WOLDIA UNIVERSITY

WOLDIA INSTITUTE OF TECHNOLOGY

SCHOOL OF MECHANICAL AND CHEMICAL ENGINEERING

DEPARTMENT OF MECHANICAL ENGINEERING

Sustainable Energy Engineering

MSc thesis on:

EXPERIMENTAL INVESTIGATION OF ESSENTIAL OIL EXTRACTION USING HYDRO-DISTILLATION FROM EUCALYPTUS (EUCALYPTUS GLOBULUS) LEAVES FOR LIQUID BIOFUEL APPLICATION

BY:

YORDANOS TARKO

MAY, 2025

WOLDIA, ETHIOPIA

WOLDIA UNIVERSITY

INSTITUTE OF TECHNOLOGY

SCHOOL OF MECHANICAL AND CHEMICAL ENGINEERING

DEPARTMENT OF MECHANICAL ENGINEERING

EXPERIMENTAL INVESTIGATION OF ESSENTIAL OIL EXTRACTION USING HYDRO-DISTILLATION FROM EUCALYPTUS (EUCALYPTUS GLOBULUS) LEAVES FOR LIQUID BIOFUEL APPLICATION

By:

Yordanos Tarko

A Thesis Submitted to the Department of Mechanical Engineering in Partial Fulfillment to the Requirements for the Degree of Master of Science in Sustainable Energy Engineering

Advisor: Semaw Kebede (PhD)

Co-Advisor: Sisay Wondmagegn (MSc)

MAY, 2025

WOLDIA, ETHIOPIA

WOLDIA UNIVERSITY

WOLDIA INSTITUTE OF TECHNOLOGY

SCHOOL OF MECHANICAL AND CHEMICAL ENGINEERING

DEPARTMENT OF MECHANICAL ENGINEERING

Approval of thesis for the defense result

I confirm here that the changes required by the examiners have been carried out and incorporated in the final thesis.

Student Name	Signature	Date
Yordanos Tarko	_________	________

As members of the board of examiners, we examined this thesis entitled “Experimental Investigation of Essential Oil Extraction Using Hydro-Distillation from Eucalyptus (Eucalyptus Globulus) Leaves for Liquid Biofuel Application" by Yordanos Tarko. We hereby certify that the thesis is accepted for fulfilling the requirements for the award of the degree of Master of Science in Sustainable Energy Engineering.

Board of Examiners

Advisor Name:	Signature	Date
Semaw Kebede (PhD)	__________	________
External Examiner Name:	Signature	Date
Ali Shemsedin (PhD)	______________	12/06/2025
Internal Examiner Name:	Signature	Date
Alemayehu Tenaw (PhD)	__________	12/06/2025
Chair Person:	Signature	Date
Shumye Girmay	__________	________
School Dean:	Signature	Date
Habtamu Adno	________	________

DECLARATION

This is to certify that the thesis titled "Experimental Investigation of Essential Oil Extraction Using Hydro-Distillation from Eucalyptus (Eucalyptus Globulus) Leaves for Liquid Biofuel Application," which I submitted to the Department of Mechanical Engineering, Studies at Woldia University in partial fulfillment of the requirements for the Master of Science degree in Sustainable Energy Engineering, is entirely original with no previous submissions made to this or any other institution that grants any other degree. I further certify that I have correctly referenced and cited all non-original work and study findings from previous research as required by law and regulations. The help and encouragement I received during my entire thesis have been duly acknowledged.

I am aware that any infraction of the academic integrity policy of the university can lead to expulsion or other disciplinary measures.

Student Name	Signature	Date
Yordanos Tarko	_________	_________
Advisor name:	Signature	Date
Semaw Kebede (PhD):	__________	_________
Co-Advisor Name:	Signature	Date
Sisay Wondmagegn (MSc):	_________	_________

ACKNOWLEDGEMENT

Firstly, I want to express my gratitude to our god for his unending miraculous blessings that I have received without asking. Second, I want to express my gratitude to Woldia University for providing this educational opportunity. Third, I would like to convey my sincere gratitude to my esteemed adviser, Dr. Semaw Kebede his insightful feedback, fatherly guidance, and advice from the beginning to the end of this project.

In addition, I am grateful to the entire mechanical engineering department for their varied expertise that enhanced my research. Finally, but just as importantly, I want to express my gratitude to co-adviser Sisay Wondmagegn for lending me his valuable time, encouraging counsel, and insightful remarks and ideas during my experimental work and documentation. Also, I would like to express my deepest gratitude to Dr. Melesse for his invaluable guidance, supervision, and assistance in selecting the research topic and his continuous advice and insightful suggestions throughout my research work.

Lastly, I would like to express my sincere gratitude to all the research-grade laboratories in the Chemistry Department and the laboratory staff of the Department of Chemical Engineering for their invaluable assistance throughout the experimental work, particularly in demonstrating the equipment and helping set up the necessary processes. Special thanks are also extended to the lab assistants of Bari Dar and the Department of Chemistry at Wollo University for their support with the FTIR analysis and bomb calorimeter, respectively. I am also thankful to all my best friends for their enthusiastic motivation and, most importantly, to my family and beloved brother for their unwavering support and encouragement, which strengthened me to accomplish my research work.

ABSTRACT

Liquid biofuels are a type of renewable energy that can be used for electricity generation and heating applications. Eucalyptus globulus leaves, a lignocellulosic biomass, contain a significant amount of cellulose suitable for biofuel production. This study focused on optimising liquid biofuel production from Eucalyptus globulus leaves using hydro-distillation. Key steps included raw material collection, proximate analysis, and biofuel extraction. The optimisation process examined the effects of temperature, particle size, and extraction time, applying a Central Composite Design within the RSM framework. The final biofuel was then characterised to assess its quality. A proximate analysis of Eucalyptus globulus leaves was conducted and revealed the following composition: moisture content (9.47%), volatile matter (76.50%), ash content (1.05%), and fixed carbon (12.98%). The experimental result indicated a maximum liquid biofuel yield of 3.40% (ml/g) at 100°C, 5 mm, and 3 hours. Under the optimum value predicted using the Central Composite Design model, the optimum yield of 3.32 % (ml/g) was achieved at 104.71°C, 4.45 mm, and 3.71 hours, and the validation value at the optimum parameter was 3.10 % (ml/g). The produced liquid biofuel exhibited desirable physicochemical and fuel properties, including a pale yellow colour, density of 0.77–0.90 g/mL, kinematic viscosity of 1.84–2.35 mm^2/s, higher heating value of 37.54 MJ/kg, mass yield of 2.56%, energy density ratio of 1.82, energy yield of 46.60, acid value of 1.24–1.82 mg KOH/g, flash point of 132 °C, and boiling point of 217.80 °C. FTIR analysis revealed key functional groups, including O–H, C–H, C=O, C=C, and C–O. The findings suggest that hydro-distilled biofuel from EGL is a viable alternative energy source and could contribute significantly to addressing fuel shortages in rural areas.

Keywords: Eucalyptus globulus leaves, Hydro-distillation, Liquid Biofuel, Central Composite Design, Characterization, optimization

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviations and symbols	Definition
%	Percentage
AC	Ash Content
ANOVA	Analysis Of Variance
ASTM	American Society for Testing and Materials
℃	Degree Celsius
CCD	Central Composite Design
CI	Confidence Interval
EGL	Eucalyptus Globulus Leaves
FC	Fixed Carbon
FTIR	Fourier Transform Infrared Radiation
G	Gram
GDP	Gross Domestic Product
GHG	Greenhouse Gas
HD	Hydro-Distillation
HHV	Higher Heating Value
LCB	Lignocellulosic Biomass
MC	Moisture Content
mgKOH/g	Milligram potassium hydroxide per gram
MJ/Kg	Mega joule per kilogram
mL/g	Milliliter per gram
Mm	millimeter
PRESS	Predicted Residual Sum of Squares
Rpm	Revolution Per Minute
RSM	Response Surface Methodology
VIF	Variance Inflation Factor
VM	Volatile Matter
cm^{-1}	Per centimeter
3D	Three-dimensional

1. INTRODUCTION

1.1. Background

Energy is a vital component of every economy, and it is crucial to ensure its supply is sustainable. Renewable energy sources are being researched to address demand shortages and provide sustainable, clean energy. Biofuels are one of these renewable resources that are still expanding and making a significant contribution to global energy consumption [1], [2].

The rising use of fossil fuels and the resulting climate change have become major global issues. As a result, countries worldwide have begun implementing energy policies that include the development of renewable energy sources [3]. Fuel is a source of economic growth. When businesses have access to reliable and affordable energy, they can produce more goods and services, which creates jobs and boosts economic activity [4].

Eucalyptus globulus is among the most widely planted eucalypt species worldwide, with an estimated global plantation area exceeding 2.5 million hectares**.** Notably, Portugal has the largest area under this species, with approximately 845,000 hectares dedicated to Eucalyptus globulus plantations. Other significant regions include Spain**,** Chile**,** Argentina**,** and parts of Australia**,** South Africa**,** and India [5].

Ethiopia is home to a vast array of native trees, which are employed for several purposes, such as traditional medicine, food, fuel, timber, agricultural equipment, and soil preservation. From an ecological standpoint, these trees are crucial for preserving resources and restoring deteriorated soil [6], [7].

In comparison to other renewable sources, the use of biomass has several disadvantages, such as deforestation, low energy density, low heating value, high moisture content, air pollution, greenhouse gas emissions, land and water use, storage and transportation issues, high costs, and lower efficiency [8].

Eucalyptus, though an exotic species, thrives in Ethiopia and across parts of Africa. Recent estimates show that Eucalyptus globulus plantations cover about 506,000 hectares in Ethiopia [9]. It is utilized for several things, such as fuel, lumber, poles, housing, and medication [10], and was introduced to Ethiopia in 1894-1895 by Mondon-Vidaillet and O'Brien, who advised Emperor Menelik II to use it [11].

However, poor management of Eucalyptus trees depletes soil nutrients, disrupts key ecological functions, and undermines essential resource production [6].

Biofuels are a renewable, eco-friendly alternative to fossil fuels that cut greenhouse gas emissions, boost air quality, support rural jobs, and enhance energy independence. However, there are also emerging challenges such as competition for land, technology, agricultural methods, crop types, governmental regulations, and water resources [1], [12]. They can change the transport and agriculture sectors of decarbonizing societies [13] and are produced from organic matter such as plants or animal waste. They are categorized based on their chemical properties, nature, biomass feedstock, conversion processes, and technologies [14].

Biofuels are found in different states, including liquid, gaseous, solid, and in different chemical forms [11]. These biofuels have shown the greatest potential for future energy supply, and to achieve energy security and sustainability [16].

They are divided into conventional and advanced types. Conventional biofuels are generated from edible crops such as corn and soybeans, whereas advanced biofuels are derived from non-food sources like algae, waste, and wood. They are further classified into four generations based on their feedstock. First-generation biofuels are made from food crops, second-generation biofuels from non-food crops, third-generation biofuels from waste materials, and fourth-generation biofuels are produced using genetically modified organisms or carbon capture technologies [17], [18]. Therefore, advanced biofuels are pricier to produce, more efficient, and emit fewer emissions than conventional biofuels [17], [19].

Biofuels can be made through various processes, including biological, chemical, and physical methods [20]. Distillation is a crucial process in the oil refining industry. It is used to produce a variety of products that are essential to our modern way of life [21]. Although liquid biofuels, such as bio-oil, have a complex chemical composition that includes phenols, aldehydes, hydrocarbons, ketones, esters, alcohols, and acids, they have unique chemical and physical characteristics [22]. High viscosity, corrosiveness, and instability are some undesirable characteristics that may result from this diverse composition. Cleaner combustion is encouraged by their high oxygen concentration. They are a more sustainable and environmentally beneficial option than traditional fuels, although they have a little lower energy content. They can frequently be mixed with gasoline or diesel [23].

Eucalyptus globulus leaves are emerging as a promising feedstock for biofuel production due to their rich chemical composition and potential for integrated valorization. While the wood of Eucalyptus globulus has been extensively studied for bioethanol production, recent research highlights the valuable bioactive compounds present in the leaves, which can contribute to sustainable biofuel and bioproduct development [9].

This study explored the use of hydro-distillation to convert Eucalyptus globulus leaves into liquid biofuels, presenting a feasible, cost-effective, and catalyst-free approach. Unlike previous methods, such as fermentation, pyrolysis, microwave extraction, or ultrasound, this technique is simpler and more sustainable. The research also focused on optimizing key variables, such as time, temperature, and particle size, that were often overlooked in prior studies, aiming to achieve the most efficient liquid biofuel production from Eucalyptus leaves.

1.2. Statement of the Problem

Ethiopia is currently facing significant challenges related to its energy sector, including rising oil costs, energy shortages, and the environmental impact of traditional biomass fuels, particularly in rural areas. The country's dependence on imported petroleum fuels exacerbates these issues, leading to high energy costs, pollution, and limited access to sustainable energy sources. With the majority of the population relying on traditional biomass for household energy, there is an urgent need to explore alternative and renewable energy solutions to mitigate the environmental and economic challenges. Eucalyptus globulus, a plant abundant in Ethiopia, particularly in the Amhara region, presents a promising biomass resource for biofuel production. However, the leaves of this plant are often underutilized and inefficiently burned, leading to environmental degradation, air pollution, and health hazards. Additionally, the leaves' resistance to natural decomposition further exacerbates soil degradation and water contamination. Current biofuel production methods primarily focus on edible crops, which contribute to food insecurity and competition for land. Thus, there is a growing need to identify non-edible biomass resources, such as Eucalyptus globulus leaves, for sustainable biofuel production.

Among various methods, hydro-distillation has shown potential as a simple, efficient, and cost-effective approach to extract biofuels from plant biomass. Despite its promising advantages, there is limited research specifically focused on the hydro-distillation of Eucalyptus globulus leaves for biofuel applications. This research aims to fill the gap by experimentally investigating the potential of hydro-distillation to produce essential oils and liquid biofuels from Eucalyptus globulus leaves. The study will explore the optimization of key parameters such as temperature, extraction time, and leaf particle size to maximize the yield and quality of biofuel produced. By investigating this alternative biofuel production method, this study seeks to contribute to the development of sustainable, non-edible, and eco-friendly energy solutions that can reduce Ethiopia's reliance on imported fuels and mitigate the environmental impacts of traditional biomass use.

1.3. Objective of the Study

1.3.1. General Objective

The main objective of this study was the experimental investigation of essential oil using hydro-distillation from Eucalyptus (Eucalyptus globulus) leaves for liquid biofuel application.

1.3.2. Specific Objectives

- ❖ To determine the proximate analysis of raw materials
- ❖ To optimize the hydro distillation process parameters (temperature, particle size, and time for the liquid biofuel production)
- ❖ To characterize the liquid biofuel product

1.4. Scope of the Study

This study focused on the experimental investigation of liquid biofuel production from Eucalyptus globulus leaves using the hydro-distillation process, aiming to evaluate the yield of liquid biofuel as a potential renewable and sustainable energy source. The scope includes the collection and preparation of raw material, the production of biofuel using eucalyptus as biomass feedstock, and the investigation of the product's characteristics. It aimed to design and optimize the extraction process by evaluating the effects of temperature, particle size, and residence time on biofuel yield. This research evaluated the potential of Eucalyptus globulus as a viable biofuel feedstock, focusing on key performance indicators such as energy density, energy yield, calorific value, viscosity, chemical composition, etc. To validate its suitability, the produced biofuel was benchmarked against findings reported in other scientific literature and standard fuel specifications. This comparative assessment provided insight into the fuel's quality and efficiency by analyzing its physicochemical characteristics with recognized benchmarks.

1.5. Significance of the Study

The significance of this study lies in its contribution to the growing global demand for sustainable and renewable energy sources. As concerns about fossil fuel depletion, environmental pollution, and climate change intensify, the exploration of alternative bio-based fuels becomes increasingly critical. This research investigates the potential of Eucalyptus globulus leaves, a widely available and fast-growing plant species, as a viable feedstock for liquid biofuel production through hydro-distillation. By evaluating the yield, properties, and applicability of the extracted liquid biofuel, the study provides valuable insights into the feasibility of utilizing eucalyptus as an eco-friendly energy resource. Furthermore, it contributes to the advancement of green technologies by promoting a low-cost, natural, and relatively simple extraction process. The findings can serve as a scientific basis for further development of biofuel production methods, support decision-making for future bioenergy projects, and encourage the sustainable use of forest biomass, particularly in regions where eucalyptus trees are abundant. If implemented at a large scale, this study could offer a renewable alternative to fossil fuels, contributing to resolving environmental pollution problems, reduce greenhouse gas emissions, and helping to reduce fossil fuel dependency as well, it has significant economic potential to create jobs for farmers, and the bio-sludge used as biochar for solid fuel.

2. LITERATURE REVIEW

2.1. Liquid Biofuel in the World

Energy is the lifeblood of the economy. It is used to power everything from factories to homes, and it is essential for economic growth [2]. World energy consumption is expected to increase by 54% between 2001 and 2025. To meet this demand, need to develop carbon-neutral and sustainable energy sources [12].

Biofuels are a promising source of renewable energy [24]. The development of biofuel has a broad range of social, environmental, and financial effects. Economically speaking, biofuels can boost farmers' earnings and generate job possibilities. Designing sustainable biofuel policies needs to find a balance between these effects [4], [7], [25]. Liquid biofuels offer several benefits, but some challenges need to be addressed. As the technology continues to develop, liquid biofuels have the potential to play a major role in the global transition to renewable and sustainable energy [14]. Its source includes various residues from woodcraft sources and farm supplies associated with edible crops, including non-edible energy crops, such as black bent, Eucalyptus, and garbage [26]. Bioethanol, bio-oil, and biodiesel are renewable, sustainable, and environmentally friendly alternatives to fossil fuels. They can help reduce greenhouse gas emissions and fight climate change [4], [16].

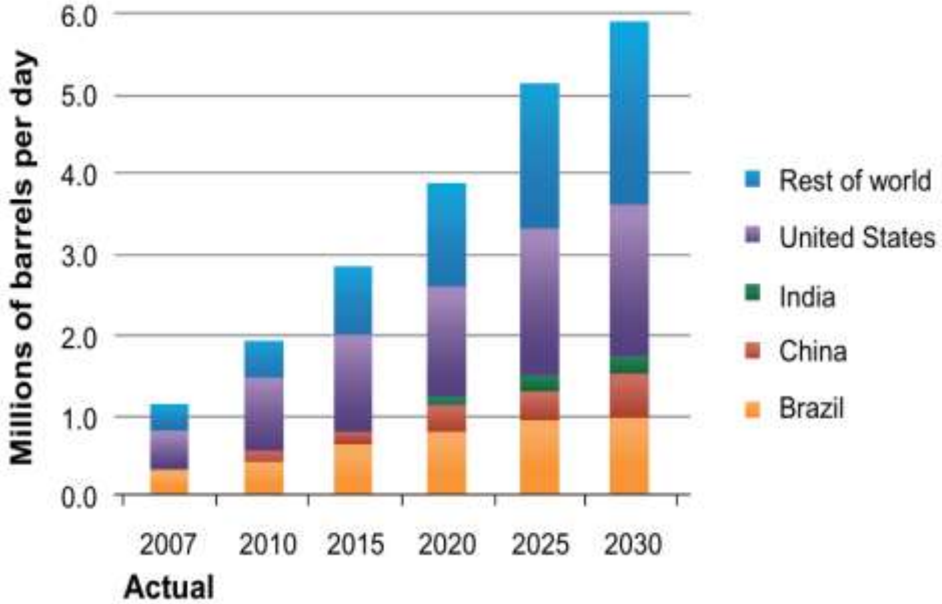


Figure 2.1 Biofuel production in the world [12]

2.2. Liquid Biofuel in Ethiopia

Ethiopia's economic and population growth has led to increased demand for petroleum products, particularly in the transportation sector. It has put a strain on the country's energy resources and infrastructure. The government is working to address this issue by investing in renewable energy and improving energy efficiency [27]. Ethiopia has the potential to produce many biofuels from its biomass and oil crops. It also features diverse geography and environmental conditions that support the cultivation of various biofuel feedstocks. Ethiopia's economy suffered from the country's growing fuel imports, which totaled $1.37 billion for 3.3 million tons in 2017, 10% higher than in 2016. The nation is shifting its focus to generating biofuels domestically despite rising costs and environmental issues [28]. Based on the literature, the most liquid biofuel produced in Ethiopia are biodiesel, bioethanol, and bio oil.

Biodiesel: Biodiesel is a renewable fuel made from plant or animal fats rich in oils. Common plant-based sources include oils from castor, corn, sunflower, cottonseed, Jatropha, soybeans, canola, palm, algae, etc., and animal-based sources include fish oil, cattle tallow, swine lard, chicken fat, duck fat, byproducts of meat processing, etc. It is a cleaner-burning fuel than petroleum diesel and helps reduce emissions in diesel engines [16], [25]. It also requires several steps, including the extraction of the fatty acids from the oil or fat by the esterification process [16].

Ethiopia's biodiesel production is still restricted to small-scale operations, but significant promise and great expectations. Several years later, there are still no major infrastructures in place. There are currently only three small factories operating: one at Bati Woreda that can produce 300 litres per day, a second 2,000 litres per day in Mekele by Africa Power Initiative, and a third 3,000 litres per day in the Southern region by Atrif Alternative. These statistics reflect how slowly the nation's biodiesel industry has developed [27].

Bio-alcohol: Bio-alcohol, such as ethanol, is produced by fermenting sugar cane, vegetable waste, agricultural waste, and starches. Butanol and propanol are produced as byproducts of this process [29]. It is also produced by thermochemical processes, biochemical processes, and direct combustion from plant material. Then, it is distilled and purified to obtain a fuel-grade product [16]. Ethiopia's annual production of ethanol is only 30 million litters, significantly lower than the 1.7 billion litter target. With 13 sugar plants total eight operating and five under construction, only two facilities are now operating, although more are planned [28].

Bio-oil: Bio-oil is produced through thermochemical and thermal processes from lignocellulosic biomass, including wood, agricultural residues, and dedicated energy crops [16]. It is a complex, energy-dense liquid comprising water and a wide array of organic compounds, such as carbonyls, amines, alcohols, hydrocarbons, and phenolic derivatives [29]. It differs from traditional fossil fuels due to its unique characteristics, such as density, oxygen content, sulfur content, viscosity, stability, water content, heating value, solid content, etc. [30].

Ethiopia's monopoly on fuel imports, the Ethiopian Petroleum Supply Enterprise (EPSE), provides big distributors with more than 90% of the market. Fuel imports are a significant economic problem as they increased by 12.5% between 2016 and 2020, reaching 3.9 million tons valued at $2.1 billion in 2019–20, and contribute to 60% of the revenue from exports [27].

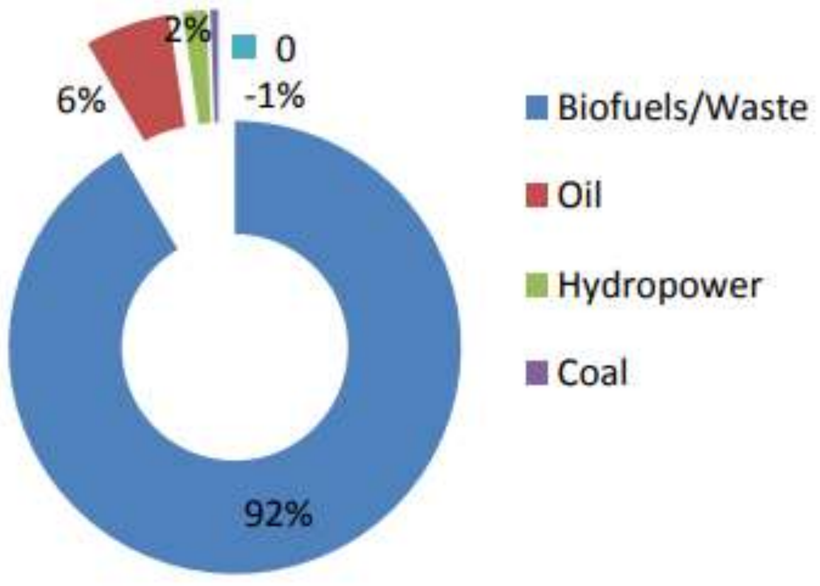


Figure 2.2 Share of Ethiopia's total primary energy supply in 2015 [28]

2.3. Overview of Biomass Energy

Biomass is a renewable energy source used to produce different forms of energy, including bioethanol, biodiesel, biomethane, biohydrogen, and bio-crude oil. It is the second-largest renewable energy source in the world after hydropower [31]. Bioenergy has positive and negative environmental impacts. It is important to consider these impacts when making decisions about its use [32].

2.4. Lignocellulosic Biomass (Woody biomass)

LCB is a promising renewable energy source that has the potential to reduce our reliance on fossil fuels like palm oil, cashew nuts, coconut, or sugarcane, etc. However, there are still some challenges that need to be addressed before it can be widely used [33], [34]. They are a major source of energy for over two billion people in developing countries, and cooking accounts for the vast majority of energy use in the household sector in Ethiopia [7]. The energy content of lignocellulosic biomasses 26% less than energy content of diesel [21].

2.4.1. Lignocellulosic Biomass (Woody Biomass) In Ethiopia

Lignocellulosic biomasses (LCBs) represent a low-cost and sustainable source of biofuel that can play a crucial role in meeting future energy demands while significantly reducing greenhouse gas emissions [16]. Ethiopia's vast agricultural sector generates a substantial volume of biomass residues with strong potential for conversion into renewable energy. However, the uneven availability and distribution of these residues across regions present challenges to the implementation of large-scale, nationwide biomass energy initiatives [35].

2.5. History of Eucalyptus in world

Eucalyptuses are a beautiful and versatile plant. They are an important part of the North America, Australia, Africa, Europe, and Indian subcontinent ecosystems used for various purposes, which are less diverse but more dense than natural forests [8].

Eucalyptus trees were introduced to East Africa in the late 19th century (early 1970s) and widely planted in the region [36]. China, India, and Brazil are the top three countries in the world for Eucalyptus cultivation. China has the largest Eucalyptus plantation area, followed by India and Brazil [37].

Eucalyptus Globulus Labill: Tasmanian blue gum (Eucalyptus globulus) is widely cultivated in many countries. These are Australia, Chile, China, Ecuador, Ethiopia, Portugal, Spain, and Uruguay [38].

Taxonomy: Eucalyptus species are flowering plants in the Plantae kingdom, with major species including Eucalyptus globulus, Eucalyptus citriodora, Eucalyptus staigeriana, and others [39], [40].

Morphology: The Eucalyptus tree is a tall, evergreen tree that can reach heights of 40–70 meters and has a straight, robust trunk that is 0.6–2 meters in diameter. It has glossy, downward-hanging leaves. Its flowers have many fluffy stamens in a range of colors, including white, cream, yellow, pink, and red [39], [40].

Table 2.1 Scientific classification of Eucalyptus [40]

Kingdom	**Plantae**	**Subclass**	**Rosidae**
Subkingdom	Tracheobionta	Order	Myrtales
Subdivision	Spermatophyta	Family	Myrtaceae
Division	Flowering plants	Genus	Eucalyptus
Class	Dicotyledons	Species	Eucalyptus globulus Labill

2.5.1. The Development of the Eucalyptus Tree in Ethiopia

Eucalyptus species were introduced to Ethiopia during the reign of Emperor Menelik II to address the country's shortage of firewood and timber. However, they also have a negative environmental impact, and their use is controversial [36]. Ethiopia has the biggest Eucalyptus plantation in East Africa. Two Eucalyptus species that grow in Ethiopia include Eucalyptus camaldulensis (Key-Baharzaf) at lower altitudes and Eucalyptus globulus (Nech-Baharzaf) at higher altitudes [41]. It covers nearly 90% of plantations in Ethiopia, with over 500,000 hectares of land planted with eucalyptus, which covers more than 0.5% of the country's land area [42].

2.6. Lignocellulosic Biomass Compositions

Lignocellulosic biomass is the main structural component of all plants, consisting of cellulose (40–50%), hemicellulose (25–30%), and lignin (15–30%) [43]. These components are dependent on species, growing environment, harvest season, collection technique, transportation, and storage conditions [44].

But other elements are found in minor percentages, such as carbohydrates, ash, pectin, and proteins [45]. Additionally, they contain over 70 elements commonly found in woody biomass, including essential metals such as phosphorus, calcium, magnesium, potassium, etc. [46].

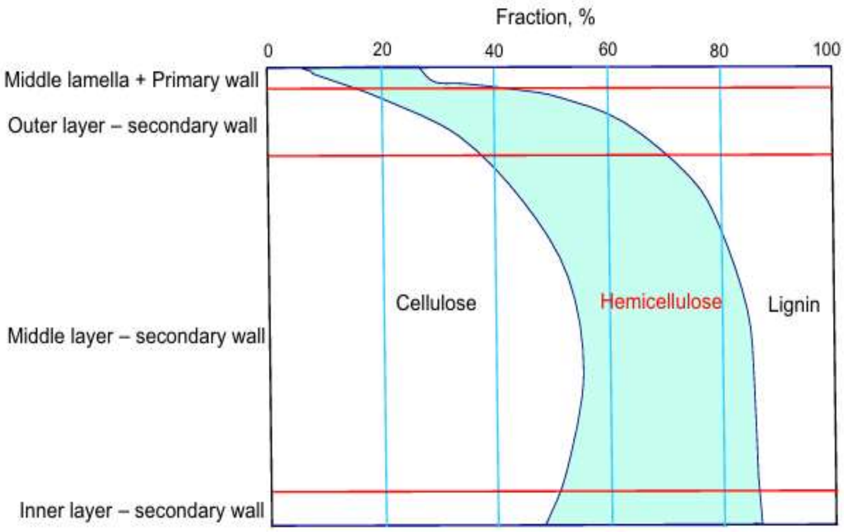


Figure 2.3 Chemical components' distribution via the cell wall of plant [46]

Cellulose: Cellulose is a long chain of sugar molecules which is linked together. The chains are very strong and tightly packed, which gives cellulose its exceptional strength. It is also very resistant to breaking down, which is why it is a major component of plant cell walls [44].

Hemicelluloses: The second most prevalent biopolymer after cellulose, hemicelluloses are sugar-based plant components found in cell walls that give structural support and are utilized to make biofuels and other goods [47]. It is a complex carbohydrate, which consists of different types of sugar molecules, including pentoses, hexoses, hexuronic acids, and deoxy-hexoses [48].

Lignin: A crucial polymer found in plant cell walls, which is a significant source of biomass energy but prevents biofuel generation. Since it is difficult to break down [49]. A crucial polymer found in plant cell walls. It is a significant source of biomass energy, but it prevents liquid biofuel generation. Since it also is difficult to break down [50].

Table 2.2 Chemical compositions of common types of wood [51]

Substrate	Cellulose (% DB)	Hemicellulose (% DB)	Lignin (% DB)
Poplar	53–54	11–19	21–34
Hemp	40–76	10–18	10–21
Eucalyptus	53–54	11–19	21-34
Beech	38–58	8-36	20-26
Aspen	44–48	18–23	19-24
Birch	39–46	25-27	26.5–28.5
Pine	37–48	13–26	23-30
Spruce	39–44	20.5–35.5	24-35

2.6.1. Nature and Chemical Composition of Eucalyptus

Numerous chemical substances, such as triterpenoids, terpenes, tannins, steroidal lactones, hydrocarbons, phenolic compounds, etc., are found in Eucalyptus globulus [40]. When Eucalyptus leaves grow, they get thicker, narrower, and darker green to indicate a change in chemical composition. Young leaves are bluish-green and broad [52]. The capacity of Eucalyptus to suppress plant development (allelopathic) may vary depending on several variables, including age, location, harvest time, weather, and extraction technique [53].

2.7. Benefits of Lignocellulosic Biomass and Biofuel

In many developing nations, wood is a vital renewable energy source, particularly in rural regions. It provides advantages, including less dependency on fossil fuels, creating jobs, local income, and assistance for regional development [54]. Lignocellulosic biomass is a prospective fuel source like wood and plant waste, because of its low cost, abundance, capacity to grow on marginal land, and low environmental impact [7]. Biofuel initiatives can help rural communities in developing nations by providing a local fuel supply for their houses, businesses, and energy needs [55].

2.7.1. Application of Eucalyptus Leaves

Ethiopia has a low per capita energy consumption rate and limited access to modern energy services, so Eucalyptus plants have been planted in the country for their many benefits [35]. Eucalyptus has several applications, including antibacterial, antifungal, antioxidant, anti-inflammatory, antiseptic, and insect-repelling properties. Pharmaceutical companies use the leaves' essential oil to relieve muscle aches and treat respiratory ailments. Medical technology is highly valued due to its numerous therapeutic advantages, especially in developing countries. Also used as a natural preservative and taste enhancer in the food business, Eucalyptus oil is used in animal feed, air fresheners, and perfumes [40], [56].

Biofuels: Advanced biofuels are more environmentally friendly because they create fewer greenhouse gas emissions during production, making them a more sustainable energy option [54]. Liquid biofuel derived from Eucalyptus globulus leaves offers valuable insights into its composition, quality, and potential applications in the energy sector. In India, Eucalyptus oil is widely accessible and shows promise as a biodiesel source. Due to its high cetane number and lack of sulfur, this biodiesel is a great substitute for conventional diesel fuel [57].

2.8. Proximate and Ultimate Analysis of Lignocellulosic Biomasses

Biomass proximate analysis measures fixed carbon, moisture, ash, and volatile matter. Pretreatment methods that enhance particle size reduction and water absorption help lower processing costs and significantly improve the conversion efficiency of biomass into valuable biofuels and biochemical [43].

Moisture Content (MC): The amount of water per unit mass of the dry solid is used to determine the moisture content of feedstock. However, the moisture content of biomass can be specified in two ways: "wet" and "dry. It can vary greatly, with agricultural leftovers containing roughly 20% water and woody biomass having about 50%. The effects of biomass's water content on its treatment, storage, and regeneration for biofuels or bio-oil [45]. In simple terms, raw biomass contains a lot of moisture, ranging from 30% to 60%, depending on factors like the crop, season, harvest time, and storage conditions [58].

Ash Content (AC): Ash content is the amount of inorganic matter left after a material is burned. It is typically expressed as a percentage of the original weight of the material. The ash content in biofuels varies based on the type of biomass used, particularly influenced by plant species, bark content, and the extent of soil contamination. In moderate amounts, ash content does not significantly impact the overall quality or performance of the biofuel [59], [60].

Volatile matter (VM): High levels of volatile matter in Eucalyptus leaves cause them to produce fumes and liquids when heated. This makes them perfect for pyrolysis and other processes since they burn mostly as gas, which produces smoke, extended flames, and less heat [60].

Fixed Carbon (FC): Fixed carbon is the part of a fuel that doesn't burn away when it's combusted. Fuels with higher fixed carbon contents burn more slowly because there's more material for the fire to consume [60].

Table 2.3 Proximate and ultimate analyses of three species of woody biomass [60]

Characteristics	**Pine**	**Holm-oak**	**Eucalyptus**
Proximate analysis, wt.%			
Volatiles Matter (VM)	71.5	70.2	74.8
Fixed carbon (FC)	6.0	17.8	13.9
Ash Content (Ash)	0.5	2.4	0.7
Moisture	12.0	9.5	10.6
Ultimate analysis, wt.% ash-free			
Carbon	51.6	51.1	52.8
Hydrogen	4.9	5.3	6.4
Nitrogen	0.9	0.9	0.4
Oxygen	42.6	42.7	40.4
HHV*, MJ/kg	20.2	19.4	21.3

2.9. Biomass Conversion Process Technologies

Biomass can be transformed into various forms of energy using different methods [55]. Biofuels are fuels made from agricultural waste or plants. They are categorized according to the feedstocks, technology, and processes [12]. Various biological resources and cutting-edge technology are used in the growing production of biofuels worldwide. Pretreatment techniques for various plant materials must be customized according to their distinct physical and chemical characteristics [16], [58].

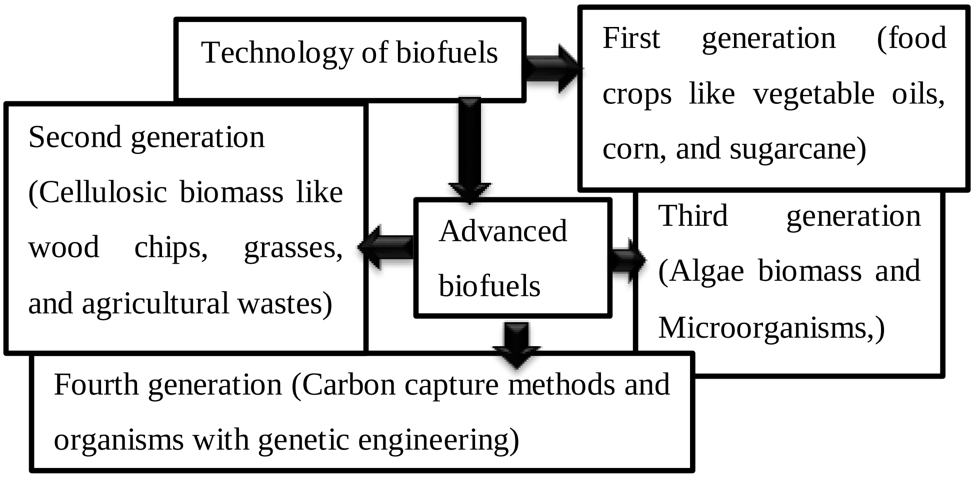


Figure 2.4 Stages of biofuel technology development [12], [16]

2.9.1. Biochemical Conversion

Fermentation, anaerobic digestion, photo fermentation, esterification, and aerobic digestion are other methods that can convert biomass into advantageous chemicals. Examples of biochemical conversion processes that can effectively convert different types of biofuels, including biogas, hydrogen, and ethanol, include gaseous or liquid biofuels that are created when enzymes break down biomass structural biopolymers using bacteria, fungi, yeast, and biochemical conversion. However, they are costly and not appropriate for portable manufacturing [16], [58].

2.9.2. Thermochemical Conversion

Various thermochemical methods can convert lignocellulosic biomass into power and other energy products like heat, syngas, biofuels, biochar, and chemicals. They involve burning, breaking down, composition, fermentation, pyrolysis, torrefaction, and liquefaction, converting to gas or liquid, and melting materials through heat [16].

2.9.3. Mechanical Conversion

Physical pretreatments usually involve grinding or milling the lignocellulosic biomass to make the particles smaller in size [61]. The main goal of these methods is to increase surface area and improve mass and heat transfer. Successfully breaking down biomass depends on factors such as equipment type, feeding speed, machine speed, screen size, and desired size reduction [62].

2.10. Conventional Techniques for Liquid Biofuel

A variety of biomass feedstocks, such as forestry and agricultural wastes like rice, maize, Gillo, sunflower, molasses, Jatropha, eucalyptus, bean, pea, white, and barley husks, can be used to generate liquid biofuels. These substances are prospective feedstocks that can be converted into biofuels such as bio-methanol, bio-ethanol, and bio-butanol. Bio-oil and biodiesel are examples of secondary products that are produced from these basic fuels. In support of the global shift to clean energy, this technique shows how various biomass sources can be effectively used to create renewable and sustainable energy options [63], [64] .

Several techniques are employed to extract useful chemicals from lignocellulosic biomass, including solvent extraction, steam distillation, and supercritical fluid extraction. These techniques have been improved recently, resulting in increased purity and yields [65].

2.10.1. Hydro-Distillation

Hydrodistillation is the most common and cost-effective method for extracting essential oils, particularly in developing countries, because of its simplicity, low operational cost, and minimal technical requirements [66]. This method extracts essential oils traditionally by evaporating them with water, materials, or solvents. It's simple, scalable, prevents chemical loss, nonanalytic, and saves more energy than other advanced methods [40], [53]. Fractional distillation enhances biocrude by lowering oxygen (53%), sulphur, and nitrogen (5-44%), which facilitates the usage of diesel engines [21].

Figure 2.5 Hydro-distillation technique (HD) [66]

2.10.2. Steam Distillation

Steam distillation extraction is an environmentally friendly method using steam as a solvent. It is in contrast to organic solvents, which create waste, need air treatment and exhaust, and may require specialized installations [83]. It is often used on farms, lacks technological advancements, and lacks access to technical suppliers for enhancing control, prediction, and process autonomy [62].

2.10.3. Solvent Extraction

Solvent extraction is used to produce oils from certain flowers, gums, resins, roses, geraniums, and others. This method involves soaking the raw material in a solvent (hexane or acetone for resins and gums) to extract the oils. It is a modern and efficient way to yield high-quality essential oils [69].

2.11. Parameters Affecting the Extraction Process

The viability of global bioenergy is influenced by geography, biodiversity, resources, technology, and economics [70]. The highest amount of liquid biofuel is found in Eucalyptus globulus leaves. that are impacted by the biofuel's properties include a variety of plant parts, picking, physical circumstances, genetics, maturity, height, root, weather conditions, and particle size [71].

The process conditions (choice of method, temperature, time, and pressure), solvent (type, volume, ratio), plant material (nature, origin, size, processing level), and equipment type (batch or continuous, material size, agitation) are all important factors that affect the synthesis of biofuel. For the production of biofuel to be feasible and high-yield, several elements must be optimized [72].

2.11.1. Temperature

Based on the previous study, high temperatures during distillation have the potential to taint the distillate, disrupt separation efficiency, and break down heat-sensitive chemicals. Therefore, temperature must be carefully managed to maintain product quality and maximize yield [25]. In the literature review, the impact of temperature on the efficiency of the hydrodistillation technique for extracting essential oils using water at 100°C was examined [73].

2.11.2. Particle Size

The size and distribution of biomass particles affect fluidization, mixing, surface area, heat transmission, storage, mass transfer, and flowability [45], [74]. The yield, quality, and efficiency of bio-oil are significantly enhanced using fine particles or catalysts, typically generated through distillation, pyrolysis, or hydrothermal liquefaction. Studies show that reducing the biomass particle size increases the production of liquid biofuels. On the other hand, reduced biofuel yields were the result of bigger particle sizes, which reduced the ability to transfer heat [34], [75].

Distillation was used to extract Eucalyptus oil from Iraqi Eucalyptus camaldulensis leaves. The parameters that were examined were the extraction temperature (100°C), agitation speed (200 rpm), water-to-leaf ratio (6:1 ml/g), and leaf particle size (0.5 cm) to achieve the highest possible oil production [76].

2.11.3. Time

Reaction during production and storage time systems are two scenarios that are investigated to determine the impact of time on liquid biofuel, including output, content, and quality [77]. Based on the previous study, longer extraction times allow the product to separate from the biomass, which usually leads to larger yields [78]. However, longer extraction times have been shown to decrease the production of liquid biofuel due to over-extraction of undesirable components, equilibrium reaching, and potential oil deterioration [29]. The previous study investigated that the essential oil by the Clevenger apparatus was maintained for 3 hours [79].

2.12. Pretreatment of Lignocellulosic Biomass

The structure of lignocellulosic biomass, which is made up of cellulose, hemicellulose, and lignin, is robust. Several pre-treatment processes are performed to change it into helpful items like biofuels. Physical methods such as extraction, drying, and grinding improve process efficiency. Biomass is broken down by heat in thermochemical processes like pyrolysis, combustion, and liquefaction. For the generation of bioenergy, microbes are used in biological processes, including fermentation and aerobic or anaerobic digestion. With the help of these pre-treatments, the energy and value contained in plant materials can be freed [29], [80].

Biofuels like biodiesel, biobutanol, and ethanol from biomass can be produced from carbon-neutral biomass like wood chips, wheat straw, corn stover, and algae. Cellulosic biomass is broken down for fermentation through pretreatment and hydrolysis; different techniques are available based on the feedstock [12]

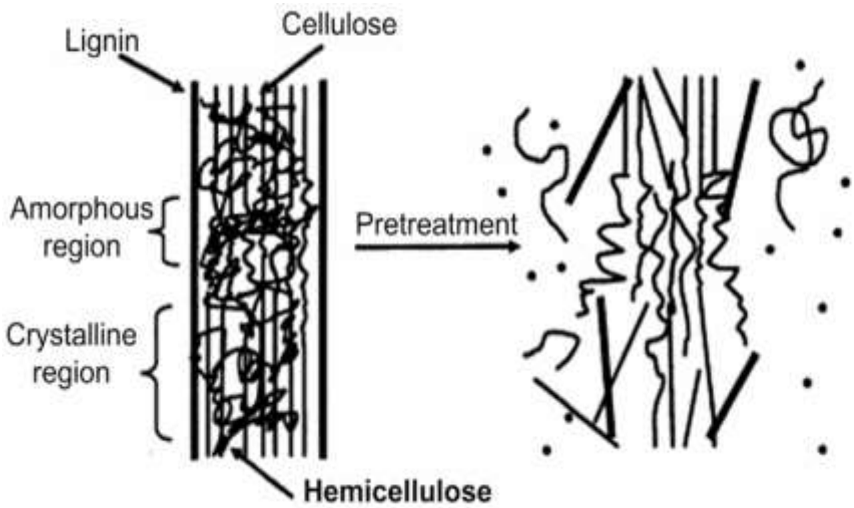


Figure 2.6 Pretreatment effect [12]

For lignocellulosic biomass (LCB) to improve enzymatic hydrolysis by decreasing its natural resistance, effective pretreatment is necessary. The best techniques should maintain the original biomass size, provide significant carbohydrates with little lignin, consume little energy, prevent inhibitors, preserve sugar content, and be economical and environmentally benign [80].

2.12.1. Chemical Pretreatment

Various chemical treatments are used to break down lignocellulosic biomass, such as dilute acid, hydrogen peroxide, ionic liquid, and various acid pretreatments like sulfuric acid and formic acid [62].

2.12.2. Biological Pretreatment

Biological pretreatments are eco-friendly, require minimal energy, and don't produce harmful byproducts. Bacteria or fungi can break down cellulose or remove lignin in the process [62]. They are green, low-energy, and clean due to aerobic and anaerobic fermentation by using bacteria or fungi to break down cellulose or remove lignin without harmful byproducts [29].

2.12.3. Physical Pretreatment

Physical pretreatment changes the surface area, particle size, crystallinity, and polymerization of biomass. However, it is inefficient and requires a lot of energy; the results vary depending on the biomass's characteristics and particle size [81].

Milling pretreatment: Physical pretreatment alters first-generation feedstocks or pre-treats second-generation wastes to be appropriate for both batch and continuous processing. It is used to enhance surface area and decrease cellulose crystallinity without releasing toxic compounds [82].

2.13. Separation Technology for Biofuels

Limitations on fossil fuels, promoting renewable energy laws, environmental concerns, and carbon emission regulations have all contributed to the rise in popularity of biofuels such as biodiesel, bioethanol, and biomethane [83]. Production costs and the range of separation technologies available, including adsorption, extraction, membrane processes, and hybrid approaches, have an impact on the product's purity [55].

Distillation processes: In biorefineries, distillation is employed to separate or dry alcohols, concentrate chemicals in pyrolysis oil, isolate volatile compounds, and purify biodiesel. It is producing liquid biofuels from plant materials such as the leaves of Eucalyptus globulus [21].

Filtration: When employing solid biomass as a feedstock, filtration and solid-liquid separation are crucial processes in biorefineries. For the separation of prehydrolyzate and post-distillation slurries, several procedures are essential [84].

2.14. Properties of the Liquid Biofuel

Several important factors affect the use, performance, and environmental impact of liquid biofuels such as bio-oil, bioethanol, and biodiesel. In contrast to oils obtained from petroleum, bio-oils have distinct physical characteristics due to their varied chemical composition, which includes phenols, aldehydes, hydrocarbons, ketones, esters, alcohols, and acids that have unwanted traits [22], [85]. Key traits include:

Viscosity: Viscosity is a key property of fuels that impacts handling, processing, and transport. It depends on chemical composition, such as water, oxygenates, and heavy molecules. Biofuels typically have higher viscosity than conventional fuels. Kinematic viscosity measures how long a set volume takes to flow through a capillary at a set temperature, indicating fuel flow behavior [86], [87]. Biofuel viscosity ranges from 1.6–264 cSt. These values are often too high for diesel engines, causing poor combustion and efficiency loss [27].

Density: An important characteristic that influences the energy content and behavior of bio-oil during processing and storage is density, which is described as mass per unit volume (kg/m^3 or g/cm^3) [45]. In biofuels, density changes are less significant than viscosity changes. Bio-crudes from pyrolysis and hydrothermal liquefaction (HTL) vary from 820 to 1200 kg/m^3 and 720 to 1250 kg/m^3, respectively. Compared to diesel, these figures are more in line with marine heavy fuel oil. More mass can be supplied with a higher density, but more energy is also needed [27].

Flash Point: The flash point of a liquid is the lowest temperature that produces enough vapor to ignite when exposed to an ignition source. It is measured using the Pensky-Martens Closed Cup or Open Cup Method and standard tests. It shows flammability and safety when handling, storing, and transporting bio-oil [23].

Acid value: Free fatty acids (FFAs) and acidic substances are measured using the acid value (also known as the acid number) for liquid biofuel. Milligrams of potassium hydroxide (KOH) needed to neutralize the acids in one gram of oil is the unit of measurement based on ASTM D-664 [88]. A greater acid value indicates increased acidity, which can result in corrosion in storage tanks, pipelines, and engines, compromising the oil's stability and usability [89].

Heating Value: The heat emitted when fuel burns completely is known as the heat of combustion. A bomb calorimeter is used to measure the heat of combustion. Fuels' heat of combustion can be expressed as gross (HHV) [23]. It can be described as the higher or lower heating value, with or without the energy in water vapor, and higher heating values for woody and agricultural residues ranged from 17 to 21 MJ/kg [45].

Chemical composition: FTIR is used to identify the different types of molecules that are present in a substance, as well as their concentrations. This information is used to determine the suitability of a substance for various purposes, including biofuel production [90].

2.15. Related studies on liquid biofuel production

Liquid biofuels have unique properties that make them appropriate for internal combustion engines. The two main forms are bioethanol and biodiesel, which are frequently combined with gasoline and diesel fuel, respectively [16]. Different conversion techniques can be used to synthesize bio-oil and bio-diesel from a range of biomass sources for use in transportation and fuel applications. As an example of its potential as a sustainable fuel, bio-oil made from palm kernel cake and Eucalyptus has been utilized efficiently in diesel engines [75], [91].

Similarly, biodiesel produced from Jatropha oil via in situ transesterification at 32°C over a period exceeding 48 hours presents a promising alternative to conventional diesel fuel. These production methods, including in situ transesterification, ethyl transesterification, and conventional transesterification, have a significant impact on determining the quality and efficiency of the resulting fuels [92]. In addition, oil palm biomass can be thermochemically converted through pyrolysis at temperatures between 400 and 500 °C to produce bio-oil, charcoal, and pyrolysis gas, thereby enhancing the variety of valuable bio-based products. Overall, both the selection of feedstock and the processing method play a critical role in determining the yield and quality of biofuels, making them viable and sustainable alternatives to conventional energy sources [93].

3. MATERIALS AND METHODS

3.1. The Study Area

The research was conducted in the rural communities surrounding Wogera Woreda, which is located in the central Gondar Zone of the Amhara Region of Ethiopia, 12°46'06.5"N latitude and 37°37'26.5"E longitude. There are 250,493 people, of whom 94% have lived in rural areas [94]. Figure 3.1 below shows the study area of this study.

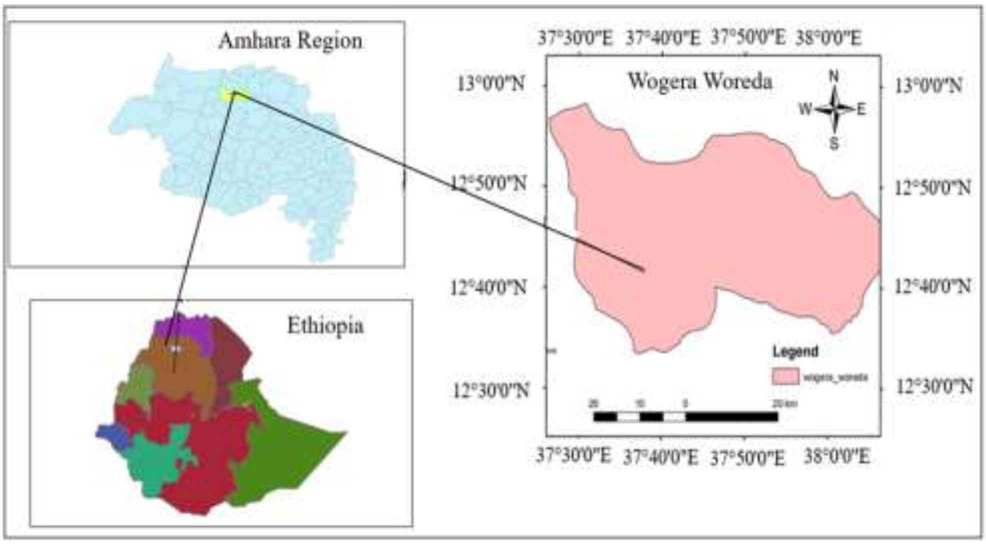


Figure 3.1 A case study area: Wogera Woreda (created by arc GIS software)

3.2. Materials

3.2.1. Equipment

The experimental work involved several stages, each supported by appropriate laboratory equipment. The hydro-distillation process used a lab-scale apparatus that provided controlled heating and condensation to extract the liquid biofuel efficiently. Following extraction, various analytical instruments were employed to characterize the biofuel's properties, ensuring accurate assessment of quality and suitability. Throughout the process, standard measurement tools maintained precise control over experimental parameters, ensuring consistency and reliability.

Ax was used to cut Eucalyptus globulus leaves (EGL) from its tree or branches; mortar was used to crush leaves into small or fine powder, in order to enhance the extraction surface area and boost extraction efficiency; viscometer was used to measure liquid biofuel's viscosity; pipettes and test tubes was used for precise measurement and handling liquid biofuel; gradual cylinder was used to measure different chemicals and liquid biofuel; hydro-distillation equipment was used to extract liquid biofuel EGL; Separatory funnel was used to separate liquid biofuel from its impurities; muffle furnace (FM 106) and dry oven (DHG-9055) was used to proximate analysis of EGL; heat mantle was used to heating source for HD setups; crucible was used to hold samples for different purpose; analytical balance (FA 2104) was used to measure samples before and after HD; and refrigerator (LR 140 S SP) was used to store raw material and sample; bomb calorimeter (BK-1A) was used to measure heating value; and FTIR (Jasco-6600) was used to analysis functional group of sample.

3.2.2. Chemicals

All reagents and chemicals used in the hydro-distillation process were of analytical grade to ensure reliability and reproducibility of results.

Water was used to clean the equipment and leaves; anhydrous sodium sulfate (200g) was applied to remove moisture from the hydrodistilled liquid biofuel; anhydrous potassium hydroxide (0.1N KOH) was used in titration to determine the acid value; and ethanol (50 ml) served as a diluent for the biofuel sample.

3.3. Methods

3.3.1. Raw Material Collection

Eucalyptus globulus leaves (EGL) were sourced from a private farm in Wogera Woreda, Central Gondar Zone, Amhara Region, Ethiopia. This location was selected due to the abundant local availability of EGL, making it an ideal site for sustainable liquid biofuel production using hydro-distillation (HD).

3.3.2. Raw Material Preparation

The Eucalyptus globulus leaves (EGL) were first carefully washed to remove dirt, dust, and other impurities that could affect the quality of the biofuel. After cleaning, the leaves were manually crushed using a mortar and pestle. This process helped to break down the leaves into smaller, uniform particles to increase the surface area for better extraction during hydro-distillation. The crushed leaves were prepared in three different particle size ranges, 3 mm, 5 mm, and 7 mm, to study how particle size affects the yield and quality of the liquid biofuel. Smaller particles tend to allow more efficient extraction, while larger particles may slow down the process.

3.3.3. Determination of Proximate Analysis of Raw Material (EGL)

Based on the ASTM E870 - 82 standards was used to perform a proximate analysis. It was used to determine the sample's physicochemical properties, including the amount of moisture, volatile matter, ash content, and fixed carbon was calculated using equation from 3.1 to 3.4. It shows how much non-combustible ash is left on the ash pit or entrained with flue gases, as well as the proportion of fuel burnt in gaseous, liquid, and solid phases.

Determination of moisture content (MC)

The ASTM E-871 standard was used to determine moisture content by dry oven in the (DHG-9055). The moisture content of the sample was determined by weighing 100g for the dry basis of the raw material and keeping it at 105°C to reach a constant weight. It had been weighed, and the differences between the wet and dry masses were noted at the end.

$$MC = \frac{M-DM}{M} \times 100 \qquad 3.1$$

Where: MC is moisture content (%), M is mass of fresh Eucalyptus globulus leaves, and DM is dry mass of Eucalyptus globulus leaves.

Determination of volatile matter (VM)

Volatile matter (%VM) was determined by weighing 5g of the dry Eucalyptus globulus leaf sample to set a crucible and placing it in a furnace (MF 106 Furnace) at a temperature of 950 °C for 7 minutes based on ASTM E-872. It had weighed the residue and the percentage of volatile matter.

$$VM = \frac{m_i - m_f}{m_i} \times 100 \qquad 3.2$$

Where: VM is the volatile content (%), m_i is the initial mass of the sample, and m_f is the final mass of the sample.

Determination of ash content (AC)

The ASTM E-1755 standard is used to determine the ash content. A 5g sample was placed in a furnace (MF 106) with a crucible; the furnace's temperature was gradually increased from 550 °C and maintained until the carbon was completely burned. Then, the ash residue was computed.

$$AC = \frac{\text{Ash residue}}{\text{DM}} \times 100 \qquad 3.3$$

Where: DM is the dry mass of EGL, and AC is the ash content (%).

Determination of fixed carbon (FC)

The percentage of fixed carbon (%FC) was the amount of carbon present in the sample. The fixed carbon is calculated by subtracting the percentages of moisture, ash, and volatile matter from 100%.

$$\% FC = 100\% - (MC + VM + AC)\% \qquad 3.4$$

3.3.4. Optimization of the Hydro Distillation Process Parameters

This research focused on the optimization of process variables to analyze three parameters (temperature, time, and particle size) to produce liquid biofuel using the State-Ease Design Expert software.

Response Surface Methodology (RSM) with Central Composite Design (CCD) is used to optimize processes by modeling quadratic and interaction effects between factors. It is used to find out optimal conditions, low experimental cost and error, increased efficiency, quadratic model fitting, and flexibility in factor levels. Hydro-distillation techniques play a vital role in enhancing the energy content of biomass.

Table 3.1 Parameters chosen for the experimental design

Factors	Unit	low	High	Reference
Temperature	°C	80	120	[66], [73], [76]
Particle size	mm	3	7	[95], [76]
Residence time	hr	1	5	[59], [79]

3.3.5. Experimental Procedures of the Hydro-Distillation (HD) Process

The hydro-distillation apparatus was set to 80, 100, and 120°C, with distillation times of 1, 3, and 5 hours. A 125g wet leaves sample was placed in the device, ensuring the sample was not fully submerged in water during the process. Because thermal processes can extract significant energy from wet Eucalyptus globulus leaves to produce liquid biofuel due to improved processing efficiency and reduced water usage. Additionally, dry leaves need other solvents to increase the extraction process.

The distillation process started to produce steam when the liquid mixture was cooled using a condenser. Finally, the liquid biofuel was separated from the liquid mixture using separatory funnels and was dried using sodium sulfate for further analysis.

3.3.6. Characterization of the Liquid Biofuel Yield

The sample was stored at 4°C until the properties of the liquid biofuel were determined. Among the key fuel-related characteristics of liquid biofuel derived from EGL, a viable non-edible feedstock for the production of biodiesel, including color, density, viscosity, heating value, mass yield, energy density ratio, energy yield, flash point, boiling point, acid value, and chemical composition, were conducted on a laboratory scale.

3.3.6.1. Physical/visual examination

The color of the liquid biofuel yield was detected with the naked eye.

3.3.6.2. Determination of density

5 ml of the liquid biofuel sample was measured using a measuring cylinder. The sample was weighed using an analytical balance (MODEL FA2104) to determine its mass. The density was calculated by dividing the mass by the volume.

$$\text{Density } (\rho) = \frac{Mass\ of\ biofuel(M)}{Volume\ of\ biofuel(V)} \qquad 3.5$$

3.3.6.3. Determination of viscosity

A capillary (Ostwald) viscometer was used to measure the viscosity of the liquid biofuel under room temperature and pressure conditions. The liquid biofuel did not exceed the top mark when drawn into the upper bulb of the viscometer. Then, the liquid was allowed to flow freely, and the amount of time (t) that passed between the two cut marks and the viscosity of the liquid biofuel sample to reach the final mark were recorded.

$$\text{kinematic viscosity } (\nu) = \frac{\boldsymbol{t_{test}}}{\boldsymbol{t_{standard}}} \times \boldsymbol{C_{standard}} \qquad 3.6$$

Where: t_{test} = time taken for the liquid to flow through the capillary tube, and $C_{standard}$ = kinematic viscosity of standard (water); $t_{standard}$ = time taken for the standard to flow through the capillary tube (water).

3.3.6.4. Determination of heating value (HV)

The ASTM D-240 test technique determines the energy content of liquid biofuels to measure the heat of burning using a bomb calorimeter [23].

The bomb calorimeter (BK-1A) was calibrated using pure ethanol as a standard material. One gram of the biofuel sample was placed inside the bomb, which is filled with pure oxygen at 25 atmospheric pressures. The bomb was filled with 2 L (2000g) of water and has reached a comfortable temperature. The fuel sample burns in a bomb, and the records change in temperature. Finally, calculate the heating value.

$$HV = \frac{mC\Delta T}{m_{sample}} \qquad 3.7$$

Where: HV = heating value (MJ/kg), C = specific heat capacity of water (J/g℃), m = mass of water (g), and m_{sample} = mass of liquid biofuel (g).

3.3.6.5. Determination of Mass yield, energy densification ratio, and energy yield

Mass yield refers to the total amount of product obtained from hydro-distillation. It is calculated by dividing the mass of the hydro-distillation product by the mass of the raw solids used in the process.

$$Mass\ yield = \frac{Mass\ of\ liquid\ biofuel\ (g)}{Mass\ of\ EGL\ (g)} \qquad 3.8$$

Where: EGL = Eucalyptus globulus leaves.

The energy densification ratio is the ratio of the calorific value of the thermally treated sample to the calorific value of the raw sample.

$$\text{Energy densification ratio} = \frac{\text{HHVof liquid biofuel}}{\text{HHV of EGL}} \qquad 3.9$$

Where HHV = higher heating value (MJ/kg) and EGL = Eucalyptus globulus leaves.

The energy yield was determined by,

Energy yield = Mass yield × Energy densification ratio 3.10

3.3.6.6. Determination of Acid Value

Based on the ASTM D-974 standard, the acid value of bio-oils is derived from biomass to measure, which is used as a sustainable energy source; the stability, quality, and usability can be determined [88]. 2 grams of the sample were measured and poured into a conical flask. Next, 25 mL of absolute ethanol was added to dissolve the liquid biofuel. Then, 2 drops of phenolphthalein were added to the solution. A burette was filled with 0.1 N KOH to add slowly to the mixture until it turned colorless, indicating the endpoint was reached.

$$\text{Acid Value } (AV) = \frac{\mathbf{V \times N \times 56.1}}{\mathbf{m}} \qquad 3.11$$

Where V = volume of KOH solution used (in mL), N = normality of KOH (mol/L) solution, 56.1 = molecular weight of KOH (g/mol), and m = mass of the liquid biofuel sample (in grams).

3.3.6.7. Determination of Flash Point

According to ASTM D-93, the Pensky-Martens Opened Cup Method was used to determine the flash point of liquid biofuel. A 5 ml sample was poured into the test cup controlled by a hot plate, with the heat gradually increasing 1℃/minute to ignite and spread the liquid's surface. The flashpoint was recorded as the lowest temperature at which ignition occurs, as measured from the digital meter.

3.3.6.8. Determination of Boiling Point

A 20 ml sample was heated to 100 °C using a heat mantle. The initial boiling point (IBP) and final boiling point (FBP) were recorded with an infrared thermometer (Nr. /HG05546) as the sample began to heat and when it was completely boiled, respectively. The boiling range was then calculated as the difference between the final boiling point (FBP) and the initial boiling point (IBP). Then, the results were compared to relevant standards.

3.3.6.9. Determination of functional group

The functional group analysis of liquid biofuel was conducted using an Fourier Transform Infrared Radiation (FT-IR) JASCO (FT/IR-6600 type A) series. It was carried out in the mid-infrared range, covering the wavenumber region of 4000-400 cm^{-1}. The spectrum resolution was 4 cm^{-1} with 2 mm/second for scanning speed.

Finally, the FTIR data and spectra graphs were collected for extra analysis and interpretation using Origin Pro ® 2024.

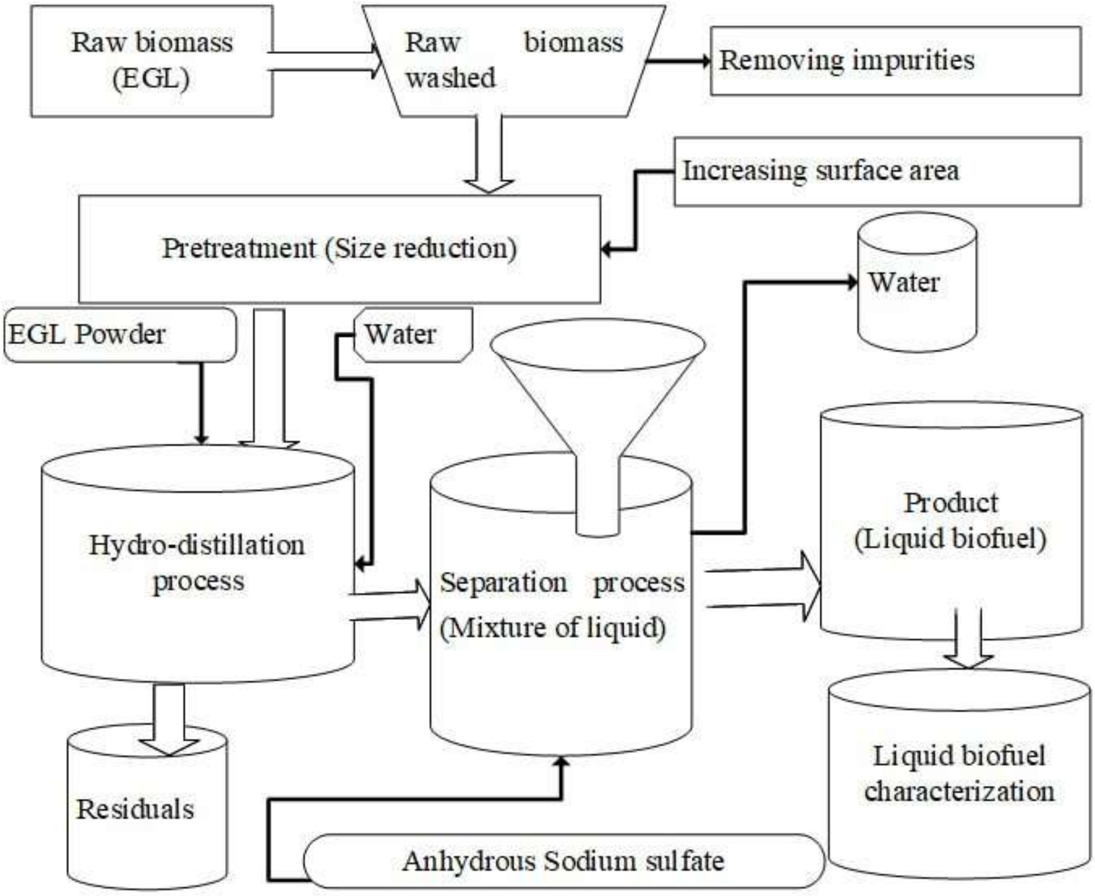


Figure 3.2 General flow diagram of the Hydro-distillation process

4. RESULTS AND DISCUSSION

4.1. Proximate Analysis of Raw Material

The moisture, ash, volatile matter, and fixed carbon contents of Eucalyptus Globulus Leaves (EGL) were investigated using an oven and furnace with different temperatures and times through lab-scale experiments (chemical engineering lab, WiT-WDU) based on ASTM-standardized methods.

Every experiment was conducted on every sample three times, and the results were obtained (dry basis %) using Equations 3.1-3.4. Proximate analysis results of the raw EGL were investigated as shown in Table 4.1.

Table 4.1 Proximate analysis of raw material (EGL)

Characteristics	**Proximate analysis (% dry basis)**		
	Present study	Previous study	Reference
Moisture content (MC)	9.47± 0.75	10.6	[60]
Volatiles Matter (VM)	76.5 ± 2	74.8	[60]
Ash Content (AC)	1.05 ± 0.1	6.09	[59]
Fixed carbon (FC)	12.98	13.9	[60]

The moisture content reported in this study was 9.47%, although a slightly higher value of 10.6% was written in the previous study [60]. This low moisture content has a higher calorific value, employed to improve combustion efficiency, reduce the energy required for water evaporation, which has an impact on combustion efficiency.

In the previous study, VM was reported at 74.8%, but in the current study, it slightly deviates to 76.5 %, which differs by 1.7 %, due to methodological or environmental differences [60].

Ash content was 1.05 %, which is significantly lower than previous reports of 6.09% [59]. It indicates the inorganic residue left after combustion and causes less fouling in distillation equipment, which could reduce maintenance expenses. However, the higher ash content leads to impurities in the liquid biofuel of quantity, quality, and suitability. The result of fixed carbon was 12.98%, referring to the solid carbonaceous residue left over after volatile matter was eliminated, slightly lower than the previous studies' fixed carbon value of 13.9%, which implies that more solid waste has more potential for use as solid fuel [60]. Since it enables more effective processing during combustion, it helps the fuel's stability and heating value.

Generally, the fundamental components of the biomass, including moisture, volatile matter, fixed carbon, and ash, were influenced by parameters such as sample preparation (drying, grinding, storage), experimental conditions (heating rate, extraction time, temperature), measurement methods (calibration, reporting), and the raw materials composition (species, growth conditions, plant parts), all of which can affect proximate analysis results. Understanding these parameters is essential for evaluating energy potential and combustion biomass behavior, which is crucial for optimizing its application in biofuel production and assessing its role in the carbon cycle and sustainable development.

The feedstock was characterized by high volatility and low ash content, suggesting its suitability for liquid biofuel production via the hydro-distillation process. This composition enhances the efficiency of the extraction, maximizing biofuel yield while minimizing undesirable residues.

4.2. Parametric Effect on Liquid Biofuel Production Via HD

Response surface methodology (RSM) tests the impact of process factors on measured responses and builds empirical models by combining statistical design and numerical optimization [96]. The process variables influencing liquid biofuel production were optimized using response surface methodology (RSM) with central composite design (CCD).

The current study examined the effects of three selected parameters (temperature, particle size, and time) of 20 tests conducted, comprising six center points, six axial points, and eight factorial tests in hydro-distillation, which influence the quantity and quality of the liquid biofuel production. It also examined how they interacted to affect the liquid biofuel production, using Design Expert ®13 to describe the conversion of Eucalyptus globulus leaves (EGL).

The result of an experimental investigation as shown in the Table 4.2 intended to assess how three process variables, such as temperature (80, 100, and 120°C), particle size (3, 5, and 7 mm), and extraction time (1, 3, and 5 hr), affect the liquid biofuel yield have been denoted with -1, 0, and +1, respectively.

Table 4.2 Factors affecting yield in the extraction process

	Factor 1	Factor 2	Factor 3	Response 1
Run	**A: Temperature ℃**	**B: Particle size mm**	**C: Extraction time hr**	**yield (ml)**
1	100	7	3	29.45
2	120	7	5	26.75
3	100	3	3	31.5
4	100	5	3	32.85
5	120	5	3	30.25
6	80	3	5	25.13
7	100	5	5	30.85
8	100	5	3	32.58
9	100	5	3	33.85
10	80	5	3	22.4
11	100	5	3	32.1
12	80	7	5	24.35
13	120	3	5	28.01
14	80	7	1	15.75
15	100	5	1	28.5
16	100	5	3	32.4
17	80	3	1	18.83
18	120	3	1	27.05
19	120	7	1	26.21
20	100	5	3	33.51

The values of the temperature at 100°C, the particle size at 5 mm, and the extraction time at 3 hours are repeated six times among the 20 experimental runs (runs 4, 8, 9, 11, 16, and 20). This repetition serves as a focal point for evaluating experimental repeatability and consistency. Under these circumstances, yields are continuously high, ranging from 32.1ml to 33.85ml; run 9 had the highest yield (33.85ml). The lowest yield was 15.75ml at 80°C, 7 mm, and 1 hour. In addition, run 2 produced 26.75ml at 5 hours, 7 mm, and 120°C. It implies that long extraction times, high temperatures, and large particle sizes restrict yield.

In this study, the essential oil yields of 2.56% w/w (or 3.4 % (mL/g)) are significantly correlated in existing literature on Eucalyptus species using hydro-distillation. Most conventional hydro-distillation studies report yields ranging from 0.60% to 1.32% w/w, depending on species, process variables, and distillation conditions [97].

4.2.1. ANOVA for Response Surface Quadratic Model

To find out which design factors have a significant effect on the quadratic model, ANOVA analysis was performed. The importance of each coefficient was assessed using P-values, which showed how much each parameter was related to the others in a linear, interactive, and quadratic way. As the p-values decrease, the relevant coefficient's significance increases. The ANOVA results for the response surface quadratic model in Table 4.3.

Table 4.3 Analysis of variance on liquid biofuel yield

Source	Sum of Squares	Degree of freedom	Mean Square	F-value	p-value	
Model	454.84	9	50.54	47.35	< 0.0001	significant
A-Temperature	101.19	1	101.19	94.80	< 0.0001	
B-Particle size	6.42	1	6.42	6.01	0.0342	
C-Residence time	35.16	1	35.16	32.94	0.0002	
AB	0.3872	1	0.3872	0.3628	0.5604	
AC	22.45	1	22.45	21.03	0.0010	
BC	0.4418	1	0.4418	0.4139	0.5345	
A^2	78.93	1	78.93	73.94	< 0.0001	
B^2	4.01	1	4.01	3.76	0.0814	
C^2	11.08	1	11.08	10.38	0.0091	
Residual	10.67	10	1.07			
Lack of Fit	8.41	5	1.68	3.71	0.0884	not significant
Pure Error	2.27	5	0.4535			
Cor Total	465.51	19				

The experimental results align with the ANOVA model, which provides P, F, and R-squared values. A P-value < 0.05 indicates statistical significance, while an R-squared value of 0.9771 shows that 97.71% of the variability in the liquid biofuel yield was explained by the model, with only 2.29% attributed to error or noise.

Adjusted R^2 value for the model is 0.9564, which is sufficiently close to the R^2 values. Additionally, the lack of a fit test was used to assess whether the model accurately captures data in the experimental domain at points not covered by the regression.

Significant model terms are indicated by "Prob> F" values less than 0.0500, which include A, B, C, AC, A^2, and C^2. The values are higher than 0.1000, and the model terms are considered non-significant when the R-squared value is 0.9771. There are many insignificant model terms (not counting those required to support hierarchy); model reduction may improve your model.

A high F-value indicates that the model is reasonably reliable, while a low p-value indicates that the model is highly significant [8]. In this study, the liquid biofuel yield model's F-value was 47.35, indicating that the regression models were dependable and significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. This relatively low probability (<10%) is troubling.

Additionally, the model's smaller degree of freedom was positive, which was desirable. Better model responses are indicated by mean square values closer to zero. The sum of square values for each factor was low, but some factors were high, which depicts little variation from the mean. The data below provides the standard deviation, mean, adjusted R-squared, and enough precision in the Table 4.4.

Table 4.4 Fit statistics

Standard Deviation	**1.03**	**R^2**	**0.9771**
Mean	28.12	Adjusted R^2	0.9564
C.V. %	3.67	Predicted R^2	0.8591
Press	65.58	Adeq Precision	22.6666

Given that the liquid biofuel yield's coefficient of R^2 is 0.9771, the predicted polynomial equations provide a very good fit to the data. R^2 values also demonstrate that the model is unable to account for roughly 2.29% of the total variance. The coefficient of determination, however, continuously rises as a variable is incorporated into the model, whether or not this extra variable is statistically significant. Conversely, the adjusted R^2 is frequently used in evaluating the obtained model because, when variables are added to the model, its value does not necessarily increase [96]. Since the discrepancy between the adjusted coefficient of determination (Adj R^2) and expected R^2 (pred R^2) is less than 0.2, the result is in reasonable agreement. Signal-to-noise ratio is represented by adequate precision (Adeq precision) greater than 4, which is a desirable value [8].

In the current investigation, the Pred R-squared of 0.8591 is in reasonable agreement with the Adj R-squared of 0.9564. The yield model's value of acceptable precision was near 22.6666, suggesting adequate signal and satisfactory fitness. The liquid biofuel yield model's p-value for lack of fit was 0.0884, which is as small as necessary for the model to be approved. As a result, this model is well-suited for this task and may be utilized to navigate the design space. An analysis of 0.9771 was fed into the model and was conducted. The goodness of fit was validated by moderately decreased standard deviation (SD) values and the R^2 value's closeness to unity (1.0).

Table 4.5 Analysis of the standard error and the confidence interval

Factor	**Coefficient Estimate**	**Degree of freedom**	**Standard Error**	**95% CI Low**	**95% CI High**	**VIF**
Intercept	32.40	1	0.3552	31.61	33.19	
A-Temperature	3.18	1	0.3267	2.45	3.91	1.0000
B-Particle size	-0.8010	1	0.3267	-1.53	-0.0731	1.0000
C-Residence time	1.88	1	0.3267	1.15	2.60	1.0000
AB	0.2200	1	0.3653	-0.5939	1.03	1.0000
AC	-1.68	1	0.3653	-2.49	-0.8611	1.0000
BC	0.2350	1	0.3653	-0.5789	1.05	1.0000
A^2	-5.36	1	0.6230	-6.75	-3.97	1.82
B^2	-1.21	1	0.6230	-2.60	0.1809	1.82
C^2	-2.01	1	0.6230	-3.40	-0.6191	1.82

Important information is given in Table 4.5 to determine the power transformation equations for calculating expected data. Intercept values and coefficients are used to create the model transformation equation.

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal, the VIFs are 1; VIFs greater than 1 indicate multi-collinearity, and the higher the VIF, the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable.

4.2.2. The Development of the Regression Model Formula

A model equation is a mathematical phrase that condenses the entire model into a single equation, hence maximizing response. The following model equation combines the response (liquid biofuel mass yield) to the process variables in terms of real value after removing the unnecessary variables.

Final equation in terms of coded factors

$$\text{Liquid biofuel yield} = 32.4019 + 3.181 * A - 0.801 * B + 1.875 * C + 0.22 * AB - 1.675 * AC + 0.235 * BC - 5.35727 * A\wedge^{2} - 1.20727 * B\wedge^{2} - 2.00727 * C\wedge^{2} \quad (4.1)$$

A given equation is a mathematical model that illustrates the relationship between three important process variables: temperature (A), particle size (B), and extraction time (C) on the liquid biofuel yield. The linear, interaction, and quadratic factors in the equation suggest a complicated and nonlinear relationship between these variables and liquid biofuel yield. First, as the positive linear coefficients show, liquid biofuel yield tends to increase with temperature and residence duration. All three variables, but particularly temperature, have negative quadratic effects, indicating that there is an ideal value beyond which additional increases will lower the yield.

In general, particle size hurts yield; as particle size increases, both its linear and quadratic coefficients contribute to a decline in output. While temperature and particle size interact modestly to boost yield, the combination of high temperature and long extraction time has a large negative effect. These interaction terms demonstrate how combinations of variables affect yield. In total, the model emphasizes that the best way to increase liquid biofuel yield is to carefully balance each variable within ideal ranges rather than just raising individual variables.

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

Using CCD based on equation 4.1, the experimental liquid biofuel output was compared to the expected value. The model predicts a biofuel yield of 32.40% at the center point, where all variables are at their medium levels (A=0, B=0, and C=0). The experimental yield is 33.85%, with a small and acceptable deviation of +1.45%, indicating that the model fits well near the center of the design space. The model predicts a yield of about 15.75% for conditions A=-1, B=-1, and C=+1. This yield accurately matches the reported minimal experimental yield, demonstrating the model's good fitness at the extreme point.

Last but not least, the model predicts a yield of 26.87% at their maximum levels (A=+1, B=+1, and C=+1), which is only 0.12% different from the experimental yield of 26.75%. In this experimental scenario, the application of the model to estimate biofuel production was confirmed by the great predictive accuracy on low, mid, and high-level variables, with non-negligible residual errors.

4.2.3. Diagnostics Graphs of Normal and Prediction

The residual analysis plot shows that most experimental data points lie along the normal probability line, with residuals clustering around the red line, indicating they follow a roughly normal distribution.

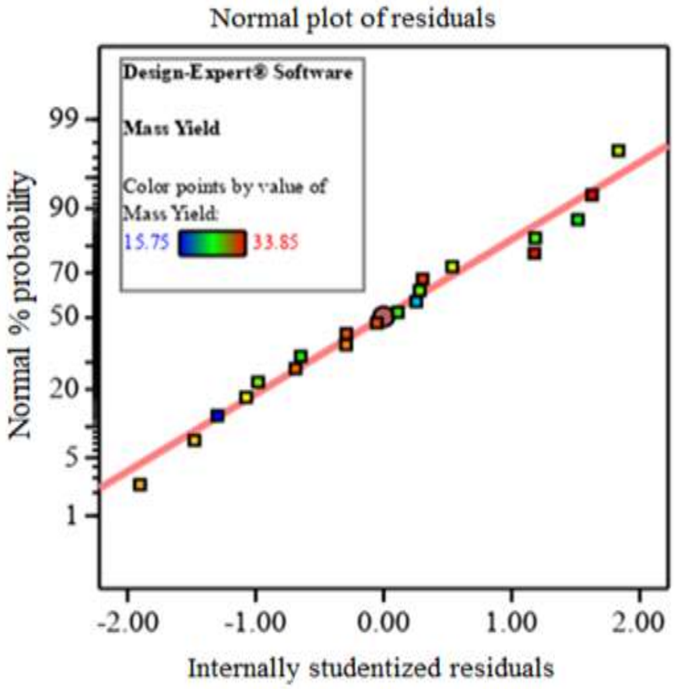


Figure 4.1 Normal probability plot

Normal probability plot of residuals supports the consistency between the experimental results and the ANOVA findings ($p < 0.05$, $R^2 = 97.71\%$) in Figure 4.1. Liquid biofuel yield values ranged from low (15.75%, shown in blue color) to high (33.85%, shown in red color). In other words, the graph points show slight deviations from the red line, particularly at the extremes, indicating minor departures from normality. However, these variations are not significant. Therefore, the ANOVA model was appropriate for fitting the experimental data, as the internally studentized residuals closely align with the normal probability plot, confirming the data's consistency with the model.

A simple analysis of the fit between experimental data and the software's predictions can be performed using the predicted vs. actual plot in Figure 4.2.

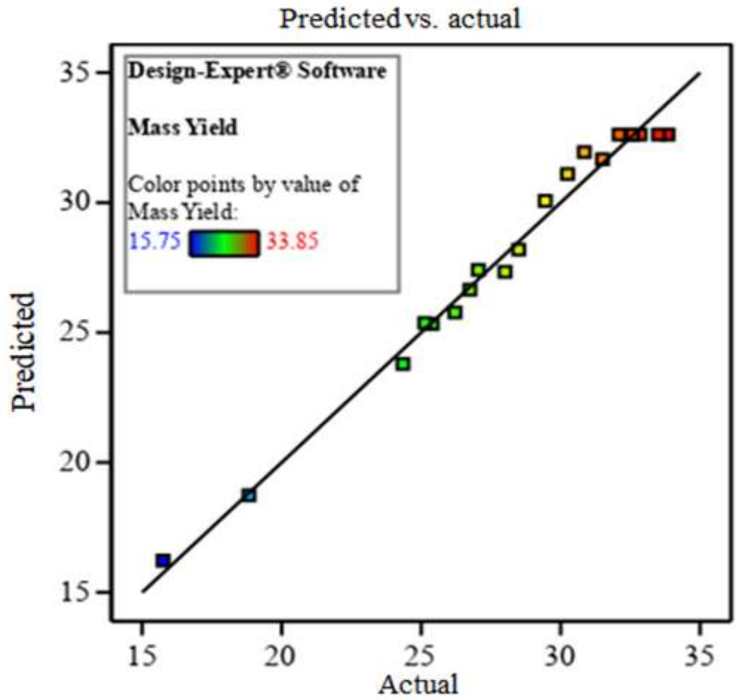


Figure 4.2 Predicted vs. actual plot

By comparing the ANOVA predicted plot versus the actual one, the experimental value can identify any consistent errors in the data. The x-axis values represent the actual or experimental results, while the y-axis values are the predictions derived from the software's equation. The results from the ANOVA predicted plot closely match the experimental value, confirming the accuracy of the model.

Based on the graph, most experimental data fall within the expected plot line, indicating an acceptable error of 2.29%. It suggests that the experimental work aligns with the ANOVA analysis model ($p < 0.05$) and that the model is well-designed. As the points closely follow the 45-degree line, the model depicts superb performance in predicting the response (liquid biofuel yield).

4.2.4. The Interaction Effect of Parameters

4.2.4.1. The effect of temperature and particle size on yield

The interaction effect of temperature and particle size on liquid biofuel yield is depicted in a 3D surface plot below.

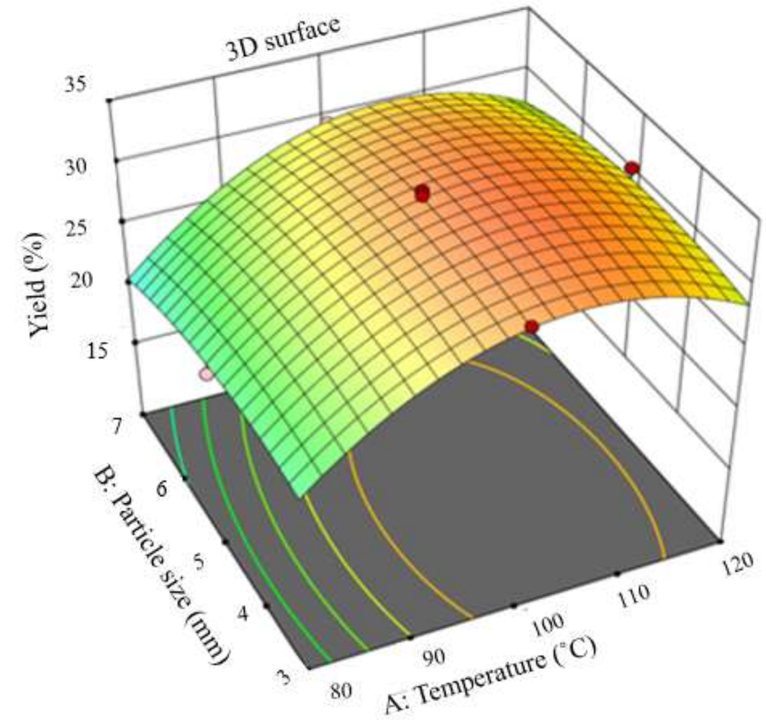


Figure 4.3 Effects of temperature and particle size on liquid biofuel

The 3D plot reveals smaller particle sizes in the middle and higher biofuel yield (yellow-red) at elevated temperatures. As temperatures decrease, yield declines (blue-green), likely due to incomplete thermal conversion and limited heat transfer, along with the growth of larger particles, as shown in Figure 4.3.

The liquid biofuel yield is governed by the complex interaction between temperature and particle size, with the maximum yield zone represented by the orange-red curve. The red data points aligning with the surface confirm the simulation's accuracy, while the yellow-green areas indicate lower value yields.

4.2.4.2. The effect of temperature and extraction time on yield

The interaction of temperature and time significantly influences liquid biofuel production, where higher temperatures and extraction times enhance yield, but excessive levels can lead to degradation and reduced efficiency [25].

The interaction effect of temperature and extraction time on the liquid biofuel yield of EGL conversion is depicted in the 3D surface plot in the Figure 4.4.

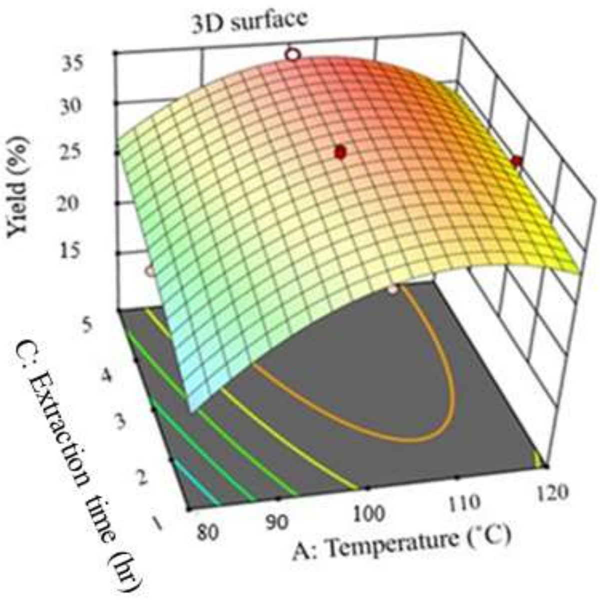


Figure 4.4 Effects of temperature and extraction time on liquid biofuel

The 3D plot displays the maximum liquid biofuel yield, indicated by yellow-red hues, at temperatures between 100 and 120 °C and extraction times of 3 to 5 hours. Conversely, blue-green indicates low yield at lower temperatures (80–100 °C) with shorter times (1–3 hours) due to the conditions are insufficient for breaking down lignocellulosic components like cellulose and lignin. This results in incomplete thermal conversion, poor depolymerisation, and low volatile release, ultimately leading to reduced biofuel yield. Validating the yield improves alongside temperature, especially once accompanied by extended extraction times [78]; the 3D lines, which reveal equivalent yield values, facilitate conceptualizing the response surface.

Increases in both parameters beyond the optimum range have a detrimental effect on yield. In the other hand, exceeding the optimal temperature or extraction time reduces liquid biofuel yield due to thermal degradation of key compounds [25], [34]. Additionally, higher energy input at extreme conditions lowers the overall efficiency of the process.

4.2.4.3. The effect of particle size and extraction time on yield

The interaction effect of extraction time and particle size on the yield conversion is depicted in the Figure 4.5.

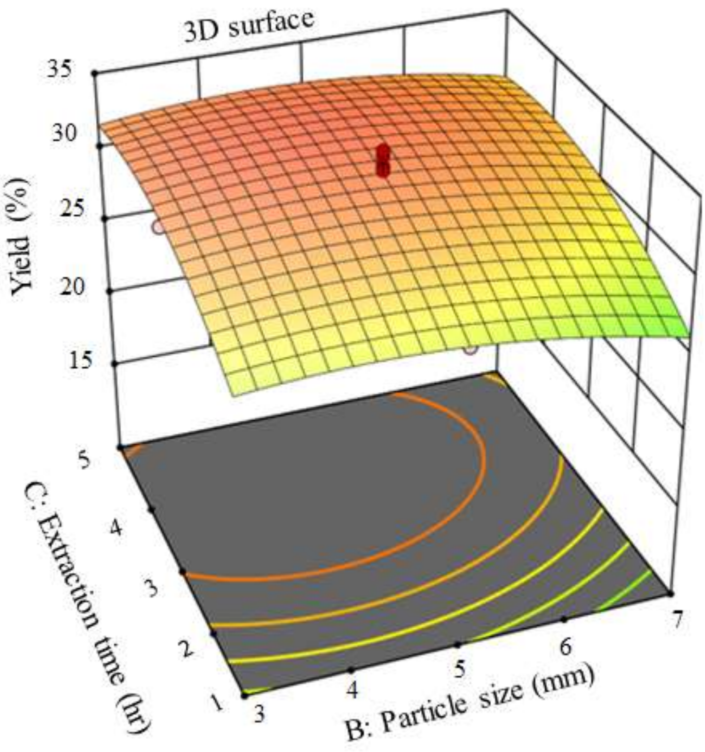


Figure 4.5 The effect of particle size and extraction time on liquid biofuel

The 3D surface plot clearly illustrates that particle size increased; the 3D surface dips downward, confirming that smaller particle sizes (3-5 mm) lead to higher liquid biofuel yield. This trend reinforces the inverse relationship between particle size and yield. It exhibits an expanding tendency as extraction time (1-5 hr) increases, signaling improved yields, particularly when combined with lower particle sizes (3-6 mm). When particle size is small, this interaction implies that prolonged extraction time intervals are more effective at boosting yield [75]. Thus, the best scenarios for optimizing yield within the range of interest are limited to optimum particles and extraction times [95].

4.2.5. Optimization of Hydro-Distillation Parameters Using RSM

The optimum liquid biofuel yield at the optimum temperature, time, and particle size was found using numerical optimization. The maximum desirability produces the most liquid biofuel for each of the three changed parameters under optimal conditions with the hydro-distillation process.

Table 4.6 Constraints for optimization of liquid biofuel yield

Name	**Goal**	**Lower Limit**	**Upper Limit**	**Lower Weight**	**Upper Weight**	**Importance**
A: Temperature	is in range	80	120	1	1	3
B: Particle size	is in range	3	7	1	1	3
C: Extraction time	is in range	1	5	1	1	3
Yield	maximize	15.75	33.85	1	1	3

Comparing the results with previous studies, along with plausible reasons for the significant difference the essential oil yield of 2.56% w/w or 33.85 mL is remarkably related values reported in previous studies on Eucalyptus species using hydro-distillation, where typical yields range between 0.5% and 2.5% w/w, depending on the species, extraction conditions, and plant material quality, product quality, experimental and human error [98].

All variables are given equal weights and relevance in the optimization, ensuring a complete strategy. When yield is maximized, certain ranges of temperature, particle size, and extraction time are maintained. Every factor is emphasized over others because each element has a weight of 1 and a significance value of 3. This develops a realistic, independent model that optimizes yield while preserving process parameters. The CCD model's optimal solution, shown in Table 4.7

Table 4.7 Optimized solution of liquid biofuel yield

Number	**Temperature**	**Particle size**	**Residence time**	**yield**	**Desirability**	
1	104.713	4.450	3.705	33.218	0.965	Selected

D values vary from 0 to 1. The high composite desirability score of 0.965 (≈1) demonstrates excellent alignment with the target response, confirming the effectiveness of the optimization to identify ideal conditions at 104.71°C, 4.45 mm, and 3.71 hours, achieving an optimum liquid biofuel yield of 33.22ml.

4.2.5.1. Validation of yield using optimized parameters

An experiment was carried out to confirm the conditions under which the CCD software. The liquid biofuel yield obtained from the hydro-distillation processes was 33.85ml at 100°C, 5 mm, and 3 hours. It was almost identical to the expected value of 33.22 ml with Design Expert ®13. The difference between the experimental and predicted values was just 0.632%. Therefore, it can be concluded that the RSM is a good indicator of how much liquid biofuel was generated using hydro-distillation.

Using the optimized parameter evaluated the predictive value of CCD to conduct experiment three times; the validation value is 30.5ml, which shows a difference of 2.718ml, which is a small difference, indicating that the prediction is accurate. The validation value describes that the model closely reflects the true behavior, with minor differences due to measurement or model limitations.

4.3. Characteristics of Liquid Biofuel Yield

Liquid biofuels differ from heavy petroleum fuel oil in the following undesired ways: high ash, water, and oxygen content (poor heating value), as well as high corrosiveness (acidity) [85]. The current study identified the liquid biofuel properties synthesized from EGL. The fuel was tested for color, density, kinematic viscosity, acid value, boiling point, flash point, and heating value. The investigation of results was contrasted with the ASTM standard value.

4.3.1. Visualization of Color

The liquid biofuel made from eucalyptus usually has a light yellow to amber appearance, which is acceptable and complies with ASTM D-6751 criteria, and floats on water, suggesting that it is less dense than water. It has the potential to be a sustainable fuel due to its color, which indicates the presence of natural components, and its clear separation. Although quality cannot be determined only by color, considerable staining could be an indicator of contaminants or deterioration. Visual inspection provides a rapid, non-instrumental way to identify problems like cloudiness, phase separation, or unexpected hue, but it cannot replace laboratory investigation.

4.3.2. Density

In the literature, the mango seed kernel and tamarind seed bio-oil densities were 0.7788 g/cm³ and 0.8011 g/cm³, respectively [99]. The temperature fluctuated between 25°C and 80°C, and the bio-oil density changed from 1.251 to 1.215 g/mL [100]. The ASTM D-4052 standard claims that liquid biofuels exhibit densities within 0.7 and 1.5 g/cm³ at temperatures from 15 to 20 °C [101].

In the current study, the density of liquid biofuel produced by hydro-distillation at 80–120°C, 1–5 hours, and 3–7 mm was computed using equation 3.5, which ranged from 0.765 to 0.895 g/ml at room temperature and pressure. The result here aligns with the assessment [99] and agree with the ASTM D-4052 standard. Stated that it is appropriate for use as fuel.

4.3.3. Viscosity

Bio-oil often has a higher viscosity than other liquid fuels, which predicts a higher internal flow resistance. Water content, oxygenated compounds, and heavier molecules are created during hydro-distillation [102]. The liquid biofuels' kinematic viscosity was affected by temperature, time, and particle size. According to published research, the kinematic viscosities of bio-oil derived from tamarind and mango seed kernels are 2.02 and 1.74 cSt, respectively [99].

In the current study, the viscosity of the produced biofuel ranged from 1.34 to 2.35 mm²/s at room temperature and pressure was computed using equation 3.6, aligning with these previous findings and supporting good vaporization and combustion properties [99]; however, this range slightly deviates from the expected ASTM D-445 standard. Nevertheless, high temperatures (around 40°C) can offset the impact of higher viscosity by improving combustion, atomization, and pumpability. The relatively low viscosity of the bio-oil also makes it easier to handle, transport, and blend with diesel or other biofuels [87].

Past studies have shown that temperature and time influence viscosity more than particle size, with temperature having the stronger inverse effect [86]. Viscosity was limited significantly with higher temperatures (80–120°C) and longer times (1–5 hours), while larger particle sizes increase it due to reduced flow. Optimizing these parameters is essential for better fuel performance.

4.3.4. Heating Value

A higher calorific value fuel is preferable for its greater heat release and improved engine performance [23]. In this study, the higher heating value (HHV) of the generated liquid biofuel and raw material was 37.54 and 20.63 MJ/kg, respectively, which was computed using equation 3.7. It was a high-quality fuel with energy content comparable to rapeseed bio-oil (33.12 MJ/kg) but lower than diesel (42 MJ/kg) [30], meeting ASTM standards. Conclude that liquid biofuel shows greater potential as an excellent sustainable fuel alternative; still, it lacks a slight diesel's energy content.

This HHV indicates a successful conversion method that produced a liquid biofuel with increased energy density by reducing the amount of oxygen and moisture. The HHV of raw EGLs (20.63 MJ/kg) was slightly different from that of Eucalyptus (21.3 MJ/kg) [60] due to plant species, climate conditions, measurement method, and ranged between the HHVs of woody and agricultural residues (17–21 MJ/kg) [45].

4.3.5. Mass Yield, Energy Densification Ratio, and Energy Yield

In this study, mass yield, energy densification ratio, and energy yield were 2.56 %, 1.82, and 46.6%, respectively, based on Equations 3.8–3.10.

The energy densification ratio (1.81) shows the biofuel is significantly more energy-dense than the original biomass, outperforming reported values for agricultural residues (1.22) [33], confirming the process's efficiency due to plant types and methods. The energy yield of 46.6% indicates efficient energy recovery, falling within the typical range of 37.1–72% for agricultural residuals [33]. The strong energy yield shows effective energy conversion, indicating the method's potential to produce high-performance, energy-efficient biofuels for practical use.

4.3.6. Acid Value of Fuel

According to ASTM D-94, the acid value (AV) of liquid biofuels derived from biomass is the amount of free fatty acids or other acidic components. It is the amount of potassium hydroxide (mgKOH) required to eliminate fatty acids from one gram of biofuel by neutralizing its acids [88].

In this study, the liquid biofuel sample has an acid value based on equation 3.11 between 1.24 and 1.82 mg KOH/g, showing mild acidity, low corrosiveness, and decent fuel stability. However, the acid value exceeds the ideal 1.0 mg KOH/g threshold set by ASTM D-974 for highly refined oils, indicating the need for further refinement to meet high-quality standards.

4.3.7. Flash Point Value

The flash point, determined at standard atmospheric pressure and room temperature according to ASTM D-93, represents the lowest temperature at which a fuel ignites when exposed to a flame or spark. It is a key indicator of flammability hazards, with a low flash point indicating higher volatility and a high flash point suggesting lower volatility [23]. Liquid biofuels with a high flash point are safer to handle and store, as they are less likely to ignite. This also reduces vapor emissions, improves engine thermal stability, and simplifies transportation by classifying the fuel as non-hazardous [30].

In this study, the flash point of the liquid biofuel derived from Eucalyptus was found to be 132 °C, which is consistent with values reported in the literature. For comparison, conventional biodiesel typically has a flash point of around 130 °C [103]. This indicates that the eucalyptus-based biofuel meets the ASTM D93 standard, which requires a minimum flash point of 40 °C. Therefore, the biofuel is considered safe for handling and storage and is suitable for use.

4.3.8. Boiling Point Value

The boiling point is an important variable for evaluating the thermal stability and volatility of liquid biofuels. Based on ASTM D-86, liquid biofuel should have a boiling point lower than 350 °C to ensure safe and efficient burning. In the current study, liquid biofuel produced from EGL using hydro-distillation showed a boiling point of 217.8 °C. This aligns with the reported biodiesel boiling point of 302°C [103] and falls within the acceptable ASTM range, confirming its suitability for fuel applications. The existence of medium-weight molecules, analogous to those present in light diesel fractions, is suggested by this moderate volatility.

The liquid biofuel is more volatile due to its lower boiling point, which might enhance combustion but may also call for cautious storage to minimize evaporation losses, handling, and combustion properties.

Table 4.8 The characteristics of the produced liquid biofuel

No.	Characteristics	Experimental values	ASTM standard
1	Color	Pale Yellow	Pale Yellow
2	Density(g/ml)	0.765-0.895	0.7-1.5
3	Viscosity (mm^2/s)	1.34-2.35	2-6
4	HHV (MJ/kg)	37.54	37-40
5	Mass yield (%)	2.56	Reported [97]
6	Energy density ratio	1.82	Reported [33]
7	Energy yield (%)	46.6	Reported [33]
8	Acid value (mg KOH/g)	1.24-1.84	< 1
9	Flash Point (°C)	132	≥ 40
10	Boiling Point (°C)	217.8	< 350

4.3.9. Analysis of Functional Group

FTIR analysis confirmed the presence of alcohols, aliphatic, phenols, amines, carbonyls, aromatics, hydrocarbons, and oxygenated compounds like acids and aldehydes. Aging at 100°C for 3 hours and 5 mm exposure caused slight wavenumber shifts, indicating chemical changes involving ethyl acetate. Upgrading processes are essential to improve the suitability of liquid biofuel, as its diverse functional groups impact fuel performance. Alcohols, ketones, and carboxylic acids can cause stability issues, acidity, and reduced heating value, while terpenes, ethers, and aromatics enhance energy content and volatility [22].

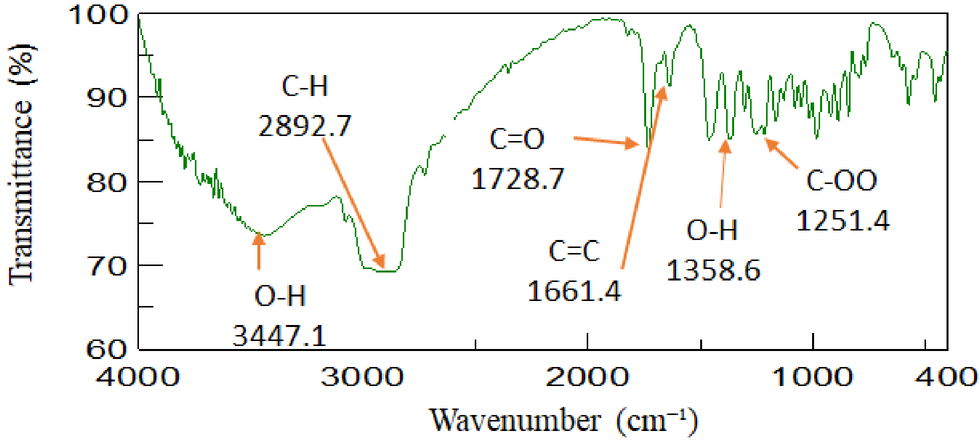


Figure 4.6 FTIR analysis for liquid biofuel product

The O–H stretching band observed between 3275–3490 cm^{-1}, with a peak at 3447.1 cm^{-1}, indicates the presence of hydroxyl groups in EGL bio-oil, likely from compounds like 1,8-cineole and α-terpineol. This aligns with literature, showing a similar but slightly shifted range from 3200-3600 cm^{-1} for methanol from EGL [90]. They used to enhance lubrication and combustion but lower energy density, lower volatility, raise acidity, and water content. In fuel blend may also cause phase separation [23].

The C–H stretching bands, indicating the presence of aliphatic groups, appear at 2850–2980 cm^{-1} with a peak at 2892.7 cm^{-1}. These fall within the literature ranges of 2800–3000 cm^{-1}, confirming consistency with reported C–H stretching vibrations [99]. It is used to boost energy content, but significantly affects emissions and fuel economy; therefore, this analysis is crucial to assess the fuel's long-term suitability [18].

The C=O stretching bands, indicative of oxygenated compounds such as carboxylic acids, ketones, esters, and aldehydes (e.g., carvone, camphor), were detected between 1665 and 1735 cm^{-1}, with a peak at 1728.7 cm^{-1}. This is consistent with previous studies, which report a C=O stretching range of 1650–1800 cm^{-1}, with a peak at 1728 cm^{-1} [90]. It has moderate stability and energy content but poor ignition quality, leading to increased carbon deposition. Its high acidity and corrosiveness can degrade engine materials, reduce combustion efficiency, and damage fuel systems. However, it enhances oxidation stability and combustion properties. It is useful in biodiesel applications, despite potentially increasing viscosity and causing incomplete combustion [30].

The C=C stretching bonds, indicating the presence of alkenes and aromatic compounds such as limonene, α-pinene, and β-pinene, show an absorbance range from 1625 to 1680 cm^{-1}, with a peak at 1661.4 cm^{-1}. It deviates slightly from the previous study's reported C=C stretching range of 1675–1600 cm^{1}. They contain excellent combustion qualities and high energy content; however, instability is due to polymerization [74].

The antisymmetrical C–O–O stretching band observed at 1251.4 cm^{-1} slightly deviates from the past study range of 1175–1280 cm^{-1}, though it still falls within the broader 1200–1300 cm^{-1} range, with a reference peak at 1286 cm^{-1} [90]. The O–H bending bond at 1358.6 cm^{-1} lies within the 1355–1455 cm^{-1} range and matches the previously reported 1250–1400 cm^{-1} range [32], indicating the presence of primary or secondary alcohols, esters, or ethers (e.g., cineole), which enhance volatility and improve ignition properties. Although these compounds enhance combustion, their high oxygen content can lead to stability issues [23].

Table 4.9 Common functional groups and their FTIR wavelengths

Functional Group	**Wavelength (Cm^{-1})**			**Compound**	**Reference**
	Present work range	peak	Previous work		
O-H stretching	3275–3490	3447.1	3200–3600	Alcohols, phenols, terpineol	[90]
C-H bending	2850–2980	2892.7	2800–3000	Aliphatic, Alkanes,	[74]
C =O stretching	1665–1740	1728.7	1650–1800	Ketones, aldehydes, carboxylic acid, esters	[90]
C = C stretching	1625–1680	1661.4	1675–1600	Aromatic compound	[74]
C-OO stretching	1175–1280	1251.4	1300-1200	Ether(1,8-cineole), alcohols, ester	[90]

Generally, functional group analysis was conducted to assess the chemical structure of the biofuel, which influences its combustion behavior, stability, and environmental performance. Key functional groups identified included O–H stretching (alcohols, phenols), C–H bending (alkanes), C=O stretching (carbonyl compounds), C=C stretching (aromatic compounds), and C–O stretching (alcohols, ethers, esters, carboxylic acids). These functional groups play a critical role in regulating important properties such as volatility, emissions, and burning efficiency, thereby enabling the use of the biofuel in domestic heating systems like boilers, stoves, and heaters.

5. CONCLUSION AND RECOMMENDATION

5.1. Conclusion

Based on the study, Eucalyptus globulus leaves (EGL) showed high volatile matter (76.5 ± 2%) and moderate fixed carbon (12.98%), suitable for biofuel conversion. Moisture content (9.47 ± 0.75%) may require drying, while low ash (1.05 ± 0.1%) minimizes slagging risks.

Using Response Surface Methodology (RSM) and Central Composite Design (CCD) in Design Expert® 13, optimal hydro-distillation conditions were identified. Yield increased with moderate temperature and time, but declined above 120 °C and 5 hr due to degradation. Larger particle sizes (6–7 mm) reduced yield. Temperature had the greatest effect on output. Proper control of these parameters enhances yield and supports sustainable biofuel production.

ANOVA results from the CCD model confirmed its statistical significance with a p-value < 0.0001. The high R^2 value of 0.9771 indicated that 97.71% of the variability in liquid biofuel yield was explained by the model, and 2.29% of the total variation was not explained by the model. The highest experimental yield was 33.85ml at 100°C, 5 mm, and 3 hours. The model predicted an optimum yield of 33.22ml at 104.71°C, 4.45 mm, and 3.71 hours, with a validation yield of 30.5 ml, emphasizing the value of process optimization.

The analysis confirmed that liquid biofuel derived from Eucalyptus globulus leaves possesses favorable physicochemical properties for household. Key performance metrics, such as a heating value of 37.54 MJ/kg, mass yield 2.56%, energy density ratio of 1.82, energy yield of 46.6, and flash point of 132 °C, indicate strong potential for safe and efficient use. Additionally, the viscosity range (1.34–2.35 mm²/s) and density (0.765–0.889 g/mL) fall within acceptable limits for biofuels, while the acid value (1.24–182 mg KOH/g) and boiling point (217.5 °C) reflect chemical characteristics influenced by production conditions. Overall, the biofuel meets ASTM standards and demonstrates promising viability as a renewable household energy source. Functional group analysis identified O–H, C–H, C=O, C=C, and C–O bonds, which influence combustion, volatility, and emissions, supporting the biofuel's suitability for domestic heating applications like stoves, heaters, and boilers.

5.2. Recommendation

The current study suggests the following recommendations based on our investigation for future work:

- ❖ The effects of temperature, particle size, and extraction time were examined; these factors are important for biomass energy enhancement; however, other important factors that impact the biomass's energy density include solvent type, feedstock-to-water ratio, climate condition, seasonal variation, and types of plant parts asses for the future work.
- ❖ To ascertain the elemental contents in the related biofuels product qualities, to determine liquid biofuels attributes for rural areas, the produced liquid biofuel was used to identify the overall physicochemical properties. Additionally, pour point, acetone number. Octane number, oxidation stability, and sulfur content for transportation applications should be conducted.
- ❖ To obtain a more comprehensive understanding of the chemical composition and physical properties of the developed product, it is recommended to utilize an additional characterization technique alongside FTIR analysis. The use of various analytical methods can provide a more complete picture of the product's characteristics and help in identifying any potential issues or areas for improvement. Therefore, it is advisable to explore other characterization methods such as Nuclear Magnetic Resonance Spectroscopy, Gas Chromatography–Mass Spectrometry, or X-ray Diffraction to complement the FTIR analysis and gain a more thorough understanding of the product.

REFERENCES

[1] B. Shehu, "Exploring Locational Criteria to Optimise Biofuel Production Potential in Nigeria," *University of Nottingham*.

[2] S. Hameer and N. Ejigu, "A prospective review of renewable energy developments in Ethiopia [version 1; peer review: 1 approved, 1 approved with," 2020.

[3] H. Chen, X. Wang, and Q. Wang, "Microalgal biofuels in China: The past, progress and prospects," *Gcb Bioenergy*, vol. 12, no. 12, pp. 1044–1065, 2020.

[4] A. P. Ingle, P. Ingle, I. Gupta, and M. Rai, "Socioeconomic impacts of biofuel production from lignocellulosic biomass," in *Sustainable bioenergy*, Elsevier, 2019, pp. 347–366.

[5] J. Costa, J. S. Silva, E. Deus, S. Pinho, J. F. Pinto, and N. Borralho, "The Genetics and Ecology of Post-Fire Eucalyptus globulus Recruitment in an Isolated Stand in Central Portugal," *Forests*, vol. 13, no. 5, 2022, doi: 10.3390/f13050680.

[6] D. M. Sandoval López, M. F. Arturi, J. F. Goya, C. A. Pérez, and J. L. Frangi, "Eucalyptus grandis plantations: effects of management on soil carbon, nutrient contents and yields," *J. For. Res.*, vol. 31, no. 2, pp. 601–611, 2020.

[7] M. F. Kedir, T. Bekele, and S. Feleke, "Problems of Mirt, and potentials of improved Gonzie and traditional open cook stoves in biomass consumption and end use emission in rural wooden houses of Southern Ethiopia," *Sci. African*, vol. 3, p. e00064, 2019.

[8] R. K. Singh, A. Sarkar, and J. P. Chakraborty, "Effect of torrefaction on the physicochemical properties of eucalyptus derived biofuels: estimation of kinetic parameters and optimizing torrefaction using response surface methodology (RSM)," *Energy*, vol. 198, p. 117369, 2020, doi: 10.1016/j.energy.2020.117369.

[9] G. B. Mosisa, N. Tassie, and M. Adula, "Current and future distribution of Eucalyptus globulus under changing climate in Ethiopia: implications for forest management," *Environ. Syst. Res.*, vol. 13, no. 1, 2024, doi: 10.1186/s40068-

024-00332-z.

[10] D. Jaleta, B. Mbilinyi, H. Mahoo, and M. Lemenih, "Eucalyptus expansion as relieving and provocative tree in Ethiopia," *J. Agric. Ecol. Res. Int.*, vol. 6, no. 3, pp. 1–12, 2016.

[11] B. Derba, "Socio-Economic Importance of Selected Indigenous and Exotic Tree Species Used in Dabat District, North Gonder, Ethiopia," 2016, *Addis Ababa University*.

[12] Y. Dahman, K. Syed, S. Begum, P. Roy, and B. Mohtasebi, "Biofuels: Their characteristics and analysis," in *Biomass, biopolymer-based materials, and bioenergy*, Elsevier, 2019, pp. 277–325.

[13] S. Prasad and A. P. Ingle, "Impacts of sustainable biofuels production from biomass," in *Sustainable bioenergy*, Elsevier, 2019, pp. 327–346.

[14] M. K. Shahid *et al.*, "Biofuels and biorefineries: Development, application and future perspectives emphasizing the environmental and economic aspects," *J. Environ. Manage.*, vol. 297, p. 113268, 2021.

[15] S. Ponce *et al.*, "Proposal of a regulatory framework for bioenergy implementation in a unified agricultural code for Ecuador," *Biofuels, Bioprod. biorefining*, vol. 16, no. 4, pp. 1116–1129, 2022.

[16] T. G. Ambaye, M. Vaccari, A. Bonilla-Petriciolet, S. Prasad, E. D. van Hullebusch, and S. Rtimi, "Emerging technologies for biofuel production: A critical review on recent progress, challenges and perspectives," *J. Environ. Manage.*, vol. 290, p. 112627, 2021.

[17] A. M. Minas, S. Mander, and C. McLachlan, "How can we engage farmers in bioenergy development? Building a social innovation strategy for rice straw bioenergy in the Philippines and Vietnam," *Energy Res. Soc. Sci.*, vol. 70, p. 101717, 2020.

[18] H. K. Jeswani, A. Chilvers, and A. Azapagic, "Environmental sustainability of biofuels : a review," 2020.

[19] S. K. Bhatia, S.-H. Kim, J.-J. Yoon, and Y.-H. Yang, "Current status and strategies for second generation biofuel production using microbial systems,"

Energy Convers. Manag., vol. 148, pp. 1142–1156, 2017.

[20] N. Hajilary, M. Rezakazemi, and S. Shirazian, "Biofuel types and membrane separation," *Environ. Chem. Lett.*, vol. 17, no. 1, 2019, doi: 10.1007/s10311-018-0777-9.

[21] A. Taghipour, J. A. Ramirez, R. J. Brown, and T. J. Rainey, "A review of fractional distillation to improve hydrothermal liquefaction biocrude characteristics; future outlook and prospects," *Renew. Sustain. Energy Rev.*, vol. 115, p. 109355, 2019.

[22] K. Jacobson, K. C. Maheria, and A. Kumar Dalai, "Bio-oil valorization: A review," *Renew. Sustain. Energy Rev.*, vol. 23, pp. 91–106, 2013, doi: 10.1016/j.rser.2013.02.036.

[23] S. Sani, M. U. Kaisan, D. M. Kulla, A. I. Obi, A. Jibrin, and B. Ashok, "Determination of physico chemical properties of biodiesel from Citrullus lanatus seeds oil and diesel blends," *Ind. Crops Prod.*, vol. 122, no. May, pp. 702–708, 2018, doi: 10.1016/j.indcrop.2018.06.002.

[24] B. Remenyik, V. László, D. Lóránt, and V. Imre, "Liquid biofuels: sustainable development analysis," *Eurasian J. Econ. Bus. Stud.*, vol. 58, no. 4, pp. 5–25, 2020.

[25] E. S. G. Khater, S. A. AbdAlla, A. H. Bahnasawy, and H. M. AbuHashish, "Improvement of the production of bio-oil and biodiesel from Egyptian Jatropha seeds by using microwave and ultrasonic," *Sci. Rep.*, vol. 14, no. 1, pp. 1–11, 2024, doi: 10.1038/s41598-024-51579-6.

[26] J. Skeer, F. Boshell, and M. Ayuso, "Technology innovation outlook for advanced liquid biofuels in transport," *ACS Energy Lett.*, vol. 1, no. 4, pp. 724–725, 2016.

[27] B. T. Teferra, "Designing and planning of Ethiopia's biomass-to-biofuel (bioethanol and biodiesel) supply chain through integrated strategic-tactical optimization model under economic and environmental dimensions," 2022.

[28] M. G. Bidir, N. K. Millerjothi, M. S. Adaramola, F. Y. Hagos, and R. C. Singh, "Assessment of Biofuel Resource Potential, Prospects, Challenges and

Utilization in Ethiopia: Sourcing Strategies for Renewable Energies-A Review,” in *IOP Conference Series: Materials Science and Engineering*, IOP Publishing, 2021, p. 12003.

[29] R. Manimaran, K. Murugu Mohan Kumar, and N. Sathiya Narayanan, “Synthesis of bio-oil from waste Trichosanthes cucumerina seeds: a substitute for conventional fuel,” *Sci. Rep.*, vol. 10, no. 1, pp. 1–12, 2020, doi: 10.1038/s41598-020-74130-9.

[30] C. Ong’era, B. Gathitu, S. Murunga, P. Kuloba, and J. Gathirwa, “Evaluation of flash point and calorific value of nanostructured rapeseed oil biodiesel as an automotive fuel,” *J. Agric. Sci. Technol.*, vol. 23, no. 1, pp. 115–124, 2023, doi: 10.4314/jagst.v23i1.8.

[31] H. Shukor, M. M. Z. Makhtar, and A. Z. Yaser, *Renewable Energy from Bio-resources in Malaysia*. Springer Nature, 2022.

[32] C. Robledo-Abad *et al.*, “Bioenergy production and sustainable development: science base for policymaking remains limited,” *Gcb Bioenergy*, vol. 9, no. 3, pp. 541–556, 2017.

[33] L. J. R. Nunes *et al.*, “Evaluation of the potential of agricultural waste recovery: Energy densification as a factor for residual biomass logistics optimization,” *Appl. Sci.*, vol. 11, no. 1, pp. 1–23, 2021, doi: 10.3390/app11010020.

[34] J. Shen, X. S. Wang, M. Garcia-Perez, D. Mourant, M. J. Rhodes, and C. Z. Li, “Effects of particle size on the fast pyrolysis of oil mallee woody biomass,” *Fuel*, vol. 88, no. 10, pp. 1810–1817, 2009, doi: 10.1016/j.fuel.2009.05.001.

[35] A. Tolessa, “Bioenergy potential from crop residue biomass resources in Ethiopia,” *Heliyon*, vol. 9, no. 2, 2023.

[36] A. Amsalu and S. Yesgat, “Effect of eucalyptus plantations on soil properties: The case of Entoto Area, Northern Addis Ababa, Ethiopia,” *J. Environ. Earth Sci.*, vol. 9, no. 6, pp. 49–62, 2019.

[37] A. Kaur and R. Monga, “Eucalyptus trees plantation: a review on suitability and their beneficial role,” *Int. J. Bio-resource Stress Manag.*, vol. 12, no. 1, pp.

16–25, 2021.

[38] J. M. Yost *et al.*, “Origins, diversity and naturalization of Eucalyptus globulus (Myrtaceae) in California,” *Forests*, vol. 12, no. 8, p. 1129, 2021.

[39] Surbhi, A. Kumar, S. Singh, P. Kumari, and P. Rasane, “Eucalyptus: phytochemical composition, extraction methods and food and medicinal applications,” *Adv. Tradit. Med.*, vol. 23, no. 2, pp. 369–380, 2023.

[40] R. Adolph, “REVIEW ON EUCALYPTUS: PHYTOCHEMICAL PROFILE, EXTRACTION TECHNIQUES, AND PHARMACOLOGICAL APPLICATIONS IN FOOD AND MEDICINE,” *Rev. EUCALYPTUS Phytochem. PROFILE, Extr. Tech. Pharmacol. Appl. FOOD Med.*, vol. 95, pp. 1–23, 2016.

[41] A. B. Madalcho, B. Lemma, M. M. Mena, and B. B. Badesso, “Is the expansion of eucalyptus tree a curse or an opportunity? Implications from a dispute on the trees ecological and economic impact in Ethiopia: a review,” *J Ecol Nat Env.*, vol. 11, pp. 75–83, 2019.

[42] D. Jaleta, B. Mbilinyi, H. Mahoo, and M. Lemenih, “Evaluation of land use/land cover changes and Eucalyptus expansion in Meja watershed, Ethiopia,” *J. Geogr. Environ. Earth Sci. Int.*, vol. 7, no. 3, pp. 1–12, 2016.

[43] A. M. Borrero-López, C. Valencia, and J. M. Franco, “Lignocellulosic materials for the production of biofuels, biochemicals and biomaterials and applications of lignocellulose-based polyurethanes: a review,” *Polymers (Basel).*, vol. 14, no. 5, p. 881, 2022.

[44] D. Haldar and M. K. Purkait, “A review on the environment-friendly emerging techniques for pretreatment of lignocellulosic biomass: Mechanistic insight and advancements,” *Chemosphere*, vol. 264, p. 128523, 2021.

[45] J. Yan *et al.*, “Characterizing variability in lignocellulosic biomass: a review,” *ACS Sustain. Chem. Eng.*, vol. 8, no. 22, pp. 8059–8085, 2020.

[46] S. Liu, “A synergetic pretreatment technology for woody biomass conversion,” *Appl. Energy*, vol. 144, pp. 114–128, 2015.

[47] W. Geng *et al.*, “The influence of lignin content and structure on hemicellulose

alkaline extraction for non-wood and hardwood lignocellulosic biomass," *Cellulose*, vol. 26, pp. 3219–3230, 2019.

[48] L. Dai *et al.*, "Recent advances in improving lignocellulosic biomass-based bio-oil production," *J. Anal. Appl. Pyrolysis*, vol. 149, p. 104845, 2020.

[49] S. Dell'Orco *et al.*, "Thermochemical biorefineries for the conversion of lignocellulosic biomass: improvements of fast pyrolysis and hydrothermal processes for fuels and chemicals precursors," 2021.

[50] T. I. Korányi, B. Fridrich, A. Pineda, and K. Barta, "Development of 'Lignin-First'approaches for the valorization of lignocellulosic biomass," *Molecules*, vol. 25, no. 12, p. 2815, 2020.

[51] M. Koubaa and Z. Maache-rezzoug, "Bioethanol Production from Woody Biomass : Recent Advances on the Effect of Pretreatments on the Bioconversion Process and Energy Yield Aspects," 2023.

[52] M. Pinto *et al.*, "Herbicidal effects and cellular targets of aqueous extracts from young Eucalyptus globulus Labill. leaves," *Plants*, vol. 10, no. 6, p. 1159, 2021.

[53] N. Z. Immaroh, D. E. Kuliahsari, and S. D. Nugraheni, "Eucalyptus globulus essential oil extraction method," in *IOP Conference Series: Earth and Environmental Science*, IOP Publishing, 2021, p. 12103.

[54] J. R. Ziolkowska, "Biofuels technologies: An overview of feedstocks, processes, and technologies," *Biofuels a more Sustain. Futur.*, pp. 1–19, 2020.

[55] N. Hajilary, M. Rezakazemi, and S. Shirazian, "Biofuel types and membrane separation," *Environ. Chem. Lett.*, vol. 17, pp. 1–18, 2019.

[56] H. D. Kumar and S. Laxmidhar, "ISSN 2229-3566 A REVIEW ON PHYTOCHEMICAL AND PHARMACOLOGICAL OF EUCALYPTUS GLOBULUS : A MULTIPURPOSE TREE Hardel Danendra kumar *, Sahoo Laxmidhar," *Int. J. Researxh Ayurveda Pharm.*, vol. 2, no. 5, pp. 1527–1530, 2011.

[57] P. Verma, M. P. Sharma, and G. Dwivedi, "Potential use of eucalyptus biodiesel in compressed ignition engine," *Egypt. J. Pet.*, vol. 25, no. 1, pp. 91–

95, 2016.

[58] A. Bychkov, E. Podgorbunskikh, E. Bychkova, and O. Lomovsky, “Current achievements in the mechanically pretreated conversion of plant biomass,” *Biotechnol. Bioeng.*, vol. 116, no. 5, pp. 1231–1244, 2019.

[59] A. Kassahun and G. Feleke, “Chemical composition and physico-chemical analysis of Eucalyptus globulus leave and oil,” *Sci. J. Chem.*, vol. 7, no. 2, pp. 36–38, 2019.

[60] S. Pang, “Advances in thermochemical conversion of woody biomass to energy, fuels and chemicals,” *Biotechnol. Adv.*, vol. 37, no. 4, pp. 589–597, 2019.

[61] D. G. Gomes, M. Michelin, A. Romaní, L. Domingues, and J. A. Teixeira, “Co-production of biofuels and value-added compounds from industrial Eucalyptus globulus bark residues using hydrothermal treatment,” *Fuel*, vol. 285, p. 119265, 2021.

[62] W.-C. Tu and J. P. Hallett, “Recent advances in the pretreatment of lignocellulosic biomass,” *Curr. Opin. green Sustain. Chem.*, vol. 20, pp. 11–17, 2019.

[63] H. Zentou, N. S. Rosli, C. H. Wen, K. Abdul Azeez, and C. Gomes, “The viability of biofuels in developing countries: Successes, failures, and challenges,” *Iran. J. Chem. Chem. Eng.*, vol. 38, no. 4, pp. 173–182, 2019.

[64] M. Usman, S. Cheng, and J. S. Cross, “Biomass feedstocks for liquid biofuels production in hawaii & tropical islands: A review,” *Int. J. Renew. Energy Dev.*, vol. 11, no. 1, pp. 111–132, 2022, doi: 10.14710/IJRED.2022.39285.

[65] A. Karapatsia, I. Pappas, G. Penloglou, O. Kotrotsiou, and C. Kiparissides, “Optimization of dilute acid pretreatment and enzymatic hydrolysis of Phalaris aquatica L. lignocellulosic biomass in batch and fed-batch processes,” *BioEnergy Res.*, vol. 10, pp. 225–236, 2017.

[66] N. A. Al-khirsan and A. M. Al-yaqoobi, “Extraction of essential oil from Eucalyptus leaves by combination of hydro-distillation and ultrasound-assisted extraction techniques,” in *AIP Conference Proceedings*, AIP Publishing, 2023.

[67] T. A. L. Silva, H. D. Z. Zamora, L. H. R. Varão, N. S. Prado, M. A. Baffi, and D. Pasquini, “Effect of steam explosion pretreatment catalysed by organic acid and alkali on chemical and structural properties and enzymatic hydrolysis of sugarcane bagasse,” *Waste and Biomass Valorization*, vol. 9, pp. 2191–2201, 2018.

[68] A. M. Mustafa, T. G. Poulsen, Y. Xia, and K. Sheng, “Combinations of fungal and milling pretreatments for enhancing rice straw biogas production during solid-state anaerobic digestion,” *Bioresour. Technol.*, vol. 224, pp. 174–182, 2017.

[69] P. Bajpai and V. Dash, “Hybrid renewable energy systems for power generation in stand-alone applications: A review,” *Renew. Sustain. Energy Rev.*, vol. 16, no. 5, pp. 2926–2939, 2012.

[70] M. A. Shaah *et al.*, “Candlenut oil: review on oil properties and future liquid biofuel prospects,” *Int. J. Energy Res.*, vol. 45, no. 12, pp. 17057–17079, 2021.

[71] P. Tan, P. Lindberg, K. Eichler, P. Löveryd, P. Johansson, and A. S. Kalagasidis, “Effect of phase separation and supercooling on the storage capacity in a commercial latent heat thermal energy storage: Experimental cycling of a salt hydrate PCM,” *J. Energy Storage*, vol. 29, p. 101266, 2020.

[72] P. Burger, H. Plainfossé, X. Brochet, F. Chemat, and X. Fernandez, “Extraction of natural fragrance ingredients: History overview and future trends,” *Chem. Biodivers.*, vol. 16, no. 10, p. e1900424, 2019.

[73] N. Z. Immaroh, D. E. Kuliahsari, and S. D. Nugraheni, “Review: Eucalyptus globulus essential oil extraction method,” *IOP Conf. Ser. Earth Environ. Sci.*, vol. 733, no. 1, 2021, doi: 10.1088/1755-1315/733/1/012103.

[74] E. S. Nelson, O. Sadare, A. Okewale, S. Iyuke, and M. Daramola, “Effect of biomass particle size on yield and composition of bio-oil produced from Empty Palm Fruit Bunch,” pp. 1–28, 2023.

[75] N. Promsampao *et al.*, “Bio-oil Production from Palm Kernel Cake Using Fast Pyrolysis Process Parameters in a Fluidized-Bed Reactor,” *Eng. Sci.*, pp. 1–13, 2024, doi: 10.30919/es1179.

[76] K. M. Abed and T. M. Naife, “Extraction of Essential Oil from Iraqi Eucalyptus Camadulensis Leaves by Water Distillation Methods,” *IOP Conf. Ser. Mater. Sci. Eng.*, vol. 454, no. 1, 2018, doi: 10.1088/1757-899X/454/1/012163.

[77] J. P. Diebold, “A Review of the Chemical and Physical Mechanisms of the Storage Stability of Fast Pyrolysis Bio-Oils,” *Nrel/Sr-570-27613*, no. January, p. 59, 2000.

[78] G. Ferrentino, S. Giampiccolo, K. Morozova, N. Haman, S. Spilimbergo, and M. Scampicchio, “Supercritical fluid extraction of oils from apple seeds: Process optimization, chemical characterization and comparison with a conventional solvent extraction,” *Innov. Food Sci. Emerg. Technol.*, vol. 64, p. 102428, 2020, doi: 10.1016/j.ifset.2020.102428.

[79] P. Moreira *et al.*, “Chemical Composition and Effect against Skin Alterations of Bioactive Extracts Obtained by the Hydrodistillation of Eucalyptus globulus Leaves,” *Pharmaceutics*, vol. 14, no. 3, 2022, doi: 10.3390/pharmaceutics14030561.

[80] S. Rezania *et al.*, “Different pretreatment technologies of lignocellulosic biomass for bioethanol production: An overview,” *Energy*, vol. 199, 2020, doi: 10.1016/j.energy.2020.117457.

[81] M. Jędrzejczyk, E. Soszka, M. Czapnik, A. M. Ruppert, and J. Grams, “Physical and chemical pretreatment of lignocellulosic biomass,” in *Second and third generation of feedstocks*, Elsevier, 2019, pp. 143–196.

[82] A. Arora, P. Nandal, J. Singh, and M. L. Verma, “Nanobiotechnological advancements in lignocellulosic biomass pretreatment,” *Mater. Sci. Energy Technol.*, vol. 3, pp. 308–318, 2020.

[83] M. F. Kedir, J. M. Onchieku, and C. J. Ntalikwa, “Developing circular economy in Eastern Africa through liquid biofuels: cases of Ethiopia, Kenya and Tanzania,” African Forest Forum (AFF), 2022.

[84] A. A. Kiss, J.-P. Lange, B. Schuur, D. W. F. Brilman, A. G. J. van der Ham, and S. R. A. Kersten, “Separation technology–Making a difference in biorefineries,” *Biomass and Bioenergy*, vol. 95, pp. 296–309, 2016.

[85] S. Xiu and A. Shahbazi, “Bio-oil production and upgrading research: A review,” *Renew. Sustain. Energy Rev.*, vol. 16, no. 7, pp. 4406–4414, 2012, doi: 10.1016/j.rser.2012.04.028.

[86] S. Thangalazhy-Gopakumar *et al.*, “Physiochemical properties of bio-oil produced at various temperatures from pine wood using an auger reactor,” *Bioresour. Technol.*, vol. 101, no. 21, pp. 8389–8395, 2010, doi: 10.1016/j.biortech.2010.05.040.

[87] D. Singh, D. Sharma, S. L. Soni, S. Sharma, and D. Kumari, “Chemical compositions, properties, and standards for different generation biodiesels: A review,” *Fuel*, vol. 253, no. May, pp. 60–71, 2019, doi: 10.1016/j.fuel.2019.04.174.

[88] L. K. E. Park, J. Liu, S. Yiacoumi, A. P. Borole, and C. Tsouris, “Contribution of acidic components to the total acid number (TAN) of bio-oil,” *Fuel*, vol. 200, pp. 171–181, 2017, doi: 10.1016/j.fuel.2017.03.022.

[89] R. A. Johnson and R. A. Johnson, “The Pennsylvania State University Earth and Mineral Sciences College of Energy and Mineral Engineering A COMPREHENSIVE EVALUATION AND DEVELOPMENT OF ALTERNATIVE BIODIESEL ANALYTICAL QUALITY TESTING METHODS A Thesis in Energy and Mineral Engineering by Sub,” no. May, 2011.

[90] O. Of, C. For, and B. Millet, “Plant Archives,” *GC-MS, FTIR NMR Anal. METHANOL Extr. EUCALYPTUS Globul.*, vol. 21, no. 1, pp. 1676–1680, 2021.

[91] H. Bousbaa, L. Tarabet, K. Naima, A. Liazid, and M. L. Tazerout, “Eucalyptus biofuel study as alternative for diesel engine,” *Int. J. Renew. Energy Technol.*, vol. 10, no. 3, pp. 247–281, 2019.

[92] P. I. Aigba, F. C. Anyadiegwu, and J. C. Ogoke, “Characterization of jatropha oil and its biodiesel,” *Adv. Environ. Stud.*, vol. 5, no. 1, pp. 376–381, 2021.

[93] A. Palamanit, P. Khongphakdi, Y. Tirawanichakul, and N. Phusunti, “Investigation of yields and qualities of pyrolysis products obtained from oil palm

biomass using an agitated bed pyrolysis reactor," *Biofuel Res. J.*, vol. 6, no. 4, pp. 1065–1079, 2019.

[94] K. A. Seyoum, B. M. Alehegn, and A. A. Abtew, "Effect of Eucalyptus Globulus Woodlot Plantation on Selected Soil Physico-Chemical Properties, Wheat Yield in Wogera District, Amhara Region, Ethiopia," *Am. J. Sci. Eng. Technol.*, vol. 12, no. 3, pp. 34–44, 2021.

[95] Z. Ma'Sum, A. Altway, and M. Mahfud, "Microwave assisted hydro-distillation (mhd) of citronella oil from lemongrass plants (cymbopogon nardus): Effect of distiller size on oil yield," *IOP Conf. Ser. Mater. Sci. Eng.*, vol. 1010, no. 1, 2021, doi: 10.1088/1757-899X/1010/1/012031.

[96] M. Barbanera, C. Pelosi, A. R. Taddei, and F. Cotana, "Optimization of bio-oil production from solid digestate by microwave-assisted liquefaction," *Energy Convers. Manag.*, vol. 171, no. June, pp. 1263–1272, 2018, doi: 10.1016/j.enconman.2018.06.066.

[97] A. Abbas *et al.*, "Hydro-Distilled and Supercritical Fluid Extraction of Eucalyptus camaldulensis Essential Oil : Characterization of Bioactives Along With Antioxidant , Antimicrobial and Antibio fi lm Activities," no. September, pp. 1–12, 2022, doi: 10.1177/15593258221125477.

[98] M. T. Jim, E. Lainez-cer, L. Aurelio, and N. Ramírez-corona, "Heliyon Effect of process variables on heating pro fi les and extraction mechanisms during hydrodistillation of eucalyptus essential oil," vol. 7, no. August, 2021, doi: 10.1016/j.heliyon.2021.e08234.

[99] M. M. Rana, M. M. Al-Mueed, M. N. Islam, and M. H. Ali, "Production and analysis of biodiesel from mango seed kernels and tamarind seeds using hybrid approach of solvent extraction and transesterification," *Results Eng.*, vol. 25, p. 103983, 2025.

[100] T. Tzanetakis, N. Ashgriz, D. F. James, and M. J. Thomson, "Liquid fuel properties of a hardwood-derived bio-oil fraction," *Energy and Fuels*, vol. 22, no. 4, pp. 2725–2733, 2008, doi: 10.1021/ef7007425.

[101] C. Ag-, B. Statements, and W. Pycnometer, "Standard Test Method for iTeh Standards iTeh Standards," vol. i, pp. 22–25, 2015, doi: 10.1520/D4052-22.2.

[102] S. Thangalazhy-Gopakumar *et al.*, “Physiochemical properties of bio-oil produced at various temperatures from pine wood using an auger reactor,” *Bioresour. Technol.*, vol. 101, no. 21, pp. 8389–8395, 2010.

[103] A. Álvarez, M. Lapuerta, and J. R. Agudelo, “Prediction of Flash-Point Temperature of Alcohol/Biodiesel/Diesel Fuel Blends,” *Ind. Eng. Chem. Res.*, vol. 58, no. 16, pp. 6860–6869, 2019, doi: 10.1021/acs.iecr.9b00843.

APPENDIXES

Appendix A: Tables

Proximate analysis of EGL was investigated at the Woldia University Institute of Technology's chemical engineering laboratory. All proximate analyses were calculated using the mathematical equations indicated in Chapter 3.

Proximate analysis for raw material (EGL)

Raw material properties	Final mass for each experimental run (g)			Characterization results of each Experimental run (%)			
	1	2	3	1	2	3	Average
MC	41.3	40.5	39.8	8.7	9.5	10.2	9.47± 0.75
VM	76.5	78.5	74.5	76.5	78.5	74.5	76.5 ± 2
AC	4.05	3.95	3.85	0.95	1.05	1.15	1.05 ± 0.1
FC	-	-	-	13.85	10.95	14.15	12.98 ± 1.55

Validation of optimum yield

parameters	Trails			Average yield (%)	
	1	2	3	experimental	predicted
Temperature (℃)	104.713	104.713	104.713	104.7	3.218
Particle size (mm)	4.450	4.450	4.450	4.45	3.218
Residence time (hr)	3.705	3.705	3.705	3.7	3.218
Yield (%)	30.75	31.55	29.25	30.5	3.218

Fit Summary

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.1100	0.0001	0.1767	-0.2145	
2FI	0.7991	< 0.0001	0.0597	-3.2759	
Quadratic	< 0.0001	0.0884	0.9564	0.8591	Suggested
Cubic	0.5757	0.0231	0.9523	-11.5364	Aliased

Select the highest order polynomial where the additional terms are significant and the model is not aliased.

Sequential Model Sum of Squares [Type I]

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Mean vs Total	15710.82	1	15710.82			
Linear vs Mean	154.80	3	51.60	2.92	0.0658	
2FI vs Linear	26.97	3	8.99	0.4575	0.7166	
Quadratic vs 2FI	249.71	3	83.24	145.50	< 0.0001	Suggested
Cubic vs Quadratic	2.59	4	0.6473	1.24	0.3868	Aliased
Residual	3.13	6	0.5220			
Total	16148.02	20	807.40			

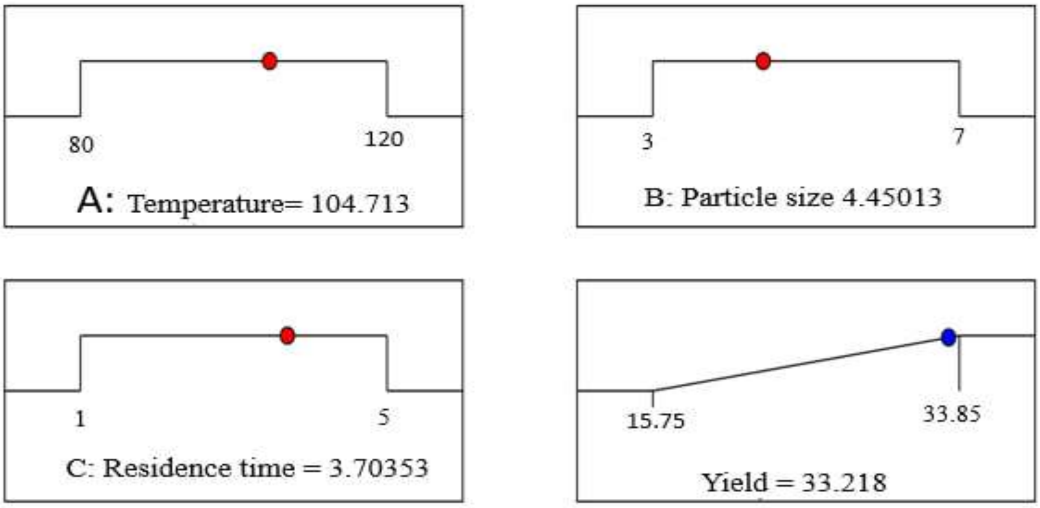
Select the highest order polynomial where the additional terms are significant and the model is not aliased.

Final Equation in Terms of Actual Factors

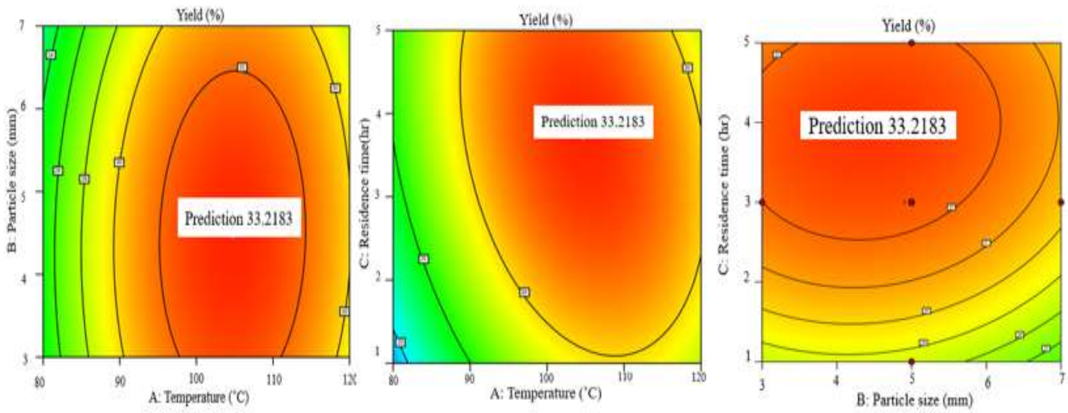
yields	=
-139.23798	
+2.93581	Temperature
+1.89143	Particle size
+7.84216	Residence time
+0.005500	Temperature * Particle size
-0.041875	Temperature *extraction time
+0.058750	Particle size *extraction time
-0.013393	Temperature^2
-0.301818	Particle size^2
-0.501818	Residence time^2

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

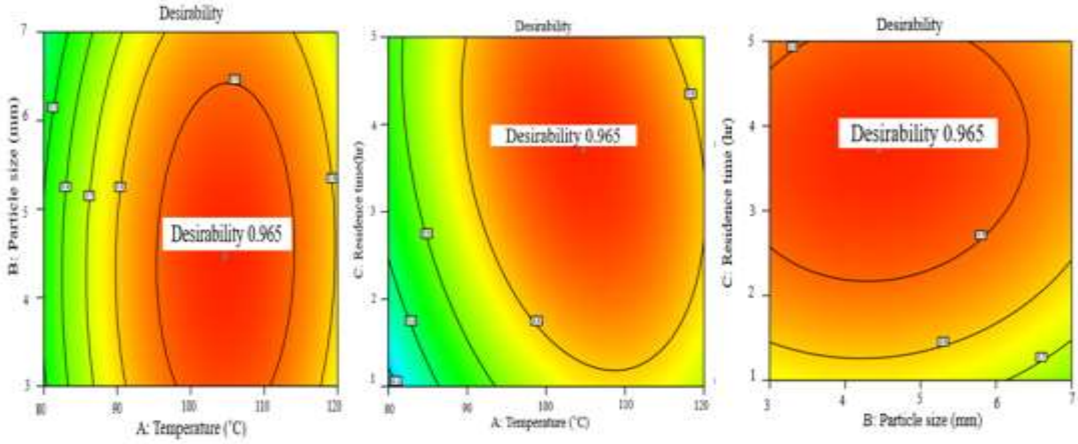
Appendix B: Graphs



Optimization constraints and solution



Yield prediction



Desirability

Appendix C: Laboratory work pictures

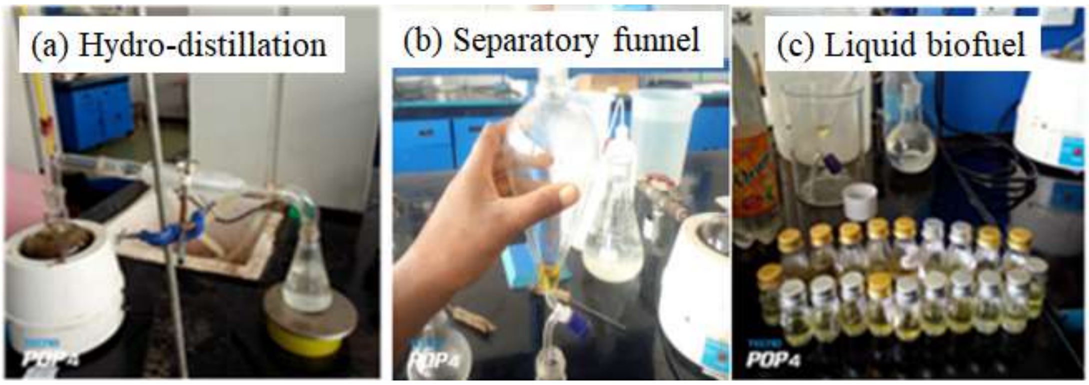
Eucalyptus tree and leaves

Raw material (EGL) pretreatment

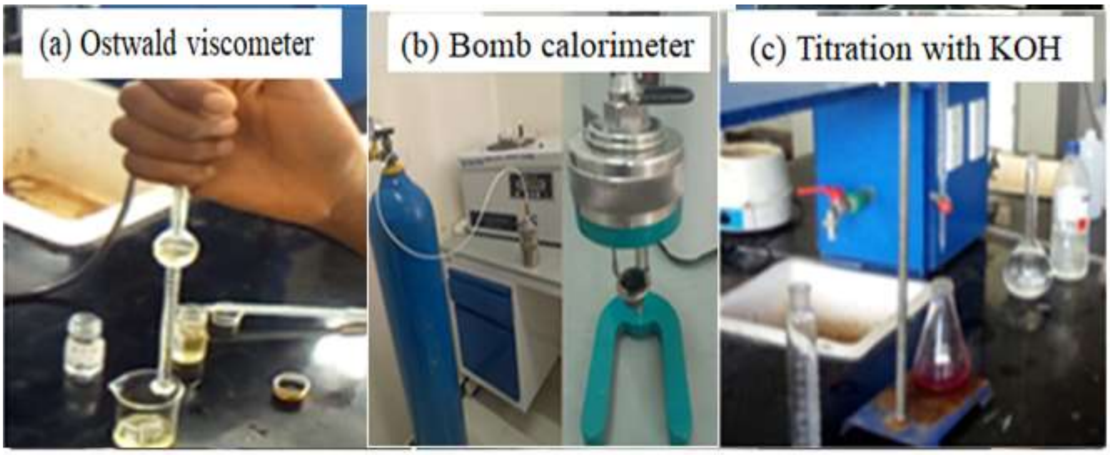
Determination of moisture content

Determination of volatile matter and ash content

Experimental procedures of the HD process and product analysis



Determination of (a) viscosity, (b) heating value, and (c) acid value



Determination of (a) flash point, (b) boiling point, and (c) functional group