order to obtain a high catalytic activity of catalyst by calcination temperature and better KOH 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 (100 ml), methanol to oil molar ratio of 12, catalyst loading of 5-wt%, reaction time of 3hr and reaction temperature of 65^0C. Catalyst with high basicity and FAME yield was selected for further investigation and its properties were studied.

3.4. Characterization of catalyst

3.4.1. Basicity of the catalyst

An acid (HCl, H_2SO_4) is a substance that increases the concentration of hydrogen ions (H^+) in a solution, usually by dissociating one of its hydrogen atoms. A base (NaOH, KOH, Ca $(OH)_2$, $Ca_3(PO_4)_2$….etc.) provides either hydroxide ions (OH^-) or other negatively-charged ions that react with hydrogen ions in solution, thereby reducing the concentration of H^+ and raising the pH. The basicity (total basic sites) of the catalyst was measured by titration method following the procedure reported in the literature (Jin et al., 2018). For each catalyst, 0.4g of sample was soaked in a beaker containing 10 mL of 0.1 N aqueous HCl and the mixture was stirred for 20 min at room temperature. The remaining acid was then titrated in the presence of three drop of phenolphthalein as indicator using 0.1 N 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:

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

3.4.2. 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, 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.

3.4.3. X-ray Diffraction (XRD) Spectroscopy

Crystalline phases of the catalyst were identified by conducting XRD measurements. Rigaku Miniflex 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 5- 70^{o} at a scanning speed of 10^{0}/min were used to record the XRD patterns of the catalyst.

3.5. Purification of waste cooking oil

3.5.1. Pre-treatment of oil

The waste cooking oil was collected from different hotels and restaurants. The waste cooking oil was filtered using vacuum pump to remove food residues and other suspended matters. Then it was washed with hot distilled water to remove the soluble impurities. The washed oil was heated at a temperature of 105^{0}C for 1 hour to remove the water content.

3.6. Two step acid-base catalyzed transterification of waste cooking oil

A two-stage process was used for the transesterification of waste cooking oil, acid-catalyzed esterification and base-catalyzed transesterification reaction. The first step was used to reduce the high free fatty acid (FFA) content of oil below 1% by converting it into esters and in the second step biodiesel was produced in the presence of alcohol and base catalyst.

3.6.1. Acid Catalyzed Esterification

Esterification was conducted to reduce FFA if FFA more than 3%. An acid-catalyzed esterification process before the base-catalyzed transesterification process will eliminate most of the free fatty acids from the waste cooking oil. A mixture of methanol and oil in 9:1 molar ratio was put into a three necked round bottom flask in an oil bath having a thermometer and magnetic stirrer attached with a water-cooled condenser and stirred at low stirring rate for 2hr at 65^{0}C followed by addition of 0.8% (v/v) Sulphuric acid. As the reaction was completed, the mixture is put in to a separating funnel for 3 hr and washed with hot distilled water until a clear water removed from the funnel. The esterified oil was put in to the oven at 105 ^{0}C for 6 hr. The esterification process was repeated until the free fatty acid of the oil is below 1%.

3.6.2. Base-catalyzed Transesterification of Waste cooking oil

For transesterification reaction, the same experimental set up was used as employed for acid pretreatment, a 1000 ml three-necked round-bottom flask fitted with a water-cooled condenser and

thermometer, mixed with a magnetic stirrer shown in (Appendix F). A known amount of catalyst was first activated by dispersing it in a known volume of methanol at 40 ^{0}C with constant stirring (200 rpm) for 30 min. After the catalyst activation, 100 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 centrifugation. The mixture was then allowed to settle for 24 hours in a separating funnel. Biodiesel was obtained at the top layer while glycerol, catalyst and any soap formed during the reaction was formed at the bottom. The biodiesel was heated at 50^{0}C to remove the remaining methanol. The biodiesel was then stored for further characterization.

3.7. Characterization of Waste cooking oil and Biodiesel

3.7.1. Determination of Moisture Content

The sample was weighed and put into crucible. It was then placed in an oven at 105^{0}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.

3.7.2. Determination of Specific gravity and Density

Hydrometer was used to measure the density of the sample according to the test method recommended by ASTM (D4052-96). The sample was filled in 250ml of graduate cylinder and then the hydrometer was injected in the sample. The specific gravity of the sample was recorded from the hydrometer screen at 15^{0}C.

3.7.3. Determination of Kinematic Viscosity

The viscosity of oil was determined using Digital viscometer. The kinematic viscosity was determined at 40°C according ASTM D445 procedure. The temperature of a water bath was set at 40°C and calibrate. 50 ml of sample was placed into the viscometer and allowed the viscometer and sample to equilibrate to the water bath for 30 minutes. The sample was keep in the water thermostat bath until it reaches the equilibrium temperature of 40^{0}C. After maintaining the equilibrium temperature, the digital viscometer tip were inserted to the sample to measure the dynamic viscosity and the reading was taken from the controller.

3.7.4. Determination of Acid Value

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. One (1 ml) of phenophitaline indicator was added in 25 ml of 1 to 1 mixture of ethanol and diethyl ether in 250 ml of beaker and then titrated by 0.1N of ethanolic KOH solution in order to neutralize the mixture.

A weighted quantity of sample was dissolved in 25 ml of 1 to 1 v/v neutralized mixture and four drops of phenolphthalein indicator was added and titrated against 0.1 N ethanolic KOH solution. The mixture 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:

$$Acid\ Value(AV) = \frac{V * N * 56.1}{m} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.2$$

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

N is concentration of ethanolic KOH

m is the mass of a sample in grams

3.7.5. Determination of Saponification Value

A weighted quantity of sample (2 g) was added to 250 ml conical flask and 25 ml of 0.5N 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 at 70^0C for one hour while stirring. Five drops of phenolphthalein indicator was added to the warm solution and then titrated with 0.5N 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:

$$Saponification\ Value(SV) = \frac{56.1 * N * (V_b - V_s)}{w} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.3$$

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

3.7.6. Determination of Iodine Value (IV)

Weighted 0.5 g of the oil into a 250 ml conical flask and add 10 ml of chloroform and 25 ml of Hanus solution then close the flask completely by Para film and leave the solution for 30 minutes with shaking continuously. Next to this, add 10 ml of 15% potassium iodide solution shake thoroughly and add 100ml of freshly boiled and cooled water and mix well. Finally the solution was titrated against 0.1 N Sodium thiosulfate solution till yellow color formed, then add 2-3 drops of starch solution where blue solution formed and then continue with titration till the blue color is disappeared (Volume (ml) of $Na_2S_2O_3$ at end point represents S). For blank, the same procedure was conducted as above but without sample, (Volume (ml) of $Na_2S_2O_3$ at end point represents B). Then the iodine value (IV) was calculated by:

$$Iodine\ Value\ (IV) = \frac{12.69 * N * (V_B - V_S)}{w} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.4$$

Where, V_B 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)

3.7.7. 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). 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 biodiesel. 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.

3.7.8. Determination of fatty acid Composition

The fatty acid composition of sample was determined using Gas Chromatography-Mass Spectrometer (GC-MS) in JIJE Chemical private limited company. The analysis was performed with Agilent 7890A/5975C GC-MS system. The chemical composition of the studied samples were performed using 7890B GC (Gas chromatograph) with a direct capillary column DB-5MS,

(30 m × 0.25 mm x 0.25µm film thickness). The column oven temperature was initially held at 60^0C for 3 minutes, and then increased by 30 °C/min to 180 °C, also increased by 5 ^{o}C/min to 195 °C for 13 min, at 5 °C/min to 240 ^{o}C for 10 min. The GC injector temperature was set up to 250 °C with sample Injection volume of 1µL. Helium was used as a carrier gas at a constant flow rate of 1 ml/min. EI mass spectra were collected at 70 eV ionization voltages over the range of amu (m/z) 40–500 in full scan mode. The ion source and transfer line temperatures were set at 230 and 230°C respectively. The components were identified by comparison of their retention times and mass spectra from mass spectral database.

3.7.9. Determination of Reaction Kinetics

To determine the kinetics of the reaction, the effect of reaction temperature and time were studied on biodiesel yield. Assumed that the catalyst was used in sufficient amount with respect to oil to shift the reaction equilibrium 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 (Birla et al., 2012). Assuming that the reaction to be a single step transesterification, the rate law for forward reaction can be expressed by Eq 3.5

$$TG + 3MeOH \xrightarrow{yields} 3ME + G \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.5$$

$$-r = \frac{d[TG]}{dt} = k' * [TG] * [MeOH]^3 \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.6$$

Where: $[TG]$ – Concentration of triglycerides

$[MeOH]$ – Concentration of methanol

K' - the equilibrium rate constant

This overall reaction follows a second order reaction rate law. However, due to the high molar ratio of methanol to oil, the change in methanol concentration can be considered as constant during reaction. This means that by taking methanol in excess, its concentration does not change the reaction order and it behaves as a first order chemical reaction. Hence, the reaction obeys pseudo-first order kinetics (Widiarti et al., 2017).

Finally, the rate expression can be written as:

$$-r_a = \frac{-d[TG]}{dt} = k * TG \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.7$$

Where k is the modified rate constant and k = k'*[ROH] ^{3}. We assume that the initial triglyceride concentration was $[TG_0]$ at time t = 0 and at time t it fails down $[TG_t]$. The integration of Eq. 3.7 for t=0, [TG] = $[TG_0]$ and at t = t, [TG] = $[TG_t]$ gives the following equation.

$$\ln[TG]_0 - \ln[TG]_t = k * [TG] \quad \ldots\ldots 3.8$$

From mass balance for the first order reaction,

$$[TG] = [TG_0](1 - X_{ME}) \quad \ldots\ldots 3.9$$

Rearranging, $X_{ME} = 1 - \frac{[TG]}{[TG]_0}$ ……3.10

Where X_{ME} is conversion of methyl ester. In addition, in terms conversion the rate expression is also gives as

$$\frac{dX_{ME}}{dt} = (1 - X_{ME}) \quad \ldots\ldots 3.11$$

Which on integration and rearrangement gives,

$$-\ln(1 - X_{ME}) = k * t \quad \ldots\ldots 3.12$$

The activation energy of transesterification reaction was calculated using the Arrhenius equation.

$$k = k_0 e^{\frac{-E_a}{RT}} \quad \ldots\ldots 3.13$$

$$lnk = \frac{-E_a}{RT} + lnk_0 \quad \ldots\ldots 3.14$$

3.8. Design of Experiment

To design the experiment and to obtain suitable predictive model and optimum reaction conditions of transesterification reaction using KOH-impregnated calcined fish bone catalyst, Design Expert 7.0.0 software was used. The design of experiment selected was response surface method coupled with Box-Behnken Design (BBD). RSM can be used to determine the significance and the optimum settings of operating parameters that result in an optimum response over a predefined range of operating conditions and the Box-Behnken method used to decreases the number of required experiments. The Box-Behnken design does not contain any point at the vertices of the cubic region created by the upper and lower limits for each variable. This could be advantageous when the points on the corners of the cube represent factor level combinations that are impossible to test due to physical process constraints or prohibitively expensive. Its "missing corners" may be useful when the researcher should avoid combined factor extremes. This property prevents a potential loss of data in those cases (Selvaraj & Sivakumar, 2013). This design consisted of seventeen randomized experiments with five replicates at the center point to minimize error. In order to maximize the biodiesel/fatty acid methyl ester yield the following variables were varied such as methanol to oil ratio, catalyst dosage and reaction time. The reaction temperature of 65^0C near boiling point of methanol and stirring speed of 600 rpm was chosen to obtain maximum FAME yield at atmospheric pressure based on literature on heterogeneous alkali transesterification (Gnanaprakasam et al., 2013). Lower stirring speed shows lower product formation and higher stirring speed favors formation of soap this is due to the reverse behavior of transesterification reaction. 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 were mentioned as follow. The lower level of time was 2 hr. since below that, the reaction rate is incomplete and the upper level of time was 4 hr above that, the reaction time leads to the reduction of product (biodiesel) due to the reversible reaction of transesterification resulting in loss of esters as well as soap formation.

Biodiesel yield could be elevated by introducing an excess amount of methanol to shift the equilibrium to the right hand side, Levels of methanol to oil ratio and catalyst dosage was 6:1 – 12:1 and 3 – 5 wt. percentage respectively.

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 molar ratio	A	-	6	9	12
Catalyst dosage	B	Wt%	3	4	5
Reaction time	C	Hr.	2	3	4

4. RESULT AND DISCUSSION

4.1. Selection of the highest catalytic activity of catalyst

The KOH-impregnated calcined fishbone catalyst was prepared by impregnation of KOH on the calcined fishbone. The fishbone was used as a support of catalyst that is calcined at different temperature of 800, 900 & 1000 ^{0}C. The Impregnated catalyst was prepared by loading of KOH at a concentration of (5, 10 & 15 wt%.) on the supported catalyst followed by drying and calcination of the impregnated catalyst at 600 ^{0}C in order to activate the catalyst. The efficiency of prepared catalysts were examined by its catalytic conversion of the triglyceride to methyl ester in the transterification reaction and basicity of the catalyst by acid-base titration method. The transterification reaction was carried out over the solid base catalyst (5 wt %) with 12:1 methanol to oil molar ratio at reaction time of 3 hr. and 600-rpm stirrer speed at 65 ^{0}C reaction temperature, and the FAME yield is listed below in table 4.1.

From table and figure, 4.1 it was observed that as the fishbone was calcined at different temperature its catalytic activity increases compared to the pure fish bone. As the temperature increases from 800 to 900 ^{0}C its catalytic activity increases. When the calcination temperature was further increased to 1000 ^{0}C, it resulted in the suppression of catalytic activity due to the high sintering rate of the catalyst. Hence, the best catalytic performance of the catalyst was obtained at a calcination temperature of 900^{0}C. Sintering rate of catalyst reduced basicity of the catalyst. Thus, the experimental results showed that calcination at 900 ^{0}C is considered to be the most perfect, appropriate and optimum temperature for the calcination of this particular catalyst.

The impregnated catalyst shows high catalytic activity than the supported catalyst. By increasing the amount of KOH loading, biodiesel production increased. When the loaded KOH was less than 10 wt %, it did not provide enough catalytic activity because of formation of least basic active site for FAME conversion. However, when the amount of KOH was increased from 10 wt % onward, the catalyst provided weak catalytic activity due to agglomeration of extreme active sites. These excessive active sites also caused poor scattering of K^+ on the surface of calcined bones and made some active sites unreachable to the substrate. Thus, the KOH loading of 10 wt % could be designated as the best value for solid catalyst and it was selected for further investigation on FAME yield.

Table 4.1: Basicity and methyl ester yield of all the prepared catalysts

Catalyst name	Basicity (mmol/g)	FAME (%)
N	0.52	16.0
F_{800}	4.51	31.2
$F_{800}I_5$	6.43	54.4
$F_{800}I_{10}$	9.87	69.0
$F_{800}I_{15}$	8.53	62.1
F_{900}	6.31	52.0
$F_{900}I_5$	9.16	68.3
$F_{900}I_{10}$	12.34	88.12
$F_{900}I_{15}$	10.91	79.2
F_{1000}	5.93	49.1
$F_{1000}I_5$	7.14	56.03
$F_{1000}I_{10}$	9.28	68.56
$F_{1000}I_{15}$	9.04	64.3

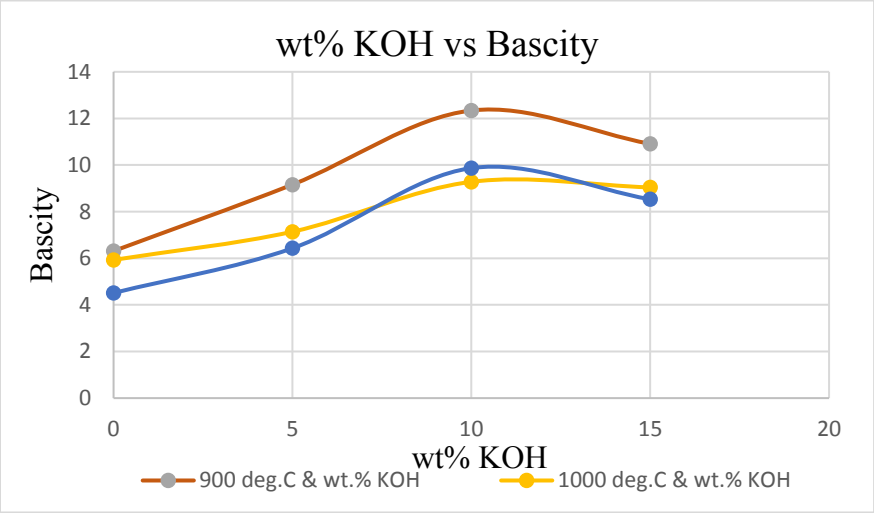


Figure 4.1: Comparison of basicity versus wt.% of KOH for the prepared catalysts

4.2. Catalyst characterizations

4.2.1. Fourier Transform Infrared Spectroscopy Analysis

Fourier transform infrared spectroscopy analysis is used to investigate the functional groups of catalysts. Analysis was conducted on the surface of catalyst using FTIR. Figure 4.2 shows the FTIR plots for fishbone raw material, calcined fishbone catalyst and impregnated KOH calcined fishbone catalyst. The FT-IR spectrum of powdered fishbone showed absorption bands from 3000-3600 cm^{-1} that is due to the stretching vibrational bands of the O–H group associated with the physically absorbed water. In addition, the O-P-O bending mode at absorption band of 567-610 cm^{-1} and P-O stretching mode at absorption band of 1000-1100 cm^{-1} were also detected (Goto & Sasaki, 2014). Besides, the characteristic absorption of the bands at 1410 - 1490 cm^{-1} and 860-880cm^{-1} shows the presence of carbonate ion (Coates, 2000).

In this study, the powdered fishbone shows different absorption bands. The O-H stretching vibration occur at absorption band of 3413.1 cm^{-1} due to presence of water molecule in raw fishbone. The spectra of raw fishbone and heated ones are obviously different due to the changes in their chemical bonds during heat treatment. Through visual observation, the color of bone particles changes from yellowish white to white after calcination. This color changes implies the decomposition of organic substance (e.g., proteins and collagen) in heated bone powder. For affirmation the FT-IR band of 1641.13cm^{-1}, which corresponds to Amide I of collagen that is present in the raw fish bone is completely eliminated in calcined samples.

In the support catalyst, many absorption peaks are transformed due to calcination. The hydrogen bonded, O-H stretching, broad peak is transformed to Ca - O-H group of sharp and strong peak that had an absorption band of 3571.54 cm^{-1}. The vibration peak at 2882.02 cm^{-1} was transformed to 2924.76 cm^{-1} and that at 2516.82 cm^{-1} was disappeared completely. In addition, the broad peak at 1421.06 cm^{-1} and sharp peak at 876.06 cm^{-1} of absorption bands of carbonate ions of the raw fishbone is converted in to a high intensity absorption peak of 1043.50 cm^{-1} of phosphate ion in the support catalyst.

For the solid base catalyst, the addition of KOH affected the structure of the support. The formation of O-H stretching vibration peak at 3642.49 cm^{-1} absorption band due the presence of potassium hydroxide. A high absorption peak of carbonate compound and phosphate compound was showed

at an absorption band of 1462.10 $cm^{-1}$and 1047.6 cm^{-1} respectively. New peak at 869.49 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). In the support and impregnated catalyst, the peaks at 570.72, 601.9 and 633.1 cm^{-1} were attributed to the Ca^{2+} group. As studied $PO4^{3-}$ and Ca^{2+} group are the basic material of (β-$Ca_3(PO_4)_2$) with pores on the surface (Widiarti et al., 2017).

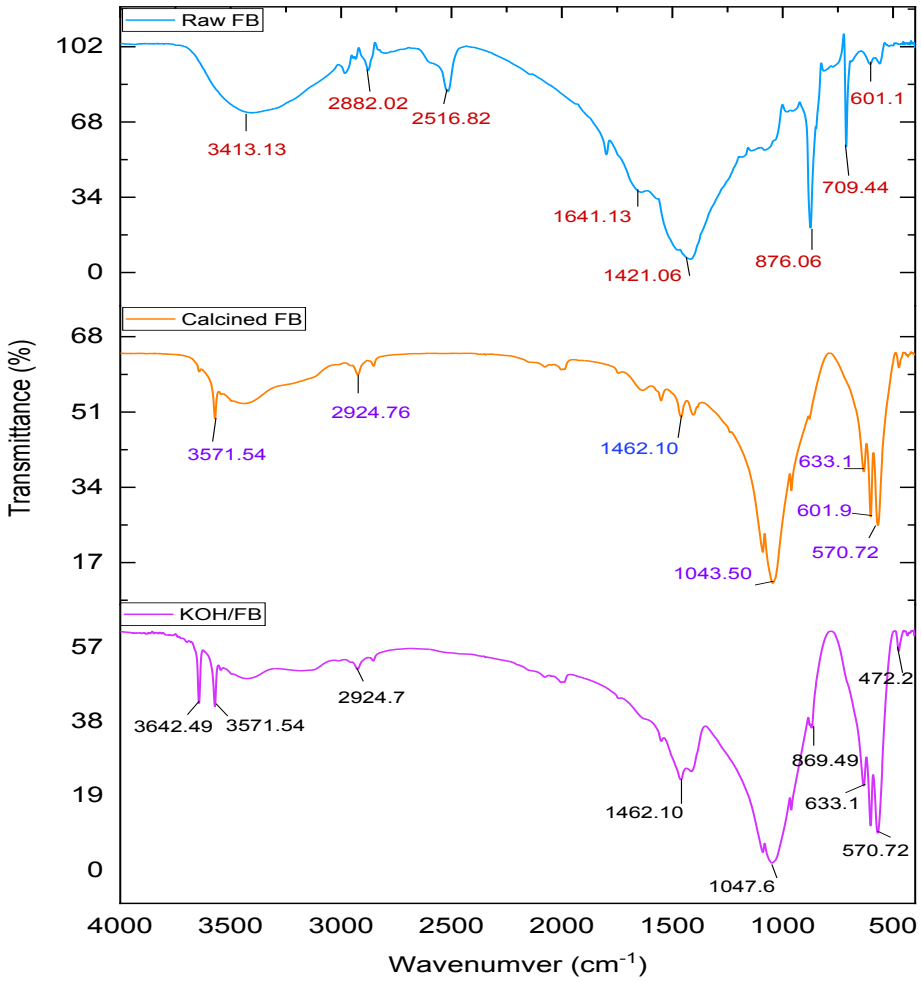


Figure 4.2: FTIR spectra of fishbone raw material, Calcined fishbone and Imp KOH/Fishbone

4.2.2. X-Ray Diffraction (XRD)Analysis

The XRD-patterns of pure, calcined and KOH impregnated calcined fish bones are illustrated in figure 4.3. The XRD pattern of the raw fish bone exhibit peaks at 26.2^0, 29.28^0, 40.1^0 and broad peak at $2\theta^0 = 30\text{-}35^0$, the patterns indicate the presence of calcium in the fish bone. Similar research was reported by (Lesbani, Okta, Sitompul, Mohadi, & Hidayati, 2016) . The peaks were compared with the Joint Committee on Powder Diffraction Standards (JCPDS). Diffraction 2θ from JCPDS for calcium oxide being used is at 32.2^0, 37.3^0, 53.8^0, 64.1^0, and 67.3^0 (Lesbani et al., 2016). The XRD pattern of the supported/calcined fish bone and the impregnated catalyst indicates the presence of calcium oxide at 32.2^0, 52.5^0 and 53.6^0. Furthermore, the peaks at 10.86^0, 21.78^0, 25.88^0, 32.92^0, 39.82^0, 46.72^0, 46.32^0, 51.43^0, 57.61^0, 60.42^0, 62.94^0 and 65.12^0 are attributed to the presence of hydroxyapatite. The peaks at 28.15^0, 29.17^0 and 31.81^0 are attributed to β-Ca_3 $(PO_4)_2$ phase, thus confirm the transformation of hydroxyapatite into β-Ca_3 $(PO_4)_2$ that could act as a base catalyst for transesterification reaction (Farooq & Ramli, 2015). The supported and impregnated catalyst shows the presence of hydroxyapatite at peaks of 11.3^0, 22.2^0, 26.4^0, 33.36^0, 40.1^0, 42.5^0, 47.2^0 and 51^0 and show β-Ca_3 $(PO_4)_2$ at a peak of 29.1^0, 29.1^0 and 29.4^0.

Fish bones when loaded with KOH, clearly shows a new phase of $KCaPO_4$ at 30.9^0, however no other peaks of KOH was detected in the synthesized catalyst and that might be due to the interaction of KOH with calcined fish bones and the formation of $KCaPO_4$ (Nisar et al., 2017).

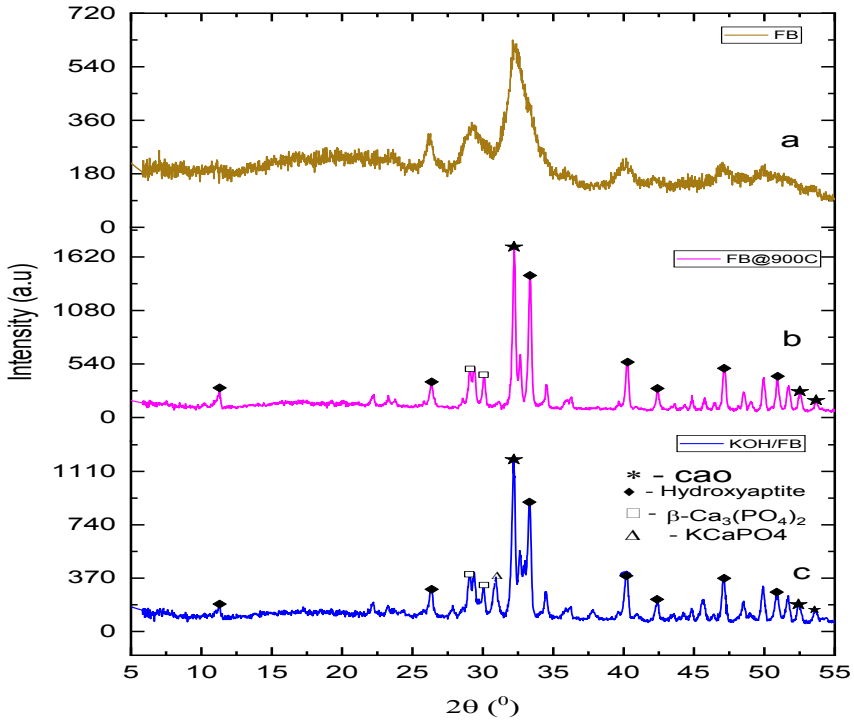


Figure 4.3: XRD patterns of raw fish bone (a), calcined fish bone (b), and KOH impregnated calcined fishbone catalyst (c)

4.2.3. Crystalline size determination

The XRD data was plotted in Match!3 software to identify the compositions present in the sample on their peak width/ angle and peak position and peak height. The software also identify peak lists in order to determine the crystalline size of the catalyst.

To calculate crystallites size and average crystallites size from XRD data using Scherer equation.

$$D = \frac{K * \lambda}{\beta cos\theta}$$

Where: D = Crystallite size β = FWHM (radians)

K = 0.9 (Scherer constant) θ = peak position (radians)

λ = 0.15406 nm (wave length of x-ray sources)

The crystallite size of the calcined fishbone and the impregnated catalyst was 45.74 nm and 35.94 nm respectively that is determined in Appendix C.

4.3. Characterization of waste cooking oil and biodiesel

4.3.1. Physico-chemical and fuel properties of waste cooking oil and biodiesel

The density, kinematics viscosity, moisture content, acid number, saponification value, iodine value and flash point was determined in the experiment by using different instruments and titration methods. The properties were determined based on standard procedures as given in appendix C. The obtained results were shown in table 4-2:

Table 4.2: Physico-chemical characteristics of waste cooking oil and FAME along with American and European Standards

Properties	**Unit**	**Waste cooking oil**	**FAME**	**FAME Standard**	
				ASTM D6751	**EN 14214**
Density @15^{0}C	Kg/m^3	910	882	-	860-900
Kinematic viscosiy@40^{0}C	mm^2/s	39.23	4.104	1.9 – 6.0	3.5 – 5.0
Moisture content	w/w %	0.0253	0.01295	-	-
Acid value	mg KOH/g oil	4.2	0.0935	≤ 0.8	≤ 0.5
Free fatty acid	mg KOH/g oil	2.1	0.04675	-	-
Saponification value	mg KOH/g	199.155	-	-	-
Iodine value	gI_2/100g	121.2	95.56	-	≤ 120
Flash point	^{0}C	-	172	≥ 130	≥ 120

4.3.2. Fourier Transform Infrared Spectroscopy Analysis

FT-IR spectra of the waste cooking oil and the biodiesel produced from this oil are illustrated in Figure 4.4. The waste cooking oil indicates a peak at 3691.52 cm^{-1} conforming the stretching and bending vibration of O-H bonds due to the presence of water molecules. The antisymmetric and symmetric stretching vibrations of C-H in CH_2 and CH_3 groups can be confirmed by the presence of peaks at 2960.53 cm^{-1} and 2855.33 cm^{-1} respectively. The strong peak present at 1746.48 cm^{-1} is attributed to the presence of C=O stretching vibration of carbonyl groups that is present in the triglycerides. The peaks at 1480-1350 cm^{-1} region confirmed the bending vibrations of CH_2 and CH_3 aliphatic groups. The antisymmetric and symmetric bending vibrations of C-H in CH_2 and CH_3 groups can be confirmed by the presence of peaks at 1462.04 cm^{-1} and 1383.27 cm^{-1} respectively.

In the biodiesel the absence of a broad peak in the region of 3200 - 3600 cm^{-1} proposes that there is no water contents in it. Moreover, the peak at 1744.66 cm^{-1} confirmed the stretching vibration of C=O present in the esters and peaks present in the range of 1300-1000 cm^{-1} conforming to that of the C-O stretching vibrations. The FT-IR spectra of the waste cooking oil and biodiesel produced from it are comparable to each other because of the presence of triglycerides and esters. However, small differences were observed where the peaks appeared at 1746.48, 1383.27, 1178.56 and 726.40 cm^{-1} in the waste cooking oil were shifted to 1744.6, 1360.2, 1170.7 and 722.53 cm^{-1} in the biodiesel respectively. So, the disappearance of the peaks from the spectrum of the waste cooking oil at 1462.04, 1094.31, 957.86 and 904.68 cm^{-1} and formation of new peaks at 1434.78, 1193.6, 1019.06, 882.6 and 844.48 cm^{-1} in the produced biodiesel sample evidently confirm the conversion of waste cooking oil into biodiesel.

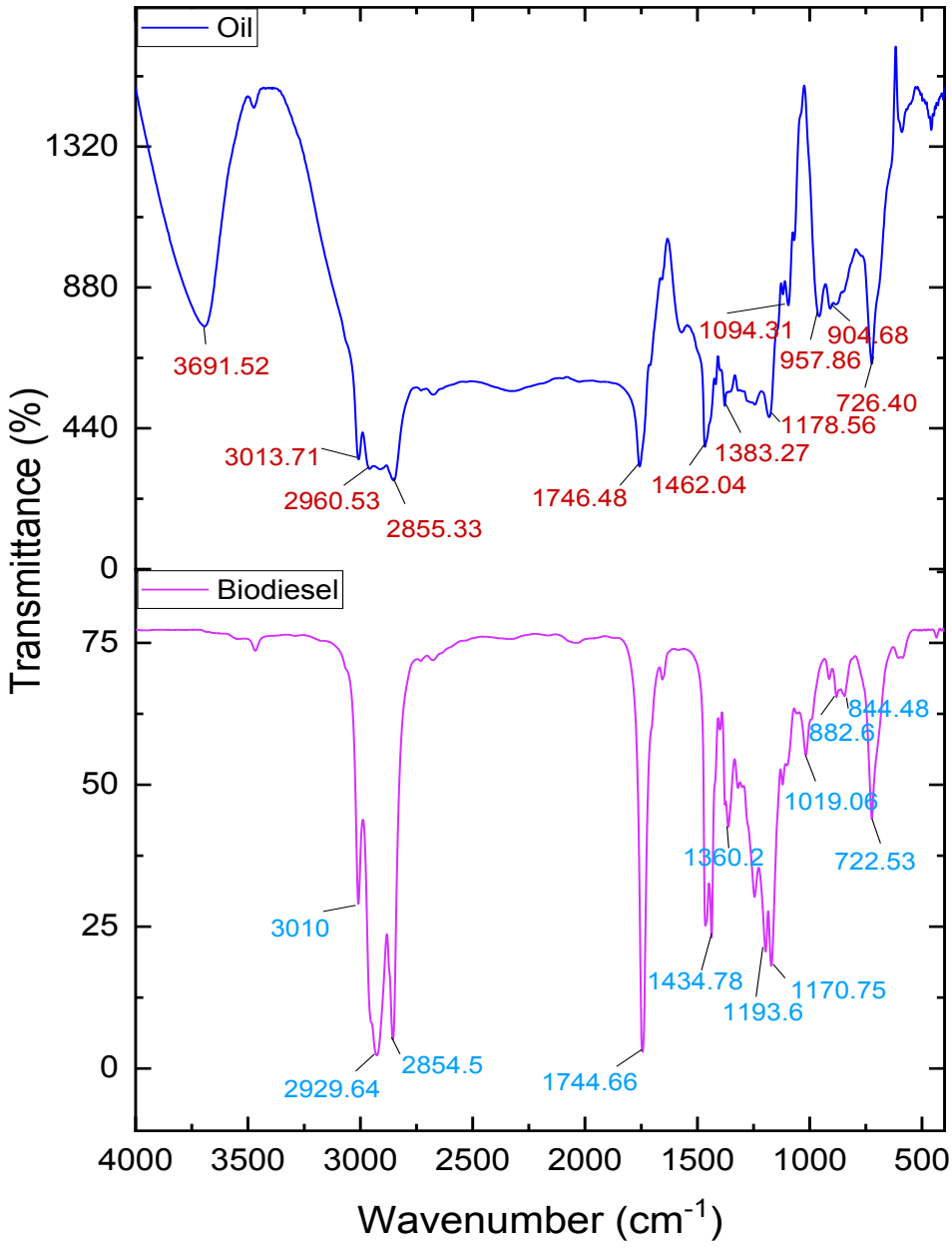


Figure 4.4. FT-IR spectrum of waste cooking oil and biodiesel

4.3.3. GC-MS Characterization of biodiesel

The fatty acid methyl ester composition was identified using GC-MS instrument and it was illustrated in table 4.3. The major fatty acid components present in the biodiesel was linoleic acid 45.54 % followed by Elaidic acid 32.66%. The saturated fatty acids (18.2 %) in the sample included Myristic acid, Palmitic acid, Stearic acid, Margaric acid and Behenic acid. The monounsaturated fatty acids identified were Palmitoleic acid 0.11 %, Elaidic acid 32.66%, Oleic acid 0.91 %, Gonodoic acid 1.03 %, Nonadecanoic acid and Erucic acid 0.09 %, whereas polyunsaturated fatty acids identified in the biodiesel was Linoleic acid 45.54 %. From the total 81.61 % of unsaturated fatty acids have been identified of which 36.07 % was monounsaturated and 18.2 % was saturated.

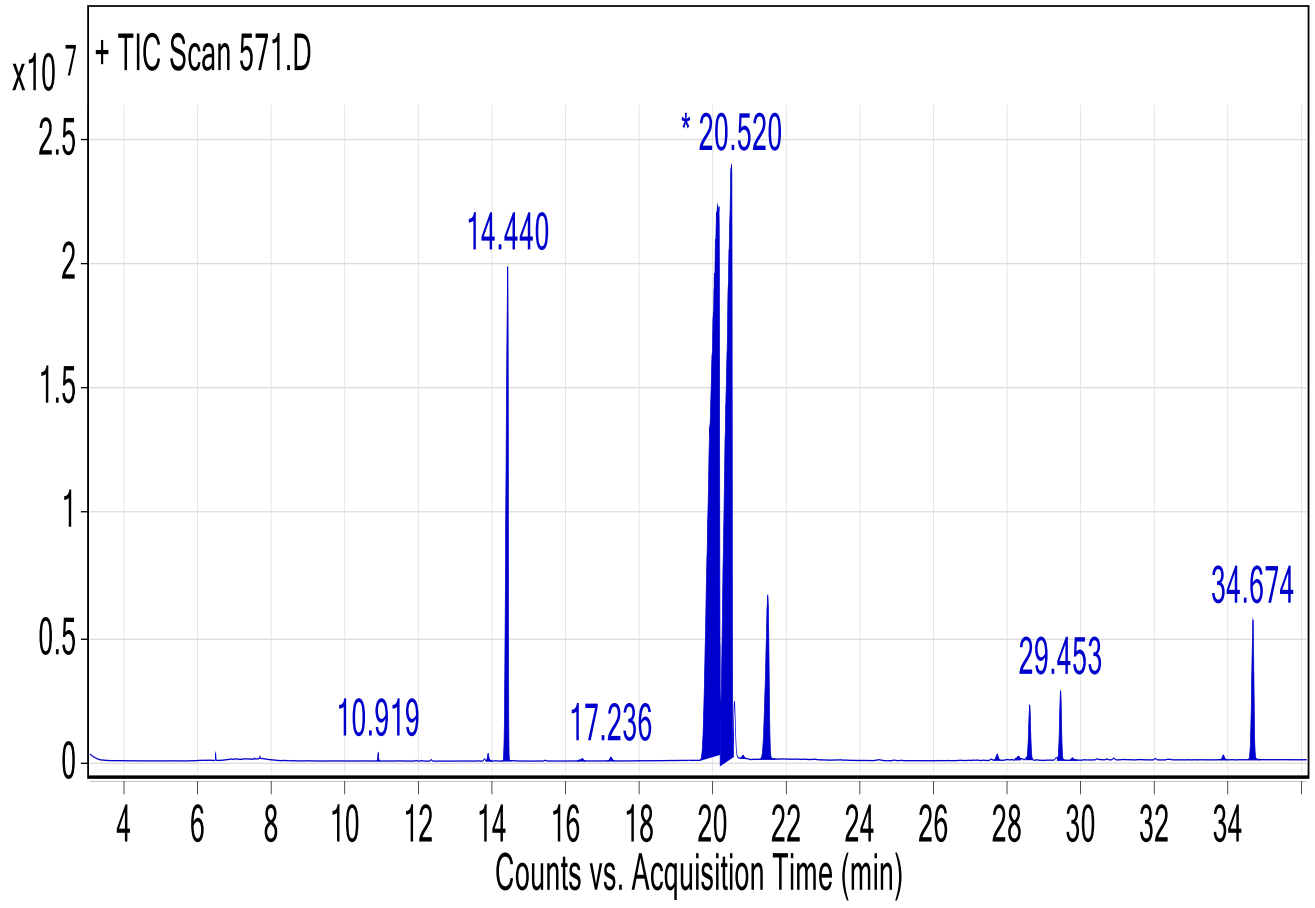


Figure 4.5: Fatty acid composition of methyl ester from GC-MS

Table 4.3: GC-MS identification of major components of the produced methyl ester

S/N	IUPAC/Systematic name	Other name	RT	Area	Formula	Area, %
1	Methyl tetradecanoate	Methyl myristate	10.92	547882.00	$C_{15}H_{30}O_2$	0.06
2	9-Hexadecenoic acid, methyl ester, (Z)-	Methyl palmitoleate	13.91	933942.00	$C_{17}H_{32}O_2$	0.11
3	Hexadecanoic acid, methyl ester	Palmitic acid, methyl ester	14.44	85326650.00	$C_{17}H_{34}O_2$	10.04
4	Heptadecanoic acid, methyl ester	Methyl margarate	17.24	615557.00	$C_{18}H_{36}O_2$	0.07
5	9-Octadecenoic acid, methyl ester, (E)-	Methyl elaidate	20.52	277442340.00	$C_{19}H_{36}O_2$	32.66
6	9-Octadecenoic acid (Z)-, methyl ester	Methyl oleate	20.59	7765635.00	$C_{19}H_{36}O_2$	0.91
7	Methyl stearate	Methyl stearate	21.50	43958479.00	$C_{19}H_{38}O_2$	5.17
8	cis-Methyl 11-eicosenoate	Methyl cis-11 eicosenoate	28.61	8773320.00	$C_{21}H_{40}O_2$	1.03
9	Methyl 18- methyl nonadecanoate		29.45	10769992.00	$C_{21}H_{42}O_2$	1.27
10	13-Docosenoic acid, methyl ester, (Z)-	Methyl erucate	33.87	760425.00	$C_{23}H_{44}O_2$	0.09
11	Docosanoic acid, methyl ester	Methyl behenate	34.67	24299403.00	$C_{23}H_{46}O_2$	2.86
12	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Methyl linoleate	20.13	386890944.00	$C_{19}H_{34}O_2$	45.54

The type and concentration of fatty acids has an outstanding effect on the specific physico-chemical properties of biodiesel. Structural features that influence the physical fuel properties of a fatty ester molecule include chain length, degree of unsaturation and branching of the chain.

Important fuel properties of biodiesel that are influenced by the fatty acid composition are viscosity, Cetane index (ignition quality), heat of combustion, cloud point, oxidative stability, and lubricity. Fatty acid alkyl esters with long chain fatty or saturated acids show higher Cetane number, better oxidative stability, increased heat of combustion and poor cloud point. Esters prepared with unsaturated fatty acids show lower Cetane number, better cold flow properties and suffer oxidative stability (Koria & Nithya, 2012). Therefore, the produced biodiesel had a good cloud point, pour point and viscosity.

4.4. Statistical analysis and model fitting for base catalyzed transterification

In this paper, the relationship between the response (biodiesel yield) and the factors (methanol to oil ratio, catalyst dosage and reaction time) were studied and evaluated in statistical analysis process using ANOVA and RSM model fitting by using design expert 7.0.0 software. Using the Box-Behnken design 17-experimental runs were done. The actual and predicted values of the biodiesel yield and the ANOVA were shown in table 4.4 and 4.5 respectively.

Table 4.4: Box-Behnken experimental design matrix, experimental and estimated data for three level- three-factors response surface analysis

Run	Methanol to oil ratio	Catalyst dosage wt%.	Reaction time, hr.	Biodiesel yield, %		Residuals
				Experimental	Predicted	
1	9	3	2	84.56	84.26	0.30
2	12	3	3	89.6	90.20	-0.60
3	12	4	4	87.25	86.96	0.29
4	12	4	2	90.74	90.44	0.03
5	6	4	4	69.20	69.50	-0.30
6	6	3	3	70.40	70.41	-0.013
7	6	4	2	71.43	71.72	-0.29
8	9	5	2	88.70	89.02	-0.31
9	9	4	3	96.70	95.92	0.78
10	9	4	3	96.20	95.92	0.28
11	9	4	3	95.20	95.92	-0.72
12	12	5	3	91.40	91.39	0.013
13	9	4	3	97.10	95.92	1.18
14	9	4	3	94.40	95.92	-1.52
15	6	5	3	75.60	75.00	0.60
16	9	5	4	84.00	84.30	-0.30
17	9	3	4	83.60	83.29	0.31

4.4.1. Analysis of variance (ANOVA)

The statstical analysis of the ANOVA was given in table 4.5. From the ANOVA of response surface quadratic model for FAME conversion, the Model F-value of 173.74 and Prob > F of <0.0001 implied that the model was significant. For the model terms, values of Prob > F less than 0.05 indicated that the model terms were significant. In this case A, B, C, A^2, B^2, and C^2 are significant model terms (all have Prob > F less than 0.050). This indicated that the methanol to oil ratio, catalyst dosage, reaction time, and their quadratic terms affect the yield much significantly. However, the interaction terms were found to be insignificant. Because values greater than 0.1000 indicate the model terms were, therefore, insignificant. The insignificance of the interaction variables indicated that the increasing and decreasing of one variable in the process doesn't affect the other variables.

As it was obseved the p-value of methanol to oil ratio was less than 0.0001. This indicate that molar ratio is the most significant in determining the model than the rest. In the same manner the catalyst dosage and the raction time have a considerable effect on the model.

The variation between the model prediction and the extra points is compared with the pure error to test the lack of fit. In the statistical output, the lack of fit should NOT be significant. A small F-value and high p-value (greater than 0.1) are good in this test. In table 4.5 it was observed that the "Lack of Fit F-value of 0.39 and p-value of 0.7645" that implies the Lack of Fit is not significant relative to the pure error. This indicated that the model desirably represent the actual relationships of reaction parameters, which are well within the selected ranges. Non-significant lack of fit is good because we want the model to fit.

Table 4.5: Analysis of variance for response surface quadratic model

ANOVA for Response Surface Quadratic Model

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	1420.92	9	157.88	173.74	< 0.0001 *significant*
A-Methanol to oil ratio	654.50	1	654.50	720.23	< 0.0001
B-Catalyst dosage	16.65	1	16.65	18.32	0.0037
C-Reaction time	16.19	1	16.19	17.81	0.0039
AB	2.89	1	2.89	3.18	0.1177
AC	0.40	1	0.40	0.44	0.5298
BC	3.50	1	3.50	3.85	0.0906
A^2	409.76	1	409.76	450.92	< 0.0001
B^2	78.03	1	78.03	85.87	< 0.0001
C^2	172.46	1	172.46	189.79	< 0.0001
Residual	6.36	7	0.91		
Lack of Fit	1.45	3	0.48	0.39	0.7465 *not significant*
Pure Error	4.91	4	1.23		
Cor Total	1427.28	16			

4.4.2. Development of Model Equation

The model equation that correlate the response variables (FAME yield) with the transterification process variables in terms of the actual values after excluding the insignificant terms was given below. Design expert software has been suggested to select quadratic model in the regression analysis because the highest values of adjusted R^2 and predicted R^2 were obtained and the model is not aliased that is shown in table 4.6.

Table 4.6: Fit summary of the response

Sources	**Sequential P-value**	**Lack of fit P-value**	**Adjusted- R^2**	**Predicted -R^2**	
Linear	0.0315	0.0005	0.3619	0.2347	
2FI	0.9923	0.0003	0.1781	-0.3095	
Quadratic	**< 0.0001**	**0.7645**	**0.9898**	**0.9783**	**Suggested**
Cubic	0.7645		0.9862	-	Aliased

Table 4.7: Regression coefficients and significance of response surface quadratic model

Factor	**Coefficient Estimate**	**Df**	**Standard Error**	**95% CI Low**	**95% CI High**	**VIF**
Intercept	95.92	1	0.43	94.91	96.93	
A-Methanol to oil ratio	9.04	1	0.34	8.25	9.84	1.00
B-Catalyst dosage	1.44	1	0.34	0.65	2.24	1.00
C-Reaction time	-1.42	1	0.34	-2.22	-0.63	1.00
AB	-0.85	1	0.48	-1.98	0.28	1.00
AC	-0.31	1	0.48	-1.44	0.81	1.00
BC	-0.93	1	0.48	-2.06	0.19	1.00
A^2	-9.86	1	0.46	-10.96	-8.77	1.01
B^2	-4.30	1	0.46	-5.40	-3.21	1.01
C^2	-6.40	1	0.46	-7.50	-5.30	1.01

The quadratic predicted model of the biodiesel yield in terms of the coded factors is shown in Equation 4.1 below:

Final Equation in Terms of Coded Factors:

$$FAME = 95.92 + 9.4 * A + 1.44 * B - 1.42 * C - 0.85 * A * B - 0.31 * A * C - 0.93 * B * C - 9.86 * A^2 - 4.30 * B^2 - 6.40 * C^2 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 4.1$$

Where: A = Methanol to oil ratio

B = Catalyst dosage, wt%

C = Reaction time, hr.

4.4.3. Model adequecy check

The developed quadratic model has to be examined for its consistency and how it correlates the experimental variables to the response variables. The regression model was found to be reasonably significant with the correlation coefficients of determination of R-Squared, adjusted R-Squared and predicted R-Squared having a value of 0.9955, 0.9898 and 0.9783 respectively.

Table 4.8: Model adequacy measurements

Std. Dev.	0.95	R-Squared	0.9955
Mean	86.24	Adj R-Squared	0.9898
C.V. %	1.11	Pred R-Squared	0.9783
PRESS	30.92	Adeq Precision	36.133

The quality of the model developed could be evaluated from their coefficients of correlation. The value of R-squared for the developed correlation is 0.9955 it reveals that the model is in a good agreement with the experimental data and can be used to predict the response accurately.

The "Pred R-Squared" of 0.9783 is in reasonable agreement with the "Adj R-Squared" of 0.9898 that is less than 0.2 differences as one might expect. The “Adeq Precision" measures the signal to disturbance ratio due to random error. A ratio greater than four is desirable. Biodiesel yield has ratio of 36.133, which indicates an adequate signal.

The predicted versus actual values of FAME yield obtained using the developed model equation as shown in figure 4.6. the graph of predicted versus experimental values shows that the model was achieved in fitting the correlation between the reaction parameters to the response yield with the correlation coefficients $R^2 = 0.9955$.

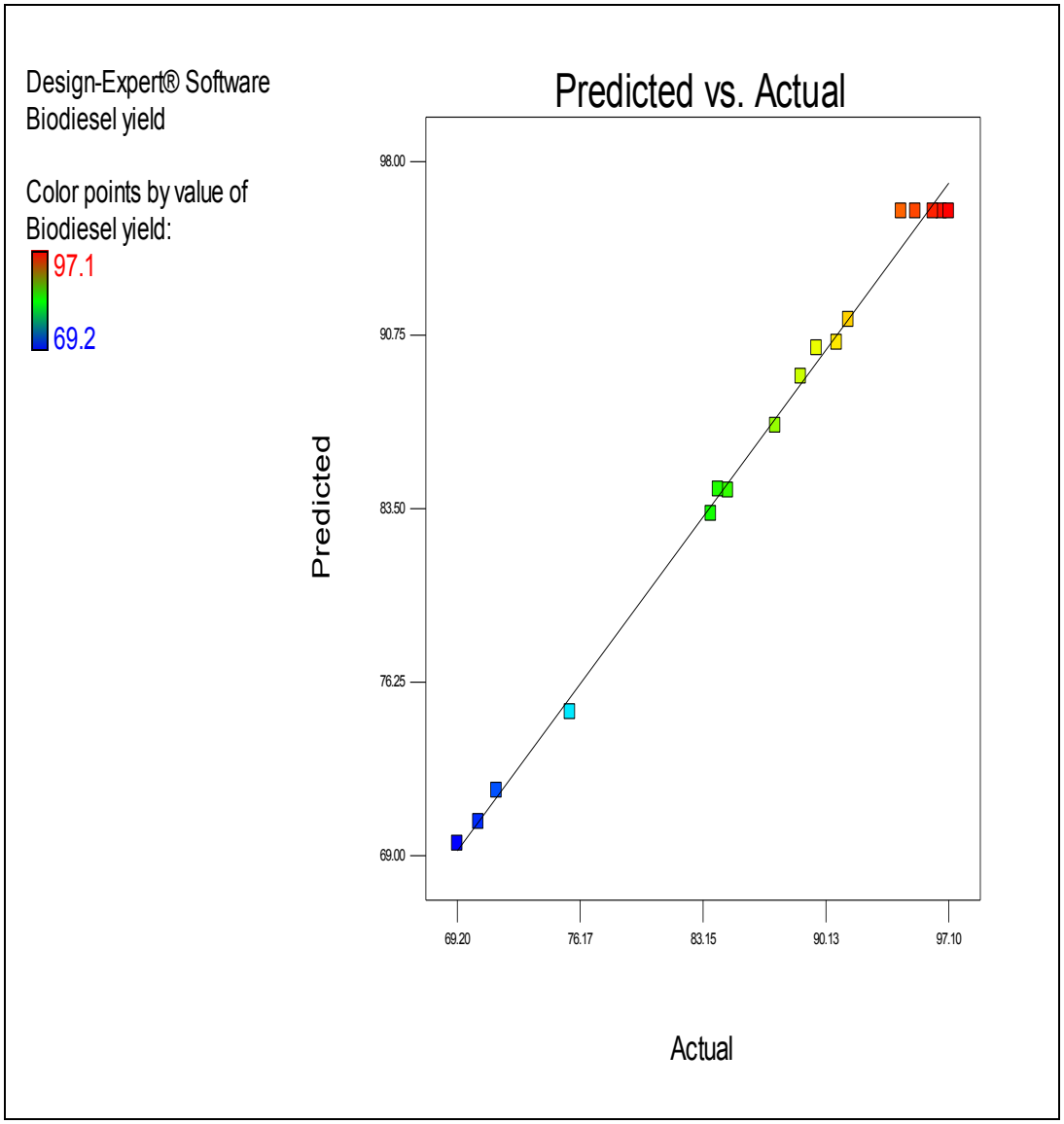


Figure 4.6: Comparison of predicted versus actual response value

As shown in fig.4.7 below, the normal probability plot indicates the residuals following by the normal % probability distribution, in the case of this experimental data the points in the plots shows fitted to the straight line in the figure, this shows that the quadratic polynomial model satisfies the assumptions analysis of variance (ANOVA) i.e. the error distribution is approximately normal.

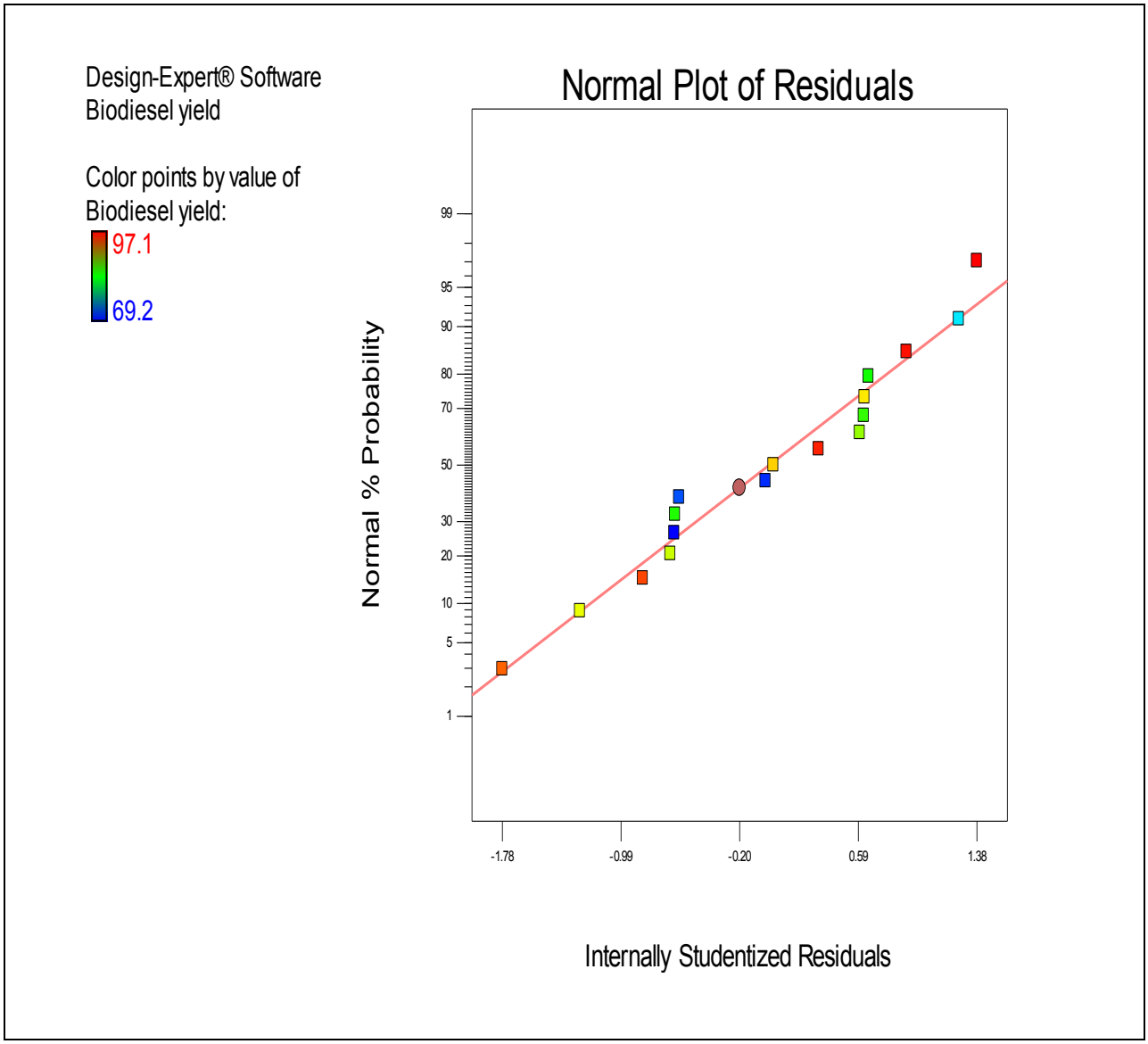


Figure 4.7: The graph of normal probability of residuals

4.5. Effect of process variables on transterification reaction

4.5.1. Effect of indiviual variables on FAME yield

i. Effect of methanol to oil ratio

The molar ratio of methanol to oil is one of the important factor that affects the conversion of triglycerides to esters. Stoichiometricaly, three moles of methanol is required for each moles of triglyceride. Heterogeneous catalysts are known to be slow in transesterification reaction rate when compared to homogeneous catalysts. However, the reaction rate of such catalysts (solid) could easily be improved through the usage of excess methanol. In this paper, the effect of methanol to oil molar ratio (6 to 12) was studied on biodiesel yield at constant reaction time of 3 hr. and catalyst dosage of 4wt% as shown in figure 4.8.

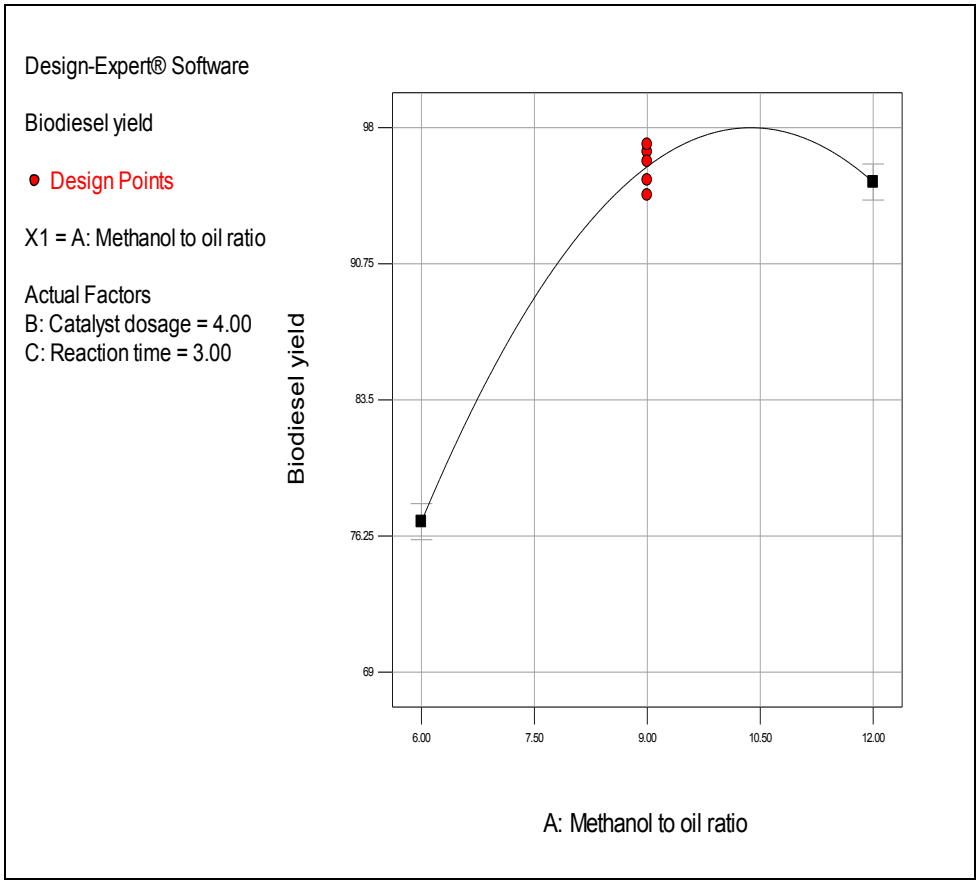


Figure 4.8: Effect of methanol to oil molar on biodiesel yield

In figure 4.8 shown that as the molar ratio of methanol to oil increases the biodiesel yield increases and maximum at molar ratio of 10.5. 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 biodiesel yield possibly due to the dilution of the oil with the excess alcohol, which cover active site of the catalyst (catalyst deactivation) and difficulty in the separation of biodiesel from glycerol, was faced due to high amount of methanol. Hence, excessive use of methanol decreased the conversion by shifting the equilibrium in the reverse direction.

ii. Effect of catalyst dosage

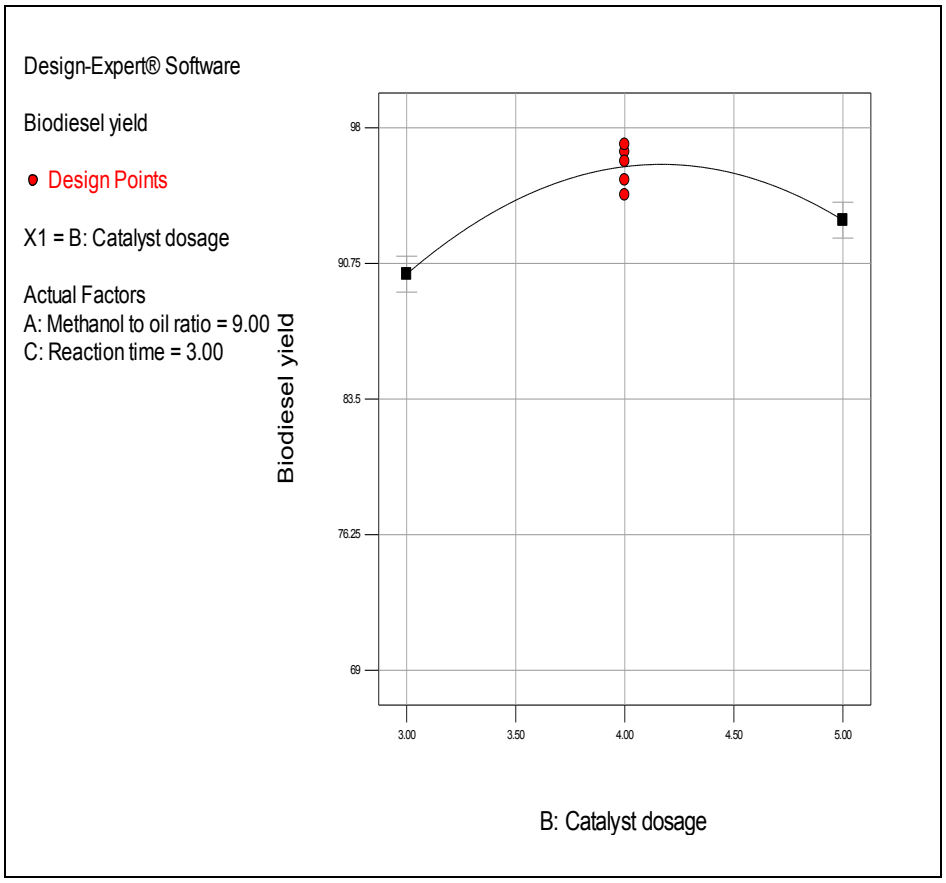


Figure 4.9: Effect of catalyst dosage on biodiesel yield

The effect of catalyst dosage was observed on the biodiesel yield at constant reaction time of 3hr and methanol to oil molar ratio of 9:1 as shown in the figure 4.9. As the catalyst amount increases from 3wt%. to 4wt%. the biodiesel yield is increased, however beyond 4wt% the biodiesel yield

decreses due to an increase in viscosity of the mixture leads to diffusion resistance between the oil, methanol and catalyst. The viscous nature of the mixture leads in difficulty of product separation, formation of soap and decreases the FAME yield.

iii. Effect of reaction time

Reaction time is one of the important parameter that has a great effect on the FAME yield. In this study, the Transterification reaction time in the range of (2 to 4) hr. on biodiesel yield was investigated and the results at constant methanol to oil molar ratio of 9:1 and catalyst amount 4wt% is shown in Figure 4.10. The FAME yield increases as the reaction time increases and a maximum yield of 97.1 attained at 3hr, and on further increasing the reaction time beyond 3 hr. results in adsorption of products on the active catalytic site and gives lower FAME yield.

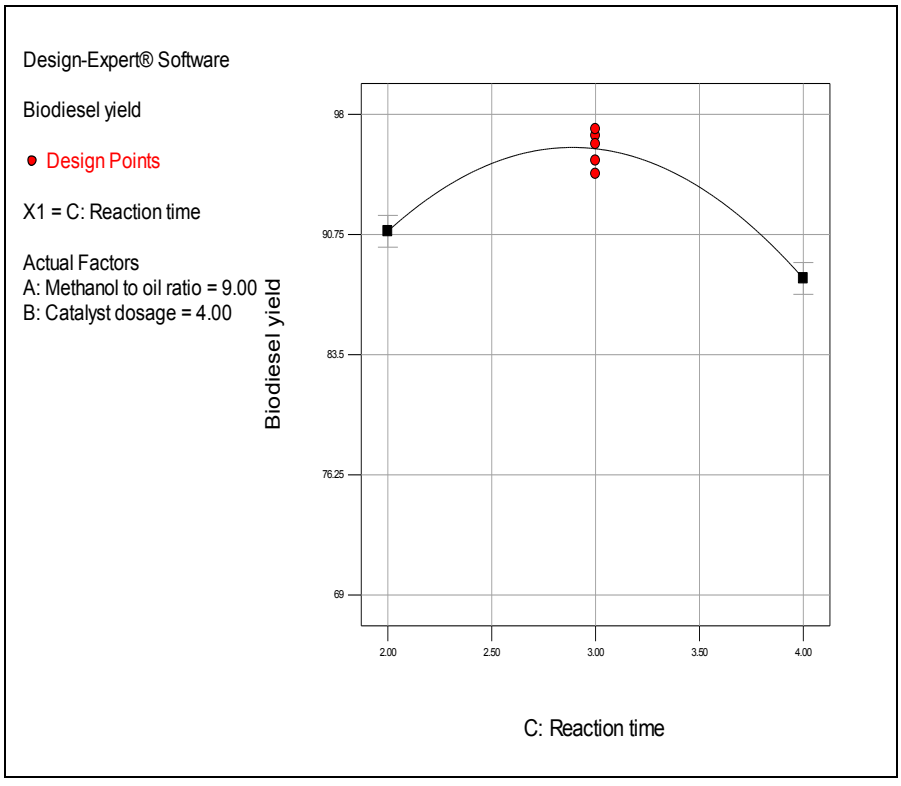


Figure 4.10: Effect of reaction time on biodiesel yield

4.6. Optimization of process variables in transterification reaction

In Response Surface Methodology (RSM) the process variables were optimized in order to maximize the biodiesel yield at optimum material cost and time. The optimum conditions for the three factors, i.e. molar ratio of methanol to oil, catalyst loading and reaction time 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. Optimization of the process was conducted by setting the parameters and response with their high and low values were listed in table 4.9 in order to identify the optimum condition.

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 parameters as shown in table 4.10, two experiments were conducted in the laboratory in order to validate the optimum conditions, FAME yield of 97.46% was obtained, and the results are closely related with the data obtained from optimization analysis using desirability functions.

Table 4.9: Optimization of process parameters

Name	**Goal**	**Lower Limit**	**Upper Limit**
Methanol to oil ratio	is in range	6	12
Catalyst dosage	is in range	3	5
Reaction time	is in range	2	4
Biodiesel yield	Maximize	69.2	97.1

It can be explained from the result KOH-impregnated calcined fishbone catalyst has the potential to serve as a solid base catalyst for transesterification of oils into FAMEs.

Table 4.10: Optimization results

Methanol to oil ratio	Catalyst dosage (wt.%)	Reaction time (hr.)	Yield (%)		Desirability
			Predicted	Measured	
10.56	3.96	2.7	97.8026	97.46	1.0

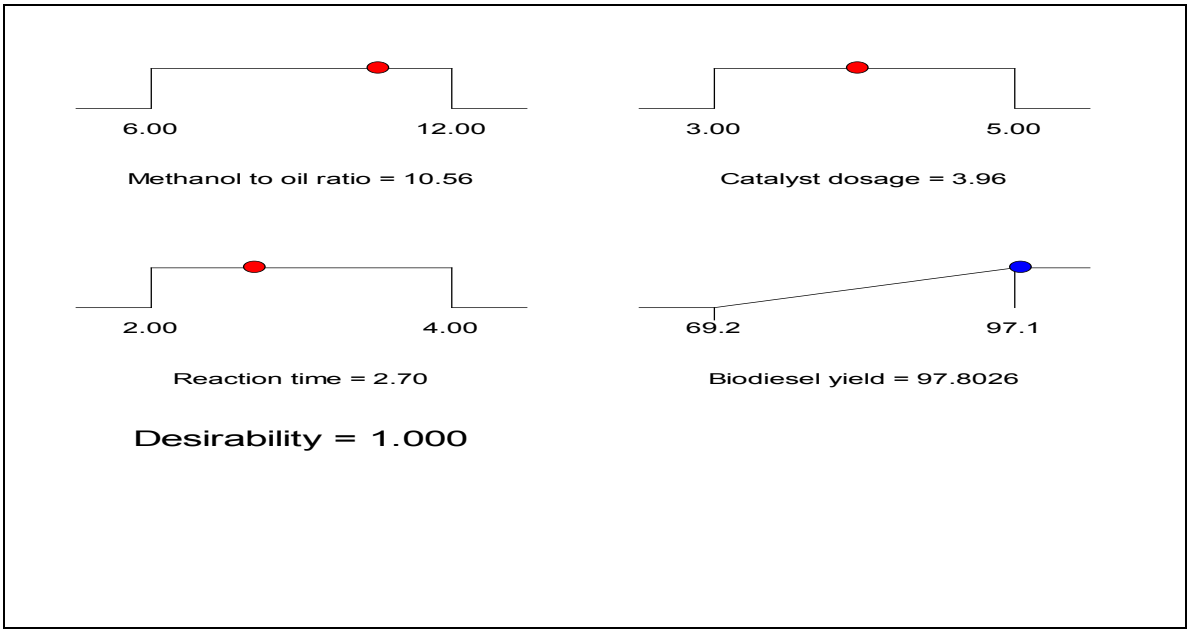


Figure 4.11: Optimization operating condition of biodiesel production.

4.7. Determination of reaction kinetics for base-catalyzed Transterification reaction

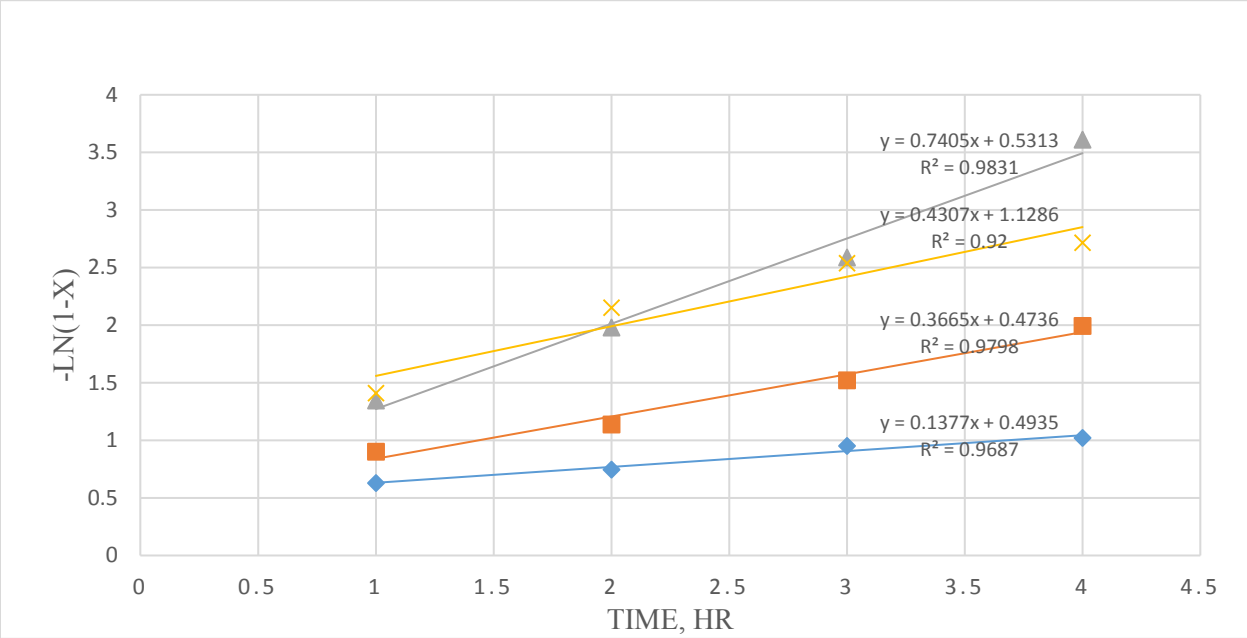
To determine the kinetics of base-catalyzed transesterification reaction of waste cooking oil with methanol (10.56:1 methanol to oil molar ratio) in the presence of KOH-impregnated calcined fish bone catalyst (3.96 wt%) at different temperatures and time under the stirring speed of 600 rpm were investigated. 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. The biodiesel yield at different time and temperature is shown in Table 4.11:

Table 4.11: Biodiesel yield at different temperature and time

Temp, K / Time, h	323	328	333	338
1	46.8	52.6	61.4	64.0
2	59.4	68.0	78.2	86.4
3	74.0	86.2	92.5	97.3
4	75.6	88.4	92.1	93.4

Temp, K	323	328	333	338
Time, hr	ln(1-x)	ln(1-x)	ln(1-x)	ln(1-x)
1	0.6311	0.9014	1.347	1.4106
2	0.7465	1.1394	1.9805	2.154
3	0.95192	1.52326	2.5902	2.5383
4	1.02165	1.9951	3.612	2.3434

The figure *-ln (1-X_{ME}) versus reaction time plot at different temperatures* plotted using equation (3.12)



Where the lines plot at different temperature as assigned below,

◆ - 323K ■ - 328K ▲ - 333K X - 338K

Figure 4.12: -ln (1-XME) versus reaction time plot at different temperatures

Table 4.12: Reaction rate constants for the transesterification

T(^{0}K)	K(hr^{-1})	R^2	lnk	1/T * 10^3
323	0.1377	0.9687	-1.9826	3.1
328	0.3665	0.9798	-1.003756	3.05
333	0.4307	0.92	-0.8423434	3.003
338	0.7405	0.9831	-0.30043	2.96

From Fig. 4.12, the value of reaction rate constant k was determined at different temperature and shown on table 4.12. The activation energy of transesterification reaction was calculated using the Arrhenius equation (3.14)

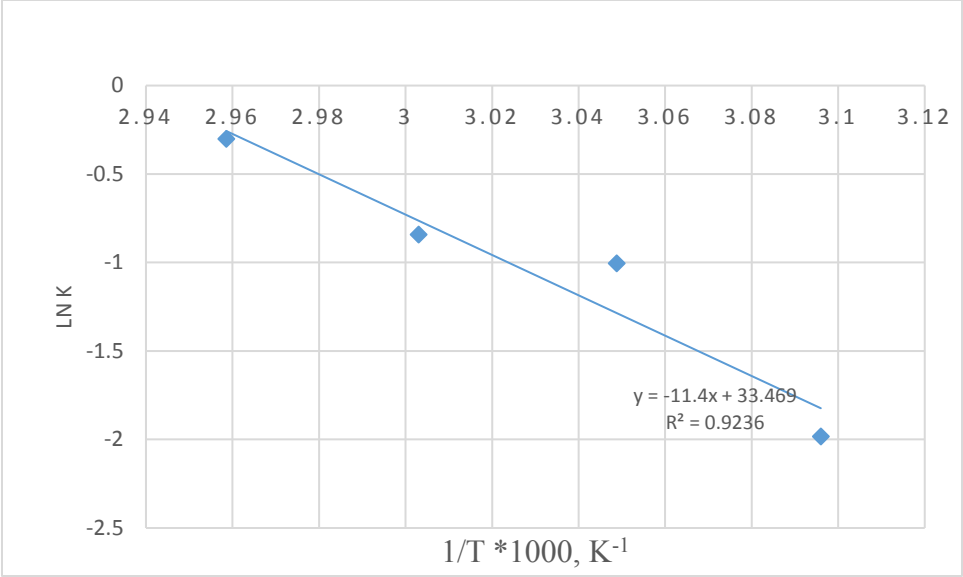


Figure 4.13: Arrhenius plot lnk versus 1/T*103 for transesterification of WCO

The slope (-11.4) and intercept (33.469) of the graph between lnk and 1/T $*10^3$ (Fig. 4.13) give the values of activation energy and frequency factor. The activation energy (Ea) determined from graph was 94.77 kJ/mol and the frequency factor (k_0) was $3.43*10^{14}$ hr^{-1}. Therefore, the rate constant for base-catalyzed transesterification reaction in the presence of KOH-impregnated calcined fish bone catalyst was $k = 3.43 * 10^{14} \exp(\frac{-11400}{T})$.

4.8. Catalyst Reusablity

Reusability study of KOH impregnated calcined fishbone catalyst was done to ensure its applicability as recycled catalyst in transesterification reaction. The solid catalyst was separated from the biodiesel mixture via centrifugation after the transesterification was completed. The catalyst was then washed with methanol to eliminate the impurities such as glycerol and reuse in the transesterification after it had undergone recalcination at 600^0C. The reusability of the calcined catalyst was studied for five sequential transesterifications, each using the same optimal operating parameters as per suggested in the numerical study. The result as represented in Figure 4.14 depicted that the KOH impregnated calcined fishbone catalyst can be reuse up three times with small significant drop in catalytic activity. However, in the fourth and fifth runs, a reduction in biodiesel yield was noticed. Although the biodiesel yield decreased with each catalyst reuse, it was still above 70% after the fifth consecutive uses. The efficiency of the catalyst decreases due to

deactivation of the catalyst that is caused by the adsorption of impurities on the catalyst surface and 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, which is reaction of gas or vapor with solid particle to produce inactive phase. Any leached species from the solid catalyst will be retained in the liquid phase medium and can facilitate the reaction. Thus, if leaching has occurred then without the solid phase catalyst the reaction will still proceed with the leached species. Furthermore, reuse of the catalyst after filtering would also give rise to a lower catalytic activity if leaching has occurred.

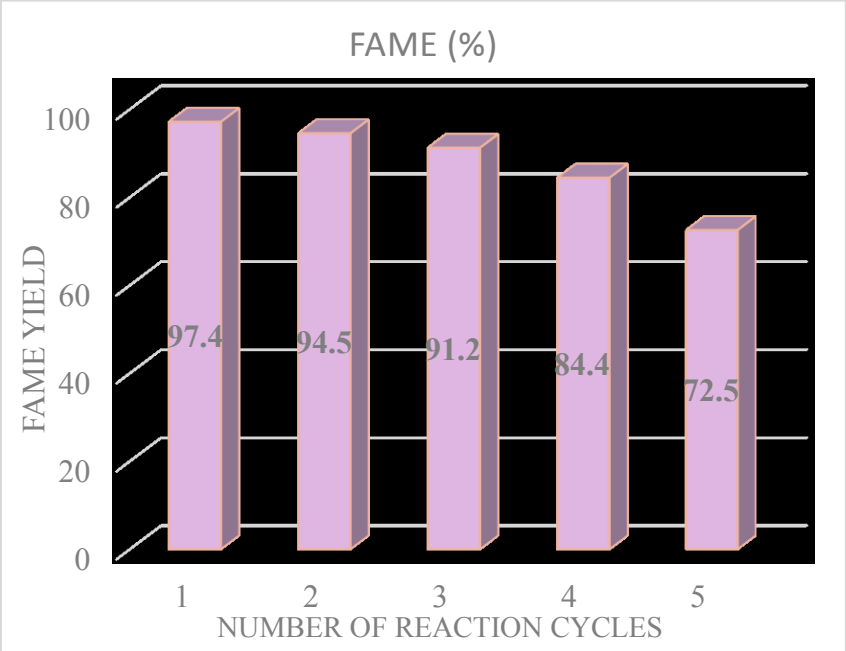


Figure 4.14: Catalyst Reusability

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

A heterogeneous solid base catalyst was synthesized by wet impregnation of potassium hydroxide catalyst on calcined fish bones followed by drying and re-calcination of the catalyst at 600^0C.

The solid base catalyst was prepared at different calcination temperature with different weight percent of KOH impregnation solution while calcination time was kept constant at 3hr. The calcination temperature and KOH impregnation ratio was varied from 800 to 1000^0C and 5 to 15wt.% respectively. The basicity and FAME yield of all the synthesized catalyst was determined. Based on the result, catalyst prepared at 900^0C calcination temperature and 10-wt% KOH loading ($F_{900}I_{10}$) was selected for further characterization using FT-IR and XRD for testing the activity of the catalyst. The XRD analysis showed that a new phase of $KCaPO_4$ compound was formed in the impregnated catalyst this due to the impregnation of potassium ion in the calcined fishbone.

The Box-Behnken design of response surface methodology was successfully used in optimizing the reaction conditions for biodiesel production. The prepared catalyst showed good catalytic activity in the transesterification of waste cooking oil and the maximum yield of 97.46% was obtained under optimal reaction conditions of methanol to oil molar ratio of 10.56:1, catalyst loading of 3.96 wt% and reaction time of 2.7 hr by maintaining the other parameters constant such as reaction temperature at 65^0C and mixing intensity at 600rpm. Under these optimal conditions, the synthesized catalyst can be recovered and reused up to three times with a small significant loss in catalytic activity and FAME yield.

The empirical value of yield obtained from RSM (97.8026%) was comparable to that obtained experimentally (97.46%). The relationship between the predicted and experimental yield indicated that the values were in reasonable agreement and the data fit well with the model by giving a good estimate of response for the system in the range studied. Results indicated that a second order quadratic model was the best fit for the experiments conducted. The model can be considered statistically significant at 95% confidence, with all three independent variables having an effect on the yield and R^2 of the model was found to be 0.9955.

The produced biodiesel under the optimal reaction conditions were characterized by using GC-MS, FT-IR and its physio-chemical and fuel properties were within the American (ASTM D6751) and European (EN 14214) Standard specifications.

Thus, the utilization of waste fish bones are not only reduce the daily waste to landfill but also can become the alternate cheap catalyst for biodiesel production.

5.2. Recommendation

KOH-impregnated calcined fishbone catalyst was an efficient catalyst for transterification of waste cooking oil with methanol in to biodiesel.

The following recommendation had improve the synthesis of solid base catalyst by the researchers

- In this study during synthesis of the catalyst, the calcination temperature and the precursor concentration were studied. Further studying of the parameters such as calcination time and precursor type will increases the efficiency of the catalyst.
- In this studying there is a full problem for characterizing of the catalyst surface area, pore volume, particle size and surface morphology due to the a absence of the equipment's in AAiT and un-functional equipment's in other institutions of Ethiopia. I strongly recommend the AAiT institution to set up the equipment's in the laboratory for further investigation of the catalysts.
- In different solid base catalysts, leaching of a catalyst is a difficulty for its commercialization as studied so far by different researchers, so it is recommended to study on catalyst stability.
- Further study on improvement of the biodiesel yield by transesterification process parameters of alcohol type and stirring speed need to be done.

Reference

Aransiola, E. F., Ojumu, T. V, Oyekola, O. O., & Madzimbamuto, T. F. (2013). ScienceDirect A review of current technology for biodiesel production : State of the art. *Biomass and Bioenergy*, *61*, 276–297.

Atabani, A. E., Silitonga, A. S., Anjum, I., Mahlia, T. M. I., Masjuki, H. H., & Mekhilef, S. (2012). A comprehensive review on biodiesel as an alternative energy resource and its characteristics. *Renewable and Sustainable Energy Reviews*, *16*(4), 2070–2093.

Avhad, M. R., & Marchetti, J. M. (2016). Innovation in solid heterogeneous catalysis for the generation of economically viable and ecofriendly biodiesel : A review, *4940*(January).

Banković-Ilić, I. B., Miladinović, M. R., Stamenković, O. S., & Veljković, V. B. (2017). Application of nano CaO–based catalysts in biodiesel synthesis. *Renewable and Sustainable Energy Reviews*, *72*(May), 746–760.

Barabás, I., & Todoru, I. (n.d.). Biodiesel Quality, Standards and Properties.

Birhanu, A., & Ayalew, S. (2017). A Review on Potential and Status of Biofuel Production in Ethiopia, *5*(X), 82–89.

Birla, A., Singh, B., Upadhyay, S. N., & Sharma, Y. C. (2012). Bioresource Technology Kinetics studies of synthesis of biodiesel from waste frying oil using a heterogeneous catalyst derived from snail shell. *Bioresource Technology*, *106*, 95–100.

Chakraborty, R., Bepari, S., & Banerjee, A. (2011). Bioresource Technology Application of calcined waste fish (Labeo rohita) scale as low-cost heterogeneous catalyst for biodiesel synthesis. *Bioresource Technology*, *102*(3), 3610–3618.

Chhetri, A. B., Watts, K. C., & Islam, M. R. (2008). Waste Cooking Oil as an Alternate Feedstock for Biodiesel Production, 3–18.

Coates, J. (2000). Interpretation of Infrared Spectra , A Practical Approach, 10815–10837.

Farooq, M., & Ramli, A. (2015). Biodiesel production from low FFA waste cooking oil using heterogeneous catalyst derived from chicken bones. *Renewable Energy*, *76*, 362–368.

Gashaw, A., & Teshita, A. (2014). Production of biodiesel from waste cooking oil and factors affecting its formation : A review, *3*(5), 92–98.

Gebremariam, S. N., & Marchetti, J. M. (2017). *Biodiesel production technologies : review* (Vol. 5).

Gnanaprakasam, A., Sivakumar, V. M., Surendhar, A., Thirumarimurugan, M., & Kannadasan, T. (2013). Recent Strategy of Biodiesel Production from Waste Cooking Oil and Process Influencing Parameters : A Review, *2013*.

Goto, T., & Sasaki, K. (2014). Effects of trace elements in fish bones on crystal characteristics of hydroxyapatite obtained by calcination, (April).

IR-frequencies. (n.d.).

Jazie, A. A., & Sinha, H. P. A. S. K. (2013). Transesterification of peanut and rapeseed oils using waste of animal bone as cost effective catalyst.

Jin, H., Kolar, P., Peretti, S. W., Osborne, J. A., & Cheng, J. J. (2018). and Activity of Sodium-Impregnated Oyster Shell Catalysts for Transesterification.

Khalili, R. G. R., & Salehi, D. Y. (2016). Waste Animal Bone as Support for CaO Impregnation in Catalytic Biodiesel Production from Vegetable Oil. *Waste and Biomass Valorization*.

Koria, L., & Nithya, G. (2012). Analysis of Datura stramonium Linn . biodiesel by gas chromatography - mass spectrometry (gc-ms) and influence of fatty acid composition on the fuel related characteristics, *4*(1), 6–9.

Lesbani, A., Okta, S., Sitompul, C., Mohadi, R., & Hidayati, N. (2016). Characterization and Utilization of Calcium Oxide (CaO) Thermally Decomposed from Fish Bones as a Catalyst in the Production of Biodiesel from Waste Cooking Oil, *20*(3), 121–126.

Lesbani, A., Susi, Y., Verawaty, M., & Mohadi, R. (2015). Aceh International Journal of Science and Technology Calcium Oxide Decomposed From Chicken ' s and Goat ' s Bones as Catalyst For Converting Discarded Cooking Oil to be Biodiesel, *4*(April), 7–13.

Mahiber, M. (n.d.). Rapid Assessment of Biofuels Development Status in Ethiopia And Proceedings of the National Workshop on Environmental Impact Assessment and Biofuels.

Moser, B. R. (2009). Biodiesel production , properties , and feedstocks, 229–266.

Mutreja, V., Singh, S., & Ali, A. (2011). Biodiesel from mutton fat using KOH impregnated MgO as heterogeneous catalysts. *Renewable Energy*, *36*(8), 2253–2258.

Nisar, J., Razaq, R., Farooq, M., Iqbal, M., Ali, R., Sayed, M., & Shah, A. (2017). Enhanced biodiesel production from Jatropha oil using calcined waste animal bones as catalyst. *Renewable Energy*, *101*, 111–119.

Patil, P. D., Gude, V. G., Reddy, H. K., Muppaneni, T., & Deng, S. (2012). Biodiesel Production from Waste Cooking Oil Using Sulfuric Acid and Microwave Irradiation Processes, *2012*(January), 107–113.

Raqeeb, M. A., & Bhargavi, R. (2015). Biodiesel production from waste cooking oil, *7*(12), 670–681.

Raut, S. N., Jadhav, A. P., & Nimbalkar, R. P. (2016). Preparation of heterogeneous catalyst derived from natural resources for the transesterification of biofuel. *International Research Journal of Engineering and Technology*, *3*(9), 196–204. Retrieved from www.irjet.net

Sani, Y. M., Daud, W. M. A. W., & Aziz, A. R. A. (2013). Biodiesel Feedstock and Production Technologies : Successes , Challenges and Prospects.

Sanli, M. C. H. (2008). Biodiesel production from various feedstocks and their e V ects on the fuel properties, 431–441.

Selvaraj, T., & Sivakumar, D. (2013). Application of Box Behnken design to optimize the parameters for turning Inconel 718 using coated carbide tools, *4*(4).

States, U., Term, S., & Outlook, E. (2018). U.S. Biodiesel Industry Update (01/17/2016), (September 2016), 2016–2018.

Sulaiman, S., & Amin, M. H. M. (2016). Fish Bone-Catalyzed Methanolysis of Waste Cooking Oil, *11*(2), 245–249.

Sulaiman, S., Khairudin, N., Jamal, P., Alam, M. Z., Zainudin, Z., & Azmi, S. (2014). Characterization of Fish Bone Catalyst for Biodiesel Production, *8*(5), 464–466.

Suwannasom, P., Tansupo, P., & Ruangviriyachai, C. (2016). Environmental Effects A bone-based catalyst for biodiesel production from waste cooking oil. *Energy Sources, Part A:*

Recovery, Utilization, and Environmental Effects, *38*(21), 3167–3173.

Tan, Y. H., Abdullah, M. O., Kansedo, J., Mubarak, N. M., Chan, Y. S., & Nolasco-hipolito, C. (2019). Biodiesel Production from Used Cooking Oil using Green Solid Catalyst Derived from Calcined Fusion Waste Chicken and Fish Bones. *Renewable Energy*.

Widiarti, N., Wijayati, N., Budi, S., & Kusuma, W. (2017). Jurnal Bahan Alam Terbarukan Catalytic Activity of Calcium Oxide from Fishbone Waste in Waste Cooking Oil Transesterification Process.

Yaakob, Z., Mohammad, M., Alherbawi, M., & Alam, Z. (2013). Overview of the production of biodiesel from Waste cooking oil. *Renewable and Sustainable Energy Reviews*, *18*, 184–193.

APPENDICES

Appendix A: Composition of waste cooking oil

Table A-1: Fatty acid profile of waste cooking oil

Fatty acid compositions	Structure	Wt.%
Oleic acid	(C18:1)	52.9
Palmitic acid	(C16:0)	20.4
Linoleic acid	(C18:2)	13.5
Stearic acid	(C18:0)	4.8
Palmitoleic acid	(C16:1)	4.6
Myristic acid	(C14:0)	0.9
Eicosenic acid	(C20:1)	0.84
Linolenic acid	(C18:3)	0.8
Arachidic acid	(C20:0)	0.12
Erucic acid	(C22:1)	0.07
Tetracosanic acid	(C24:0)	0.04
Behenic acid	(C22:0)	0.03

Source: (Chhetri, Watts, & Islam, 2008).

Table A-2: Main feed stocks of biodiesel

Edible oils	Non-edible oils	Animal fats	Other sources
Soybeans Rapeseed Safflower Rice bran oil Barley Sesame Groundnut Sorghum Wheat Corn Coconut Canola Peanut Palm Sunflower	Jatropha curcas Mahua Pongamia Camelina Cotton seed Karanja Cumaru Neem seed Jojoba Passion seed (Passiflora edulis) Moringa (Moringa oleifera) Tobacco seed Rubber seed tree Salmon oil Coffee ground (Coffea arabica) Nagchampa	Pork lard Beef tallow Poultry fat Fish oil Chicken fat	Bacteria Algae (Cyanobacteria) Microalgae (Chlorellavulgaris Poplar Tarpenes Switchgrass Miscanthus Latexes Fungi

Source : (Moser, 2009)

Appendix B: Standard Specifications of Biodiesel

Table B-1: American standard specification of biodiesel (ASTM D6751-02)

Property	**Method**	**Limits**	**Units**
Flash point	D93	130 min	^{0}C
Kinematics viscosity,40^{0}C	D445	1.9-6.0	mm^2/s
Acid number	D664	0.50 max	mg KOH/g
Water and sediment	D2709	0.050 max	% volume
Sulfated ash	D874	0.020 max	wt%
Total sulfur	D5453	0.05 max	wt%
Copper strip corrosion	D130	No.3 max	-
Cetane number	D613	47min	-
Cloud point	D2500	Report	^{0}C
Carbon residue	D4530	0.050 max	wt%
Free glycerin	D6584	0.020	wt%
Total glycerin	D6584	0.240	wt%
Phosphorus	D4951	0.001 max	wt%
Vacuum distillate end point at 90% distilled	D1160	360^{0}C max	^{0}C

Source: (Moser, 2009)

Table B-1: European Committee for Standardization EN 14214 biodiesel fuel standard: 2009

Property	**Method**	**Limits**	**Units**
Ester content	EN 14103	96.5 min	% (mol/mol)
Density at 15 °C	EN ISO 3675, EN ISO 12185	(860 – 900)	kg/m^3
Viscosity at 40 °C	EN ISO 3104, ISO 3105	(3.50 – 5.00)	mm^2/s
Flash point	EN ISO 3679	120 min.	°C
Sulphur content,	EN ISO 20846, EN ISO20884	10.0 max	mg/kg
Carbon residue (10 % distillation residue)	EN ISO 10370	0.30 max	% (mole/mole)
Cetane number	EN ISO 5165	51 min	
Sulfated ash	EN ISO 3987	0.02 max	% (mol/mol)
Water content	EN ISO 12937	500 max	mg/kg
Total contamination	EN 12662	24 max	mg/kg
Copper strip corrosion (3 h, 50°C)	EN ISO 2160	1	Degree of corrosion
Oxidation stability, 110°C	EN 14112	6.0 min	H
Acid value	EN 14104	0.50 max	mg KOH/g
Iodine value	EN 14111	120 max	g I_2/100 g
Linolenic acid content	EN 14103	12.0 max	% (mol/mol)
Polyunsaturated (≥4 double bonds) methyl esters	EN 14103	1 max	% (mol/mol)
Methanol content	EN 14110	0.20 max	% (mol/mol)

MAG content	EN 14105	0.80 max	% (mol/mol)
DAG content	EN 14105	0.20 max	% (mol/mol)
TAG content	EN 14105	0.20 max	% (mol/mol)
Free glycerol	EN 14105 EN 14106	0.020 max	% (mol/mol)
Total glycerol	EN 14105	0.25 max	% (mol/mol)
Group I metals (Na, K)	EN 14108 EN 14109	5.0 max	mg/kg
Group II metals (Ca, Mg)	EN 14538	5.0 max	mg/kg
Phosphorous content	EN 14107	10.0 max	mg/kg

Source: (Moser, 2009)

Appendix C: Experimental Results

Table C1: Catalyst screening

An efficient catalyst were selected based on its basicity and high FAME content. The basicity was calculated using eq.3.1

No.	Catalyst name	Catalyst weight (g)	Initial volume of titrant (ml)	Final volume of titrant (ml)	Volume difference of titrant (ml) V_2	Volume analyte (ml) , V_{HCl}	Volume difference (ml)	Basicity (mmol/g)	FAME yield (%)
1	N	0.04	50	40.21	9.79	10	0.21	0.525	16.0
2	F_{800}	0.04	50	41.80	8.20	10	1.80	4.50	31.2
3	$F_{800}I_5$	0.04	50	42.60	7.40	10	2.60	6.50	54.4
4	$F_{800}I_{10}$	0.04	50	43.90	6.10	10	3.90	9.75	69.0
5	$F_{800}I_{15}$	0.04	50	43.40	6.60	10	3.40	8.50	62.1
6	F_{900}	0.04	50	42.50	7.50	10	2.50	6.25	52.0
7	$F_{900}I_5$	0.04	50	43.65	6.35	10	3.65	9.125	68.3
8	$F_{900}I_{10}$	0.04	50	44.94	5.06	10	4.94	12.35	88.12
9	$F_{900}I_{15}$	0.04	50	44.36	5.64	10	4.36	10.90	79.2
10	F_{1000}	0.04	50	42.37	7.63	10	2.37	5.925	49.1
11	$F_{1000}I_5$	0.04	50	42.85	7.15	10	2.85	7.125	56.03
12	$F_{1000}I_{10}$	0.04	50	43.71	6.29	10	3.71	9.275	68.56
13	$F_{1000}I_{15}$	0.04	50	43.62	6.38	10	3.62	9.05	64.3

Table C2: Moisture content of waste cooking oil and biodiesel

The moisture content of the waste cooking oil and the biodiesel were calculated using the equation

$$Moisture\ content, \% = \frac{w_1 - w_2}{w_1} * 100\% \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots i$$

Where, W_1 is the weight of the sample before drying

W_2 is the weight of the sample after drying

Types of sample	Run	Sample weight (g)			Moisture content, %	Average Moisture content, %
		w_1	w_2	w_1-w_2		
WCO	1	2	1.999465	0.000535	0.02675	0.0253
	2	2	1.999523	0.000477	0.02385	
FAEE	1	2	1.999758	0.000242	0.0121	0.01295
	2	2	1.999724	0.000276	0.0138	

Table C3: Density of oil and biodiesel

The density was measured using hydrometer at 15^0C. A 15^0C of oil and biodiesel was placed in 250ml of graduate cylinder and insert the hydrometer. Then read the specific gravity of the sample on the hydrometer and the density was determined by the following formula.

$$SG = \frac{\rho_{sample}}{\rho_{water}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots ii$$

$$SG_{Oil} = 0.91$$

$$SG_{FAME} = 0.882$$

$$SG = \frac{\rho_{Oil}}{\rho_{Water}}, \qquad \rho_{Oil} = 0.91 * 1000\frac{kg}{l} = 910\frac{kg}{L} = 0.91\frac{g}{ml}$$

$$SG_{FAME} = \frac{\rho_{FAME}}{\rho_{Water}}, \qquad \rho_{FAME} = 0.882 * 1000\frac{kg}{L} = 882\frac{kg}{L} = 0.882\frac{g}{ml}$$

Table C4: Kinematic viscosity of oil and biodiesel

Dynamic viscosity of oil and biodiesel were measured at 40^0C using digital viscometer. Then the kinematic viscosity was calculated by the following equation:

$$\nu = \frac{\mu}{\rho} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots iii$$

Where, μ is dynamic viscosity, mpa.sec

υ is kinematic viscosity, mm^2/s

ρ is density, kg/m^3

$$\upsilon_{oil} = \frac{\mu_{oil}}{\rho_{oil}} = \frac{35.7\ mpa.s}{0.91\frac{g}{ml}} = 39.23\frac{mm^2}{s}$$

$$\upsilon_{FAME} = \frac{\mu_{FAME}}{\rho_{FAME}} = \frac{3.62\ mpa.s}{0.882\frac{g}{ml}} = 4.104\frac{mm^2}{s}$$

Table C5: Acid value of WCO and FAME

Acid value indicate the mass of potassium hydroxide (KOH) in milligrams that is required to neutralize the acidic constituents in one gram of sample. The acid value was calculated using eq.3.2

Types of sample	Run	Initial volume of KOH,ml	Titration volume of KOH, ml	Average titer value, ml	Acid value mgKOH/g
WCO	1	0	3.76	3.74	4.19628
	2	0	3.72		
FAME	1	0	0.21	0.2	0.0935
	2	0	0.19		

$$AV = \frac{56.1 * 0.1N * Titere\ value}{Weight\ of\ sample}$$

$$AV_{oil} = \frac{56.1g/mol * 0.1mol/L * 3.74ml}{5g} = 4.19628\ mgKOH/g$$

$$AV_{FAME} = \frac{56.1g/mol * 0.1mol/L * 0.2ml}{12g} = 0.0935\ mgKOH/g$$

Table C6: Saponification value of WCO

Sample	Initial volume of HCl consumed (ml)	Finial Volume of HCl consumed (ml)	Volume difference, (Vb-Va)	Average vol. difference, Vb – Va
Sample 1	0	14.9		
Blank 1	0	29.0	14.1	14.2
Sample 2	0	16.2		
Blank 2	0	30.5	14.3	

$$SV = 56.1 * 0.5N_{HCl} * (V_b - V)_a / w$$

$$SV = 56.1 \frac{g}{mol} * 0.5 \frac{mol}{L} * 14.2ml/2g$$

SV = 0.199155 = 199.155 mgHCl/g oil

Table C7: Iodine value of WCO and FAME

Iodine value is the mass of iodine in grams that is consumed by 100 grams of a sample.it is used to determine the amount of unsaturation in fatty acids.

Types of sample	Run	Initial Volume of $Na_2S_2O_3$, ml	Finial Volume of $Na_2S_2O_3$, ml	Volume difference V_B-V_S, ml	Iodine value, $gI_2/100g$
Waste cooking oil	Sample 1	0	31.4		
	Blank 1	0	78.5	47.1	121.2
	Sample 2	0	33.6	48.4	
	Blank 2	0	82	Avg. = 47.75	
Biodiesel	Sample 1	0	28.4		
	Blank 1	0	65.5	37.1	95.56
	Sample 2	0	29.7	38.2	
	Blank 2	0	67.9	Avg. = 37.65	

$$IV_{WCO} = \frac{126.9\frac{g}{mol} * 0.1\frac{mol}{L} * 47.75ml * \frac{1L}{1000ml}}{0.5g} = \frac{12.69g * 0.1 * 47.75}{0.5 * 100g} = 121.189\frac{g}{100gI2}$$

$$IV_{FAME} = \frac{12.69\frac{g}{mol} * 0.1\frac{mol}{L} * 37.65ml * \frac{1L}{1000ml}}{0.5g} = \frac{12.69g * 0.1 * 37.65}{0.5 * 100g} = 95.56\,{}^{g}/_{100gI_2}$$

Table C8: GC-MS analysis of biodiesel from waste cooking oil

JIJE LABOGLASS Pvt. Limited Company	JIJE Analytical *Testing* Service Laboratory	Doc. No: JATSL/F5.10-3.1	Page 1 of 1	
			Ver. No: 04	Effective Date: July 08, 2017
	Analytical Test Report			
Customer Name:	AAiT	Test Report No:	142	
Contact Person:	Kidist Getachew	Report Date:	03/06/2019	
Sample Type:	Biodiesel	Test Request No:	Not Specified	
Sample Source:	Waste Cooking Oil	Tel (Mob):	+251 920 15 70 95	
Sample Collected By:	Customer	Fax:	Not Specified	
Sample Collection Date:	21/04/2019	E-mail:	kgetch07@gmail.com	
Sample Receiving Date:	21/05/2019	Tested By:	LE-02	
Sample Condition:	Normal	Dates Tested:	01/06/2019	

Lab No: J-0571/2105/19, Customer Code: Biodiesel – Waste Cooking Oil, Test Method: GC-MS

S/N	Name	RT	Area	Formula	Area %
1	Methyl tetradecanoate	10.92	547882.00	C15H30O2	0.06
2	9-Hexadecenoic acid, methyl ester, (Z)-	13.91	933942.00	C17H32O2	0.11
3	Hexadecanoic acid, methyl ester	14.44	85326650.00	C17H34O2	10.04
4	Heptadecanoic acid, methyl ester	17.24	615557.00	C18H36O2	0.07
5	9-Octadecenoic acid, methyl ester, (E)-	20.52	277442340.00	C19H36O2	32.66
6	9-Octadecenoic acid (Z)-, methyl ester	20.59	7765635.00	C19H36O2	0.91
7	Methyl stearate	21.50	43958479.00	C19H38O2	5.17
8	9,12-Octadecadienoyl chloride, (Z,Z)-	27.73	999441.00	C18H31ClO	0.12
9	cis-Methyl 11-eicosenoate	28.61	8773320.00	C21H40O2	1.03
10	Methyl 18-methylnonadecanoate	29.45	10769992.00	C21H42O2	1.27
11	Z,Z,Z-4,6,9-Nonadecatriene	29.78	403799.00	C19H34	0.05
12	13-Docosenoic acid, methyl ester, (Z)-	33.87	760425.00	C23H44O2	0.09
13	Docosanoic acid, methyl ester	34.67	24299403.00	C23H46O2	2.86
14	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	20.13	386890944.00	C19H34O2	45.54

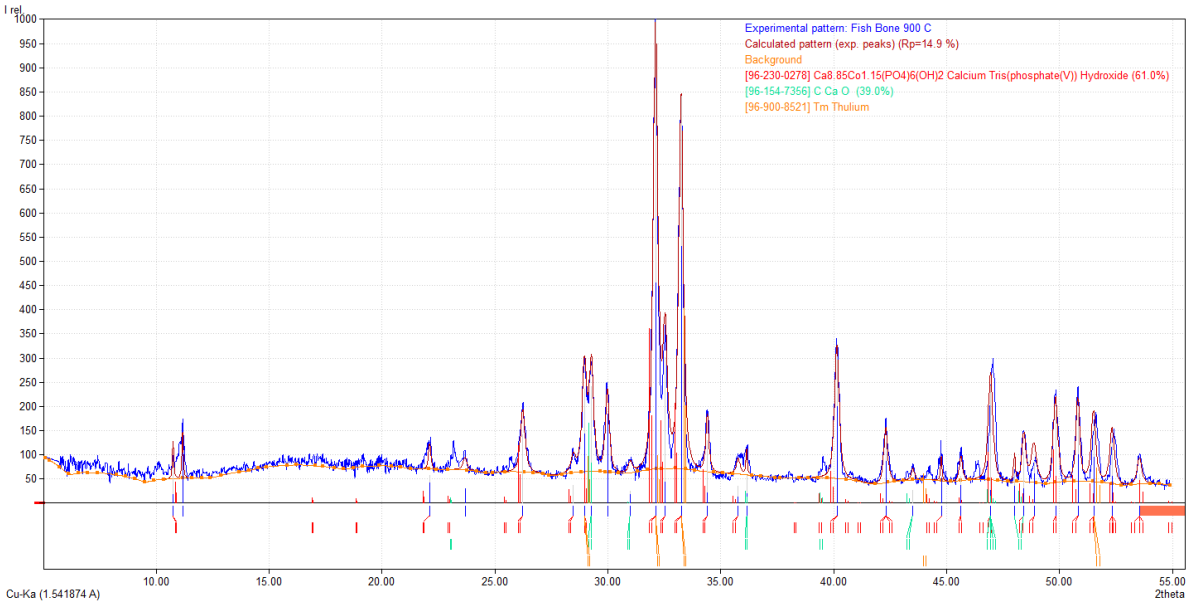
Remark:

1. This test report is only for specific sample (s) which has been tested by JIJE Analytical *Testing* Service Laboratory.

Verified by			*Authorized by*		
Name	**Signature**	**Date**	**Name**	**Signature**	**Date**
Gemechu Sorsa			**Mulugeta Terefe**		03/06/19
Technical Signatory			*Laboratory Manager*		

Tel : +251-372 07 01/ 02
Fa x : +251-011-372 07 03
P.O. Box: 70077
Email: info@jijelaboglassplc.com
Web site: www.jijelaboglassplc.com
Physical Address: Nifas Silk Lafto Sub City, Woreda 01, Lebu, Foziana Building, Infront of Varnero Real Estate, Addis Ababa, Ethiopia.

For Calcined fishbone (a)



For KOH impregnated calcined fishbone catalyst (b)

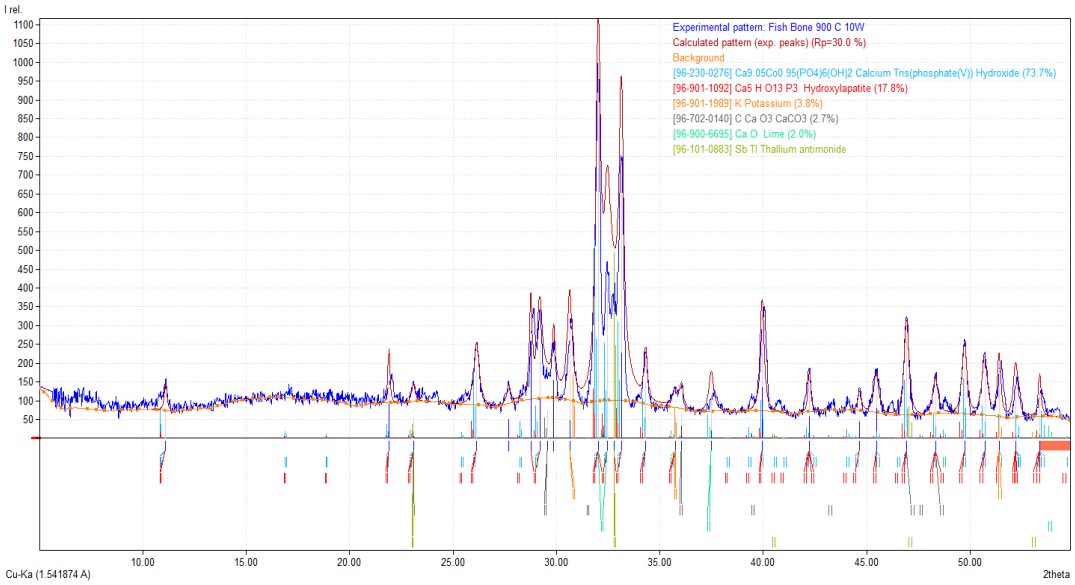


Figure C1: XRD graph of calcined fishbone and KOH impregnated in calcined fishbone catalyst for determination of crystallite size

Table C9: Crystallite size of calcined fishbone

2θ	**FWHM**	**θ**	**β = Radian (FWHM)**	**Cos (Radian(θ))**	$D = \frac{K*\lambda}{\beta cos\theta}$ **, nm**
10.75	0.08	5.375	0.001396	0.995603	99.7421858
11.17	0.08	5.585	0.001396	0.995253	98.83220977
22.11	0.12	11.055	0.002094	0.981444	64.97392793
23.67	0.2	11.835	0.003491	0.978742	38.87705797
26.24	0.24	13.12	0.004189	0.973897	32.23715659
28.47	0.24	14.235	0.004189	0.969295	32.08484231
28.97	0.24	14.485	0.004189	0.968213	32.04902142
29.27	0.24	14.635	0.004189	0.967555	32.02723596
29.98	0.2	14.99	0.003491	0.965971	38.36976337
31	0.2	15.5	0.003491	0.96363	38.27679411
32.12	0.24	16.06	0.004189	0.960973	31.8093477
32.54	0.24	16.27	0.004189	0.959952	31.77557107
33.25	0.24	16.625	0.004189	0.958198	31.71750199
34.41	0.16	17.205	0.002793	0.955253	47.43001476
35.77	0.2	17.885	0.003491	0.951675	37.80189976
36.15	0.08	18.075	0.001396	0.950651	94.40309847
40.14	0.24	20.07	0.004189	0.939274	31.09110258
42.31	0.2	21.155	0.003491	0.932608	37.04451888
43.51	0.16	21.755	0.002793	0.928777	46.11546626
44.76	0.08	22.38	0.001396	0.924679	91.82396485
45.64	0.16	22.82	0.002793	0.921728	45.76545155
46.95	0.24	23.475	0.004189	0.917234	30.3615491
48.01	0.08	24.005	0.001396	0.91351	90.7148392
48.42	0.2	24.21	0.003491	0.912049	36.22788666
48.87	0.28	24.435	0.004887	0.910431	25.83117179
49.83	0.24	24.915	0.004189	0.906934	30.02059948
50.81	0.2	25.405	0.003491	0.903298	35.88029623
51.51	0.24	25.755	0.004189	0.90066	29.8129413
52.33	0.24	26.165	0.004189	0.897528	29.7092544
53.54	0.24	26.77	0.004189	0.892822	29.55347591
					Davg = 45.74534 nm

Table C10: Crystal size of KOH impregnated in calcined fishbone

2θ	**FWHM**	**β=Radian(FWHM)**	**θ**	**Cos(Radian θ)**	$\boldsymbol{D = \frac{K * \lambda}{\beta cos\theta}, nm}$
11.11	0.16	0.002792527	5.555	0.995303734	49.88608463
21.91	0.12	0.002094395	10.955	0.981776742	67.43122507
23.09	0.2	0.003490659	11.545	0.979767819	40.5416919
26.14	0.32	0.005585054	13.07	0.974094508	25.486134
27.7	0.2	0.003490659	13.85	0.97092575	40.91089874
28.77	0.16	0.002792527	14.385	0.968648235	51.25886214
29.2	0.28	0.004886922	14.6	0.96770917	29.31920212
29.56	1	0.017453293	14.78	0.966912497	8.216140588
29.88	0.16	0.002792527	14.94	0.966196331	51.38894107
30.65	0.28	0.004886922	15.325	0.96444219	29.41851885
32.03	0.28	0.004886922	16.015	0.961189501	29.51807185
32.47	0.36	0.006283185	16.235	0.960123081	22.98400062
32.78	1.04	0.018151424	16.39	0.959363238	7.962301591
33.14	0.24	0.00418879	16.57	0.958472029	34.53538884
34.32	0.2	0.003490659	17.16	0.955484572	41.5720423
35.74	0.32	0.005585054	17.87	0.951755205	26.08433664
36.05	0.2	0.003490659	18.025	0.950921592	41.77152502
37.49	0.28	0.004886922	18.745	0.946958177	29.96168306
39.95	0.24	0.00418879	19.975	0.939841766	35.21997577
42.23	0.16	0.002792527	21.115	0.932859256	53.22539924
44.66	0.2	0.003490659	22.33	0.925010909	42.94159635
45.47	0.28	0.004886922	22.735	0.922302181	30.76265171
46.93	0.28	0.004886922	23.465	0.917303485	30.93028776
48.33	0.28	0.004886922	24.165	0.912370354	31.09752596
49.73	0.28	0.004886922	24.865	0.907301041	31.27127543
50.71	0.32	0.005585054	25.355	0.903671899	27.47225314
51.41	0.2	0.003490659	25.705	0.901039174	44.08403786
52.21	0.28	0.004886922	26.105	0.89798918	31.59554856
53.37	0.16	0.002792527	26.685	0.893488989	55.57069749
					Davg = 35.94546 nm

Appendix D: Required Calculations for the Experiment

D-1: Feed material requirement

In the Transterification reaction the required amount of methanol and solid base catalyst were calculated based on the volume of oil.

❖ Calculation of Molecular weight of Oil

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

$$M_{Oil} = \frac{56.1 * 1000 * 3}{SV - AV}$$

$$M_{Oil} = \frac{56.1 * 1000 * 3}{199.155 - 4.19628} = 863.26\ g/mol$$

❖ Methanol to oil molar ratio calculation

The following basic formula works for all cases:

$$Methanol\ to\ oil\ molar\ ratio = \frac{number\ of\ mole\ of\ methanol}{number\ of\ mole\ of\ oil} = \frac{n_{Methanol}}{n_{Oil}} \dots\dots\dots\dots (1)$$

$$Number\ of\ mole\ (n) = \frac{Given\ mass\ (m)}{Molecular\ weight\ (M)} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (2)$$

$$Density\ (\rho) = \frac{Mass\ (m)}{Volume\ (V)} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots (3)$$

Substitute equation (2&3) in to equation (1) to calculate the volume of methanol

$$Methanol\ to\ oil\ molar\ ratio = \frac{(\frac{\rho * V}{M})_{Methanol}}{(\frac{\rho * V}{M})_{oil}}$$

$$V_{Methanol} = molar\ ratio * V_{Oil} * (\frac{\rho}{M})_{Oil} * (\frac{M}{\rho})_{Methanol}$$

Where: Density of methanol = 0.792 g/ml

Molecular mass of methanol = 32.04 g/mol

Density of oil = 0.91 g/ml

Molecular mass of oil = 863.26 g/mol

Volume of oil = 100 ml

❖ Catalyst to oil weight ratio calculation

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

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

Mass of oil is calculated from density and volume of oil.

Appendix E: Fourier Transform Infrared Radiation (FTIR) Standard Functional Groups with their frequencies

Characteristic IR Absorption Frequencies of Organic Functional Groups

Functional Group	Type of Vibration	Characteristic Absorptions (cm-1)	Intensity
Alcohol			
O-H	(stretch, H-bonded)	3200-3600	strong, broad
O-H	(stretch, free)	3500-3700	strong, sharp
C-O	(stretch)	1050-1150	Strong
Alkane			
C-H	stretch	2850-3000	Strong
-C-H	bending	1350-1480	Variable
Methyl C-H	asym./sym. stretch	2970–2950/2880–2860	
Methyl C-H	asym./sym. bend	1470–1430/1380–1370	
Alkene			
=C-H	stretch	3010-3100	Medium
=C-H	bending	675-1000	Strong
C=C	stretch	1620-1680	Variable
Alkyl Halide			
C-F	stretch	1000-1400	Strong
C-Cl	stretch	600-800	Strong
C-Br	stretch	500-600	Strong
C-I	stretch	500	Strong
Alkyne			
C-H	stretch	3300	strong, sharp
	stretch	2100-2260	variable, not present in symmetrical alkynes
Amine			

N-H	stretch	3300-3500	medium (primary amines have two bands; secondary have one band, often very weak)
C-N	stretch	1080-1360	medium-weak
N-H	bending	1600	Medium
Aromatic			
C-H	stretch	3000-3100	Medium
C=C	stretch	1400-1600	medium-weak, multiple bands

Analysis of C-H out-of-plane bending can often distinguish substitution patterns			
Carbonyl	Detailed Information on Carbonyl IR		
C=O	stretch	1670-1820	strong

(Conjugation moves absorptions to lower wave numbers)

Ether			
C-O	Stretch	1000-1300 (1070-1150)	Strong
Nitrile			
C-N	Stretch	2210-2260	Medium
Nitro			
N-O	Stretch	1515-1560 & 1345-1385	Strong, two bands

IR Absorption Frequencies of Functional Groups Containing a Carbonyl (C=O)

Functional Group	Type of Vibration	Characteristic Absorptions (cm-1)	Intensity
Carbonyl			
C=O	stretch	1670-1820	strong
(conjugation moves absorptions to lower wave numbers)			
Acid			
C=O	stretch	1700-1725	Strong
O-H	stretch	2500-3300	strong, very broad
C-O	stretch	1210-1320	Strong
Aldehyde			
C=O	stretch	1740-1720	Strong
=C-H	stretch	2820-2850 & 2720-2750	medium, two peaks
Amide			
C=O	stretch	1640-1690	Strong

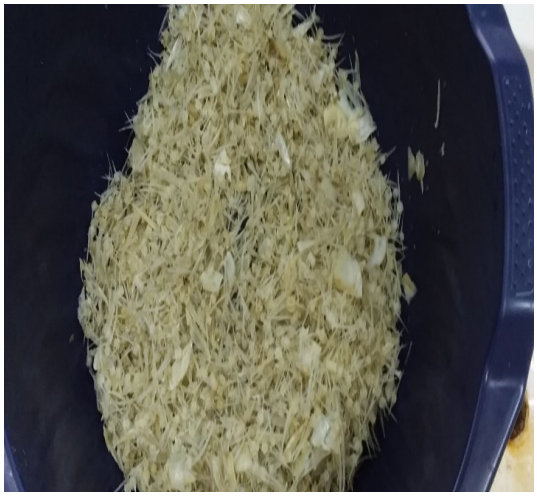
N-H	stretch	3100-3500	unsubstituted have two bands
N-H	bending	1550-1640	
Anhydride			
C=O	stretch	1800-1830 & 1740-1775	two bands
Ester			
C=O	stretch	1735-1750	Strong
C-O	stretch	1000-1300	two bands or more
Ketone			
acyclic	stretch	1705-1725	Strong
cyclic	stretch	3-membered – 1850 4-membered - 1780 5-membered - 1745 6-membered - 1715 7-membered - 1705	Strong
α,β-unsaturated	stretch	1665-1685	Strong
aryl ketone	stretch	1680-1700	Strong

IR Absorption Frequencies of Inorganic Functional Groups

Functional groups	**Characteristic Absorptions (cm^{-1})**	**Intensity**
Carbonate ion	1490-1410/880-860	Strong, broad/ weak to medium, narrow
Phosphate ion	1100-1000	Strong
Sulfate ion	1130-1080/680-610	Strong, broad/ weak to medium, narrow
Nitrate ion	1380-1350/840-815	Strong, broad/ weak to medium, narrow
Silicate ion	1100-900	Strong

Source: ("IR-frequencies," n.d.)

Appendix F: Laboratory equipment and experimental sample photos



Raw fishbone

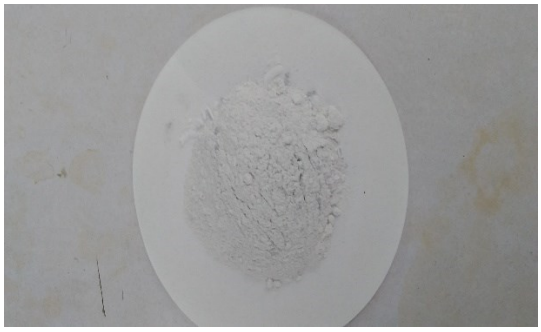
Dried fishbone in an oven

Grinding of fishbone

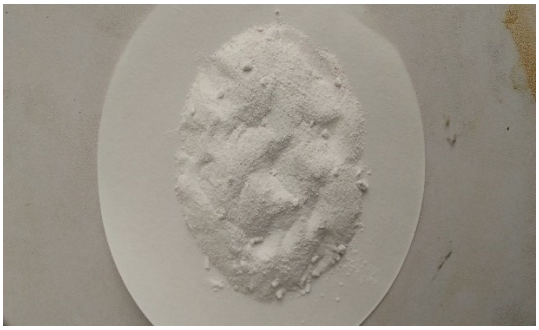
Sieving of raw fishbone

Calcination of raw fishbone

Powder raw fishbone

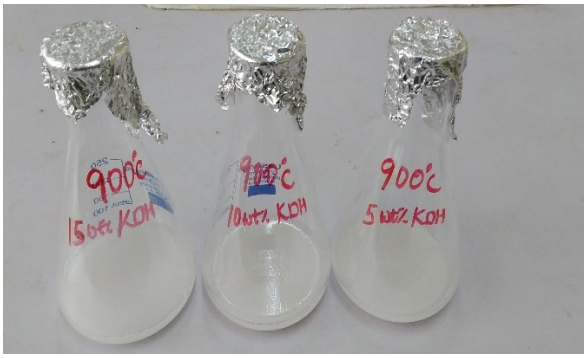


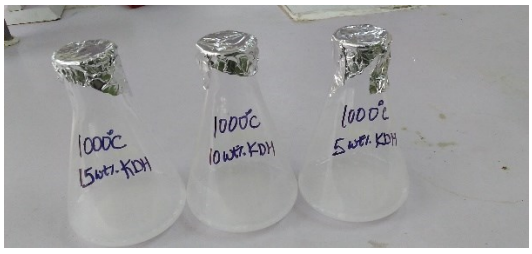
Fish bone calcined at 800^0C



Fish bone calcined at 900^0C

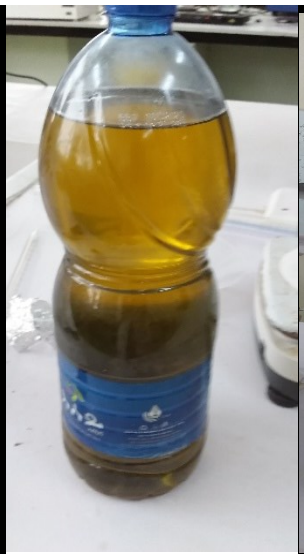
Fish bone calcined at 1000^0C



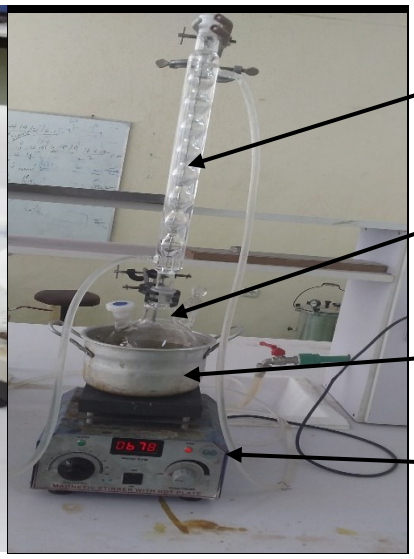


Impregnation of different KOH conc. in 800^0C, 900^0C and 1000^0C calcined fishbone

Impregnation process

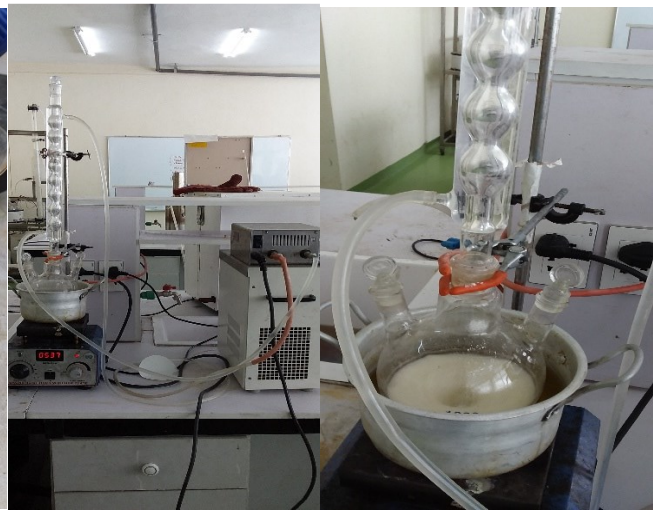


Purified waste cooking oil

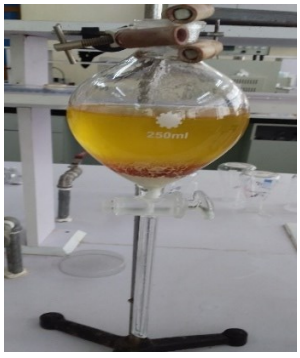


Esterification process

Purification of esterified oil



Transterification reaction



After completion of the reaction

purified product