MICROWAVE-ASSISTED BIODIESEL SYNTHESIS FROM NEEMOIL USING A BIO-WASTE DERIVED FROM COW BONE AND RICE BRAN

BY

OLAWUYI ZAINAB OPEYEMI

ENG1905031

A PROJECT SUBMITTED TO THE DEPARTMENT OF CHEMICAL
ENGINEERING, FACULTY OF ENGINEERING, UNIVERSITY OF BENIN, BENIN
CITY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD
OF BACHELOR'S DEGREE IN CHEMICAL ENGINEERING
(B.ENG.)

FEBRUARY, 2025

MICROWAVE-ASSISTED BIODIESEL SYNTHESIS FROM NEEMOIL USING A BIO-WASTE DERIVED FROM COW BONE AND RICE BRAN

BY

OLAWUYI ZAINAB OPEYEMI

ENG1905031

A PROJECT SUBMITTED TO THE DEPARTMENT OF CHEMICAL ENGINEERING, FACULTY OF ENGINEERING, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR'S DEGREE IN CHEMICAL ENGINEERING
(B.ENG.)

FEBRUARY, 2025

CERTIFICATION

This is to certify that this research project was carried out by Olawuyi Zainab Opeyemi, with matriculation number ENG1905031 in the Department of Chemical Engineering, University of Benin, Benin City, Edo State Nigeria.

-------------------------------	---------------------------------
ENGR. PROF. C.E AKHABUE	Date
Project Supervisor	

-----------------------------------	--------------------------------
Engr. Prof. S.E. Uwadiae	Date
Project Coordinator	

-------------------------------------	------------------------------
Engr. Prof. (Mrs) E.T. Akhihiero	Date
Head of Department	

---------------------------------	--------------------------------
External Examiner	Date

DEDICATION

This work is dedicated to Almighty Allah for His blessings upon me, and to my family members for their support and encouragement throughout my academic journey.

ACKNOWLEDGEMENT

I want to express my sincere appreciation to Almighty Allah for granting me strength and knowledge to successfully complete with my project.

I am very grateful to my project supervisor, Engr. Prof. Christopher E. Akhabue, for his knowledge and academic foundation he provided during my undergraduate program. His insightful advice, direction, inventiveness, and corrections contributed to the success of this research work in countless ways.

Special appreciation goes to my friends, Cynthia and Naomi for their constant support and encouragement.

Finally, I appreciate my family for their prayers, sacrifices, and moral support, which have been a major source of inspiration.

ABSTRACT

This research examined the optimization of microwave-assisted biodiesel synthesis from neem oil by utilizing a bifunctional catalyst derived from waste cow bone and rice bran. The bifunctional catalyst was developed by combining the acid and basic precursors to facilitate simultaneous esterification and transesterification reaction. Rice bran was carbonized and treated with 1.5 M H_2SO_4 to produce the acid precursor, while cow bone was calcined and treated with 1.5 M KOH to produce the basic precursor. These precursors were then combined incorporating the wet impregnation technique.

Neem oil characterization revealed an acid value of 17.67 mg KOH/g, a free fatty acid (FFA) content of 8.835%, a saponification value of 196.35 mg KOH/g and a calculated molecular weight of 941.91 g/mol showing that it is suitable for a high FFA feedstock that requires a bifunctional catalytic approach.

Response Surface Methodology (RSM) was used for process optimization in order evaluate the effects of key reaction variables and identify the ideal conditions for optimizing biodiesel yield.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

NOMENCLATURE

AV- Acid Value

FFA- Free Fatty Acid

FAME- Fatty Acid Methyl Ester

$CaCO_3$- Calcium Carbonate

NaOH- Sodium Hydroxide

KOH-Potassium Hydroxide

HCl-Hydrogen Chloride

H_2SO_4- Sulphuric Acid

FTIR- Fourier Transform Infrared Spectroscopy

SEM- Scanning Electron Microscopy

BET- Brunauer-Emmett-Teller

XRD- X-ray Diffraction

XRF- X-ray Fluorescence

MW- Molecular Weight

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of Study

The world economy continues to give energy the attention it merits because of the crucial roles it plays in people's everyday lives, particularly in the sectors of manufacturing, transportation, and power generation. The majority of the energy utilized globally comes from conventional fuels that are sourced from fossil fuels. Although fossil fuels are the backbone of the global economy and the key to the success of other sectors, they are the main source of energy consumed globally, with the disadvantage that prolonged use of fossil fuels can be detrimental to the environment. It is now vital to look for economic and ecologically friendly alternative energy sources everywhere .(Falowo et al., 2019).

Finding a clean, sustainable fuel that may lessen dependency on fossil fuels will undoubtedly affect how energy is used now and in the future because of the environmental issues related to their use and their limited supply. The necessity for energy security and growing awareness of the destructive effects of fossil fuels on the environment and global warming have made biodiesel and other renewable and eco-friendly fuels more and more attractive (Abiodun et al., 2021). The cost of feedstock has a big influence on how profitable biodiesel production is. Feedstock costs can account for as much as 88% of the total cost of manufacturing biodiesel. Therefore, making biodiesel with non-edible oils or leftover cooking oil can save a lot of money. Unfortunately, the high concentrations of free fatty acids (FFA) in waste cooking oils and other inedible oils saponify the alkali catalyst, making it difficult to separate the products and reducing the amount of biodiesel produced. Additionally, the wastewater generated during product purification pollutes the environment, therefore it needs to be cleaned either before it is released into the environment or before it can be utilized again (Akhabue et al., 2020).

Selecting the right catalyst is another crucial step in the transesterification process. It depends on the oil's free fatty acid (FFA) content. High FFA oil is preferred in acid-catalyzed reactions, whereas the opposite is true with reaction catalyzed by alkali. Catalysts that are homogeneous or heterogeneous have been used to produce biodiesel. For industrial applications, homogeneous catalysis is often favored; nevertheless, it has some disadvantages, such as high cost, sensitivity to FFA and wet feedstock that causes soap formation, and excessive washing, which raises production costs. Nonetheless, there is still growing interest in alternative bio-based waste heterogeneous catalysts for the manufacture of biodiesel. The quantity and biogenic nature of the catalysts set them apart. They retain active sites with few unit operations during the manufacture of biodiesel, making them extremely efficient, economically viable, ecologically friendly, and easily recyclable while lowering the overall cost of production. There have been several published research on heterogeneous catalysts based on bio-waste that exhibit intriguing catalytic properties. It has been demonstrated that these catalysts have significant concentrations of potassium and calcium following high-temperature calcinations. The catalysts may be made from easily accessible bio-wastes at little or no cost, and they are a type of waste-to-wealth management. They are also recoverable, recyclable, and use less water during the washing process. The studied bio-waste-based catalysts are categorized into shell-based, trunk-based, peel-based, and husk-based types. Shell-based catalysts encompass materials such as Capiz shell, chicken eggshell, ostrich eggshell, crab shell, oyster shell, mussel shell, coconut shell, rubber seed shell, palm kernel shell, cockle shell, and obtuse horn shell. Trunk-based catalysts include oil palm trunk and pawpaw trunk. The peel-based category consists of unripe and ripe banana peel, sulfonated banana peel, banana stem, unripe and ripe plantain peel, and tucuma peels. Meanwhile, husk-based catalysts comprise cocoa pod husk, kola nut pod husk, elephant tree, and rice husk. Fly ash and bones are two more naturally occurring waste-based catalysts (Betiku et al., 2024). While many researchers have employed heterogeneous catalysts to produce biodiesel through transesterification, catalysts utilized in the simultaneous processes of esterification and transesterification are unique in nature. They have both basic and acidic characteristics, making them bi-functional(Ali et al., 2018).

An innovative and effective technique for producing biodiesel from vegetable oils is microwave-assisted transesterification. In both commercial and research settings, transesterification is the most popular method for creating biodiesel employing affordable and environmentally benign catalysts. With this method, the reaction time is shortened, the reaction rate is increased, and more biodiesel is produced by heating the reaction mixture with microwaves. Microwaves are superior to conventional transesterification methods in a number of ways, including higher yields, less energy use, and less waste generation (Akhtar et al., 2023). Heat is transferred by microwaves far more effectively than by conventional heating techniques, which speeds up the reaction. The microwave-irradiated method is a cost-effective, environmentally responsible, and energy-efficient method of producing biodiesel. During microwave irradiation, direct energy transfer to the reactants can improve and speed up chemical reactions (Ruatpuia et al., 2023).

1.2 Problem Statement

The increasing global demand for renewable and sustainable energy sources has underscored the need for eco-friendly alternatives to fossil fuels. Biodiesel stands out as a promising option due to its biodegradable nature and lower greenhouse gas emissions (Demirbas, 2009). However, its widespread adoption remains limited by high production costs, with feedstock expenses accounting for approximately 80–85% of the total manufacturing cost (Gülşen et al., 2014). Using edible oils as feedstock would create competition with the food supply, while non-edible oils pose challenges due to their high FFA content, necessitating additional pretreatment steps that raise costs. Catalyst production expenses are a key factor in biodiesel synthesis, and in the past decade, heterogeneous catalysis has surpassed traditional homogeneous catalysis for producing biodiesel from non-edible oils. The limitations of homogeneous catalysis—such as inefficiency in handling excessive feedstock, saponification issues, high catalyst consumption, and difficulties in product separation, purification, and environmental impact—have contributed to this shift. (Semwal et al., 2011).

1.3 Relevance of The Study

This study aims to identify critical factors and develop an effective strategy to enhance biodiesel production from neem seed oil using simultaneous esterification and transesterification processes. By optimizing key parameters such as catalyst concentration, reaction time, temperature, and methanol-to-oil ratio, high-yield biodiesel with superior quality and high methyl ester purity can be achieved. The widespread reliance on fossil fuels has led to significant pollution concerns due to high energy demands in both industrial and domestic sectors. Consequently, the need for renewable energy sources with a lower environmental impact than conventional diesel fuels is growing. An ideal alternative fuel should be technically viable, economically competitive, environmentally sustainable, and readily available, with a comprehensive understanding of biodiesel properties. Beyond being biodegradable and non-toxic, biodiesel also exhibits lower emissions compared to diesel fuel.

1.4 Aim and Objectives

The objective of this research is to optimize the microwave-assisted biodiesel production from neem oil using a bi-functional catalyst synthesized from waste cow bone and rice bran, employing Response Surface Methodology.

The objectives of the research work are;

1. Preparation and characterization of bi-functional catalyst from waste cow bone and rice bran

2. Characterization of neem seed oil
3. Response methodology optimization of microwave assisted production from neem seed oil using bi-functional derived from agricultural waste.
4. Characterization of biodiesel produced using response surface methodology
5. Studying the effect of the process variables on the biodiesel production process

1.5 Scope of Study

This current study investigates biodiesel production from neemoil using bi-functional catalyst made from cow bone and rice bran. To achieve this objectives, the work entails the following:

1. Collection, washing, pulverization and preparation of waste cow bones and rice bran into heterogeneous bi-functional catalyst.
2. Characterization of neem oil
3. Characterization of unused heterogeneous bi-functional catalyst
4. Production of biodiesel by transesterification process using the characterized neem oil.
5. Efficient time management using microwave assisted technique
6. Optimization of the biodiesel production
7. Characterization of the biodiesel from the sample produced.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 ENERGY AND SOURCES OF ENERGY

Energy plays a crucial role in sustaining and enhancing the current phase of human life. Modern technology, including household appliances, automobiles, and communication devices, as well as equipment used in banking, healthcare, entertainment, and various other sectors, all depend on energy for operation. Energy sources are generally classified into two main categories: renewable and non-renewable. Non-renewable energy, often referred to as conventional sources, includes fossil fuels such as crude oil, nuclear power, and natural gas. Although these energy sources are finite and will be eventually depleted, they remain the most advanced sources for powering vehicles and generating electricity. Due to significant advancements and substantial investments, non-renewable energy sources have achieved a much higher efficiency level compared to renewable alternatives, along with extensive possibilities for utilization. (Dahlke et al., 2021).

The demand for energy is rising rapidly due to the swift growth of the global population and advancements in technology, while existing energy resources with limited reserves continue to diminish. Biomass, derived from plant materials, has been identified as a key renewable energy source worldwide, offering a viable alternative to supplement the depletion of fossil fuel reserves. Additionally, the fact that the amount of pollutants produced by petroleum-based fuels has been increasing overtime is one of its drawbacks. Long-term use of different energy sources results in global warming. Environmental emissions are becoming more apparent and leaving behind flared gases, oil and gas exploration spills on the land, and an increase in heatwaves worldwide (Bello et al., 2022). The health of the current and future generations is currently at jeopardy due to these GHG emissions. In order to fight the consequences of global warming and climate change, it is imperative that pollution be reduced and greenhouse gas emissions be mitigated through the use of green remediation technology. In the light of this, the present world push for low-carbon emission sources has signaled that clean, renewable energy sources will account for the majority of future energy production (Bello et al., 2022). While electricity is primarily produced by other renewable energies like solar, hydro and wind, energy from biomass, especially biodiesel can help meet the transportation sector's need for bulk liquid fuel and lower CO2 emissions.

In recent years, biodiesel has become increasingly popular as a replacement for diesel in engines. The conversion of vegetable oil into biodiesel is a straightforward process that offers significant environmental benefits. Vegetable oil derived from plants serves as the ideal raw material for biodiesel production due to its high conversion rate from pure triglycerides to fatty acid methyl esters and its rapid reaction time. The most common method for biodiesel production is the transesterification reaction, which involves the reaction of vegetable oil with an alcohol to produce fatty acid alkyl esters and glycerol (Chhetri et al., 2008).

Some of the differences between fossil fuels and biodiesel

- The major sources of biofuels are from plants and organic residues while fossil fuels are gotten from organisms that have died for millions of years.
- Biofuels have no hazardous effect on human health; fossil fuels on the other hand contain toxic substances and byproducts such as carbondioxide which is one of the major causes of respiratory problems in the human body.
- Biofuels can be produced from safer processes which are environmentally friendly while fossil fuels are obtained from unsafe processes like drilling and milling.

Table 2.1 differences in properties of biodiesel and petroleum diesel

S/N	**Property**	**Biodiesel**	**Petroleum Diesel**
1	Cetane No	51-62	44-49
2	Lubricity	Greater than diesel	Lower
3	Biodegradability	Good	Poor
4	Toxicity	Non-toxic	Highly toxic
5	Oxygen	11% free oxygen	Very low
6	Aromatics	No aromatics	18-22%
7	Sulphur	None	0.05
8	Cloud point	Slightly more	------
9	Flash point	300-4000F	125
10	Spoil point	None	High
11	Heating value	2-3% higher than diesel	-------
12	Renewable supply	Renewable	Non-renewable
13	Alternative fuel	Yes	No
14	Production process	Chemical reaction	Reaction

2.2 OVERVIEW OF BIODIESEL

2.2.1 History of Biodiesel

Biodiesel is a type of mono-alkyl ester derived from long-chain fatty acids found in vegetable oils or animal fats, which are reacted with alcohol, with or without the use of a catalyst. The production of biodiesel occurs through the transesterification process, where the oil reacts with alcohol, yielding biodiesel and a catalyst as a by-product (Humphery et al., 2017). As an alternative fuel, biodiesel presents several advantages over fossil fuels. Unlike conventional diesel, it does not emit sulfur or net carbon dioxide and generates lower levels of carbon monoxide, particulate matter, smoke, and hydrocarbons while increasing oxygen content. The higher oxygen concentration enhances combustion efficiency, leading to reduced emissions (Atabani et al., 2012). Therefore, biodiesel not only reduces greenhouse gas emissions but also environmentally friendly and free from pollutants. Additionally, it allows diesel engines to operate more quietly and produce less smoke (Rozina et al., 2022).

On August 10, 1893, Rudolf Diesel used vegetable oil (peanut oil) as fuel in a diesel engine. Earlier, in 1853, a group of researchers successfully converted vegetable oil into methyl ester through the transesterification process. The realization that vegetable oils could power diesel engines fostered a sense of energy independence, particularly in oil-producing African nations during the 1940s. In China, tung oil and other vegetable oils were utilized to create versions of gasoline and kerosene. Similarly, during World War II, fuel shortages spurred India to explore the conversion of various vegetable oils into diesel. The United States also demonstrated interest in biodiesel research, specifically evaluating cottonseed oil as a diesel fuel. Notably, automobile pioneer Henry Ford played a key role in integrating agriculture with the automobile industry, exemplified by the development of the “soybean car” in 1941. According to the Benson Ford Research Center, only one prototype of this experimental vehicle was built before production was halted due to World War II. The car weighed 2,000 lbs—1,000 lbs lighter than the steel-bodied vehicles of that time—making it more fuel-efficient. Ford’s innovation was evident in a vehicle partially made from soybeans and powered by ethanol derived from corn. However, production never resumed after the war, highlighting the challenges of sustaining innovation. Since the 1950s, interest in converting vegetable oils into biodiesel has been influenced more by geographical and economic considerations than by fuel shortages.

For example, the USA is a leading producer of soybean oil, while Europe generates significant quantities of canola oil, which largely dictates the type of oil used for biodiesel in these regions. Additionally, in remote areas where refining and distributing fossil fuels pose challenges, vegetable oil-based biodiesel offers a sustainable and practical solution to meet fuel energy needs. Moreover, biodiesel sources have expanded to include used vegetable oil from the food service industry and animal fats from slaughterhouses. However, further research is necessary to discover new oil crops to accommodate the growing demand for biodiesel (Songstad et al., 2019).

In 1937, Belgian scientist G. Chavanne was granted a patent titled "Procedure for the Transformation of Vegetable Oils for Their Uses as Fuels" (Belgian Patent No. 422,877). Then, in 1977, Brazilian scientist Expedito Parente filed the first patent for an "industrial process for biodiesel." The development of biodiesel progressed further when an Austrian company, Gaskoks, established the first biodiesel pilot plant and industrial-scale facility in 1987. In 1991, the first biodiesel standard was introduced, followed by the German standard (DIN 51606) in 1997. The ASTM D6751 standard was published in 2002, and in October of the following year, Europe introduced the DIN EN 14214 biodiesel standard. In September of the next year, Minnesota became the first U.S. state to sell diesel fuel containing 2% biodiesel. Later, in October 2008, ASTM released the first biodiesel blend specification standard, and the latest version of the European standard EN 14214 was published in November 2008 (Atabani et al., 2012).

Fig 1: soybean car

(http://www.thehenry ford.org/research/soybeancar.aspx#)

2.2.2 Biodiesel Properties

Over the past century, the advancement of various production catalysts, numerous feedstocks, and multiple techniques in biodiesel production has been driven by the observed complementary fuel properties. The characterization of these fuel properties plays a crucial role in determining fuel quality. The key properties of biodiesel include density, viscosity, flash point, calorific value, cloud point, oxidation stability, and cetane number (Mahmudul et al., 2017).

1. **Appearance and odour**

The hues of biodiesel range from golden to dark brown. Except when it is created from used cooking oil or animal fat, biodiesel has no smell.

2. Density and API gravity

Fuel density (ρ) refers to the mass per unit volume measured in a vacuum. Since temperature significantly affects density, quality standards require it to be measured at 15°C. Fuel density directly influences performance, as key engine characteristics such as heating value and viscosity are closely linked to it. Additionally, density affects both combustion and atomization quality. Variations in density alter the mass of fuel entering the combustion chamber, which in turn impacts the fuel's energy content, modifying the fuel/air ratio and engine power. This is particularly important in diesel engines, where fuel is metered by volume through injectors and pumps. Consequently, fuel density is the primary factor determining the mass of fuel delivered into the internal combustion chamber.

According to ASTM D-6751, the standard fuel density at 15°C falls within the range of 0.86 to 0.9 g/cm^3. Diesel quality is often assessed using API gravity, a measure that helps determine fuel characteristics (Elgharbawy et al., 2021). The API gravity is calculated using the following equation, as outlined by the American Petroleum Institute guidelines:

$$\text{API gravity} = (141.5/\text{sp.gr}) - 131.5$$

3. Acid Number

The acid number represents the amount of acid in a sample, measured in milligrams, required to titrate it to a specific endpoint. It directly measures the free fatty acids present in B100 biodiesel. The presence of free fatty acids in the fuel can lead to engine part corrosion, which is often associated with water content in the biodiesel (Giakoumis, 2013). A high acid number indicates a significant concentration of water and free fatty acids, requiring additional processing to eliminate these impurities. The acid number is determined using the ASTM-D664 standard.

4. Kinematic Viscosity

Viscosity is a crucial factor in selecting fuel, as it directly affects the combustion quality of internal combustion (IC) engines, including atomization and spray characteristics. Additionally, viscosity serves as an indicator for monitoring biodiesel quality during storage. At low temperatures, high viscosity negatively impacts fuel flow, while excessively low viscosity can lead to increased wear and leakage due to inadequate lubrication. Conversely, high viscosity contributes to incomplete combustion and operational difficulties in cold weather, as viscosity increases with decreasing temperature. In extreme cases, biodiesel may become excessively thick or even solidify at low temperatures (Isioma et al., 2013).

The higher viscosity of biodiesel results in the formation of larger droplets during injection, which further deteriorates combustion efficiency and increases exhaust emissions whereas low viscosities may not provide enough lubrication for the precision fit of fuel injection pumps, increasing wear or causing leaks.(*Cennatek Bioanalytical Services, (2013). Kinematic Viscosity According to Astm D445. Retrieved June 13, 2013 - Google Search*, n.d.). Compared to diesel fuel, biodiesel exhibits a significantly higher kinematic viscosity due to its larger molecular mass and complex chemical structure. Research has shown that biodiesel's kinematic viscosity is approximately 10–15 times higher than that of diesel fuel. Sajjadi et al. also reported that vegetable oils are 9–17 times more viscous than petroleum diesel, while biodiesel is about 1.6 times more viscous. Kinematic viscosity is measured using ASTM D445 and EN ISO 3104 test methods. According to ASTM D6751 and EN 14214 standards, biodiesel's kinematic viscosity ranges between 1.9–6 mm^2/s and 3.5–5 mm^2/s, respectively (Mahmudul et al., 2017).

5. Flashpoint

Flashpoint is the lowest temperature at which a fuel's vapors ignite under specific test conditions at a barometric pressure of 101.3 kPa (760 mmHg). In biodiesel, flashpoint is influenced by residual alcohol content and is used to assess and classify the material's flammability (Giakoumis, 2013). A higher flashpoint indicates delayed combustion, which can lead to carbon buildup and increased emissions of gaseous pollutants (Kumar et al., 2023).

Generally, biodiesel is less volatile and has a higher flashpoint than conventional diesel due to its high concentration of long-chain fatty acids (Orhevba et al., 2016).

6. Water content

Water contamination poses a significant challenge in biodiesel production and usage, primarily affecting fuel cleanliness and performance. Water can exist in two forms: dissolved water and suspended water droplets. While biodiesel is generally considered insoluble in water, it absorbs significantly more water than diesel fuel. Compared to diesel, which can contain about 50 ppm of dissolved water, biodiesel can absorb nearly three times more, reaching approximately 1500 ppm (Mahmudul et al., 2017)

Sanford et al. and Atabani et al. reported that when the water content in 100 mL of biodiesel is below 0.005 vol%, it is recorded as <0.005 vol%. Water in biodiesel can lead to several issues, including reduced heat of combustion, corrosion of fuel system components such as fuel pumps, injector pumps, and fuel tubes, as well as fuel filter blockages. Additionally, water contamination can promote microbial growth, allowing fungi and bacteria to thrive at the interface between fuel and water. The standard limits for water content in biodiesel are measured using ASTM D2709 and EN ISO 12937, with a maximum allowable limit of 0.05% by volume (Mahmudul et al., 2017).

7. Sulphur content

This process involves measuring the total sulfur content in liquid hydrocarbons with boiling points ranging from 25 to 400°C. The viscosity of the substance is typically evaluated between 0.2 and 20 cSt (mm^2/s) at room temperature. Biodiesel feedstock generally contains only trace amounts of sulfur. However, testing can reveal contamination from protein-containing materials, residual catalysts from the reaction, or substances involved in sulfur neutralization during production (Giakoumis, 2013).

8. Cetane Number

This test evaluates the ignition quality of diesel by comparing it with reference fuels tested in a standardized engine, assessing how easily the fuel ignites during combustion. In biodiesel, ignition issues are rarely significant since most common fatty esters have cetane numbers around or above 47 (Giakoumis, 2013). The cetane number (CN) is an indicator of a fuel's ignition quality—the higher the CN, the easier and faster the fuel ignites when injected into an engine. Due to its higher oxygen content, biodiesel generally has a higher CN value, ranging from 46 to 60, which enhances combustion efficiency (Lemma et al., 2020).

9. Cloud Point

The cloud point is the temperature at which wax crystals first appear in a liquid as it cools under specific conditions. This property is particularly important in low-temperature environments and applies to all diesel fuels. Biodiesel generally has a higher cloud point than conventional diesel, making it more susceptible to solidification in cold weather. The formation of wax crystals in pure biodiesel (B100) can be slowed down by incorporating additives that function through different mechanisms. Another approach to lowering the cloud point involves blending feedstock oils with varying fatty acid content, combining high and low saturated fatty acids to improve cold flow properties (Giakoumis, 2013).

Heating Value

The heating value of a fuel refers to the amount of thermal energy released per unit quantity when the fuel is burned, and the combustion products are cooled back to the initial temperature of the reactants. It is a measure of the fuel's energy content, indicating its efficiency in providing usable energy during combustion (Sivaramakrishnan & Ravikumar, 2011)

A key characteristic of a fuel is its higher heating value (HHV), which represents the total heat released when one gram of fuel undergoes complete combustion, producing CO_2 and H_2O at its initial temperature and pressure. HHV serves as an indicator of the fuel's energy content and is particularly important for evaluating vegetable oils, animal fats, and their derivatives as potential alternative fuels (Fassinou, 2012).

2.2.3 Advantages of Biodiesel

The advantages of using biodiesel as a substitute for diesel include:

1. Biodiesel is an eco-friendly liquid fuel that produces significantly lower emissions than conventional diesel, making it a cleaner and more sustainable alternative.
2. Biodiesel can be used in existing diesel engines without modification for blends up to B20, while higher blends may require minor adjustments to ensure optimal performance and compatibility.
3. Biodiesel is a more cost-effective alternative to diesel and can serve as an "on-farm fuel," allowing farmers to cultivate oilseed crops, produce biodiesel, and utilize it directly for agricultural operations.
4. Biodiesel can enhance vehicle performance due to its high cetane number, which exceeds 100, indicating superior ignition quality and improved combustion efficiency.
5. Due to its inherent clarity and purity, biodiesel can be used without the need for additional lubricants, unlike conventional diesel engines.
6. Biodiesel helps mitigate the environmental impact of waste by utilizing discarded materials as its feedstock. Since it is derived from waste products such as used cooking oils and animal fats, it does not contribute to environmental pollution. Instead of disposing of these substances, converting them into biodiesel provides a sustainable and eco-friendly alternative.
7. Biodiesel is an energy-efficient fuel option. When compared to conventional diesel, its production requires significantly less energy. Unlike petroleum diesel, which involves

drilling, transportation, and refining, biodiesel production is a simpler and less time-consuming process. This makes it a more sustainable and cost-effective alternative.

8. Biodiesel is a locally produced fuel, making it a more cost-efficient alternative. Since it does not rely on imported petroleum, there is no need to pay tariffs or additional taxes associated with oil imports. This allows countries to achieve greater energy independence, as biodiesel can be produced using locally available resources.

2.2.4 Disadvantages of Biodiesel

1. Higher production cost compared to conventional diesel.

2. Requires large-scale agricultural land for feedstock production.

3. Can cause fuel filter clogging due to higher viscosity.

4. May degrade rubber seals and hoses in older engines.

5. Limited availability and distribution infrastructure.

2.2.5 Standards for Biodiesel

A biofuel must be of high quality in order to be used successfully and without technical problems over the long term. Numerous factors that reflect the physical and chemical characteristics of biodiesel impact its quality. The quality of biodiesel can be affected by several different circumstances.

Several factors influence biodiesel quality, including the feedstock's quality, the fatty acid composition of the original vegetable oil or animal fat, the production process and its components, as well as postproduction conditions, handling, and storage. Since modern diesel engines are typically designed to operate on biodiesel, its physicochemical properties should closely resemble those of conventional diesel fuel. To ensure biodiesel meets the necessary standards for use, strict fuel specifications such as ASTM D6751 in the United States and EN 14214 in the European Union have been established.

Table 2.2 Standards for biodiesel fuels include ASTM D6751 and EN 14214 (Sakthivel et al., 2018)

BIODIESELPROPERTY SPECIFICATION					
Property	Units	ASTM D6751		EN 14214	
		Tests	Limits	Tests	Limits
Flash point	°C	ASTM D93	130 minimum	EN ISO 3679	101 minimum
Cloud point	°C	ASTM D2500	−3 to −12	–	–
Pour Point	°C	ASTM D97	−15 to −16	–	–
Cetane number		ASTM D613	47 minimum	EN ISO 5165	51 minimum
Density at 15 °C	Kg/m^3	ASTM D 1298	880	EN ISO 3675/ 12185	860–900
Kinematic viscosity at 40 °C	mm^2/s	ASTM D445	1.9–6.0	EN ISO 3104	3.5–5.0
Iodine number	g I_2/100 g	–	–	EN 14111	–
Acid number	mg KOH/g	ASTM D664	0.5 maximum	EN 14104	0.5 maximum
Saponification value	mgKOH/g	ASTM D5558	370 maximum	–	–
Boiling point	°C	ASTMD7398	100–615	–	–
Ash content	%Mass	–	–	–	–
Water and sediment	%Mass	ASTM D 2709	0.005 vol% Maximum	EN ISO 12937	500 mg/kg

2.2.6 Biodiesel Purification

The fatty acid methyl ester (FAME) mixture produced through the transesterification process must undergo purification to meet the required quality standards for biodiesel. Purification of biodiesel offers enhanced efficiency, and protects the engine from deterioration. Once crude biodiesel is obtained, residues such as glycerol, soap, water, a catalyst, and triglycerides must be removed. One of the most important steps in the manufacturing of biodiesel is purification. Adsorbents based on membranes, ion exchange, and water washing are the most widely used technology for biodiesel purification. The efficacy of engine performance is dependent on this purifying technique. High amounts of free fatty acids can cause deposit accumulation in storage tanks and even injectors, so shortening engine life. Moreover, cars' engines may deteriorate due to the excessive water content. As a result, purifying crude biodiesel might be difficult and raise operational costs for biodiesel production (Wan Osman et al., 2023). Therefore, FAME must undergo washing, neutralization, and drying. Successive water-washing steps help remove residual methanol, catalyst, and glycerin, as these contaminants are water-soluble. However, caution is necessary to prevent emulsion formation, which can reduce process efficiency. The first wash uses acidified water to neutralize the ester mixture, followed by two additional washes with pure water. Afterward, any remaining water must be removed through a drying process. Once dried, the purified product can be characterized as biodiesel according to international standards. Alternatively, ion exchange resins or silicates can be used for purification. The glycerin obtained from the chemical reaction is of low quality and has no commercial value in its raw form. Therefore, it must undergo purification after phase separation. However, this process is not cost-effective for small-scale production due to the low glycerin yield. In contrast, large-scale production plants benefit from purification, as it not only produces high-quality glycerin but also allows for methanol recovery from both FAME and glycerin, reducing overall biodiesel production costs. The continuous growth of biodiesel production is driving research into new applications for glycerin in the development of high-value-added products.

2.3 FEEDSTOCK OF BIODIESEL

Various feedstocks have been utilized for biodiesel production, including plant oils, animal fats, algae, and waste oils. Both edible and non-edible plant oils serve as potential sources, with over 350 oil-bearing plants identified for biodiesel production (Atabani et al., 2012). However, for biodiesel production to be sustainable and cost-effective, the ideal oil plants must be well-suited to local conditions, have high oil content, contain suitable fatty acid compositions, require minimal agricultural inputs, produce mature seeds at a uniform rate, and thrive in marginal lands unsuitable for food crops. In developed countries, edible oilseed crops are primarily used as biodiesel feedstock to reduce greenhouse gas emissions and decrease dependence on non-renewable petro-diesel imports. Using edible oils as biodiesel feedstock is unsustainable, as it compromises food security. Additionally, biodiesel derived from edible oils is not economically competitive with petro-diesel. Cultivating non-edible oil plants, on the other hand, can help mitigate climate change by reducing greenhouse gas emissions. Therefore, for industrial-scale biodiesel production to be cost-effective, the use of non-edible oils is essential (Lemma et al., 2020). Non-edible oilseed plants can be grown on marginal or waste lands with minimal cultivation costs, as they produce high yields with little effort. These plants thrive in arid and semi-arid regions, requiring less fertile soil and minimal rainfall (Rozina et al., 2022). Key factors in biodiesel production include selecting an appropriate feedstock, choosing the right catalyst, and employing efficient synthesis techniques. Among these, feedstock selection significantly impacts the overall cost of biodiesel (Kumar & Sharma, 2011).

Biodiesel feedstocks are categorized into first-generation and second-generation based on their edibility. First-generation biodiesel feedstock, primarily used in developed countries include edible oils such as palm oil, soybean oil, sunflower oil, mustard oil, rapeseed oil, peanut oil, and coconut oil. These were initially popular as there were no concerns about food being used for fuel. However, second-generation biodiesel feedstock, which are non-edible, are now been explored to address food security concerns. Common second-generation feedstock include waste cooking oil, neem oil, pongamia oil, cottonseed oil, tallow, beauty leaf oil, and moringa oil (Anwar, 2021).

2.3.1 First Generation Biodiesel

First-generation biodiesels are derived from edible feedstocks such as rapeseed oil, soybean oil, coconut oil, corn oil, palm oil, sunflower oil, mustard oil, olive oil, and rice oil. Initially, these feedstocks were widely used due to their availability and relatively simple conversion process. However, the major drawback of using edible oils for biodiesel production is the potential impact on food supply, leading to increased food costs. Additionally, factors such as environmental adaptability, high production costs, and limited cultivation areas have restricted their viability, prompting a shift toward alternative, non-edible sources for biodiesel production (Monika et al., 2023).

2.3.2 Second Generation Biodiesel

Second-generation biodiesels are produced from non-edible feedstocks, including neem oil, jatropha oil, nagchampa oil, karanja oil, Calophyllum inophyllum oil, rubber seed oil, and Mahua indica oil. The challenges associated with first-generation biodiesel feedstocks have led researchers to explore non-edible alternatives. Second-generation biodiesels offer several advantages, including being environmentally friendly, having lower production costs, reducing competition with food supplies, and requiring less agricultural land for cultivation. One of the primary benefits of second-generation biodiesel is that it does not rely on food crops, which helps prevent food shortages and ensures that agricultural land is primarily used for food production. Additionally, these feedstocks can be grown on marginal or non-arable lands, reducing the need to convert fertile farmland for biofuel production. This makes second-generation biodiesel a more sustainable and economically viable alternative to first-generation biofuels. Despite these advantages, second-generation biodiesel feedstocks also have some drawbacks. The yield of certain non-edible plants, such as jatropha, jojoba, and karanja, is relatively low, making large-scale production challenging. Due to these lower yields, there is often pressure to cultivate these crops on agricultural land, which can indirectly impact food production and economic stability. Furthermore, the production process for second-generation biodiesel generally requires a higher amount of alcohol, which can increase costs and complicate the biodiesel synthesis process. To address these challenges, researchers are actively seeking new alternative solutions that are more economically viable, widely available, and easier to produce at a larger scale. The focus is on improving the efficiency of biodiesel production while ensuring sustainability and minimal environmental impact (Singh et al., 2019).

2.3.3 Third generation biodiesel

Third-generation biodiesel is produced from microalgae and waste oils, offering a more sustainable alternative to previous generations of biofuels. The primary advantages of third-generation biodiesel include a reduced greenhouse gas effect, a higher growth rate and productivity, minimal competition for agricultural land, a higher oil yield percentage, and a lesser impact on the food supply. These factors make it a promising solution for addressing the limitations of first- and second-generation biodiesel feedstocks.

However, third-generation biodiesel also presents significant challenges. One of the major drawbacks is the high initial investment required for cultivation, harvesting, and processing. The production process also depends on sunlight availability, making it location-dependent. Additionally, large-scale production remains a challenge due to the complexities involved in cultivating and processing algal biomass. Extracting oil from microalgae is another major hurdle, requiring advanced and cost-intensive technologies.

Currently, research efforts are focused on improving the efficiency of biodiesel production from algal biomass, with a particular emphasis on increasing the production rate and optimizing the oil extraction process. The main sources of third-generation biodiesel include fish oil, animal fat, microalgae, and waste cooking oil. These feedstocks address many of the issues associated with earlier biodiesel generations, such as food security concerns, land use conflicts, environmental adaptability, and economic feasibility. In extreme conditions, certain algal species can survive with high lipid content, making microalgae a potential future source for third-generation biodiesel. Waste oils like used cooking oil, waste fish oil, and waste animal tallow oil are also key feedstocks, reducing waste management burdens and water pollution. Currently, animal fats from pork, beef, goat, and poultry are emerging as reliable sources for biodiesel production (Singh et al., 2019).

2.4 THE PRODUCTION OF BIODIESEL

2.4.1 Biodiesel production techniques

Worldwide, significant efforts are being made to enhance vegetable oil properties to match those of diesel fuel. The primary challenges associated with crude vegetable oils include high viscosity, low volatility, and polyunsaturated characteristics. These issues can be addressed through four main methods: pyrolysis, direct use and blending, micro-emulsion, and transesterification. (Atabani et al., 2012).

Of these, transesterification is a straightforward and often used process to effectively manufacture biodiesel. This process produces the chemical product known as biodiesel, which is methyl or ethyl esters, from the reaction of animal and vegetable oils, such as edible or non-edible oils containing triglycerides, with alcohol and a catalyst. Depending on the catalyst used, a significant contribution to the contribution to the production of high-quality biodiesel and its yield % in varying reaction times is made by the catalyst (Basumatary et al., 2024). Every technique has benefits and drawbacks of its own. Transesterification is a cheap process, but it needs high temperatures and a catalyst. The thermal process of pyrolysis can make biodiesel from vegetable and animal fats, but it uses a lot of energy and yields a lot of byproducts. The high purity of biodiesel produced by the micro-emulsion process is an advantage, although separation and purification steps are extra. Overall, considerations including cost, effectiveness and environmental impact influence the choice of biodiesel production technology.

Direct Use and Blending

Using vegetable oils directly in diesel engines is not ideal due to several inherent issues. Although vegetable oils share some properties with biodiesel, they require chemical modification before being used in engines. Research on their use has expanded in recent decades, though experiments date back nearly a century. While some diesel engines can run on pure vegetable oil, turbocharged direct injection engines, such as those in trucks, face significant challenges. Energy consumption with pure vegetable oils is comparable to diesel fuel, and short-term use has been successful with oil-to-diesel ratios between 1:10 and 2:10 (Gashaw & Teshita, 2014).

Pyrolysis

Pyrolysis is the thermal breakdown of organic materials without oxygen, often with a catalyst. It can be applied to vegetable oils, animal fats, fatty acids, or their methyl esters. Researchers have explored pyrolysis of triglycerides to produce diesel-compatible fuels. This process yields alkanes, alkenes, alkadienes, aromatics, and carboxylic acids. Pyrolysis is considered efficient, simple, waste-free, and environmentally friendly. According to Sharma et al., it produces fuel with a high cetane number, low viscosity, and acceptable sulfur, water, and sediment levels.

However, it results in high ash content, carbon residues, and pour points, making some properties undesirable. (Atabani et al., 2012).

Micro-emulsification process (micro-emulsified fuel)

A micro-emulsion is a colloidal dispersion of two normally immiscible liquids, stabilized by one or more ionic amphiphiles, with microstructures typically ranging from 1 to 150 nm (Atabani et al., 2012). Oil micro-emulsification involves blending oil with another liquid fuel, such as diesel or biodiesel, with or without surfactants. However, surfactants are usually required to prevent phase separation between diesel, neem oil, and solvents like ethanol, methanol, 1-butanol, 2-octanol, butanol, and hexanol. Emulsification with alcohol is considered a promising method to address the high viscosity of vegetable oils. The micro-emulsion process is simple, energy-efficient, and does not generate waste. Additionally, NOx emissions from emulsified oil combustion are lower compared to other fuels. However, studies indicate that using micro-emulsified oils in engines can lead to issues such as carbon deposits, incomplete combustion, lubricating oil thickening, and injector needle sticking (Ewunie et al., 2021).

Trans-esterification

Trans-esterification is a chemical process that converts glycerol esters in vegetable oils into alkyl monoesters, which form biodiesel. This reaction occurs when vegetable oil is combined with alcohol in the presence of an acidic or alkaline catalyst. Common alcohols used include methanol, ethanol, and butanol, producing methyl, ethyl, or butyl esters, respectively. Trans-esterification is the most widely used method for producing biodiesel, which can be utilized directly or blended with diesel for use in diesel engines. This process is widely employed to lower viscosity and molecular weight while reducing most exhaust emissions. It also enhances volatility and cetane number while maintaining the heating value of triglycerides. Additionally, trans-esterification improves biodegradability, provides inherent lubricity, increases the flash point, and ensures a domestic source of feedstock for biodiesel production (Bello et al., 2012).

Table 2.3 differences in different production method of biodiesel from Awogbemi et al., 2021

Production methods	Advantages	Disadvantages
Pyrolysis	Simple procedure Environmentally friendly Generation of byproduct High output efficiency	Requires high temperature Expensive equipment needed Product contains sulfur Contaminated product
Micro-emusification	Straightforward procedure Enhanced cetane rating Lower emissions of NOx, CO, and other pollutants 4o	Decreased volatility and stability Formation of carbon residue Partial combustion
Direct use/blending	Simple procedure	Increased smoke and CO emissions Carbon buildup in engines Incomplete combustion Residue formation
Trans-esterification	Effortless procedure Large-scale industrial production Biodiesel properties similar to PBD fuel	Several separation steps Expensive catalysts required Wastewater production Elevated moisture and impurity content

2.5 HEATING METHOD FOR TRANSESTERIFICATION

2.5.1 Supercritical and subcritical alcohol transesterification

The transesterification of vegetable oil using non-catalytic supercritical methanol presents an innovative approach to biodiesel production. Under supercritical conditions, the transesterification reaction is completed within minutes, whereas conventional catalytic transesterification requires several hours. Typically, the reaction between triglycerides (non-polar molecules) and alcohol (a polar molecule) is heterogeneous, forming two liquid phases at standard processing temperatures due to their incomplete miscibility. However, in supercritical conditions, the mixture transitions into a single homogeneous phase, significantly enhancing the reaction rate by eliminating interphase mass transfer limitations. Another advantage of utilizing supercritical conditions is that the alcohol serves not only as a reactant but also as an acid catalyst. Kusdiana and Saka presented a reaction mechanism for vegetable oil in supercritical methanol, proposing that the alcohol molecule directly attacks the carbonyl atom of the triglyceride due to the high pressure. In the supercritical state, depending on temperature and pressure, hydrogen bonding is significantly reduced, allowing methanol to exist as a free monomer. The transesterification proceeds through a methoxide transfer, leading to the formation of fatty acid methyl esters and di-glycerides. Similarly, di-glycerides undergo transesterification to produce methyl esters and mono-glycerides, which are further converted into methyl esters and glycerol in the final step. Notably, the presence of water in the reaction system does not impact the yield of methyl esters during supercritical alcohol transesterification (Andrade et al., 2011).

2.5.2 Microwave assisted transesterification

The application of microwave heating as a tool in preparative chemistry continues to expand. Microwave irradiation can significantly reduce reaction times and enhance product yields. As an alternative energy stimulant, microwave irradiation is also utilized for biodiesel production. It works by activating the smallest degree of variance in polar molecules and ions, such as alcohol, through a continuously changing magnetic field. This fluctuating electric field interacts with molecular dipoles and charged ions, inducing rapid rotation and generating heat through molecular friction (Nezihe et al., 2007). Biodiesel preparation using microwave irradiation provides a fast and efficient route to this valuable biofuel, offering several advantages, including a short reaction time, a low oil-to-methanol ratio, simplified operation, a substantial reduction in by-products, and lower overall energy consumption (Andrade et al., 2011).

2.5.3 Ultrasound assisted transesterification

Ultrasound has proven to be an effective tool for enhancing reaction rates across various reacting systems. It has successfully increased conversion efficiency, improved yields, and even altered reaction pathways in biological, chemical, and electrochemical processes. Ultrasound refers to sound waves with frequencies beyond the range detectable by the human ear. While human hearing typically ranges from 16 Hz to about 18 kHz, ultrasound generally falls between 20 kHz and 100 MHz. Like other sound waves, ultrasound propagates by alternately compressing and expanding the molecular spacing of the medium, creating cycles of compression and rarefaction.

When a significant negative pressure gradient is applied to a liquid, the molecular spacing may exceed the critical distance required to maintain its integrity, leading to the formation of voids or cavitation bubbles. At high ultrasonic intensities, small cavities can expand rapidly due to inertial effects, eventually collapsing violently. This collapse releases energy, resulting in both chemical and mechanical effects. The disruption of the phase boundary due to cavitation promotes emulsification, as ultrasonic jets force one liquid into another.

Low-frequency ultrasonic irradiation can be particularly beneficial for transesterification of triglycerides with alcohol. Ultrasonication provides both the mechanical energy necessary for mixing and the activation energy required to initiate the transesterification reaction. This process enhances the reaction rate and increases biodiesel yield from vegetable oils and animal fats. The ultrasonic-assisted transesterification method offers several advantages over conventional mechanical stirring, including a shorter reaction time, reduced energy consumption, an optimized molar ratio of methanol to triglycerides, and overall simplicity (Andrade et al., 2011).

2.6 FACTORS AFFECTING BIODIESEL PRODUCTION

2.6.1 Amount of FFA content in the feedstock and moisture content

The fatty acid composition of a biodiesel source is a crucial factor in determining the most effective production method (Mahmudul et al., 2017). The presence of moisture in the oil increases free fatty acid (FFA) levels. For a complete reaction, oils should have less than 3% FFA content (Gashaw & Teshita, 2014). If the FFA content exceeds 1%, additional alkali catalyst is required to neutralize it. Water has a more detrimental effect than FFAs, as it promotes soap formation and frothing, increasing viscosity. Furthermore, gel and foam formation obstructs glycerol separation from biodiesel. FFAs and water negatively impact transesterification by causing soap formation, consuming the catalyst, and reducing its effectiveness. Additionally, they lower methyl ester yield (Mathiyazhagan & Ganapathi, 2011).

A high FFA content in oil triggers saponification (formation of soap and water), reducing catalyst efficiency, forming gels, increasing biodiesel viscosity, and complicating glycerol separation (Demisu, 2021). Commercial crude oils and fats contain significant FFAs that react with base catalysts, forming saponified products during base-catalyzed transesterification, necessitating extensive and costly purification. Saponification not only depletes the alkali catalyst but also creates emulsions, making downstream biodiesel recovery and purification challenging. Oil quality directly influences the transesterification process, with high-FFA oils requiring different production methods than low-FFA oils. Therefore, chemical analysis of FFA content and consumption is essential (Jain & Sharma, 2010).

2.6.2 Molar ratio of alcohol and type of alcohol

The choice of alcohol(s) for base-catalyzed transesterification depends on their efficiency, operational performance, and cost. Lower alcohols such as methanol (CH_3OH), ethanol (C_2H_5OH), and butanol (C_4H_9OH) are commonly used. Among them, methanol is the most widely used due to its higher availability and lower cost compared to ethanol, making methanol-based biodiesel the dominant commercial product. Additionally, methanol is preferred over ethanol because of its lower boiling point, which simplifies the recovery of unreacted alcohols. In contrast, using isopropanol or ethanol in transesterification leads to azeotrope formation with water molecules, making alcohol-water separation in the distillation process more difficult.

In the base-catalyzed transesterification reaction, one mole of fatty acid triglyceride reacts with three moles of alcohol (typically methanol) to yield three moles of alkyl (methyl) ester and one mole of tri-hydroxy alcohol (glycerol). The oil-to-alcohol molar ratio significantly influences the conversion rate of triglycerides into biodiesel. According to Le Chatelier's principle, increasing reactant concentration enhances product formation. Therefore, raising alcohol concentration accelerates biodiesel production. Maximum triglyceride conversion occurs at a 1:6 oil-to-alcohol molar ratio. Beyond this optimal ratio, the conversion efficiency remains unchanged, but excessive alcohol complicates recovery (Demisu, 2021).

2.6.3 Reaction Time

The conversion of fatty acid esters increases with reaction time. Initially, the reaction progresses slowly due to the mixing and dispersion of alcohol and oil, but it then accelerates rapidly. Maximum ester conversion is achieved within 90 minutes. Extending the reaction time beyond this does not increase biodiesel (mono-alkyl ester) yield. Instead, prolonged reaction time reduces the final product due to the reversible nature of transesterification, leading to ester loss and soap formation (Gashaw & Teshita, 2014).

2.6.4 Reaction temperature

The reaction temperature significantly influences base-catalyzed transesterification. Increasing the temperature enhances the reaction rate and improves biodiesel yield while also reducing the time needed for maximum reactant conversion. However, the temperature must remain below the alcohol’s boiling point, as exceeding it causes alcohol vaporization and bubble formation, disrupting the transesterification process. Conversely, maintaining a temperature too far below the alcohol’s boiling point increases the viscosity of fatty acid alkyl esters (biodiesel). Therefore, the optimal reaction temperature is between 63-68°C to achieve the highest yield of the desired product (Demisu, 2022).

2.6.5 Agitation

Agitation speed is crucial in biodiesel (mono-alkyl ester) formation, as it enhances the reaction by improving the mixing of oil and catalyst. Lower stirring speeds result in lower product yield, while excessively high stirring speeds promote soap formation due to the reverse nature of the transesterification reaction (Demisu, 2022).

2.6.6 Type and amount of catalyst

The selection of a catalyst for transesterification depends on factors such as the type of feedstock (edible or non-edible oil), operating conditions (temperature and pressure), required catalytic activity (high surface area), and the catalyst's cost and availability. The catalyst's weight significantly affects the reaction and, consequently, the biodiesel yield. A proper increase in catalyst concentration enhances the number of active sites, leading to higher biodiesel production (Omar & Amin, 2011). However, excessive catalyst loading increases slurry viscosity, resulting in poor reaction mixtures (I.W. Khan et al., 2020).

2.7 CATALYSTS IN BIODIESEL PRODUCTION

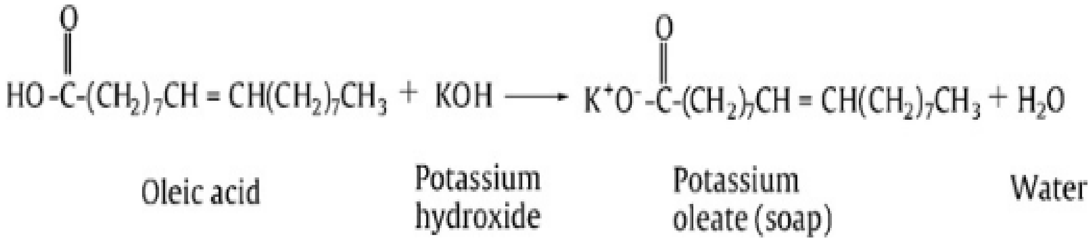
Materials, either natural or artificial, called catalysts can speed up processes without being consumed themselves. In catalytic processes, the activation energy is essential, and the purpose of catalysts is to lower the activation energy. The lowest amount of energy needed for the re action to initiate is called activation energy. Catalysts are classified into two types: homogeneous catalysts, which have the reactants and catalysts in an identical phase, and heterogeneous catalysts, which have the reactants and catalysts in separate phases. The general reaction of transesterification for biodiesel commences with the attachment of free fatty acid onto the Lewis acid site of the catalyst creating a carbon-positive ion. The nucleophiles in the methanol attack the electrophilic carbon. Concomitant esterification and transesterification reactions are performed with bi-functional catalysts that have both acidic and alkaline active sites. The acidic and basic active sites absorb triglycerides and alcohol respectively on their surface as the reaction commences (Parameshwar et al., 2024).

2.7.1 Homogeneous catalysts

Homogeneous catalysts operate in the same phase as the reactants, whether in liquid or gaseous form. Typically, they dissolve in a solvent along with the substrate and can function as either acids or bases. Common homogeneous acid and base catalysts used in esterification and transesterification include sulfuric acid (H_2SO_4), hydrochloric acid (HCl), sodium hydroxide (NaOH), and potassium hydroxide (KOH).

a) Homogeneous base catalyst

Homogeneous base catalysts are widely studied for transesterifying vegetable oil into FAME due to their affordability and availability. Several catalysts, such as KOH, NaOH, and $NaOCH_3$, have been used for FAME synthesis (Nisar et al., 2017). NaOH and KOH demonstrate excellent catalytic activity, enabling high biodiesel yield and minimal reaction time under ambient conditions. However, a drawback of this process is water formation as a byproduct, which reduces biodiesel yield. Sodium methoxide and potassium methoxide offer better performance since they do not produce water. Alkaline catalysts are unsuitable for oils with high FFA content (>2 wt%) but are effective for refined vegetable oils with low FFA content (0.5–2 wt%) (Changmai et al., 2020).



$$HO-\overset{\overset{\displaystyle O}{\|}}{C}-(CH_2)_7CH=CH(CH_2)_7CH_3 + KOH \longrightarrow K^+O^- -\overset{\overset{\displaystyle O}{\|}}{C}-(CH_2)_7CH=CH(CH_2)_7CH_3 + H_2O$$

Figure 2. 1 Saponification

b) Homogeneous acid Catalysts

Acid catalysts exhibit greater tolerance in processing waste oils for biodiesel production compared to base catalysts. In two-step transesterification, acids are preferred as the initial catalyst, followed by a base, to achieve better results, particularly when working with organic substrates. Homogeneous acid catalysts are suitable for biodiesel synthesis from renewable feedstocks such as animal fat, grease, and waste cooking oil. However, using acid catalysts for biodiesel production has several drawbacks, including slow reaction rates and the need for larger amounts of methanol. Iram et al. conducted a study using a raw material with a methanol-to-vegetable oil molar ratio of 2:1 and produced biodiesel through transesterification. It was observed that a higher methanol-to-vegetable oil molar ratio was necessary to bring the acidic value of the catalyst within the required range for transesterification. Besides slow reaction rates, homogeneous acid catalysts are corrosive, generate large volumes of wastewater, and require complex separation processes for catalyst recovery and reuse. Additionally, they tend to leave residual acid, resulting in substandard biodiesel production. These challenges have led to the development of heterogeneous acid catalysts for biodiesel synthesis.

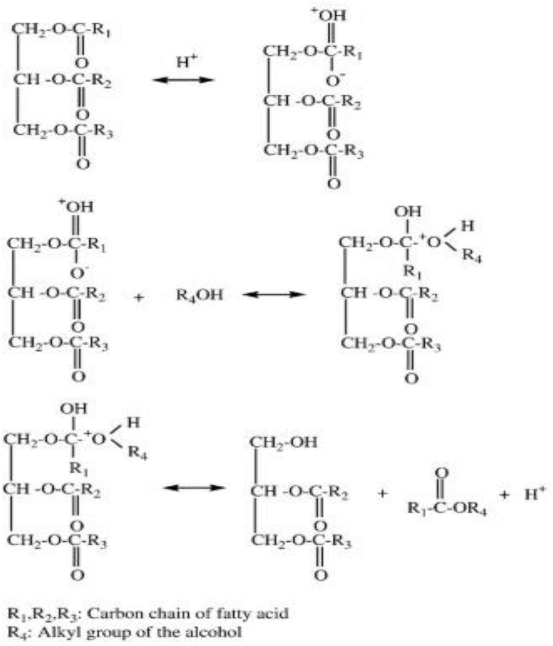


Figure 2. 2 Homogeneous acid-catalysed reaction mechanism for the transesterification of triglycerides

2.7.2 Heterogeneous acid and base-catalyst

Homogeneous catalysts have demonstrated higher efficiency in transesterification for biodiesel production. However, several challenges are associated with their use, including high energy consumption, the formation of unwanted soap as a byproduct due to reactions with free fatty acids (FFAs), the expensive process of separating the homogeneous catalyst from the reaction mixture, and the generation of large amounts of wastewater during the separation and cleaning of the catalyst and reaction products. A promising alternative to overcome these issues is the use of heterogeneous catalysts. Heterogeneous catalysts offer the advantage of easier separation from the reaction products, reducing the complexity of the purification process. Additionally, undesired saponification reactions can be prevented by using heterogeneous acid catalysts. These catalysts enable the transesterification of vegetable oils and animal fats that contain high levels of FFAs, such as deep-frying oils collected from restaurants and food processing industries. The use of solid catalysts in biodiesel synthesis has the potential to significantly lower production costs, as they can be reused multiple times, reducing the need for fresh catalyst input. Furthermore, heterogeneous catalysts allow for the simultaneous execution of both transesterification and esterification reactions, making the biodiesel production process more efficient and cost-effective.

2.7.3 Bio-based Catalyst

A bio-based or ‘green’ catalyst refers to a type of catalyst obtained from natural sources such as biomass. Recent trends indicate that utilizing naturally occurring biological sources of calcium and carbon has the potential to serve as an effective heterogeneous catalyst for the transesterification of vegetable oil. This approach is highly promising, as it enables the production of a highly efficient bio-based heterogeneous catalyst. Solid catalysts derived from biomass offer an environmentally friendly alternative, as they are non-toxic, non-corrosive, and eliminate wastewater production. Additionally, these catalysts are primarily sourced from biomass, which is not only abundant but also considered a low-cost material. Furthermore, disposal issues are minimal since the catalyst itself is biodegradable, reducing concerns about environmental pollution. This study examines the development of heterogeneous base and acid catalysts derived from biomass for biodiesel production, highlighting their advantages in sustainability, efficiency, and cost-effectiveness. This study presents the sources of catalysts, their preparation methods, and their performance. The research aims to offer valuable and informative insights into the current state of biomass-derived heterogeneous catalysts, contributing to future advancements in biodiesel processing and production. Bio-based waste materials serve as significant sources of essential elements such as calcium and potassium, which can be utilized as active phases in the preparation of heterogeneous catalysts for biodiesel production (Farooq et al., 2023).

2.8 COW BONE

Cow bone serves as a valuable source of a heterogeneous catalyst for biodiesel production due to its high calcium content. It contains calcium phosphate (hydroxyapatite), which can be transformed into calcium oxide (CaO) through high-temperature heating (calcination). This process generates a basic catalytic site essential for the transesterification reaction. The calcium oxide derived from cow bone functions as a solid heterogeneous catalyst, allowing for easier separation from the reaction mixture after biodiesel synthesis. Additionally, cow bone is an abundant and cost-effective option for transesterification reactions, making it a practical choice for biodiesel production. Furthermore, its use contributes to the valorization of waste animal material, helping to reduce the environmental impact of abattoir waste disposal.

Unlike certain traditional catalysts such as sodium hydroxide, the calcium oxide obtained from cow bone is a solid heterogeneous catalyst, which simplifies its separation from the reaction mixture after biodiesel production. To convert calcium phosphate into calcium oxide when using cow bone as a catalyst, processes such as cleaning, grinding, and high-temperature calcination are necessary. Research has shown that properly prepared cow bone catalysts can achieve high biodiesel yields in the transesterification process.

2.9 RICE BRAN

Rice bran is the outer layer of the rice grain, removed during the milling process to produce white rice. It is a byproduct of rice milling and consists of the husk, germ, and endosperm. Rice bran contains ash, which serves as a source of inorganic components such as calcium, magnesium, and silica, essential for developing solid acid catalysts. These solid acid catalysts play a crucial role in catalyzing the transesterification of oils with high free fatty acid content. This makes rice bran a suitable material for biodiesel production, particularly from oils containing elevated levels of free fatty acids.

For oils with high free fatty acids (FFA), solid acid catalysts are preferred for biodiesel production and rice bran’s bioactive compounds, minerals, and high fatty acid content can help form these catalysts, facilitating the transesterification process in a more sustainable and efficient manner.

Because of the unique composition of rice bran which includes triglycerides, antioxidants, and bioactive compounds, it serves an adequate candidate as a catalyst precursor for biodiesel production. Its rich content of triglycerides allows it to be used directly in transesterification processes, while its other chemical components contribute to the enhancement of catalytic activity and biodiesel quality.

In biodiesel production, rice bran can either serve as a feedstock for biodiesel production (due to its oil content) or as a precursor for producing solid acid catalysts.

The Guardian reports that the Central Bank of Nigeria (CBN) has significantly increased the nation's rice milling capacity by facilitating over 58 new integrated mills in the past seven years. As of January 2022, Nigeria's rice milling capacity reached 3 million metric tonnes annually, up from fewer than 350,000 metric tonnes in 2015. This expansion is largely attributed to the CBN's Anchor Borrowers' Programme (ABP), introduced in 2015, which has positively impacted rice cultivation and processing. CBN Governor Godwin Emefiele highlighted that the number of integrated mills grew from fewer than 10 in 2015 to over 68 by January 2022, with plans to commission an additional 10 mills within the year. Emefiele emphasized the importance of food security for economic development and encouraged millers to invest in rice paddy cultivation to ensure a steady supply of raw materials for their operations.

CHAPTER THREE

3.0 MATERIALS AND METHODS

Neem oil was utilized in this study and was sourced from a local vendor. The reagents employed in this research were of analytical grade with high purity and were purchased from a local vendor in Benin City, Edo State.

3.1 RAW MATERIALS

Table 3.1 Raw materials

MATERIALS	SOURCE	USES
Waste Cow bone	UBTH abattoir, Benin City, Edo State.	It serves as a feedstock for the primary precursor of the heterogeneous bi-functional catalyst.
Rice Bran	Danesi & Sons Rice Milling Factory, Auchi, Edo State.	It serves as the feedstock for the acid precursor of the heterogeneous bi-functional catalyst.
Neem oil	Local vendor	It is a feedstock for biodiesel production

3.1.1 Reagents

Materials used in this study are of high analytical grade and they are as follows;

Table 3.2: Reagents

Reagents	Formula	Molecular Weight (g/mol)	Maker	Uses
Potassium hydroxide	KOH	56.1	Labtech Tech	It is used for the pretreatment of basic precursor and in determining the acid value of neem oil
Acetic acid	CH_3COOH	60.05	Guangbang Guanghua Chemical Factory Co Ltd	Determination of the peroxide value of the neem oil
Benzene	C_6H_6	78.11	Guangbang Guanghua Chemical Factory Co Ltd	It is for the determination of the acid values of neem oil
Ethanol	C_2H_5OH	46.07	Guangbang Guanghua Chemical Factory Co Ltd	It is used for determining acid and saponification values of neem oil
Chloroform	$CHCL_3$	119.37	Loba Chemie Pvt Ltd	It is used for determination of the peroxide of neem oil
Phenolpthlein	$C_{20}H_{14}O_4$	318.31	Spectrum Reagent and Chemical Pvt, Ltd	As indicator
Sulphuric acid	H_2SO_4	98	Guangbang Guanghua Chemical Factory Co Ltd	It is for the pre-treatment of the acid precursor
Distilled water	H_2O	18.02	Central Research Lab, University of Benin	For washing the apparatus
Methanol	CH_3OH	32.04	Guangbang Guanghua Chemical Factory Co Ltd	Used for during the transesterification reaction of biodiesl production

3.1.2 Apparatus

Table 3.3 Apparatus

Apparatus	**Uses**
Measuring cylinder	For measuring the volume of reagents and solutions.
Beakers	For mixing and heating reagents and solutions.
Volumetric flask	Used to prepare standard solutions such as potassium hydroxide, sodium thiosulfate and dilute HCl solutions.
Spatula	For measuring and transferring small amount of substance such as potassium hydroxide and sodium thiosulfate salt.
Weighing balance	For measuring the mass of reagents such as KOH, KI.
Conical flask	For mixing and heating solutions.
Burette	For measuring and dispensing liquids such as KOH solution and dilute HCl during titration.
Pipette	For measuring and transferring small amount of reagents.
Retort stand	For attaching clamps that holds the burette during titration.
Magnetic stirrer	For thorough mixing of neem oil, catalyst and methanol during transesterification reaction
Oven	For proper drying of rice bran and cow bone before calcination.
Muffle furnace	For calcining cow bone and carbonizing rice bran.

3.2 METHODS

3.2.1 Preparation of Catalyst

Basic Precursor

Waste cow bones were collected from UBTH abattoir, Benin city. The waste cow bones were cleaned thoroughly to remove any attached organic matter, and rinsed with distilled water. The cleaned waste cow bones were sundried to remove moisture. The dried bones were ground into fine powder using a grinding mill and then sieved using a 0.6 mm sieve to obtain a consistent particle size for catalytic activity. The ground cow bone powder was calcined in a muffle furnace at 800°C for 4 hours to decompose organic content and produce calcium oxide (CaO). The calcined samples were removed before room temperature was reached and were immediately placed in a desiccator to cool. The samples were kept in an airtight container to avoid reaction with moisture before use. The calcined cow bone powder was soaked in a solution of 1.5M of KOH solution for 48 hours. The soaked bones powder was thoroughly washed with distilled water and sundried for 1 hour then later oven dried at temperature of 150°C for 1 hour.

Acid Precursor

The rice bran was collected from a rice mill in Auchi, Edo State. The rice bran collected was dried at 110C for 2 hours to remove moisture. The dried rice bran was sieved using a 0.425 mm sieve to remove large impurities and rice husk in the rice bran, this was done to ensure uniform particle size. The sieved rice bran was placed into a metallic crucible and then placed into a muffle furnace. The temperature was set which increased gradually to 200°C and this temperature was maintained for 4 hours. After carbonization, the furnace was turned off and the rice bran was allowed to cool slowly inside the furnace. The resulting powder was soaked in a solution of 1.5M H_2SO_4 for 48 hours then washed thoroughly with distilled water and sundried for 1 hour after which was oven dried at 150°**C** for 1 hour.

Catalyst Impregnation

The acid and base precursors were properly mixed in an equal ratio in a beaker with some addition of distilled water to yield a wet mixture. This mixture was sundried for 1 hour and then dried in the oven at 150°C for 1 hour. The dried catalyst was then calcined in a muffle furnace at 700°**C** for 3 hours. This gave a bi-functional catalyst for the biodiesel production.

3.3 CATALYST CHARACTERIZATION

3.3.1 Surface Morphology of the Processed Catalyst

Scanning Electron Microscopy (SEM) is a highly effective analytical technique used to examine materials at high magnifications and produce detailed images. It operates by directing a focused electron beam from an electron gun onto the surface of a sample, leading to the emission of high-energy electrons. The electron beam is concentrated on a specific area of the sample with the aid of an objective lens. To ensure optimal image quality, various parameters such as accelerating voltage, aperture size, and working distance (the gap between the sample and the electron gun) can be fine-tuned. SEM detects two types of electrons: backscattered electrons, which highlight contrast based on differences in chemical composition, and secondary electrons, which depict the surface's topography.

When the electron beam interacts with the sample, it initiates multiple interactions that lead to the emission of photons and electrons from or near the surface. These emitted signals provide crucial insights into the sample's surface features, chemical composition, crystalline structure, and the distribution of its components. The comprehensive data derived from these interactions facilitates an in-depth analysis of the sample's properties. (Omidi et al., 2017)

3.3.2 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectroscopy is a widely used technique for analyzing the chemical properties and structure of various materials, including biological samples. In industrial applications, it is a standard method for quality control and is often the first step in material examination. Changes in absorption band patterns can indicate alterations in material composition or the presence of contaminants. FTIR microanalysis is particularly useful for identifying the source of defects in products detected during visual inspection. It is effective for analyzing large surface areas and small particles, typically ranging from 10 to 50 microns, to determine their chemical composition.

When a sample is subjected to infrared radiation (ranging from 10,000 to 100 cm^{-1}) in an FTIR instrument, a portion of the radiation is absorbed while the rest passes through. The absorbed radiation induces vibrations or rotations in the sample's molecules, generating a spectrum at the detector that serves as a chemical fingerprint, typically covering the range of 4000 to 400 cm^{-1}. Each molecule or chemical structure produces a distinct spectral fingerprint, making FTIR a highly effective technique for chemical identification. (FTIR Analysis | RTI Laboratories, n.d.)

3.3.3 BET Surface Area Analyzer

The Brunauer-Emmett-Teller (BET) theory is a fundamental technique for determining the specific surface area of materials by examining the physical adsorption of gas molecules onto solid surfaces. It is especially valuable for studying systems that involve multilayer adsorption. Nitrogen is the most commonly used adsorbate in BET analysis, typically performed at its boiling point. However, other gases such as water, carbon dioxide, and argon are also occasionally used to measure surface area under varying conditions. The specific surface area obtained through BET analysis depends on the adsorbate molecule and its adsorption cross-section, as it is a scale-dependent property without a single universal value. (Nasrollahzadeh et al., 2019).

Porous materials are often used in chemical reactions and separation processes. The BET theory, derived from the Langmuir monolayer adsorption model, is widely applied to measure the specific surface area of porous materials, including both crystalline and amorphous structures. This method is essential for evaluating the performance of porous materials based on empirical gas absorption data. (Pourhakkak et al., 2021)

3.3.4 Electron Dispersive X-Ray Spectroscopy (EDX)

Energy-dispersive X-ray spectroscopy (EDS, EDX, or XEDS) is an analytical technique utilized for elemental analysis and chemical characterization of materials. This method allows for mapping the elemental composition of specific areas or determining the distribution of elements across a surface. EDX is effective in analyzing quasi-bulk samples, individual particles, or targeted regions on filters or deposits, providing valuable insights into a sample's composition. (Ismail et al., 2019).

Energy-dispersive X-ray spectroscopy (EDX) functions by detecting X-rays emitted when electrons interact with a sample. Due to the unique atomic structure of each element, this interaction produces distinct peaks on the X-ray spectrum. EDX detectors are commonly integrated with scanning electron microscopy (SEM) instruments to analyze the elemental composition of nanoparticles. The detector measures both the energy and quantity of X-rays released from the sample, allowing for precise identification and quantification of elements within the material. (Akintelu et al., 2023).

3.3.5 X-Ray Diffraction (XRD)

X-ray diffraction (XRD) is a versatile technique used for phase and elemental analysis, texture analysis, and stress measurements. While it is primarily used for crystalline samples, XRD can also assess the crystallinity of polymers. Recent advancements have expanded its application to thin film analysis. The technique is based on Bragg’s law of diffraction, which describes how X-rays interact with atomic planes in a crystalline structure to produce a diffraction pattern. (Nasrazadani & Hassani, 2016).

XRD is particularly valuable for characterizing polymeric nanocomposites, as it helps determine bonding types and the degree of crystalline organization in amorphous materials. The technique can identify microstructural changes and variations in interlayer spacing, providing critical insights into the material's structural properties and performance. XRD is essential for optimizing the design and application of nanocomposites in various fields. (Nasrazadani & Hassani, 2016).

3.4 CHARACTERIZATION OF NEEM OIL

Neem oil was utilized in this study and sourced from a local vendor. To assess its suitability for biodiesel production, the oil underwent a series of characterization analyses. These analyses were conducted to determine key physicochemical properties that influence its effectiveness as a feedstock for transesterification. The following tests were performed.

3.4.1 Determination of Acid value/ Free Fatty Acids

The Acid Value (AV) indicates the concentration of free fatty acids (FFA) in an oil sample. It is defined as the milligrams of potassium hydroxide (KOH) needed to neutralize the FFAs in one gram of oil. This value was determined through titration using a standard KOH solution.

A 250 ml conical flask was used to weigh 2g of oil. Then, 10 ml of benzene and 10 ml of ethanol were added to the flask and thoroughly mixed. Two drops of phenolphthalein were introduced into the mixture. The solution was then titrated with 0.05M potassium hydroxide until a permanent pink color was observed.

Calculation:

$$\text{Acid Value} = \frac{Titration\ value \times KOH\ Normality \times 56.1}{Weight\ of\ Neem\ oil\ used}$$

Where;

Titration value= $T - T_S$

Where: T = Titrate value of the neem oil

T_s = Titrate value of blank solution.

The weight percentage of fatty acids present in the oil is known as the free fatty acid (FFA) content, can be calculated using

FFA (%) = $\frac{Acid\ Value}{2}$

3.4.2. Determination of Density

This is the mass of a substance per unit volume. For determination of the density of neem oil, 50mL density bottle of known weight was filled with neem oil. The new weight of the density bottle was noted, the weight of neem oil contain ed in the 50mL bottle is gotten by subtracting the known weight of the density bottle from its new weight. The density is calculated with the Equation below, measured in Kg/m^3.

Density = $\frac{Weight\ of\ NEEM\ \ OIL}{Volume\ of\ NEEM\ \ OIL}$

3.4.3 Peroxide Value

A sample of fat or oil that has undergone oxidation can be used to measure the rancidity of the sample using the peroxide value. After dissolving 2.5g of the neem oil in 30ml of deionized water, 30ml of acetic acid: chloroform (3:2), and 30ml of acetic acid, the mixture was exposed to an excess of iodide using 1.5ml potassium iodide in a saturated solution. The iodide was converted to iodine by the peroxide present, and the iodine was then titrated to endpoint using sodium thiosulphate of 0.1N and an indicator of 1mm of starch solution. The peroxide value was calculated using the Meq/kg formula.

Peroxide Value= $\frac{\mathbf{10\times(N_1-N_2)}}{\boldsymbol{Weight\ of\ NEEM\ OIL}}$

Where: N_1 = Sodium Thiosulphate Blank titrate

N_2 = Sodium Thiosulphate titrate of neem oil

3.4.4 Saponification Value

This value represents the average molecular weight of all the fatty acids present and is expressed as the amount of KOH in milligrams needed to saponify 1g of fat. To determine the saponification value of 1g of neem oil, alcoholic KOH was prepared. In a round-bottom flask, 50 ml of the alcoholic KOH was added to the weighed oil sample. Using a condenser and heating mantle, the mixture was refluxed for an hour, then cooled and titrated with 0.5M HCl using phenolphthalein as an indicator.

Saponification Value= $\frac{56.1 \times M \times (T_B - T_N)}{Weight\ of\ NEEM\ OIL}$

Where: T_B = Blank titration

T_N= NEEM OIL titration

M = Molarity of HCL

3.4.5 DETERMINATION OF THE IODINE VALUE

The proportional degree of un-saturation in oil components, as measured by the uptake of halogen, is expressed as the iodine value. Iodine Value offers an estimate of these quality criteria since the degree of un-saturation has a relationship with the melting point and oxidative stability. There is more un-saturation and increased oxidation susceptibility with increasing iodine values.

The iodine value of neem oil was determined by weighing 1g of the oil into a conical flask, followed by the addition of 10 ml of chloroform ($CHCl_3$). The mixture was gently heated and then allowed to cool for 10 minutes. Next, 25 ml of Wij's reagent (iodine trichloride solution) was added to the flask and swirled vigorously. The mixture was kept in the dark for 30 minutes to prevent unwanted reactions with sunlight. After the specified time, titration was performed against sodium thiosulphate until a yellow color appeared. Subsequently, 20 ml of 10% KI and 150 ml of distilled water were added. The resulting yellow mixture was further titrated against sodium thiosulphate using a 1% starch solution as an indicator, until the color changed from indigo/black to colorless, marking the endpoint. A blank test was carried out under the same conditions for accuracy.

The value can be calculated with the Equation below:

$$\textbf{Iodine Value} = \frac{\mathbf{12.69 \times N \times (V_1 - V_2)}}{\boldsymbol{Weight\ of\ neem\ oil}}$$

Where: V_1 = Sodium Thiosulphate Blank titrate

V_2 = Sodium Thiosulphate titrate of neem oil

N = Normality of Sodium Thiosulphate (Na_2SO_3)

3.5 OPTIMISATION OF BIODIESEL PRODUCTION.

The biodiesel was produced using waste cooking oil, methanol and a bi-functional catalyst which was synthesized from cocoa pods and clam shells. The waste cooking oil is first sieved to remove particles, then the oil is measured using an electrical weighing balance into a round bottom flask and the catalyst and methanol is added as stated by the optimizing condition. It is then placed inside the microwave; the magnetic stirrer and microwave are then set to the various optimizing conditions. The reaction takes place producing the biodiesel which is placed in a centrifuge to separate the glycerol, biodiesel and catalyst before putting it into a separating funnel. It is then left for some time inside the separating funnel before being separated and washed with warm water then heated using a magnetic stirrer.

Plate 3. 1 Microwave aided biodiesel production setup

Plate 3. 2 Product within separating funnel

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 OIL CHARACTERIZATION

4.1.1 PHYSIOCHEMICAL PROPERTIES OF NEEM OIL

Thorough physicochemical characterization of neem oil was conducted as in the previous chapter according to ASTM standard. The analytical results, meticulously compiled in Table 4.1, presents the results obtained.

Table 4.1 physiochemical properties of neem oil

Properties	Values
Acid Value (mg KOH/g)	17.67
FFA (%)	8.835
Saponification Value (mg KOH/g)	196.35
Molecular Weight (g/mol)	941.91
Peroxide Value (mol/kg)	5.0
Density	0.924
Iodine value (mg KOH/g of oil)	88.83

According to the analysis carried out, the acid value and the derived FFA content of the neem oil was gotten to be 17.67 mgKOH/g and 8.835% simultaneously. These values categorically classified the neem oil as a high acid feedstock because it surpassed the conventional threshold of 2 mgKOH/g are considered normal (Falowo, 2021). This implies that just esterification reaction would not produce biodiesel but it should be followed by tranesterification reaction which would be carried out simultaneously. The saponification value of 196.35 mg KOH/g determines the average chain length of the fatty acids present in the triglyceride molecules. This value derived from the experiment fell within the range for many plant-based oils and was used in calculating the average molecular weight of 941.91 g/mol using the standard formula. The molecular weight (941.91g/mol) calculated was used to determine the stoichiometric molar ratio of methanol used to complete transesterification, forming the basis for the experimental design of the methanol-to-oil ratios investigated in this research.

The iodine value of 88.83 mg I_2/g derived during the experiment indicates the degree of unsaturation in the oil. This intermediate value indicated a balanced composition of saturated and unsaturated fatty acid chains. For biodiesel production, oils with high iodine value (highly unsaturated oils) yield biodiesel with improved cold flow properties but poorer oxidative stability, while oils with low iodine value (highly saturated oils) yield biodiesel with excellent oxidative stability but poor cold flow (Knothe, 2005). The neem oil's iodine value gotten from experiment was average which is a potential oil for producing biodiesel.

The peroxide value of 5.0 meq/kg indicates a relatively low level of primary oxidation products, suggesting the oil was reasonably fresh or well-preserved. The density of 0.924 g/cm^3 is consistent with typical vegetable oils and influences reactor volume calculations and energy inputs for mixing. In summary, the thorough characterization established that neem oil, while plentiful and inedible, poses a considerable technical challenge due to its elevated FFA content, thereby substantiating the fundamental research hypothesis of utilizing a specifically engineered bifunctional catalyst for its conversion.

4.2 MODELLING OF SIMULTANEOUS ESTERIFICATION AND TRANS ESTERIFICATION

4.2.1. STATISTICAL DESIGN AND ANALYSIS OF VARIANCE (ANOVA)

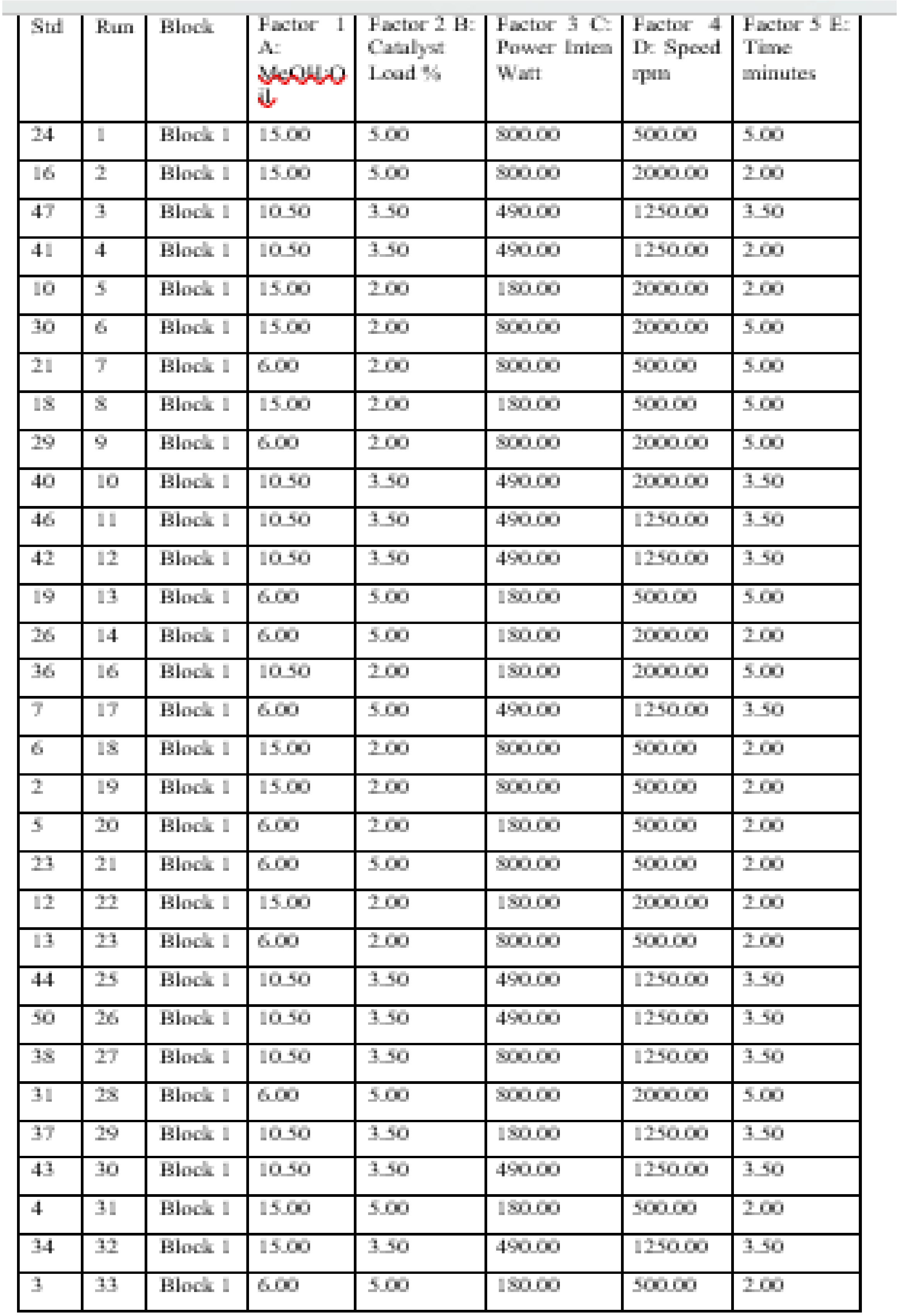
The optimization of biodiesel production from neem oil using a bifunctional catalyst necessitates a systematic approach to comprehend the complex interactions between various process factors.

Traditional univariate optimisation methods, which involve varying on factor at a time while keeping others constant are inefficient for multi factor systems. These approaches often fail to discover the interaction effects between variables, require an excessive number of experimental runs, and fail to establish true optimal conditions. Response Surface Methodology (RSM) was used as it is a strong statistical framework for experimental design, optimization, and modeling in order to get around these limitations.

A Central Composite Design (CCD) Is a popular second-order design in Response Surface Methodology (RSM), which was employed in this study to effectively explore the five-dimensional experimental space. Compared to a full three-level factorial design, the CCD requires fewer experimental points and is especially useful for estimating quadratic response surfaces. The five independent variables investigated along with their respective ranges.

The complete CCD matrix, comprising 50 randomized experimental runs (including factorial points, axial points, and center points for estimating pure error), is presented in Table 4.2. The design was generated and analyzed using Design Expert® software version 13.0, which facilitates model fitting, analysis of variance (ANOVA), and graphical optimization.

Tables 2: Central Composite Design (CCD) Matrix for the Optimization of Microwave-Assisted Biodiesel production from Neem Oil



Std	Run	Block	Factor 1 A: MeOH:Oil	Factor 2 B: Catalyst Load %	Factor 3 C: Power Inten Watt	Factor 4 D: Speed rpm	Factor 5 E: Time minutes
24	1	Block 1	15.00	5.00	800.00	500.00	5.00
16	2	Block 1	15.00	5.00	800.00	2000.00	2.00
47	3	Block 1	10.50	3.50	490.00	1250.00	3.50
41	4	Block 1	10.50	3.50	490.00	1250.00	2.00
10	5	Block 1	15.00	2.00	180.00	2000.00	2.00
30	6	Block 1	15.00	2.00	800.00	2000.00	5.00
21	7	Block 1	6.00	2.00	800.00	500.00	5.00
18	8	Block 1	15.00	2.00	180.00	500.00	5.00
29	9	Block 1	6.00	2.00	800.00	2000.00	5.00
40	10	Block 1	10.50	3.50	490.00	2000.00	3.50
46	11	Block 1	10.50	3.50	490.00	1250.00	3.50
42	12	Block 1	10.50	3.50	490.00	1250.00	3.50
19	13	Block 1	6.00	5.00	180.00	500.00	5.00
26	14	Block 1	6.00	5.00	180.00	2000.00	2.00
36	16	Block 1	10.50	2.00	180.00	2000.00	5.00
7	17	Block 1	6.00	5.00	490.00	1250.00	3.50
6	18	Block 1	15.00	2.00	800.00	500.00	2.00
2	19	Block 1	15.00	2.00	800.00	500.00	2.00
5	20	Block 1	6.00	2.00	180.00	500.00	2.00
23	21	Block 1	6.00	5.00	800.00	500.00	2.00
12	22	Block 1	15.00	2.00	180.00	2000.00	2.00
13	23	Block 1	6.00	2.00	800.00	500.00	2.00
44	25	Block 1	10.50	3.50	490.00	1250.00	3.50
50	26	Block 1	10.50	3.50	490.00	1250.00	3.50
38	27	Block 1	10.50	3.50	800.00	1250.00	3.50
31	28	Block 1	6.00	5.00	800.00	2000.00	5.00
37	29	Block 1	10.50	3.50	180.00	1250.00	3.50
43	30	Block 1	10.50	3.50	490.00	1250.00	3.50
4	31	Block 1	15.00	5.00	180.00	500.00	2.00
34	32	Block 1	15.00	3.50	490.00	1250.00	3.50
3	33	Block 1	6.00	5.00	180.00	500.00	2.00

22	34	Block 1	15.00	2.00	800.00	500.00	5.00
33	35	Block 1	6.00	3.50	490.00	1250.00	3.50
15	36	Block 1	6.00	5.00	800.00	2000.00	2.00
48	37	Block 1	10.50	3.50	490.00	1250.00	3.50
32	38	Block 1	15.00	5.00	800.00	2000.00	5.00
39	39	Block 1	10.50	3.50	490.00	500.00	3.50
14	40	Block 1	15.00	2.00	800.00	2000.00	2.00
1	41	Block 1	6.00	2.00	180.00	500.00	2.00
25	42	Block 1	6.00	2.00	180.00	2000.00	5.00
8	43	Block 1	15.00	5.00	800.00	500.00	2.00
27	44	Block 1	6.00	5.00	180.00	2000.00	5.00
49	45	Block 1	10.50	3.50	490.00	1250.00	3.50
9	46	Block 1	6.00	2.00	180.00	2000.00	2.00
28	47	Block 1	15.00	5.00	180.00	2000.00	5.00
20	48	Block 1	15.00	5.00	180.00	500.00	5.00
17	49	Block 1	6.00	2.00	180.00	500.00	5.00
35	50	Block 1	10.50	2.00	490.00	1250.00	3.50

The targeted response variable for all runs was the percentage production of biodiesel (FAME), which was determined gravimetrically after purification. However, as explained in the following sections, the predominant outcome seen across all trials was a reaction failure, resulting in the recovery of unreacted oil and methanol. Despite the execution of the 50 planned experimental runs, no quantifiable conversion of neem oil to biodiesel was noticed. Instead, two immiscible liquid phases, the upper phase was primarily composed of unreacted methanol, while the lower phase contained the original neem oil, with no sedimented glycerol phase.

As a result, while the intended experimental matrix reflects a theoretically acceptable approach to optimization, the subsequent statistical modeling (ANOVA, regression model fitting, and factor significance testing) was unable to be completed due to the lack of variation in the responses. The lack of a measurable product yield made it impossible to build a meaningful response surface model to describe the link between process factors and biodiesel production efficiency for this specific catalyst system under the conditions investigated. This conclusion is crucial since it shows that the catalyst was unsuccessful within the CCD's broad working window. It shows that the factor with the most dominating effect on the process outcome was not one of the five variables evaluated in the RSM design.

4.2.3 ROOT-CAUSE ANALYSIS OF CATALYST INEFFICACY

The catalytic system is clearly the source of the issue, as evidenced by its inability to manufacture biodiesel despite changes in all important process parameters. In order to reduce the activation energy of the transesterification reaction, the manufactured catalyst was unable to supply the required active sites. Several connected and tenable hypotheses are produced by a multifaceted research into potential failure mechanisms:

a) Incomplete Thermal Activation and Phase Transformation: The basic idea behind producing an active basic catalyst from cow bone is that its main mineral component, hydroxyapatite, and any related calcium carbonate must completely decompose thermally into calcium oxide (CaO) through high-temperature calcination. Calcination at 800°C for four hours was required per the protocol. However, severe temperature excursion was caused by the instability in the electrical power supply throughout this long, high-temperature process. Improper calcination yields partial CaO which is insufficient for the esterification and transesterification reactions. According to Amesho et al., 2022, CaO is well known for its stability and catalytic efficiency and as a solid base catalyst for transesterification. However, $CaCO_3$ displays relatively low basicity under mild reaction conditions. Improperly calcined catalysts lack the density of strong basic sites (O^{2-} anions) required to extract a proton from methanol and create the methoxide ion (CH_3O^-), the major nucleophile in the base-catalyzed transesterification pathway.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

This research project aimed at development of a sustainable and optimized process for biodiesel production converting agricultural wastes (rice bran and cow bones) into a bifunctional heterogeneous catalyst, using non-edible, high free fatty acid neem oil as feedstock, and driving the transesterification reaction with energy-efficient microwave irradiation. The study was carried out methodically, comprising the synthesis of the catalyst, the thorough physicochemical characterization of the feedstock, and the development of an advanced experimental framework utilizing Response Surface Methodology (RSM) for process optimization.

The conclusive outcome of this study was that the synthesized bifunctional catalyst derived from cow bone and rice bran was unable to catalyse the transesterification of neem oil under the tested microwave-assisted conditions, which resulted to no yield of biodiesel across all 50 experimental runs. This result was because of the catalyst partial activation during the crucial calcination stage which was caused by unstable power supply. Because of this insufficiency, the requisite phase transformation to active calcium oxide (CaO) was not achieved and that yielded a catalyst with poor textural properties. Consequently, the catalyst lacked the required strength and density of active sites (acidic and basic sites) required to facilitate the esterification and transesterification reactions simultaneously.

The overall strategy of employing waste-derived bifunctional catalysts for microwave-assisted biodiesel production from the difficult feedstock remains scientifically sound and worthy of further investigation, this thesis concludes, even though the particular catalyst formulation prepared under the specified conditions was ineffective. The value of this work lies not in a successful optimization result but rather in the clear identification of a critical failure point and the provision of a strong experimental and analytical framework upon which successful future iterations can be built.

5.2 RECOMMENDATION

Based on the analysis of the experimental results and challenges faced in this thesis, the following recommendations are proposed for future research and improvements:

1. Further work should prioritize absolutely stable thermal activation. Using a programmable muffle furnace with a reliable power backup is important. The calcination profile should incorporate controlled heating ramps and sufficient dwell duration at the target temperature.
2. Hot calcined catalyst should be transferred into a sealed container within a glove bag purged within inert gas and later store in a dessicator. This helps to prevent the rapid surface re-carbonation and hydration that deactivates CaO.

REFERENCES

Abiodun, O., Esther, O., Oluwadamilola, A., Emmanuel, A., Abiodun, M., Enamhanye, A., Latinwo, L. M., & Betiku, E. (2021). Energy Conversion and Management : X Influence of nature of catalyst on biodiesel synthesis via irradiation-aided transesterification of waste cooking oil-honne seed oil blend : Modeling and optimization by Taguchi design method. *Energy Conversion and Management: X*, *12*, 100119. https://doi.org/10.1016/j.ecmx. 2021.100119

Akhabue, Osa-Benedict, E. O., Oyedoh, E. A., & Otoikhian, S. K. (2020). Development of a bio-based bifunctional catalyst for simultaneous esterification and transesterification of neem seed oil: Modeling and optimization studies. *Renewable Energy*, *152*, 724–735. https://doi.org/10.1016/j.renene. 2020.01.103

Akhtar, R., Hamza, A., Razzaq, L., Hussain, F., Nawaz, S., Nawaz, U., Mukaddas, Z., Jauhar, T. A., Silitonga, A. S., & Saleel, C. A. (2023). Maximizing biodiesel yield of a non-edible chinaberry seed oil via microwave assisted transesterification process using response surface methodology and artificial neural network techniques. *Heliyon*, *9*(11), e22031. https://doi.org/10.1016/j.heliyon.2023.e22031

Akintelu, S. A., Oyebamiji, A. K., Olugbeko, S. C., Afolabi, D. O., David, D. M., Banda, L. O. L., & Kaka, M. O. (2023). Mycosynthesis of nanobiomaterials and their wound healing, antimicrobial, and biofilm inhibitory activities. *Bioengineered Nanomaterials for Wound Healing and Infection Control*, 325–371. https://doi.org/10.1016/B978-0-323-95376-4.00017-4

Ali, B., Yusup, S., Quitain, A. T., Alnarabiji, M. S., Kamil, R. N. M., & Kida, T. (2018). Synthesis of novel graphene oxide/bentonite bi-functional heterogeneous catalyst for one-pot esterification and transesterification reactions. *Energy Conversion and Management*, *171*, 1801–1812. https://doi.org/10.1016/J.ENCONMAN.2018.06.082

Amesho, K. T. T., Lin, Y. C., Chen, C. E., Cheng, P. C., & Shangdiar, S. (2022). Kinetics studies of sustainable biodiesel synthesis from Jatropha curcas oil by exploiting bio-waste derived CaO-based heterogeneous catalyst via microwave heating system as a green chemistry technique. *Fuel*, *323*(February), 123876. https://doi.org/10.1016/j.fuel.2022.123876

Andrade, J. E., Pérez, A., Sebastian, P. J., Eapen, D. (2011). A review of bio-diesel production processes. *Biomass and bioenergy*, 35, 1008–1020. https://doi:10.1016/j.biombioe.2010.12.037

Atabani, A. E., Silitonga, A. S., Badruddin, I. A., Mahlia, T. M. I., Masjuki, H. H., Mekhilef, S. (2012). A comprehensive review on biodiesel as an alternative energy resource and its characteristics. *Renewable Energy and Sustainable Energy Reviews*, 16, 2070–2093. https://doi.10.1016/j.rser.2012.01.003

Atadashi, I. M., Aroua, M. K., Abdul Aziz, A. R., & Sulaiman, N. M. N. (2013). The effects of catalysts in biodiesel production: A review. *Journal of Industrial and Engineering Chemistry*, 19(1), 14–26. https://doi.org/10.1016/j.jiec.2012.07.009

Awogbemi, O., Kallon, D. V. Von, & Aigbodion, V. S. (2021). Trends in the development and utilization of agricultural wastes as heterogeneous catalyst for biodiesel production. *Journal of the Energy Institute*, *98*(May), 244–258. https://doi.org/10.1016/j.joei.2021.06.017

Basumatary, B., Atmanli, A., Azam, M., Basumatary, S. F., Brahma, S., Das, B., Brahma, S., Rokhum, S. L., Min, K., Selvaraj, M., & Basumatary, S. (2024). *Catalytic Efficacy , Kinetic , and Thermodynamic Studies of Biodiesel Synthesis Using Musa AAA Plant Waste-Based Renewable Catalyst. 2024.*

Bello, E. I., & Anjorin, S. A. (2012). Fatty Acid Compositions of Six Nigeria's Vegetable Oils and their Methyl Esters. *Research Journal in Engineering and Applied Sciences* 1 vol. 3 pg. 166–170.

Bello, U., Agu, C. M., Ajiya, D. A., Mahmoud, A. A., Udopia, L., Lawal, N. M., Abubakar, A. A., & Muhammad, M. (2022). Biodiesel, In a Quest for Sustainable Renewable Energy: A Review on Its Potentials and Production Strategies. *Journal of Chemical Reviews*, 4(3), 272–287. https://doi.org/10.22034/jcr.2022.343497.1175

Betiku, E., Okpalaeke, K. E., Sotunde, B. A., Ojumu, T. V. (2025). Fluted pumkpin waste disposal as a green solid bioalkaline catalyst for neem seed oil biodiesel synthesis. *Biomass and Bioenergy*, 193 (2025) 107525. https://doi.org/10.1016/j.biombioe2024.107525

Changmai, B., Sudarsanam, P., & Rokhum, L. (2020). Biodiesel Production using Heterogeneous Catalysts: A Review. *Catalysis Reviews*, 62(1), 18-65. https://doi.org/10.1080/01614940.2019.1682839

Chhetri, A. B., Tango, M. S., Budge, S. M., Watts, K. C., & Islam, M. R. (2008). Non-edible plant oils as new sources for biodiesel production. *International Journal of Molecular Sciences, 9(2), 169*–180.

Demirbas, A. (2009). Political, economic and environmental impacts of biofuels: A review. *Appl. Energy*, *86*(SUPPL. 1), S108–S117. https://doi.org/10.1016/j.apenergy.2009.04.036

Elgharbawy, A. S., Sadik, W. A., Sadek, O. M., & Kasaby, M. A. (2021). A review on biodiesel feedstocks and production technologie. *Journal of the Chilean Chemical Society*, 65(1), 5098–5109. https://doi.org/10.4067/S0717-97072021000105098

Ewunie, G. A., Morken, J., Lekang, O. I., Yigezu, Z. D. (2020). Factors affecting the potential of Jatropha curcas for sustainable biodiesel production. *Renewable and Sustainable*

Energy Reviews. https://doi.org/10.1016/j.rser.2020.110500

Fadillah, G., Fatimah, I., Sahroni, I., Musawwa, M. M., Mahlia, T. M. I., & Muraza, O. (2021). Recent progress in low-cost catalysts for pyrolysis of plastic waste to fuels. *Catalysts*, 11(7). https://doi.org/10.3390/catal11070837

Falowo, O. A., Oloko-oba, M. I., & Betiku, E. (2019). Chemical Engineering & Processing : Process Intensification Biodiesel production intensification via microwave irradiation-assisted transesterification of oil blend using nanoparticles from elephant-ear tree pod husk as a base heterogeneous catalyst. *Chemical Engineering & Processing: Process Intensification*, *140*(January), 157–170. https://doi.org/10.1016/j.cep.2019.04.010

Fassinou, W. F. (2012). Higher heating value (HHV) of vegetable oils, fats and biodiesels evaluation based on their pure fatty acids' HHV. *Energy*, *45*(1), 798–805. https://doi.org/10.1016/J.ENERGY.2012.07.011

Gashaw, A., & Teshita, A. (2014). Production of Biodiesel from Waste Cooking Oil and Factors Affecting its Formation: A review. *International Journal of Renewable and Sustainable Energy*, 3(5), 92-98. https://doi.org/10.11648/j.ijrse.20140305.11

Giakoumis, E. G. (2013). A Statistical Investigation of Biodiesel Physical and Chemical Properties, and their corelation with the degree of Unsaturation. *Renewable Energy*, 50, 858-878. https://dx.doi.org/10.1016/j.renene.2012.07.040

Gülşen, E., Olivetti, E., Freire, F., Dias, L., & Kirchain, R. (2014). Impact of feedstock diversification on the cost-effectiveness of biodiesel. *Applied Energy*, *126*, 281–296. https://doi.org/10.1016/J.APENERGY.2014.03.063

Humphery, I., Obot, N. I., & Chendo, M. A. C. (2017). Utilization of some Non Edible for Biodiesel Production. 7(1), 1–6.

Isioma, N., Muhammad, Y., Sylvester, O., Innocent, D., & Linus, O. (2013). Cold Flow Properties and Kinematic Viscosity of Biodiesel. *Universal Journal of Chemistry*, *1*(4), 135–141. https://doi.org/10.13189/ujc.2013.010402

Ismail, A. F., Khulbe, K. C., & Matsuura, T. (2019). RO Membrane Characterization. *Reverse Osmosis*, 57–90. https://doi.org/10.1016/B978-0-12-811468-1.00003-7

Jain, S., & Sharma, M. P. (2010). Kinetics of acid base catalysed transesterification of Jatropha curcas oil. *Bioresource Technology*, 101(20), 7701–7706. https://doi.org/10.1016/j.biotech.2010.05.034

Khan, I.W., Naeem, A., Farooq, M., Mahmood, T., Ahmad, B., Hamayum, M., Ahmad, Z., & Saeed, T. (2020). Catalytic conversion of spent frying oil into biodiesel over raw and 12-tungsto-phosphoric acid modified clay. *Renewable Energy*, 155, 181–188. https://doi.org/10.1016/j.renene.2020.03.123

Kumar, A. & Sharma, S. (2011). Potentaial non-edible oil resources as biodiesel feedstock: an indian perspective. Renew Sustain Energy Rev 2011; 15(4):1791-800

Kumar, S., Singhal, M. K., & Sharma, M. P. (2023). Analysis of oil mixing for improvement of biodiesel quality with the application of mixture design method. *Renewable Energy*, 202, 809-821. https://doi.org/10.1016/j.renene.2022.11.097

Kusdiana, D., & Saka S. (2004). Effects of Water on Biodiesel Fuel production by Supercritical Methanol Treatment. *Biochem Biotechnol* 113-116:794-891

Lemma, D. B., Abasiga, M. A., & Kebede, A. B. (2020). Production of biodiesel from mixed castor seed and microalgal oils: Characterization and Optimization Studies. *Research Square*, 2022, 1–30

Mahmudul, H. M., Hagos, F. U., Mamat, R., Abdul Adam, A., Ishak, W. F. W., Alenzi, R. (2017). A Review: Production, Characterization and Performance of Biodiesel as an Alternative Fuel in Biodiesel Engine. *Renewable and Sustainable Energy Reviews*, 72, 497–509. https://dx.doi.org/10.1016/j.rser.2017.01.001 1364

Mathiyazhagan, M. & Ganapathi. (2011). Factors Affecting Biodiesel Production. Research in Plant Biology, 1(2): 01-05

Mohammad Anwar (2021). Biodiesel Feedstocks Selection Strategies Based on Economic, Technical and Sustainable Aspects. *Fuel*, 283, 119–204. https://doi.org/10.1016/j.fuel.2020.119204

Monika, Banga, S. & Pathak, V. V. (2023). Biodiesel Production from Waste Cooking Oil: A Comprehensive Review on the application of heterogenous Catalyst. *Energy Nexus*. https://doi.org/10.1016/j.nexus.2023.100209

Nasrazadani, S., & Hassani, S. (2016). Modern analytical techniques in failure analysis of aerospace, chemical, and oil and gas industries. *Handbook of Materials Failure Analysis with Case Studies from the Oil and Gas Industry*, 39–54. https://doi.org/10.1016/B978-0-08-100117-2.00010-8

Nasrollahzadeh, M., Atarod, M., Sajjadi, M., Sajadi, S. M., & Issaabadi, Z. (2019). Plant-Mediated Green Synthesis of Nanostructures: Mechanisms, Characterization, and Applications. *Interface Science and Technology*, *28*, 199–322. https://doi.org/10.1016/B978-0-12-813586-0.00006-7

Omidi, M., Fatehinya, A., Farahani, M., Akbari, Z., Shahmoradi, S., Yazdian, F., Tahriri, M., Moharamzadeh, K., Tayebi, L., & Vashaee, D. (2017). Characterization of biomaterials. *Biomaterials for Oral and Dental Tissue Engineering*, 97–115. https://doi.org/10.1016/B978-0-08-100961-1.00007-4

Nezihe, A., & Danisman, A. (2007). Alkali Catalyzed Transesterification of Cottonseed Oil by

Microwave Irradiation. Fuel, 86, 2639-2644. https://doi.org/10.1016/j.fuel.2007.05.021

Nisar, J., Razaq, R., Farooq, M., Iqbal, M., Khan, R. A., Sayed, M., Shah, A., & Rahman, I. ur. (2017). Enhanced biodiesel production from Jatropha oil using calcined waste animal bones as catalyst. *Renewable Energy*, *101*, 111–119. https://doi.org/10.1016/j.renene.2016.08.048

Omar, W. N. N. W., & Amin, N. A. S. (2011). Optimization of heterogeneous biodiesel production from waste cooking palm oil via response surface methodology. *Biomass and Bioenergy*, 35(3), 1329-1338.

Orhevbva, B., Adebayo, S., & Salihu, A. (2016). Synthesis of Biodiesel from Tropical Almond (Terminalia Catappa) Seed Oil. *Current Research in Agricultural Sciences*, 3(4), 57–63. https://doi.org/10.18488/journal.68/2016.3.4/68.4.57.633

Parameshwar, S., Agnihotri, M., Bhan, U., Nangan, S., Verma, D., Rajendran, S., Jain, S. (2024). Developments in the Utilization of heterogeneous catalysts for biodiesel generation. *Journal of the Taiwan Institute of Chemical Engineers*, https://doi.org/10.1016/j.jtice.2024.105810

Pourhakkak, P., Taghizadeh, A., Taghizadeh, M., Ghaedi, M., & Haghdoust, S. (2021). Fundamentals of adsorption technology. *Interface Science and Technology*, *33*, 1–70. https://doi.org/10.1016/B978-0-12-818805-7.00001-1

Rizwanul Fattah, I. M., Ong, H. C., Mahlia, T. M. I., Mofijur, M., Silitonga, A.S., Ashrafur Rahman, S. M., & Ahmad, A. (2020). State of the Art of Catalysts for Biodiesel Production. *Frontiers in Energy Research*, 8(June), 1–17. https://doi.org/10.3389/fenrg.2020.00101

Rozina, Ahmad, M., Elnaggar, A. Y., Teong, L. K., Sultana, S., Zafar, M., Munir, M., Hussein, E. E., Abidin, S. Z. U. (2022). Sustainable and eco-friendly synthesis of biodiesel from novel and non-edible seed oil of Monotheca buxifolia using green nano-catalyst of calcium oxide. *Energy Conversion and Management: X* 13 (2022) 100142. https://doi.org/10.1016/j.ecmx.2021.100142

Ruatpuia, J. V. L., Halder, G., Mohan, S., Gurunathan, B., Li, H., Chai, F., Basumatary, S., & Lalthazuala Rokhum, S. (2023). Microwave-assisted biodiesel production using ZIF-8 MOF-derived nanocatalyst: A process optimization, kinetics, thermodynamics and life cycle cost analysis. *Energy Conversion and Management*, *292*(July), 117418. https://doi.org/10.1016/j.enconman.2023.117418

Sajjadi, B., Raman, A. A. A., Arandiyan, H. (2016). A comprehensive review on properties of edible and non-edible vegetable oil-based biodiesel: composition, specifications and prediction models. *Renew Sustain Energy Rev* 2016;63:62-92

Sakthivel, R., Ramesh, K., Purnachandran, R., & Mohamed Shameer, P. (2018). A review on the properties, performance and emission aspects of the third generation biodiesels. *Renewable and Sustainable Energy Reviews*, *82*(November 2017), 2970–2992. https://doi.org/10.1016/j.rser.2017.10.037

Sanford, S. D., Shah, P. S., MAV, C. W., & Meier, G. R. (2009). Feedstock and Biodiesel Characteristics Report; 2009.

Semwal, S., Arora, A. K., Badoni, R. P., & Tuli, D. K. (2011). Biodiesel production using heterogeneous catalysts. *Bioresource Technology*, *102*(3), 2151–2161. https://doi.org/10.1016/J.BIORTECH.2010.10.080

Sivaramakrishnan, K., & Ravikumar, P. (2011). Determination of Higher Heating Value of Biodiesels. *International Journal of Engineering Science and Technology*, *3*(11), 7981–7987. http://www.ijest.info/docs/IJEST11-03-11-099.pdf

Wan Osman, W. N. A., Badrol, N. A. I., & Samsuri, S. (2023). Biodiesel Purification by Solvent-Aided Crystallization Using 2-Methyltetrahydrofuran. *Molecules*, *28*(3). https://doi.org/10.3390/MOLECULES28031512

APPENDIX A

- ACID VALUE OF NEEM OIL

Acid Value = $\frac{N*56.1056*(T_v-T_b)}{weight\ of\ NEEM\ OIL\ (g)}$

Where;

N = normality of KOH solution

56.1g/mol = molecular weight of KOH

T_v = Titration volume of KOH solution with neem oil

T_B = Titration volume of KOH solution without neem oil (blank solution)

2.g of neem oil sample

Initial reading = 0ml

Final reading = 12.8ml

Blank reading = 0.2ml

AV = $\frac{0.05*56.1056*(12.8-0.2)}{2\ (g)}$

= 17.67 mgKOH/g

FFA present = $\frac{Acid\ Value}{2}$

$\frac{17.67}{2}$ = 8.835 mgKOH/g

- PEROXIDE VALUE OF NEEM OIL

Peroxide value (PV) = $\frac{10*N*(N_1-N_2)}{weight\ of\ neem\ oil}$

Where;

N_2 = Titre value using neem oil

N_1 = Titre value of blank test

N = normality of sodium thiosulphate

Initial reading = 0ml

Final burette reading = 32.40 ml

Blank reading = 32.35ml

PV = $\frac{10*0.1*(32.40-32.35)}{1}$

=5meq/kg

- SAPONIFICATION VALUE OF NEEM OIL

Saponification Value = $\frac{\frac{56.1056g}{mol} * N * (T_b - T_j)}{weight\ of\ neem\ oil\ (g)}$

Where;

N = Normality of HCL solution

T_b = Titre value of blank test

T_j = Titre value of neem oil sample

Initial reading = 0ml

Final reading = 32.5ml

Blank reading = 39.5 ml

SV = $\frac{\frac{56.1g}{mol} * 0.5 * (39.5 - 32.5)}{1(g)}$

= 196.35 mgKOH/g

Molecular weight = $\frac{168300}{(Saponification\ Value - Acid\ Value}$

$\frac{168300}{(196.35 - 17.67)}$ = 941.91 g/mol

- DENSITY

$\rho = \frac{weight\ of\ oil}{volume\ of\ bottle}$

$\frac{46.2}{50ml}$ = 0.924