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(ENERGY AND CLIMATE SIENCE STREAM)

ENHANCED *CHLORELLA VULGARIS* SPECIES DERIVED BIODIESEL PRODUCTION THROUGH TRANSESTERIFICATION REACTION USING HETEROGENEOUS NANOCATALYST.

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A THESIS SUBMITTED TO THE COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES CENTER FOR ENVIRONMENTAL SCIENCE OF ADDIS ABABA UNIVERSITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN ENVIRONMENTAL SCIENCE.

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Declaration

This is to certify that the thesis prepared by Mesfin Gebreyohanes Kerala "Enhanced *Chlorella vulgaris* Algae Species Derived Biodiesel Production through Transesterification Reaction Using Heterogeneous Nano-catalyst." Which is submitted to environmental scince of the College Natural and Computational Sciences of Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Science in Environmental Science, complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

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Abstract

Enhanced *Chlorella vulgaris* Species Derived Biodiesel Production through Transesterification Reaction using Heterogeneous Nano Catalyst.

Mesfin G/yohans Kerala

The goal of the research project is to produce biodiesel utilizing oil from the Chlorella vulgaris species using a heterogeneous potassium impregnated calcium oxide (K/CaO) catalyst that is made from calcite with an average size of 38.1906 nm. Chlorella vulgaris Oil extraction from harvested, dried, and ground up microalgae was carried out using a microwave-assisted n-hexane and methanol mix solvent extraction method, and the physicochemical properties were determined. Microalgae were isolated and cultured in BBM under illumination of 2500lux light intensity from non-heat releasing white florescence 12:12 hour dark and light cycle. The density, kinematic viscosity, acid value, saponification value and free fatty acids were recorded as 0.87 g/ml, 35.51mm2/s, 1.93mgKOH/g of oil, 212.81mg/g of oil, and 0.88% respectively. The factors that affect the biodiesel yield were investigated. An optimum yield of 94% biodiesel was obtained at reaction temperature of 50 ^{0}C, 5% catalyst load and 13:1 alcohol to oil molar ratio and 60 minute time. The biodiesel's physicochemical qualities were assessed, and the outcomes were contrasted with ASTM criteria. Density at 20^{o}C (811kg/m^{3}), kinematic viscosity (4.7 mm2/s), acid value (0.459mgKOH/g of oil), free fatty acid (0.23%), and flash point (124 ^{o}C) were listed as the physicochemical characteristics of the biodiesel produced. The findings suggested the possibility of algal oil as a feedstock for the biodiesel sector, which could be used as an alternative fuel source, and the fuel qualities were found to be within ASTM criteria.

Key Words: *Algal Biomass, algal oil, Biodiesel, Microwave assisted solvent Extraction, nano-Hetrogeneous catalyst, K doped CaO catalyst, Transesterification & ASTM.*

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ACRONYMS

CO_2……...Carbon Dioxide

GHG…….Greenhouse Gases

O_2 …….Oxygen

CaO …..…….Calcium Oxide

B20…….Petroleum diesel to biodiesel blend

B100……...Petroleum diesel to biodiesel blend

EPA……Environmental Protection Authority

ASTM……American Society for Testing Material

Ph……...Potential of Hydrogen

NaOH……Sodium hydroxide

$CaCl_2$……Calcium Chloride

PER……..Protein Efficiency Ratio

MAG……Mono Glyceride

DAG……Diglyceride

TAGs……Triglyceride

UV……Ultraviolet radiation

DNA……Dinucleic Acid

HPH……High Pressure Homogenizer

MAE……Microwave Assisted Extraction

UAE……Ultrasound Assisted Extraction

PEF……Pulsed Electric Field

FAME……..……Fatty Acid Methyl Ester

FFA……Free Fatty Acid

TPD……Temperature Programed Desorption

XRD……X-ray Diffraction

SEM……Scanning Electromicrosopy

TEM……Transmission Electromicrosopy

FTIR……Fourier Transform Infrared

BJH……..Burret Pyner Hlenda

BET…….Brunuur Emett Teller

TGA……Termogravimetric Analysis

HFRR……..High frequency reciprocating RIg

CP ……Cloud point

HCl…….Hydro Chloric Acid

BBM……Bolds Basal Medium

RPM……Revolution per Minute

AV…….Acid value

SV……..Saponification value

N……Normality

MW……Molecular weight

GC…….Gas Chromatography

NaOH……Sodium Hydroxide

FWHM…….Full width at half maximum or half width

CHAPTER ONE

1. INTRODUCTION

1.1.Background

One of the most crucial pillars of a nation's social and economic development is its energy supply. Petrochemical sources, like coal and natural gas, provide 79.9% of the world's energy consumption, meeting the majority of its energy needs. Only 20.1 percent of the total comes from nuclear, hydro, biofuel, and other sources (IEA, 2016) which are renewable. The petrochemical sources are currently being utilized at a rapid rate and have limited reserves (IEA, 2010), (2017). The rapidly growing population, rising prosperity, and the resulting increase in fuel use has resulted in a rapid increase in the need for energy. By 2050, commercially extractable conventional oil reserves will run out due to anticipated increases in energy use (Ho S-H et al., 2012).

Environmental pollution, deterioration, and global warming are caused by massive carbon dioxide (CO_2) and greenhouse gas (GHG) emissions from various anthropogenic activities (Bankovi et al., 2017, Yang, X. et al. 2018) and largely contribute to climate change. Furthermore, environmentally friendly pollution reduction is a critical global concern (Sekomo CB, 2012). Therefore, it is imperative to lower these emissions, which can be done by concentrating on mitigating strategies like the deployment of renewable energy systems. Searching for a non-fossil fuel-based energy system that is affordable, environmentally benign, sustainable, and renewable is inevitable.

According to Ellabban (2014), renewable resources are those that replenish themselves naturally within a human timescale. Examples of renewable resources include sunlight, wind, rain, tides, waves, bioenergy, and geothermal heat. This type of energy source is renewable, in contrast to fossil fuels, which run out much more quickly.

Ethiopia's energy system is defined by the predominance of biomass fuel, which accounts for 91.5 percent of total energy consumption. Petroleum and electricity, which make up the majority of modern energy sources, account for 7.4 and 1.1 percent, respectively. Fuel requirements will increase in lockstep with population expansion and industrialization.

Ethiopia is especially susceptible to problems with oil supply and price on the international market because it is a net importer of petroleum. It exists to assist nations in achieving their top goals for substituting some of the imported petroleum with alternative fuels (Mahiber, M., 2008). The country's ongoing development program and national fuel security depend on increasing gasoline use and demanding substitution with locally produced fuels, such as biodiesel. Ethiopia's strategy for developing and using biofuels aims to ensure food self-sufficiency, import substitution, and an improvement in the country's balance of payments, all while delivering fuels derived from locally generated biofuel (MME, 2007).

Biofuels are one of the renewable energy sources obtained from organic matter. Biofuels are divided into two main categories: I) primary biofuels such as firewood, wood chips, pellets, animal waste, forest and crop residues, and landfill gas, and (ii) secondary biofuels such as bioethanol, butanol, biodiesel, and hydrogen (2012, Russo D et al.) Second generation biofuels of secondary biofuel categories include Jatropha, cassava, and Miscanthus bioethanol/biodiesel production, as well as bioethanol, bio-butanol, and/or syndiesel production from lignocellulosic materials like straw, wood, and grass (Dragone G, et al., 2010) (Sims RE, et al.,2010).

The first generation biofuel production systems have significant economic and environmental limits, as well as increased pressure on lands needed for food production to support the world's ever-growing population, which is predicted to reach 9.2 billion by 2050. The fundamental disadvantage of second-generation biofuel production is the requirement for a considerable amount of land for cultivation, as well as the fact that woody parts of plants do not compete with food production (Schenk PM, et al., 2008). The drawbacks of first and second generation biofuels can be overcome by considering microalgae, which is considered as third generation of secondary biofuel category and can generate huge amounts of biomass and, as a result, biofuels on much smaller areas, as a viable alternative energy supply (Demirbas A. 2009).

Biofuel production from natural renewable sources such as microalgae, which can be produced from natural resources (light, water, and O_2/CO_2), is widely regarded as one of the most sustainable options for biodiesel and bioethanol production, with the potential to

replace fossil fuels in a cost-effective and GHG-reducing manner (Ndimba BK et al., 2013; Georgianna DR and Mayfield SP. 2012).

Microalgae are unicellular photosynthetic organisms that use light energy H_2O and carbon dioxide (CO_2) to produce food. They have a higher photosynthetic efficiency than other photosynthetic species. Microalgae farming does not require arable land, can grow considerably faster than other biofuel crops, and has more energy per unit weight than other biofuel crops. Additionally, they can produce up to 40 times more oil per acre than conventional biofuel plants. In comparison to other plant-based feedstocks, microalgae have lately demonstrated to be a potential characteristic due to their rapid growth rates and lipid productivities. Several microalgae, including *Chlorella vulgaris*, *Nannochloropsis sp*, *Scenedesmus sp*, and others, have the ability to accumulate intracellular lipids. The major strategy to algal product manufacture, according to Blair et al., (2013), is to find a suitable medium for *C. vulgaris* growing (for mass or small scale culturing). The most essential nutrient affecting biomass growth and fat accumulation is nitrogen (Griffiths & Harrison, 2009; Wang et al., 2014).BBM (Bold Basal Medium) is the best suited for *C.vulgaris* culture and cultivation.

One low-carbon mitigation strategy that has been investigated is biodiesel, a liquid biofuel. Because of this, switching to biodiesel from petroleum fuels may be a practical approach to lower CO2 and other GHG emissions, especially in the transportation industry. This will help to solve environmental problems including global warming and climate change (Osman et al, 2020). Through the use of basic or acidic catalysts in the Transesterification reaction, microalgae oil can be converted into biodiesel. Because calcium oxide (CaO) is readily available, inexpensive, non-corrosive, and has a high basic resistance, it is a viable catalyst for the trans-esterification of biodiesel synthesis. Its low cost also contributes to its minimal environmental effect. As a result of its lengthy, high catalytic activity and the fact that it only requires moderate conditions for the reaction to occur, this solid catalyst is commonly utilized. CaO can be found in calcite, oyster shells, snail shells, bones, crab shells, and ostrich and chicken egg shells, among other places.

The major goals of this research was to make biodiesel from chlorella vulgaris microalgae and to see if lime could be used as a precursor for catalyst development and biodiesel

synthesis utilizing *Chlorella vulgaris* microalgal oil. The impacts of four parameters on biodiesel yield was investigated: Methanol to oil ratio, temperature, time, and catalyst concentration. Furthermore, ideal values for all process parameters was determined in order to achieve maximum biodiesel yield. The biodiesel produced was characterized in order to ascertain its fuel qualities. The effect of transesterification reaction parameters on biodiesel production from *C.Vulgaris* microalgae oil was determined in this study. Calcium Oxide Nano catalyst was synthesized from lime by calcination and enhanced catalytic activity by potassium doping techniques and characterized by various instrumentation techniques such as X-ray diffraction (XRD), Fourier transforms infrared spectroscopy (FTIR), and Scanning electron microscope (SEM), and applied for biodiesel production from *C.vulgaris* microalgae oil by transesterification process using methanol. The optimization of reaction parameters were carried out via Taguchi experimental design using Minitab statistical software. The produced biodiesel were also characterized according to ASTM D6751 (American society for Testing and Materials) protocols to determine fuel properties.

To the best of our knowledge, little research has been done on heterogeneous catalysts made from K-doped CaO that are used in triglyceride transesterification. To enhance the biodiesel synthesis process, more investigation is required, especially with regard to the catalyst, alcohol, and feedstock used. Thus, the main objective of this study is to optimize the key transesterification reaction parameters and increase the yield of biodiesel from the feedstock of Chlorall vulgaris microalgae oil by using CaO and K-doped CaO nanoparticles generated from limestone.

1.2.Statement of the Problem

The hunt for alternative fuels has become more important as the price of petroleum, crude, and products has risen, as have environmental concerns about air pollution generated by the combustion of fossil fuels. The use of food crops (such as corn and maize) for biofuel production may induce price inflation, resulting in food insecurity. Alternative and non-edible agricultural products must be investigated to address such issues.

In comparison to blue-green algae, green microalgae produce a greater amount of biofuel. *Sendesumes* Species, *Chlorella* Species, and *Chlorococcum* Species and are discovered to be biodiesel feedstock (Mondal and colleagues, 2017).

Many experts believe that using algae biofuels might cut GHG emissions from 101, 000 g of CO_2 equivalent per million British thermal units (BTU) to 55,440 g. According to the United States Environmental Protection Agency Act, biodiesel made from microalgae has the ability to meet the Environmental Protection Agency's Renewable Fuel Standard (RFS 2007) requirement (EPA). (Mondal and colleagues, 2017).

Another issue is the possibility of a future energy catastrophe as a result of the depletion of fossil resources. The continued use of fossil fuels as a primary source of energy is universally acknowledged as unsustainable. As a result, securing new energy supplies is critical before the world is faced with an energy catastrophe (Takisawa et al., 2014).

Currently, the globe is dealing with global warming, nonrenewable fossil fuel depletion, and environmental pollution. To address these issues, alternative energy sources that are renewable, economically viable, and ecologically friendly must be prioritized (ensöz et al., 2000).

1.3.Objective

1.3.1. General Objective

Producing biodiesel from microalgae (*Chlorella vulgaris*) using enhanced heterogeneous nano catalyst (CaO from lime) and then doped by KCl.

1.3.2. Specific Objectives

- Isolating ,culture and harvesting micro algae
- Extract and Characterize microalgae oil
- Synthesize and characterize lime based CaO Nano catalyst which is then doped by Potassium(K)

- Synthesize Biodiesel from Chlorella Vulgaris microalgae oil through trans esterification reaction using enhanced lime based CaO nano catalyst and optimize reaction parameters

1.4.Research questions

- At which parameter combination can optimum yield of biodiesel can be achieved
- How much milligram oil yield can be extracted per gram of the microalgae chlorella vulgaris?
- How much of the extracted oil converted to diesel product?

1.5.Significance of the Study

With rising population and economic expansion, Ethiopia's demand for modern energy sources such as petroleum fuels is expanding. The country spends much of its foreign earnings to import all of its petroleum fuel requirements. To address the aforementioned issue, it is critical to concentrate on environmentally friendly renewable and locally produced energy sources, such as biodiesel.

The problems with biodiesel production can be solved with the help of this research. Reliance on imported petroleum and the energy crisis are lessened by the dependable, renewable, and domestic distribution of biodiesel produced from locally accessible resources.

In order to solve problems with biodiesel production, this research is crucial. The production of biodiesel from locally accessible resources is a dependable, sustainable, and domestic process that lessens dependency on imported petroleum and the energy crisis.

Algae are the world's fastest-growing plants. Algae are an important source of biomass. Algae will be competitive as a biofuel source in the future. Algae species may be better suited to different forms of fuel than others. Algae can be cultivated practically everywhere, even in sewage or saline water, and it doesn't require fertile soil or food crops, and processing takes less energy than the algae itself. Algae can be a more effective and

environmentally friendly alternative to oil-based energy. Algae are among the world's fastest-growing plants, with oil accounting for over half of their weight. Biodiesel for vehicles, trucks, and airplanes can be made from this lipid oil. Microalgae develop at a significantly faster rate than terrestrial plants. Oil from algae is projected to yield between 20,000 and 80,000 l per acre per year, which is 7–31 times more than the next best crop, palm oil. Microalgae lipid and fatty acid concentration varies depending on cultivation conditions. The majority of current research on oil extraction is concerned on using microalgae to produce biodiesel from algal oil. Biodiesel from algae can be produced nearly as quickly as oil from crops grown on land.

In order to ensure the development of an environmentally friendly alternative form of energy from non-edible algal biomass, which can be abundantly farmed and grown on places unsuitable for higher plants utilizing waste waters discharged from businesses and families, it is essential to carry out this research. The research will also serve as a foundation for future studies in the field of heterogeneous catalysts to increase biodiesel output, as well as a source of information for anyone interested in conducting additional research and creating biodiesel from fresh water microalgae. It also provide smart ideas that enlighten policy makers to consider biofuel from microalgae in the national strategy of renewable energy development for economic, social and environmental benefits of the society as a whole.

1.6.Scope of the Study

The goal of this study is to look into the possibilities of making biodiesel from chlorella vulgaris algae. To get the end product, there will be five essential steps: micro algae culture, oil extraction, and conversion of the oil into biodiesel using tran's esterification reaction method, which involves manufacturing nano-hetrerogenous catalysts out of calcite. To determine the impacts of the alcohol-to-oil molar ratio and catalyst loading on biodiesel yield, the transesterification process will be carried out. The ideal parameters were calculated based on the end product yield. Finally, the manufactured biodiesel will be tested for physicochemical qualities in accordance with ASTM standards.

CHAPTER TWO

2. LITERATURE REVIEW

2.1.Bioenergy

Energy obtained from biomass is known as bioenergy. A raw material that is biological in origin, biomass does not include material that has become fossilized or embedded in geological formations. Biomass can be immediately turned into different types of energy, such as heat or light, or it can be transformed into solid, liquid, or gaseous fuels (ISO, 2015).

2.2.Biofuel and its feed stocks

Natural biofuels, primary biofuels, and secondary biofuels are the three different categories of biofuels. Examples of natural biofuels derived from organic sources are vegetable and animal waste, as well as organic landfill gas. In contrast, primary biofuels are fuel-woods that are mostly utilized for brick kilns, cooking, heating, and electricity production. Secondary biofuels produced by digesting biomass include bioethanol and biodiesel, which are utilized in the transportation industry. Three groups comprise the so-called generations of secondary biofuels: first, second, third, and fourth (Sheetal R., Mahesh M., 2015).

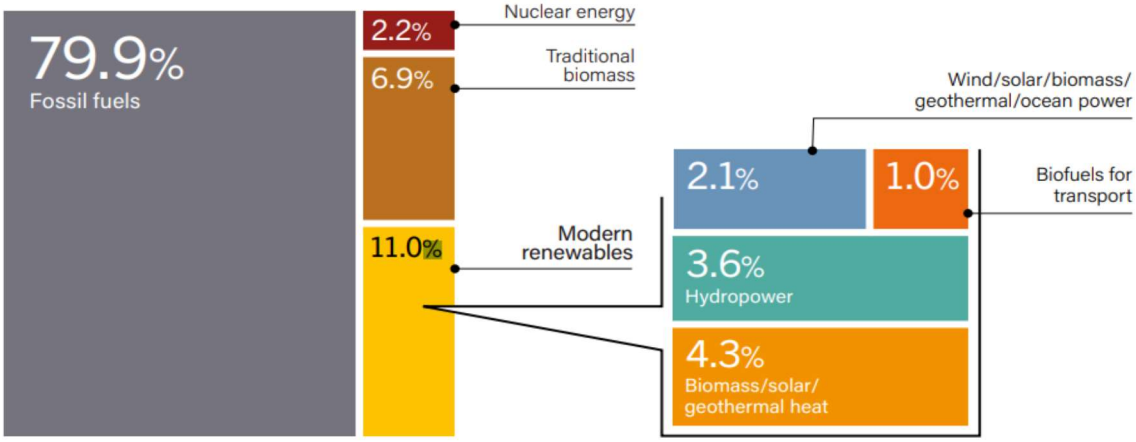

Figure 1 Estimated renewable share of total final energy consumption

Biofuels generated from sugar, starch, vegetable oil, or animal fats using traditional technologies are referred to as first-generation fuels. These liquid biofuels are made out of

previously existing fuels comprised of pure plant oil (PPO) derived from oil crops, biodiesel produced by etherifying PPO or leftover vegetable oils, and bio-ethanol produced by fermenting sugar or starch crops.

Second-generation biofuels have an impact on the global climate, ecosystems, and the carbon cycle. Using biomass on a large scale for second-generation biofuels requires a consistent supply of grasses, wood, and "plant waste." The removal of organic wastes from fields will force farmers to use more nitrate fertilizers, which will raise emissions of nitrous oxide, cause nitrate overloading, and have detrimental consequences on freshwater, land, oceans, and biodiversity. Additionally, it's probably going to speed up topsoil erosion. There have already been significant losses in biodiversity and a decrease in the amount of carbon stored by forests as a result of clearing dead and dying trees from managed forests.

On the other side, planting perennial crops on millions of hectares of land for bioenergy will greatly increase demand for both food production and natural ecosystems. To mitigate the problems typically associated with land-based biofuel feedstock, recommendations have been made for the use of third generation biofuel sources, which require a great deal less land and can be utilized to lower CO2 emissions into the environment. It has been suggested that biofuel made from algae, or more specifically Aquatic Microbial Oxygenic Photoautotroph (AMOPS), is a more sustainable resource that might satisfy global fuel needs without endangering food supply. Algal biofuel seems to have the best possibility of being the only renewable energy source that can both supply the worldwide need for transportation fuels and address the issue of carbon build-up and global warming (Ullah & Associates, 2014)

2.3.The algae biofuel opportunity

Microalgae are photosynthetic prokaryotic or eukaryotic microorganisms with a unicellular or basic multicellular structure that allows them to develop quickly and survive in severe environments. Cyanobacteria (Cyanophyceae) are examples of prokaryotic microorganisms, while green algae (Chlorophyta) and diatoms are examples of eukaryotic microalgae (Bacillariophyta). More than 100,000 strains of algae are thought to exist (Martins et al, (2010). More than 15,000 novel compounds have been found by chemical

analysis obtained from algal biomass. Most of these microalgae species generate carotenoids, anti-oxidants, fatty acids, enzymes, polymers, peptides, toxins, and sterols. Half of the organic carbon produced on Earth is created by algae, which generate 52,000,000,000 tons of organic carbon year net primary generation (Munir et al., 2013).

Algal production facilities can be grouped together on otherwise unproductive, unarable land, and they can utilize salt and waste water sources that other plants cannot use. These are the main benefits of using microalgal organisms as a renewable energy option in a range of industrial applications. They also grow quickly, with cell doublings of 1-4 per day, and have a higher solar conversion efficiency than most terrestrial plants (Pienkos et al., 2010). Over 50 years of research has shown that many microalgae species have the ability to produce a variety of chemical intermediates and hydrocarbons that can be turned into biofuels. Lipids, carbohydrates, and proteins are the three principal macromolecular components that may be recovered from microalgae biomass. These chemical components can be transformed into alcohols, diesel, methane, and hydrogen, among other fuels. Lipids contain by far the highest energy content of the three primary microalgae fractions. (Pienkos et al. 2010, Pienkos et al., Pienkos et al., Pienko several species of microalgae are high in oil; in ideal harvest conditions, the oil content of some microalgae is about 75% of their dry weight. (Martins et al. 2010, Martins et al., Martins et al., Martins (Shumbulo and Demeke 2018).

The yield of algal oil, and thus the production of biodiesel, is the most important distinguishing feature. According to some estimations, algal oil yields more than 200 times the production of the best-performing plant/vegetable oils (per acre). Many microalgae species can be driven to accumulate large amounts of lipids, often greater than 60% of their dry biomass, resulting in rapid growth and high productivity. According to Ahmad, Khan, and Yasar 2013, Scenedesmus species microalgae have been shown to have a high lipid content of up to 55 percent of their dry weight, as well as the ability to remove nutrients from wastewater (2018, Shumbulo and Demeke).

2.3.1. Microalgae species

Microalgae may flourish in almost any kind of ecosystem on Earth, from the icy tundra of Scandinavia to the scorching desert soils of the Sahara (Lee RE., 2008). They can grow in both fresh and salt water. If production units were built with intelligence, there would be no deforestation, no competition with agricultural land, and no conflict with food production (Singh A, et al., 2011). With more than 40.000 species already described or studied, microalgae have a very high biodiversity (Hu Q, et al., 2008).

2.3.2. *Chlorella vulgaris* microalgae

Chlorella is a type of coccoid green alga that belongs to the phylum *chlorophyta* and the family's *trebouiophyceae* and *chlorellaceae* (Krienitz et al. 2004). Chlorella species can be found in a variety of environmental circumstances, ranging from mild too harsh. They can be found in freshwater lakes and ponds, as well as marine and brackish water. Some chlorella species can be found in hot springs (Sakai et al. 1995) where the temperature is higher than 100 degrees Celsius. They can also be found in regions that have extreme weather, such as the arctic and Antarctic (Ahn et al. 2012; shukla et al.2013)

2.3.2.1.Morphological futures

Chlorella vulgaris is a microscopic organism with a size of 2 to 10 µm and with a structure similar to that of higher plants, because it contains; a cell wall, mitochondria, and chloroplasts the latter being necessary to carry out photosynthesis (Safi et al., 2014).

Regarding the cell wall, it is the main defense against biotic and abiotic factors of the microalgae. At the beginning of the cell wall formation, its thickness is approximately 2 nm and as the microalgae matures, the thickness increases until it reaches a thickness of 21 nm (Yamamoto et al., 2004). As for the mitochondria, it can be mentioned that they are in charge of carrying out the metabolic processes with which the microalgae will obtain its energy, necessary to carry out all the growth and maintenance processes. The mitochondria of *Chlorella vulgaris* are made up of double membranes, proteins and phospholipids. Finally, *Chlorella vulgaris* only contains a single chloroplast which is composed of phospholipids and consists of two membranes where the first is permeable to certain

metabolites and some ions, but the second membrane is highly selective and its function is the transport of proteins. In addition to this function, the synthesis of starch granules is possible within the chloroplast (Safi et al., 2014). Within the chloroplasts are the thylakoids where the chlorophyll is found, with which it is possible to capture energy through radiation and that it is used by the organism to create its own food.

2.3.3. Lipid content and fatty acid of microalgae

Every biodiesel feedstock's fatty acid composition and lipid concentration should be taken into account when producing biodiesel, in general. According to Atabani et al. (2013), the yield and quality of biodiesel produced are significantly impacted by the fatty acid composition and lipid content. The most important characteristics of biofuel, such as cetane number (ignition quality), oxidative stability, cold-flow characteristics, and iodine value, are determined by the structure of fatty esters. The length of the carbon chain, its level of unsaturation, and the alcohol moieties that make up a fatty ester are additional factors that affect the fatty ester qualities. In order to produce biodiesel, a microalgae species needs to have a proper fatty acid (FA) composition and high lipid productivity. The fatty acids found in microalgae can either be saturated or unsaturated. The number and location of double bonds inside the carbon chain may fluctuate throughout unsaturated fatty acid types. MUFAs (monounsaturated fatty acids) only have one double bond, but PUFAs (polyunsaturated fatty acids) have two or more. Individual fatty acids are classified as dienoic, trienoic, tetraenoic, pentaenoic, and hexaenoic depending on the number of double bonds they contain. Fatty acids can also be classified as either x3 PUFA or x6 PUFA, depending on where the first double bond is located from the terminal methyl end (x) of the carbon chain (x3 indicates the third carbon from the end of the fatty acid, and x6 indicates the sixth carbon from the end) (Basova, 2005; Hu et al., 2008). Some species of algae can synthesize medium-chain fatty acids such as C10, C12, and C14 (>C20), whereas other species can only produce very-long-chain fatty acids.

While biodiesel produced from feed stocks high in PUFAs has good cold flow characteristics, they are susceptible to oxidation, which could cause instability issues during prolonged storage. Biodiesel produced from saturated fat has superior oxidative

stability, higher cetane, poor low-temperature properties, and they are more likely to gel at ambient temperatures. According to study, high-quality biodiesel should have relatively low quantities of both long-chain saturated fatty acid methyl esters (FAME) and polyunsaturated FAME for excellent low-temperature operability and oxidative stability (Basova, 2005; Hu et al., 2008).

2.4.Growth efficiency of microalgae

2.4.1. Autotrophic growth

Cultivating microalgae most commonly involves autotrophic development. Microalgae may create their own nourishment by using light energy and carbon dioxide from the surrounding environment to carry out photosynthesis. Many microalgae are particularly efficient solar energy converters and can be cultivated in naturally lighted situations (such as open ponds) or artificially, in bioreactors (Perez-Garcia et al. 2011). The autotrophic culture mode has significant drawbacks, including extended cultivation times and low biomass yields, despite the fact that it can use inorganic carbon and energy directly from the environment. Furthermore, autotrophically produced microalgae in bioreactors require a high surface area and shallow depth to offer adequate light exposure by growing close to the light source's surface. Furthermore, both outdoor and indoor bioreactors require time and money to maintain. Autotrophic production of microalgae is an expensive technique because to the high space requirements and ongoing need for light (Knothe, 2010). Furthermore, critics frequently express worries about the negative environmental consequences of using high amounts of inorganic nutrients (Dhull et al. 2014).

2.4.2. Heterotrophic growth

When photosynthesis is impossible or when light is experimentally eliminated and/or energy-producing reactions occur in the dark, microalgae develop heterotrophically. In this situation, microalgae receive carbon via reducing carbon from other organisms or through alternate organic processes (Crane and Grover 2010). Heterotrophic growth for microalgae has the following advantages: In addition to increased growth rates and biomass yields, it is a straightforward process with a less expensive and simpler bioreactor design; it can also

be scaled up more easily without encountering issues with surface area or large landmass requirements; and finally, it can be customized to change the biomass composition by adjusting the culture medium for specific metabolic and biosynthetic pathways (Barclay etal. 1994; Rosenberg et al. 2008; Zheng et al. 2012; Perez-Garcia and Bashan 2015).Heterotrophic cultures also have a number of drawbacks: I) The metabolic diversity of microalgae species that can grow heterotrophically is limited; ii) organic substances such as glucose and glycerol require additional cost and energy; iii) are more susceptible to contamination; iv) excessive levels of organic substrate can inhibit growth; and v) are unable to produce light-induced metabolites (Borowitzka 1999). Lipids, polyunsaturated fatty acids, pigments, and carotenoids are metabolic products derived by heterotrophic culture of microalgae. Depending on the type of sugar employed (glucose, glycerol, or sucrose), lipid production in heterotrophic growth can be up to four times more than in autotrophic development (Li et al.2014). When glucose is used as a carbon source in conjunction with nitrogen deficiency, pigments and carotenoids such as lutein and astaxanthin are generated (Ip and Chen 2005a; Ip and Chen 2005b).

2.4.3. Mixotrophic growth

Mixotrophic cultivation is a growth strategy in which microalgae utilize organic or inorganic supplies and light in various combinations at the same time (Crane and Grover 2010). This type of growth allows microalgae to be more physiologically versatile because it can meet carbon and energy needs from both organic and inorganic sources, as well as light (Chen et al. 2011). Mixotrophic growth has a number of advantages, including: I Higher growth rates in comparison to autotrophic and heterotrophic modes by reducing growth cycles and increasing biomass production; ii) A longer exponential growth phase; iii)A reduction of the photo-inhibitory effect; iv)Flexibility for switching between growth modes (such as from heterotrophic to autotrophic mode), and v)Protection from photo-oxidative damage stimulated by accumulating oxygen in enclosed photo-bioreactors (kruger and muller-luner 2011).

2.5.Biofuel from microalgae

In the 1970s, algae was investigated as a possible replacement fuel source for fossil fuels, but high production costs and constraints hampered commercial development of algal-based fuel production. Following studies, which began in the 1980s and have intensified in the last 15 years, show that research advances are allowing microalgae's commercial potential to transition from aquaculture, fine chemicals, and health food to fuel production.

2.5.1. Biodiesel

Biodiesel is created by the mono-alcoholic trans-esterification process, which combines triglycerides with a mono-alcohol (most frequently methanol or ethanol) with the help of alkalis, acids, or enzymes. It has been used to fuel cars in backyard and commercial settings and has combustion characteristics similar to diesel. There have been considerable technological developments in the trans-esterification process. Currently, biodiesel is produced using plant and animal oils. This agricultural approach will soon face competition for land resources from the food industry (Demibras, 2010).

2.5.2. Bio-oil and bio-syngas

The vapor phase, the liquid phase, and the solid phase are formed when biomass is treated at high temperatures without oxygen. The liquid phase is composed of a complex substance called bio-oil. Depending on the kind of feedstock utilized and the processing parameters, bio-oil content varies substantially. For example, bio-oils obtained from a commercial wood biomass feed derived from beech wood primarily contain phenols, alcohols, and carbonyls, with concentrations varying significantly depending on pyrolysis conditions, whereas bio-oils obtained from rice husk primarily contain formic acid (7.69 percent), b-hydroxybutyric acid (2.31 percent), toluene (5.00 percent), benzoic acid, 3-methyl (1.15 percent), 1,2-benzenedicarboxybutyric acid (2.31%), toluene (5.00%), benzoic acid, 3-methyl (1.15%), 1,2-benzenedicarboxylic acid (1.22%), and other organic compounds.

The entire energy to biomass ratio in a well-managed pyrolytic process may reach 95.5 percent. This category includes two different technological approaches: (1) pyrolysis, where pyrolytic liquids (bio-oils) are the main output; and (2) gasification, where "syngas"

is the main output. It has been demonstrated that bio-oils can be used to generate electricity by internal and external combustion, as well as cofiring with fossil diesel or natural gas in Stirling engines, organic Rankine cycles, steam cycles, and other engines. However, they have a variety of disadvantages that make them unsuitable for use as transportation fuels, such as a high oxygen concentration, poor heat content, high viscosity at low temperatures, and chemical instability. Studies have been done to overcome this limitation and improve the bio-oils' quality.

Most research to far have been focused on using conventional biomasses sourced from forestry and agriculture. Wood and wood wastes were predicted to account for 64% of all biomass energy in the year 2000, followed by municipal solid waste (MSW) (24%), agricultural waste (5%), and landfill gases (4%). Recent research has been done on the viability of producing bio-oil from microalgae biomass. In general, it has been discovered that microalgae bio-oils are of higher quality than wood bio-oils.

2.5.3. Bio-Hydrogen

The precious fuel hydrogen finds applications in fuel cells, coal liquefaction, and bitumen upgrading, among other heavy oil applications. Hydrogen can be produced via a wide range of biological processes, including the steam reformation of bio-oils, the dark and photofermentation of organic materials, and the photolysis of water made possible by specific microalgae species.

2.6. Comparison between biodiesel production from algae & vegetables

It is evident that the accelerated cultivation of terrestrial plant biomass for biofuels will have an exceptionally large land footprint (Table 1), which in turn affects the production of food crops (Martins et al. 2010). However, quantifying the land use changes associated with intensive biofuel feedstock production depends on many assumptions.

Table 1: Biodiesel production potential of algae and vegetables

Source	Oil yield(l/ha)	Area to produce global oil demand
Corn	172	1540
Cotton	325	15002

Soya bean	446	10932
Mustard seed	572	8524
Sunflower	952	5121
Canola	1190	223
Rapeseed	1190	4097
Oil palm	1892	2577
Jatrofa	1950	819
Algae(10 gm/m^2/day at 30% TAG)	12000	406
Algae(50 gm/m^2/day at 50% TAG)	98500	49

2.7.Biodiesel production from microalgae

The upstream step, which involves isolating the algae and producing biomass, and the downstream step, which involves drying the biomass and synthesizing diesel, are the two primary procedures needed to produce microalgal biodiesel. As will be covered below, there are also a number of steps and material requirements.

2.7.1. Upstream process

2.7.1.1.Isolation and culturing of algae

Depending on how much of the culture is required, different materials are employed to cultivate algae. Erlenmeyer flasks with input and outflow tubes are used for aeration in small-scale experiments. Microalgae use photosynthetic mechanisms, just like all plants, to take in inorganic carbon and convert it to organic matter. Since light is the source of the energy for this reaction, its intensity, spectrum composition, and photoperiod must all be taken into account. The relevance of light intensity varies greatly depending on the algal culture's depth and cell density; at higher depths and densities, more light is required to penetrate the culture (1,000 lux is suitable for Erlenmeyer flasks; 5,000–10,000 is needed for larger volumes). Both natural and fluorescent lighting are options. Photo-inhibition may occur in circumstances of extreme light intensity, such as in direct sunlight or a small container near artificial light. It's also advisable to avoid overheating due to both natural and artificial lighting. Despite the fact that cultivated phytoplankton can develop

continuously in the presence of light, artificial illumination must continue at least 18 hours every day. Fluorescent lights that are white and bright can be employed in controlled conditions. Sunlight is essential to outdoor cultures for illumination. The metabolic rate of an organism is frequently influenced by temperature. Controlled rooms are commonly kept at low temperatures (18–23 °C). When scaling up for mass production, algal starters or inoculate previously cultivated in controlled rooms should be transported early in the morning to reduce stress caused by abrupt temperature changes. Depending on the culture media, the species, and the strain grown, the ideal temperature for growing phytoplankton varies, but is frequently between 20 and 24°C. Microalgae that are widely cultivated can withstand temperatures of 16 to 27 °C. Ethiopia has a temperature that is favorable for growing algae, as illustrated in Figures 5 and 6 below.

Temperatures below 16°C limit the growth of many species, whereas temperatures above 35°C kill others. Algal cultures can be chilled by either using refrigerated air conditioning systems to control the air temperature or by trickling cold water over the surface of the culture jar. The majority of farmed algal species have a pH range of 7 to 9, with 8.2 to 8.7 being the ideal range; this affects algae growth as well. Failure to maintain a suitable pH may result in a culture entirely collapsing due to the disruption of numerous cellular processes. Aerated culture is used to achieve the latter. The addition of carbon dioxide causes a high-density algae culture to grow more quickly. To compensate for an elevated pH that may reach limiting levels of up to pH 9 during algal growth.

Algae will develop considerably more quickly if you feed them extra carbon in the form of the gas carbon dioxide (CO_2). Compressed gas cylinders are used to distribute CO_2, and only a tiny amount (about 0.5%) of CO_2 is necessary in the air supplied to the culture. To ensure that the amount of CO_2 injected maintains the culture's pH between 7.8 and 8.0, a flow meter should be used. To measure pH, use a pH meter or indicator sheets that change color when the pH changes. Using an in-line filter device with a pore size of 0.3 millimeter to 0.5 micron before entering the culture will help keep other, potentially contaminating organisms out.

On the other hand, because some crucial nutrients can occasionally only be present in trace amounts, natural waters are suboptimal for long-term algae development in lab cultures.

The concentrations of these components are principally controlled by dynamic equilibrium, which is upset as soon as water is collected. The constant and luxuriant development of algae in the lab was unsustainable in lakes, ponds, and the sea. Mineral salts must be added to natural waters in order to make them drinkable. At the time, it was innovative to utilize enriched culture media, in which specific conditions were enforced to encourage the growth of specific organisms. Algal colonies must be nourished to make up for the ocean's nutritional deficits. The macronutrients nitrate, phosphate, and silicate are examples. For the growth of diatoms, which need silicate to create an exterior shell, this substance is specifically used. Three vitamins that are categorized as micronutrients include thiamin (vitamin B1), cyanocobalamin (vitamin B12), and biotin. The majority of algae can grow on the Guillard's F/2 medium and the Walne medium, two enrichment media that have been extensively utilized (Krishna et al, 2012). You can use centrifugation or the washing method to isolate the desired species: By repeatedly centrifuging or washing water samples, larger organisms can be isolated. Using phototactic movement as a tool the phyto flagellates can be isolated with a micropipette since they only travel in one direction. Using the agar-plating technique: The agar-medium is made by adding 1.5 percent agar to 1 liter of a suitable medium (or even plain saltwater) and sterilizing the mixture in an autoclave at 150 pounds per square inch and 120 degrees Celsius for 15 minutes. After sterilizing the Petri plates, pour the medium into them and set the timer for 24 hours. Before autoclaving, culture tubes are correctly packed with cotton and have the medium put in up to 1/3 of the way. Micromanipulation: An algal cell will be removed from a drop of an enrichment sample. Inhale deeply into the micropipette as you observe the cell. Give the cell a drop of sterile medium and transfer it to an agar plate. Repeat the process to "wash" the cell. Bacterial contamination is less likely to happen the more times a cell is washed. On the other hand, each handling raises the possibility of cell damage. Depending on the type of algae, a different number of washes will be suitable. In a tissue culture plate, Petri dish, or culture tube, put the cell in diluted liquid. Place the culture vessel in a dimly lit space with a steady temperature. Check for growth under a microscope or wait until macroscopic growth becomes visible (3–4 weeks after transfer). This approach should produce a colonial uni-algal culture. Gradual diluting Tubes 10-1 through 10-10 should be identified by their dilution factor on the label. Aseptically add 1 mL of the enrichment sample to the

first tube (10-1), then give it a gentle stir. 1 mL of this dilution should be added to the following tube (10-2) before being gently stirred. Repeat the process with the remaining tubes (10-3 to 10-10), following the same steps. Incubation of test tubes should take place at a steady temperature and illumination: After 2-4 weeks, aseptically remove a small sample from each dilution tube and examine the cultures under a microscope. In one of the higher dilution tubes, such as 10-6 to 10-10, a unialgal culture may form. Micromanipulation can be used to make tubes with two or three different kinds of unialgal cultures (Chen et al, 2013).

2.7.1.2.Cultivation of microalgae

Microalgae can be grown in both open-culture systems like ponds or lakes and highly regulated closed-culture systems called photo bioreactors (PBRs, Figure 2). In general, photo bioreactors are more expensive and fragile than open-culture systems. According to (Richmond, 2004), the water level cannot be kept much lower than 15 cm (150 L m-2) for the microalgae to receive adequate sun energy to flourish. Ponds, on the other hand, require more energy to homogenize nutrients.

Open ponds are more vulnerable to weather since they cannot manage the water temperature, evaporation, or lighting. They may also generate significant amounts of microalgae, but they also occupy larger land areas and are more vulnerable to bacterial or other microorganism contamination (Mata et al., 2010).

Contrarily, PBRs are tightly regulated systems that may be adapted to the physiological and biological characteristics of the cultivated algal species, making it possible to cultivate algae species that are inedible in non-open ponds. PBRs are seen as being better than open ponds in a variety of aspects, including better control over culture conditions and growth factors (temperature, pH, mixing, CO_2 and O_2), Reduced CO_2 losses, avoided evaporation, increased volumetric productivity, ability to reach higher microalgae densities or cell concentrations, and provision of a more secure environment by preventing contamination or minimizing invasion by rival microorganisms are all benefits (Mata et al., 2010). Despite its benefits, PBRs have a number of problems that must be considered and fixed. Their main negatives are biofouling, oxygen accumulation, overheating, high construction and

operating expenses for growing algal biomass, scaling problems, cell injury from shear stress, and degradation of the photo-stage material.

2.7.2. Downstream

2.7.2.1.Microalgae harvesting technology

2.7.2.1.1. Centrifugation

For *C. vulgaris*, centrifugation (5000 rpm, 15 min) is the most common harvesting method due to its high efficiency (95 percent recovery), speed, and processing capacity (Converti A, et al., 2009; Cha KH, et al., 2010). Furthermore, Chlorella vulgaris form permits high centrifugal stress without compromising the organism's structural integrity. To maximize biomass recovery, additional methods such flocculation, flotation, filtration, as well as a combination of two methods, are used.

2.7.2.1.2. Flocculation

The algae cells remain spread because they have a significant negative surface charge that is difficult to eliminate during exponential growth. The negative charge of the cells reduces as they approach the stationary or declining phase, allowing them to mingle and form lumps, a process known as auto-flocculation. This behavior is associated to an increase in pH that results from the absorption of carbon dioxide, nitrate, and phosphate (Vandamme D, et al., 2012). Additionally, interactions between bacteria and algae, expelled organic compounds, and simply ceasing the flow of CO_2 are all capable of causing auto-flocculation. This method is time- and money-consuming. Microalgae cultivation is frequently quite stable, and the possibility of auto-flocculation is small and occasionally misleading. It is essential to introduce a base to elevate pH in order to expedite the coagulation process. Sodium hydroxide is the most effective solution, causing more than 90% flocculation at pH 11 and requiring Jess amounts (9 mg of NaOH per gram of dry biomass) (Vandamme D, et al., 2012; Wu Z, et al., 2012). Lime, however, seems to be the most cost-effective in an industrial setting. This mechanism relates to the precipitation of Mg2 + from hydrolyzed Mg $(OH)_2$ and the attraction of negatively charged micro algal

cells. Occasionally, flocculation is used as a pre-harvesting step to help or enhance other harvesting techniques like centrifugation or filtration (Chang Y-R, and Lee D-J. 2012; Lee D-J, et al., 2012).

2.7.2.1.3. Flotation

Although this method uses scattering micro-air bubbles to collect cells, there is very little proof of its viability to our knowledge. As the lipid content of microalgae rises, flotation may occur naturally. Cheng et al. (2010) successfully floated C. vulgaris (0.05 mg g-1 biomass) using dispersed ozone gas. As a result, unlike flocculation, this process does not involve synthetic chemicals; yet, its viability, particularly on a large scale, remains uncertain.

2.7.2.1.4. Filtration

This process involves continuously passing a filter with concentrated algal cells through a broth containing microalgae until the broth achieves the desired thickness. The low size of *Chlorella vulgaris* renders traditional filtration useless. On the other hand, micro- or ultrafiltration are more efficient. Chlorella's structure, on the other hand, provides a more significant permeation flow without the necessity for an additional unit activity like spinning while filtering because this phenomena is minor in this organism (Frappart M, et al, 2011). One of the main drawbacks of the ultrafiltration process is fouling brought on by soluble substances, such as exopolysaccharides of certain microalgae like Porphyridium. Because various elements, including filter type, transmembrane pressure, flow velocity, and turbulent flow, affect the cross-flow and growth phase of microfiltration and ultrafiltration, a compromise that takes these factors into consideration should be found. A different harvesting technique, such as flotation or flocculation, may also be utilized in conjunction with them to improve the process (Chang Y-R, et al., 2012; Lee D-J. 2012).

2.7.2.2. Microalgae oil extraction

2.7.2.2.1. Cell disruption

Because floating the cells in the extraction solvent does not easily disrupt them, cell disruption as a pre-treatment method can greatly boost the efficiency of lipid recovery. As a result, despite the process' increased processing expenses and energy requirements, this stage is generally seen as crucial. The effectiveness of various microalgae species varies significantly, therefore there isn't a single technique to cell disruption that is uniformly effective (J.R. McMillan et al., 2013). The lipids are encased within the cellular architecture of the most researched algae strains, including the cell wall and membrane (R. Halim, et al., 2013). Without the proper pre-treatment, extraction may not be efficient. Accurate methods are essential since variables like temperature, pressure, biomass growth stage, and scale have a significant impact on how effective they are. Accurate comparison of various approaches is challenging due to factors such as temperature, pressure, biomass growth stage, and size having a significant impact on the effectiveness of processes (D-Y. Kim, et al., 2016).

2.7.2.2.1.1. Mechanical disruption method

Mechanical methods that apply direct physical pressure to the cells to force them to burst include solid and liquid shear and thermal/pressure shock. The most widely used techniques for decreasing biomass are mechanical cell disruption methods that rely on shear forces, like bead-milling, high-pressure homogenizers, and grinding; these methods may be used to any kind of biomass and are easier to scale up than other state-of-the-art methods (J. Kim, et al., 2013). The high energy requirement for these approaches, however, is a significant obstacle during the scale-up of bioprocesses. Temperature management may be necessary because of how much heat they produce. In addition, they result in a non-selective discharge of cellular contents along with cell debris, complicating subsequent separation procedures (N. Grimi et al., 2013).

2.7.2.2.1.1.1. Grinding

Using a pestle and mortar to pound dry or wet microalgae may help extract their lipids, according to(Y-R. Hu, et al., 2013). However, because it is a manual process, it takes longer to finish, only works on a limited scale, and operator efficiency varies (R. P. Utomo, et al., 2013). Cryogenic grinding, which is most frequently carried out with liquid nitrogen, is another extremely effective method for rupturing wet algal cell walls. Although this low-temperature method can recover more than 29 wt% of the lipids from C. vulgaris, it is not practical for industrial oil production due to the high cost of liquid nitrogen.

2.7.2.2.1.1.2. Bead milling

Bead milling works on the same principle as grinding. For any type of biomass, the significant shear provided by quickly spinning beads provides excellent cell rupture. These days, bead mills come in two varieties: huge bead beaters that use agitated beads and smaller bead beaters for laboratory usage that use shaker jars. The fast-spinning beads create a liquid shear that strikes the biomass. If the right substance and size of beads are employed, both kinds can be highly effective. Because it uses distinct vials to lessen the possibility of environmental contamination, the bead beater is perfect for DNA extraction (P. Mercer and R.E. Armenta, 2011). The shape of the container, the pace of shaking, the diameter, the material, the number, and the biomass content all affect how successful the disruption is. Bead milling offers greater potential for larger-scale operations than grinding. The effectiveness of microscopic cells with strong cell walls, like the microalgae *C. vulgaris*, makes this strategy difficult (J.Y. Lee, 2010). However, managing this strain via alternative methods of cell disruption is equally difficult. Request an additional separation cost because the beads used in bead milling are so small in size.

2.7.2.2.1.1.3. High pressure homogenizer

For large-scale disruption of bacterial or yeast cells as well as for laboratory-scale disruption of micro algal cells, the high pressure homogenizer (HPH) is frequently utilized. Scaling is simple because it operates in a continuous way with slurry algal suspension. High pressure is applied to the microalgal cell suspension until it strikes a small nozzle output and is released into a low pressure chamber. The rotor-stator style homogenizer, on the other hand, is capable of processing biomass in liquids with low viscosity. Turbulence,

viscosities, high pressure shear, impingement of the cells on the set surface, cavitation in the liquid, and an abrupt pressure drop between the nozzle and the surrounding environment are all thought to contribute to cell disruption in HPH (R. Halim, et al., 2013; C.W. Ho, et al., 2008); although the exact mechanism is still unknown. Control of the HPH system must take into account the pressure, cell diameter, and nozzle size, among other factors. High-pressure homogenizers outperformed more conventional pre-treatment techniques like osmotic shock or enzymatic hydrolysis (E. Molina Grima, 2003).

2.7.2.2.1.2.Physical or chemical methods

2.7.2.2.1.2.1. Steam explosion

First, the biomass is heated for two to six thousand seconds to a steam temperature of 160°C to 260°C while being compressed (1 to 3.5 MPa). The cell wall's stiff fibers are then ruptured by the biomass's rapid breakdown. The cellulose bundles are defibrillated by the abrupt drop in pressure. Compared to hydrothermal liquefaction, this technique operates at a lower temperature, often between 100 and 300 degrees Celsius. Lower temperatures are recommended for pre-treating microalgae biomass with steam technology in order to avoid lipid degradation. Steam explosion methods have several advantages, including a high recovery yield, the production of hemicelluloses, the dissolution of solid wastes into individual fibers, and the use of water as a green solvent (W. Stelte, 2013). The optimal lipid yield may be (10–15%) higher than the lipid content of microalgae due to the polymerization of proteins and carbohydrates into oily composites, albeit this depends on the biomass and process requirements (A. M. Aguirre, 2014). In order to change the lignocellulosic cell wall for lipid extraction, steam explosion is therefore a cheap and effective pre-treatment (M. A. hattab Abdel Ghaly, 2015).

2.7.2.2.1.2.2. Autoclave

An autoclave (steam autoclave) operates at 121°C and 15 psig of pressure. This method is used as a sterilizer, demonstrating its effectiveness in dismantling bacteria cells. The microbial extracellular cell membrane bursts under severe heat stress, permitting the removal of the intracellular lipids. Lee et al.'s study from 2010 showed that autoclave had

successful outcomes. To remove *Chlorella vulgaris* lipids. Conversely, autoclave is unsuitable for large-scale treatment due to its lengthy treatment time and high energy usage ("M. A. hattab Abdel Ghaly, 2015).

2.7.2.2.1.3. Enzymatic hydrolysis

Enzymatic hydrolysis is a cost-effective but expensive process due to the high price of enzymes and the lengthy reaction time. The breaking of bonds in the constituents of cell walls is facilitated by the enzymes. One of the most important advantages of enzyme hydrolysis is that it lowers the activation energy of the cleavage reactions, creating one of the mildest conditions for cell disintegration. Furthermore, there are fewer unintended by-products due to increased selectivity. Additionally prevented are lipid degradation and machine corrosion. Although lysozyme has a higher yield of 16.6% than cellulose, both substances are efficient at dissolving cell walls for lipid production from microalgae (H. Taher et al., 2014). The need to maintain a steady state for an extended period of time severely restricts the commercial applicability of this technique for cell disruption when used on a larger industrial scale for enzymatic hydrolysis (H. Zheng, et al., 2011; S.U. Kadam, et al., 2013). As an intriguing alternative to conventional enzymatic hydrolysis, Chen et al. (2016) proposed co-cultivating bacteria with algae to lyse cell walls.

2.7.2.2.1.3.1. Osmotic shock, acid/ alkaline treatment

Physical or chemical treatments that just require the addition of a few chemicals and, if necessary, temperature management, like osmotic shock or acid/alkaline therapy, are particularly tempting from an operational perspective.

They are easy to scale up, need little energy input, and have affordable capital costs (D-Y. Kim, et al., 2016). On the other hand, the cost of the chemicals, their bio-toxicity, and lipid breakdown have restricted their use (E. Günerken et al., 2015). Additionally, the selectivity and suitability of the chemical are very dependent on the type of microalgae being targeted; for instance, osmotic shock is insufficient to extract lipids from microalgae cells with rigid cell walls (R. Halim et al., 2012).

To save time, energy, and space, the manufacturing of lipids also uses membrane technology (cationic polymer). (G. Yoo, et al., 2014) shown that direct interaction with the tertiary amine cations on the membrane's surface makes it easy and effective to lyse wet cells with an efficient working membrane.

2.7.2.2.1.3.2. Microwave

A few decades have passed since the invention of the microwave-aided extraction (MAE) method. It is presently utilized to extract vegetable or animal oil because it can efficiently tear cells (J.Y. Lee et al., 2010). Wavelengths of electromagnetic radiation spanning from 0.001 m to 1 m are fired into the algae slurry. Heat shock breaks the cells as a result of the algal slurry becoming uniformly heated as the medium absorbs the transferred energy. Furthermore, the internal pressure would be shocked by the water content of the cell that was evaporating (G.J. Gil-Chávez et al., 2013). When heat and pressure stress are coupled, microalgae cells can rupture effectively, especially in strains with strong mechanical resistance (F.J. Barba et al., 2015). The design of the extraction process is influenced by operational elements such as power, working volume, temperature, and the polarity, dissipation factor, and heat capacity of the solvent. Understanding the qualities of the target molecule is essential before developing a way to disrupt cells [89]. Extraction would generally be simpler with solvents that have high heat dissipation factors and dielectric constants (G.J. Gil-Chávez et al., 2013). More energy is used by other mechanical or thermal processes than by MAE. Due to the absence of a temperature gradient, MAE has fewer heat transmission requirements than other treatment methods, especially ultra-sonication. But precise temperature control might be necessary (S.U. Kadam et al., 2013). MAE is a straightforward and efficient method for lipid extraction that utilizes less solvents than conventional techniques and has the potential to be scaled up even further. Overall, the architecture of the large-scale reactor is still being worked out, and more research needs to be done on the working mechanism. If a more effective microwave absorber could be discovered, the microwave reflection tank might be used for cell disruption (H. Li et al., 2016). Using a standard microwave oven as the foundation, Chen et al. (2015) devised a continuous extraction process that recovered 96.2 percent of the oil in 80 minutes.

2.7.2.2.1.3.3. Ultra-sonication

The use of ultrasound-aided extraction (UAE) to extract lipids, proteins, and carbohydrates has been well studied. Micro-bubble cavitation in the suspending media, where micro-bubbles form and collapse close to the cells, releases mechanical energy as shock waves, creating micro-turbulence, high liquid shear, and pressure shock, is the primary method used by UAE to break the cell wall (R. Halim, et al., 2013). According to reports, the UAE has lower prices, quicker extraction times, and higher efficiency. The two factors that have the biggest effects on UAE yield are frequency and working power (M. Wang et al., 2014). High frequency is more effective and energy efficient in disrupting microalgal cells, although a combination of low frequency was also an option to conserve energy (M. Wang, et al., 2014). Additionally, because energy is quickly lost by transmittance in the UAE, the size and shape of the extractor is crucial (H. Zheng et al., 2011). UAE has demonstrated its scalability by recent innovations in installations, which are created by combining several devices. According to P. Mercer and R.E. Armenta (2011), Origin Oil (USA) uses it in conjunction with electromagnetic pulses to disturb cells for the creation of lipids.

2.7.2.2.1.3.4. Pulsed Electric Field

Pulsed electric field (PEF) assisted cell disruption is a method that was created in the 1990s. This non-thermal method uses a great deal less energy because less of it is lost as heat. Electroporation, which comprises pulsed delivery of a high intensity intermittent electric field to the cells on a time scale of microseconds, increases the permeability of microalgal cell membranes (E. Luengo, et al., 2014). The electric field's intensity can be changed to make the electroporation process either irreversible or reversible. PEF is helpful in eliminating lipids and bioactive substances from plant and algal tissues, according to research (P. Mercer and R.E. Armenta, 2011). PEF is an intriguing new technique for intracellular lipids in microalgae because of its advantages in treatment time, energy and solvent requirements, and selectivity (X. Yu, etal, 2016) M.D. Zbinden, etal. 2.13). Electrically resistant strains can be relieved by high voltage electric discharges, which are comparable to low voltage electric discharges but more powerful. The generation of localized high electric fields, shockwaves, cavitation, liquid turbulences, ozone, and

hydroxide radicals are secondary phenomena that accelerate cell death when the pulsed streamer is used (N. Grimi, et al., 2014). The algal suspensions are treated and concentrated using PEF. Soluble intracellular components were released into the suspension as a result of the PEF-induced cell disintegration for every pulse setting that was used. While the field strength had minimal impact, the efficiency of disintegration increased as the specific treatment energy grew. In suspensions with a biomass composition of 100 g dry weight per kilogram suspension, significant cell rupture needed an electrical energy input of around 1 MJ/kg dried algae. This is equivalent to 4.8% of the maximal heating value of the algae. Although the treated algae included lipids, PEF treatment only caused the spontaneous release of soluble components. PEF treatment could be employed in a bio-refinery concept where soluble algae components are eliminated before solvent extraction of lipids due to the method's selectivity (M. Goettel, et al., 2013).

2.7.2.3.Biodiesel production technologies

Direct use or blending of oils, micro-emulsion, pyrolysis, and transesterification are the most dominant technologies that enable us to use oil and fat feedstock types as fuel in diesel engines. Various researchers are currently mentioning transesterification as the most preferred method due to the higher quality of fuel obtained.

2.7.2.3.1. Micro-emulsion

One of the physical characteristics of raw vegetable oil that makes it unsuitable for use as fuel is its viscosity. Ma and Hanna (1999) state that one potential remedy for the viscosity issue in vegetable oil is micro-emulsion formation. A micro-emulsion is defined by the IUPAC as a dispersion consisting of water, oil, and surfactant(s) that is an isotropic, thermodynamically stable system with a dispersed domain diameter that can range from 1 to 100 nm, but is typically between 10 and 50 nm. A biodiesel micro-emulsion is made up of diesel fuel, vegetable oil, alcohol, surfactant, and cetane improver in the right amounts. Alkyl nitrates are utilized to improve cetane, higher alcohols are employed as surfactants, and alcohols like methanol and ethanol are used as additives to lessen viscosity (Chiaramonti et al., 2003). Mahanta and Shrivastava (2012) state that vegetable oils with an ester and dispersant (co-solvent) or vegetable oils with alcohol, a surfactant, and a cetane

improver, with or without diesel fuels, can be combined to create a micro-emulsion. All micro-emulsions containing butanol, hexanol, and octanol satisfied the maximum viscosity requirement for diesel fuel.

2.7.2.3.2. Pyrolysis and catalytic cracking

A viable method for creating liquid, activated carbon, and gaseous fuels as well as necessary substances simultaneously is biomass pyrolysis. It is a thermochemical process where biomass is heated without oxygen or partially burned with little oxygen present. The chemical makeup of the liquid fuel created via pyrolysis is similar to that of normal diesel made from petroleum. Vegetable oil that has been paralyzed has a low viscosity yet a high cetane number. They have appropriate levels of sulfur, water, sediment, and copper corrosion, and they also have acceptable levels of carbon residues, ash content, and pour points (Hoekman & associates, 2012).

2.7.2.3.3. Trans-esterification

Low-quality biodiesel is produced by the pricy processes of catalytic cracking, pyrolysis, and micro-emulsification. The most widely used method of converting oil to biodiesel is transesterification, which produces fatty acid methyl esters (biodiesel) that are physically similar to diesel fuel. This makes transesterification the best option. In addition, the technique is really easy to follow. As seen in Figure 9, transesterification transforms free fatty acids and triacyle glycerols, a raw, viscous microalgal lipid, into lower molecular weight fatty acid alkyl esters. The process of replacing an ester compound's alkoxy group with an alcohol (alcoholysis), carboxylic acid (acidolysis), or ester molecule is known as inter esterification. Only alcoholysis and inter-esterification have proven effective in the biodiesel industry. Thus, it is a reaction between the short chain alcohol and the parent oil (triglyceride) in the presence of a catalyst. Glycerol and fatty acid methyl esters (FAME) are byproducts of the procedure. Ethanol can be produced through fermentation, which increases its safety and renewable nature. Despite this, methanol is preferred to ethanol due to its lower cost, higher level of reactivity, and ability to produce a greater quantity of volatile fatty acid methyl esters. The reaction rate and yield can be increased by using the right catalyst. The catalyst could have an enzymatic, basic, or acidic composition.

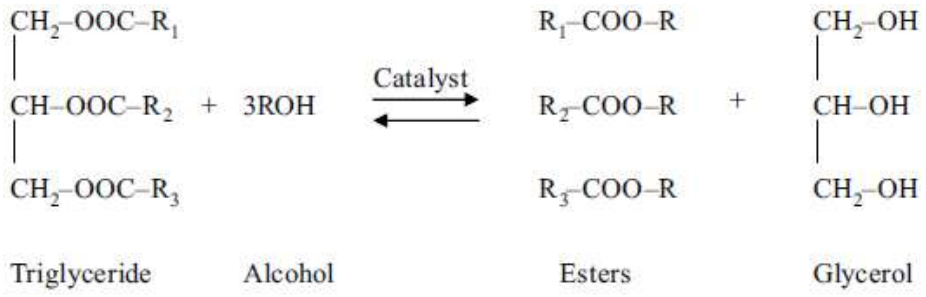

Figure 2: Transesterification of Triglyceride Process

The composition of FAME derived from vegetable oils and animal fats is typically dominated by only a few species: palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), and linolenic acid (18:3). This is despite the fact that biodiesel fuel produced by transesterification of triglycerides contains a large number of individual FAME species. (18:3). These same fatty acid families predominate in some algal-derived lipids, whilst others are more varied and contain sizeable amounts of numerous distinct FA Groups (Hoekman et al. 2012).

2.8.Biodiesel from microalgae

2.8.1. Trans-esterification

Transesterification, also known as alcoholysis, is a chemical process that produces esters and glycerol by combining alcohol with oil or fat and a catalyst. Alcohol is a reactant in the transesterification reaction, which breaks down the molecular structure of triglycerides in oil by exchanging alkyl groups between an ester and an alcohol. Three reversible reactions convert triglycerides (TGs) to diglycerides (DGs), which in turn turn to monoglycerides (MGs), and lastly, MGs to glycerol. Three ester molecules are produced from a single TG molecule with each step. Alcohols such as methanol, ethanol, propanol, butanol, and amyl alcohol can be utilized in the transesterification process. Ethanol and methanol are the most often used solvents. However, due to its low cost, methanol is the preferred option. The transesterification process between TGs and alcohol is depicted in Equation 2.1. It is common practice to employ a catalyst to quicken the reaction and boost yield. Excess alcohol is utilized to move the equilibrium to the product side of the reaction

because it is reversible. It produces glycerol as a byproduct, which has some commercial value.

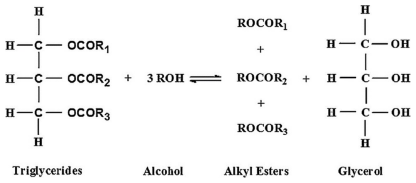

Figure 3: The General Chemical Reaction Depicting Transesterification

2.8.2. Catalysts for trans-esterification

The biodiesel yield, reaction duration, waste minimization, and separation process ease are all determined by the type of catalyst utilized. Homogeneous catalysts, heterogeneous catalysts, biocatalysts, and Nano-catalysts are the four types of catalysts used in biodiesel production (Adeyemi et al., 2011).

2.8.2.1.Homogeneous catalysts

In homogeneous catalysis, a chemical that is in the same phase as the reaction system catalyzes a series of reactions. Due to its ease of use and quick reaction time, the homogeneous catalyst is the one that is most frequently utilized in the production of biodiesel. Catalysts that are basic or acidic fall within this category. The majority of the time, solvents that are in the same phase as all of the reactants dissolve homogeneous catalysts.

2.8.2.1.1. Homogeneous base catalyst

Homogeneous base catalysts include alkali metal-based hydroxides (like sodium or potassium hydroxide); alkali metal-based oxides (like sodium and potassium methoxides); and carbonates. Base catalysts are highly active in transesterification. Because they are less expensive than alkoxides, metallic hydroxides are frequently used as catalysts. Nevertheless, their activity is lower. A base-catalyzed process has been found to proceed 4,000 times faster than an acid-catalyzed one. One well-known drawback is that oil with

high FFA content cannot be completely transformed into biodiesels; instead, it stays mostly as soap. If the FFAs are less than 5%, the process can still be accelerated with an alkali catalyst; however, more catalyst is required to make up for the catalyst that is lost to soap (De Lima et al., 2016). Most studies show that an FFA level of less than 2 weight percent is appropriate for biodiesel synthesis using a homogenous catalyst.

2.8.2.1.2. Homogeneous acid catalyst

Bronsted acids, primarily sulfonic and sulfuric acids, as well as hydrochloric acid, catalyze the esterification process. In alkyl esters, these catalysts provide extremely high yields. However, because of the slower reactions compared to those catalyzed by alkali, the process is more energy-intensive and not as profitable. Both esterification and transesterification reactions can be catalyzed by homogeneous acid catalysis, which is not impacted by the presence of free fatty acids. Notwithstanding these advantages, homogeneous base and acid catalysis have similar separation issues (Silitonga et al., 2020).

2.8.2.2.Heterogeneous catalysts

The reactants and heterogeneous catalysts are in distinct phases or states of existence. These are catalysts that frequently create active sites with their reactants throughout a process. The heterogeneously catalyzed methanolysis reaction (oil and methanol) is a highly complex three-phase reaction involving a solid (heterogeneous catalyst) and two immiscible liquid phases. There are other side reactions that take place in conjunction with methanolysis, including the saponification of glycerides and methyl esters as well as the catalytic neutralization of FFAs. Greater temperatures and higher oil/alcohol ratios are this catalysis's key drawbacks compared to homogeneous catalysis. Other benefits include ease of separation and purification, as well as the catalyst's enhanced reusability. Acid and base heterogeneous catalysts are two types of heterogeneous catalysts (Di Serio et al., 2008).

2.8.2.2.1. Heterogeneous base catalyst

When utilizing a homogeneous base catalyst, saponification makes it difficult to separate glycerol from the methyl ester layer. This problem is solved with a heterogeneous base catalyst. These catalysts also show improved catalytic activity under reasonable conditions.

Other advantages of these catalysts include non-corrosion, environmental friendliness, and simplicity in disposal. By merely removing them from the reaction environment, they can also be designed to offer improved activity, selectivity, and catalytic longevity (Calero et al., 2014).

For example, metal-based oxides are the heterogeneous catalysts for transesterification that are most frequently used. Zhang et al. (1988) demonstrated the fundamental characteristics of alkaline earth metal oxides using temperature-programmed desorption (TPD) of adsorbed carbon dioxide analysis. They found that the surface area is closely associated with the number of basic sites per unit weight and that the fundamental sites are organized as follows: CaO > MgO > SrO > BaO. Oxides from stronger basic sites facilitate the reaction more effectively. Due to its numerous benefits, which include a long catalytic life, a relatively high basic strength, high activity, and limited solubility in methanol, CaO has been the most researched metal-based catalyst for the production of biodiesel (Latchubugata et al., 2018).

2.8.2.2.2. Heterogeneous acid catalysts

They discovered that the fundamental sites are arranged as follows: CaO > MgO > SrO > BaO, and that the surface area is roughly correlated with the number of basic sites per unit weight. Stronger basic sites' oxides aid the reaction more successfully. CaO has been the most studied metal-based catalyst for the synthesis of biodiesel due to its many advantages, which include a long catalytic life, a relatively high basic strength, high activity, and limited solubility in methanol (Latchubugata et al., 2018).

2.8.2.2.3. Impregnation

The most straightforward technique for preparing catalysts is the wet impregnation approach, which is typically employed to create doped CaO catalysts. It is also the simplest way to synthesize supported catalysts and an active catalytic precursor. The impregnation of alkali metal ions onto Nano CaO particles improves the fundamental strength of CaO catalysts. The esterification and transesterification reactions benefit greatly from this system's ability to uniformly disperse the catalyst active site across the surface. Using a

solution of metal acetate, sulfate, or nitrate salts of the metal oxides to be impregnated, solid support is combined (Widiarti et al., 2019).

In a typical preparation, CaO solid carrier is first suspended in water, then the aqueous solution of the precursor is added and finally, the catalyst is calcined at the temperature between 500 °C and 900 °C in order to transform the precursor to the active form (Banković-Ilić et al., 2017)High calcination temperature favors the interaction between the carrier and the active component to form new crystals, i.e. new active sites, but overheating results in the surface sintering and the reduction of the specific surface area, i.e., the catalytic activity.

The activity of a doped CaO catalyst depends on the type of metal ion, available basic sites of CaO and interaction between metal ions and CaO. Ions of alkali metals (Li+, K+ and Na+) with various concentration are used as an impregnation precursor. (Kumar & Ali, 2013) reported that nano CaO impregnated with alkali metal ions showed a high specific surface area, compared to neat CaO; the highest specific surface area was found in the case of lithium–impregnated CaO. The basic strength of the alkali metal ion impregnating CaO and the catalytic activity toward transesterification decreases with increasing the ion size. The amount of Li+ ions impregnated onto CaO that provides the best catalytic activity is 1.5% (Kumar & Ali, 2010) or 1.75% (related to carrier amount) (Kumar & Ali, 2012), while the best amount of impregnated K+ ions is 3.5% (related to carrier amount) (Mahesh et al., 2015), i.e., 3% (related to oil amount) (Kataria et al., 2017).

2.8.2.2.4. Effect of calcination temperature

The solid catalyst's calcination process facilitates the interaction of CaO with other metal oxides, potentially increasing the catalyst's stability. Lower conversions were obtained with the CaO and transition metal ions thermally treated at 500°C and 600°C. Furthermore, the solid state reaction caused by high temperature treatment may improve the contacts between catalytic components, increasing the catalyst's stability (Kataria et al., 2017). However, because of the sintering of fine crystals and cluster aggregation, which decreased the catalyst's activity, the conversion efficiency somewhat declined as the temperature rose (Kumar & Ali, 2012).

2.9.Advantages and disadvantages of biodiesels

There are several benefits and drawbacks to using biodiesel in terms of economy, environmental issues, and fitness for use.(2016) U.S. Department of Energy According to Gulab et al. (2012), utilizing biodiesel has the following benefits:

2.9.1. Biodiesel reduces greenhouse gas emissions

When biodiesel is used instead of petroleum, there are noticeable drops in greenhouse gas emissions during the life cycle. According to Argonne National Laboratory's life cycle analysis, B100 emits 74% fewer greenhouse gases than petroleum-based diesel. Carbon dioxide (CO2) from the environment is absorbed by oil seed plants to form their stems, roots, leaves, and seeds. The oil from the oilseeds is extracted and processed into biodiesel. CO2 and other emissions are produced and released back into the atmosphere when biodiesel is utilized. Overall, the amount of CO2 emitted has minimal effect on the net CO2 concentration of the air because most of it gets repurposed by the next oilseed crop as it matures (Panwar et al., 2011).

2.9.2. Biodiesel reduces tail pipe emissions

Biodiesel appears to be totally compatible with emission control, with the exception of NOx, which appears to reduce when biodiesel is utilized. The fact that NOx emissions rise as biodiesel concentration rises could be a problem in regions where ozone levels aren't being met. (Demirbas and Demirbas 2010).

2.9.3. Biodiesel and human health

The impact of biodiesel and its mixes on human health is a widely researched topic. Diesel engine emissions include particulate matter and hydrocarbons, which can be harmful and/or cause cancer. The Mining Safety and Health Administration states that converting underground diesel vehicles' PM emissions from petroleum diesel fuel to high biodiesel mix levels (B50 to B100) dramatically decreased worker exposure. In areas where people are more exposed to diesel exhaust, even tiny amounts of biodiesel lower PM pollution and have a major positive impact on compliance and health Demirbas (2010).

2.9.4. Biodiesel improves engine operation

Even at relatively low levels, gasoline is made more lubricating and has a higher cetane number when biodiesel is added. Diesel engines rely on the lubricating properties of the gasoline to prevent moving parts—fuel pumps and injectors in particular—from malfunctioning prematurely. The ASTM D975 specification for diesel fuel was changed to include a lubricity requirement (a maximum wear scar diameter on the high-frequency reciprocating rig [HFRR] test of 520 microns) in order to account for the decreased natural lubricity of ultra-low sulfur diesel. At mix percentages as low as 1%, biodiesel can provide low-natural-lubricity diesel fuels with the lubricity they need (Demirbas, 2009).

2.9.5. Biodiesel is easy to use

All things considered, one of the main advantages of biodiesel is its ease of use. There is no need for extra equipment or equipment adjustments for blends containing B20 or less. B20 can be kept in diesel fuel tanks and pumped with equipment made specifically for that use. Although B20 does need to be handled carefully and employ safeguards when using it, most users shouldn't have any problems. (Demirbas, 2007).

There are certain drawbacks to utilizing biodiesel as a diesel fuel in addition to these benefits. Increased viscosity, reduced energy content, greater pour and cloud points, decreased engine power and speed, injector coking, engine compatibility, high cost, and increased engine wear are the main disadvantages of biodiesel. When compared to normal diesel, biodiesel has significant operating disadvantages such as reduced energy content, more copper strip corrosion, problems pumping fuel due to higher viscosity, and cold start difficulties. When biodiesel is utilized in place of pure petroleum diesel, the amount of gasoline consumed increases in proportion to the percentage of biodiesel component. This increase in fuel consumption drives up the total cost of utilizing biodiesel as a fuel because it has a greater production value than petroleum diesel.

2.10. Main factor affecting the yield of biodiesel

2.10.1. Alcohol quantity

The molar ratio of alcohol to triglycerides is one of the most important parameters determining the generation of biodiesel, according to numerous research. In order to produce 3 mol of fatty acid ester and 1 mol of glycerol, the transesterification reaction requires 3 mol of alcohol to 1 mol of triglyceride. When too much alcohol is used to make sure that the oils or fats are fully converted to esters, a greater alcohol to triglyceride ratio may result in a speedier ester conversion during the biodiesel production process. However, recovering glycerol becomes more challenging when alcohol levels are too high. For this reason, the ideal alcohol to oil ratio needs to be established empirically, accounting for the specifics of each operation.

2.10.2. Reaction temperature

Temperature significantly affects how biodiesel reacts and is produced. Raising the reaction temperature can decrease oil viscosities by accelerating the reaction rate and cutting down on the reaction time. The yield of the biodiesel product decreases when the reaction temperature exceeds the optimum range because a higher reaction temperature speeds up the triglyceride saponification reaction.

The majority of the findings indicate that methanolysis can take place close to methanol's boiling point and that when temperature rises, the reaction is more likely to go in the opposite direction since methanol is not involved.

2.10.3. Catalyst

The yield of the biodiesel product may depend on the type of catalyst and its concentration. As was already mentioned, sodium hydroxide is the catalyst for the process that is used the most frequently. However, research has shown that conducted to identify an alternative catalyst that is environmentally benign and produces higher biodiesel yields in a shorter reaction time.

2.11. Characterization of biodiesel

Test procedures for the physical and chemical properties of FAME (fatty acid methyl esters) and free fatty acid concentration play a major role in the production of biofuels. The American Society for Testing Materials (ASTM), the European Union (EN), and the Czech Republic (CSN) are the main evaluators of biodiesel quality. The biodiesel's quality ought to be up to par. Hoang (2018) reports that the ASTM (D6751) and EN 14214 biodiesel standards were compared to the density, kinematic viscosity, oxidation stability, flash point, cloud point, and fire point of biodiesel made using different catalysts. These estimates are crucial since the yield and purity of biodiesel are influenced by the feedstock's acid value and saponification value.

2.11.1. Density measurement

The process of converting mass to volume, which is necessary for the manufacture of biofuel, depends on density, which is defined as weight per unit volume. It significantly affects how well the fuel performs. Density measurements are used to find the best mix formulation for biodiesel fuel. The type of oil used and the technique of treatment are the main determinants of biodiesel density. A factor is also the constituents' molecular weight, such as the molecular weight of fatty acids. The quality of the fuel is impacted by the high density that results from a high molecular weight. Low density fuel is said to be of greater quality than higher density fuel (Hoang, 2018).

2.11.2. Kinematic viscosity

A key factor in establishing the quality of biodiesel is its viscosity. Viscosity, or the gasoline's ability to flow, affects how well fuel injection machinery performs, especially at low temperatures. When gasoline is atomized, high viscosity fuel causes large droplets to form, which can interfere with operation; higher carbonization eventually results in more emissions and smoke; and too little viscosity fuel leads to leaks and increased wear, which has a substantial impact on engine performance. When compared to diesel fuel, biodiesel has a higher viscosity, which poses issues for process equipment and design. At a temperature of 40°C, an experimental viscosity is measured using a viscometer. In

accordance with ASTM D6751 and EN 14214, the kinematic viscosities of biodiesel were 1.9-6 and 3.5-5.0 mm2/s, respectively (Huang et al., 2020).

2.11.3. Acid values

The acid value is a crucial parameter because it affects a biodiesel's quality. This is referred to as the presence of free acids in a sample. By neutralizing the sample with a certain quantity of KOH, the excess of free fatty acid that leads to soap formation and catalyst deactivation can be detected. According to Ismail and Ali (2015), feedstock acidity should typically range from 1.86 to 3.31 mg KOH/g oil.

2.11.4. Iodine value

The degree of unsaturation of a substance is measured by the iodine value. The kind and character of the feedstock also have a major role in determining how well the fuel oxidizes. The diesel fuel's iodine content needs to be less than 120 g I2/100 g of the given sample. It will identify the level of unsaturation in a certain mixture of fatty acids. Higher iodine levels are correlated with lower oxidative stability. Additionally, it will cause corrosion, have an effect on the quality of lubricant, and provide information regarding the production of sludge in fuel (Bassam et al., 2019).

2.11.5. Saponification value

The saponification value is the volume of potassium hydroxide (KOH) required in a given setting to saponify one gram of fat or oil. It calculates the fatty acid molecular weight and length. Crude esters saponify more readily than oil, with a range of 199 to 207 mg KOH/g oil. Since there are fewer free carboxylic acid groups per unit of fat or oil, longer fatty acid chains have lower saponification values. Most often, it is used to determine the average molecular weight of the sample's lipids or oil. On the other hand, the saponification values of crude feedstock and biodiesel are similar, despite small differences in average molecular weight. According to Gopinath et al. (2009), the differences between saponification values are essentially the same.

2.11.6. Cetane number

A key factor in evaluating the fuel's ability to ignite is the assessment of cetane number. It reveals the fuel's ability to automatically ignite as soon as it is injected into the fuel line as well as the fuel's quality of ignition. In contrast, low cetane numbers primarily affect engine parameters including increased smoke emission, combustion instability, and excessive deposit of unburned components in the engine (Ismail and Ali, 2015). Higher cetane numbers are equivalent to better ignition quality. As the fatty acid chain lengthens, there are more cetane molecules present. The cetane number of biodiesel is higher than that of diesel fuel.

2.11.7. Cloud and pour point

The temperature at which wax crystals become visible is known as the cloud point (CP), and it is attained when a fuel cools. It causes fuel to solidify at lower temperatures, which can clog fuel lines. Using ASTM Standards D5771, D5772, D5773, and D2500, the cold point is ascertained. Using a Differential Scanning Calorimetry (DSC) curve is a simple approach to monitor the generation of crystalline biodiesel. Engine performance will eventually be lowered by fuel agglomeration brought on by higher crystallization. Additionally, it may thicken and clog the engine's fuel filters, lowering the engine's capacity for fuel flow. Because there is a significant amount of saturated fatty acids present, the fuel crystallizes easily (Sierra-cantor and Guerrero-fajardo, 2017).

The pour point is the lowest temperature at which fuel can be consumed and starts to flow. High concentrations of saturated fatty acids in biodiesel indicate poor fuel qualities since they have a high pour point. The trans-esterification procedure significantly lowers the pour point value to provide gasoline of the highest caliber. The fuel characteristics are further affected by the increasing concentration of fatty acids that occurs after catalysis.

2.11.8. Flash point

The temperature at which a fuel will ignite when it comes into contact with flames is known as its flash point. Biodiesel fuels are safer to transport and store than fuels derived from petrochemicals because of their higher flash points. Fuels can be categorized based on low flash points, which denote significant volatility and reduce handling and transportation concerns. Diesel fuel has a lower flash point than biodiesel. The minimum and maximum temperatures for biodiesel specified by EN 14214 and ASTM 6751 are 120°C and 130°C, respectively. Impurities like oil content, moisture content, and unreacted alcohol can all have an impact on it. Thus, it indirectly contributes to determining the quality of biodiesel fuels (Rasouli Esmaeili, 2019).

Table 2: Requirement for biodiesel (B100) as listed in ASTM D6751-03

Property	ASTM methods	Limits	Units
Flash Point	D93	130.0 min	^{0}C
Water and sediment	D2709	0.050 max	% vol
Kinematic viscosity 40 ^{0}	D445	1.9-6.0	mm^2/se
Sulfated ash	D874	0.020 max	%mass
Copper strip corrosion	D5453	N.3 max	%mass
Cetane number	D130	47 min	
Cloud point	D613	Report to customer	^{0}C
Carbon Residue	D2500	0.050 max	%mass
Acid number	D4530	0.80 max	mgKOH/g
Free glycerin	D664	0.020 max	%mass
Total Glycerin	D6584	0.240 max	%mass
Phosphorus content	D65884	0.001 max	%mass
Distillation temperature 90^0	D4951	360 max	^{0}C
Recovered D90	D1160		

2.12. Optimization parameters of raw materials for increasing biodiesel production

2.12.1. Methanol to oil ratio

The kind of alcohol utilized, the type of triglycerides used, and the alcohol to oil molar ratio are the three most crucial factors in the synthesis of biofuel. The most common M:O ratio, which increases oil solubility and contact time, is 3:1. A higher ester conversion occurs more quickly when the molar ratio is high. According to stoichiometry, the esterification process requires 3 mol of alcohol and 1 mol of triglycerides in order to make 3 mol of fatty acids and 1 mol of glycerol. Since the catalyst and oil composition mostly determine the molar ratio, a substantial amount of alcohol is required to accelerate the reaction. Next, the least amount of alcohol is used to increase the generation of biofuel. Excessive alcohol application will eventually block the catalyst's active sites, favoring the opposite reaction (Gashaw et al., 2015). In the transesterification process, alcohols such as methanol, ethanol, propanol, and butanol are commonly utilized. Many experts claim that since ethanol is made from agricultural waste and shares many of the same environmental advantages as methanol—which is widely used because of its low cost—it can also be used (Hossain and Boyce, 2009).

2.12.2. Catalyst weight

This is an additional essential component in the biodiesel synthesis process. The type and amount of catalyst used are mostly determined by the reaction conditions (basic or acidic). Inadvertently, it relies on the type of feedstock that is employed. The ideal amount of catalyst is sufficient to carry out the intended reaction if the feedstock is pure, has normal moisture content, and has a high concentration of free fatty acids. However, if the feedstock is high in moisture and free fatty acids, the transesterification process is minimal since it mostly results in the generation of soap and lowers the yield of biodiesel. A larger catalyst concentration in the latter case will almost certainly increase biodiesel production. As a result, proper feedstock pre-treatment is essential for carefully controlling catalyst optimization (Gashaw et al., 2015).

For the best concentration of triglycerides, the average catalyst amount was found in the majority of studies to be between 1-6 wt percent, although it also varied significantly depending on the volume of production. It has been noted to be high in some instances, depending on the reaction conditions. It has been established that high catalyst weight generally has a detrimental effect on catalytic reaction because it imposes restrictions on mass transfer and reduces active site contact due to agglomeration (Kazemifard et al., 2018).

2.12.3. Reaction temperature

The temperature of the reaction has a big influence on increasing yield. As the temperature rises, the kinetics and reaction rate quicken, cutting the reaction time in half (Gashaw et al., 2015). Depending on the type of oil used, the trans-esterification process is often carried out below the alcohol boiling point, with an ideal temperature range of 50–60°C. The alcohol being used evaporates causes the temperature to rise. Many organizations state that it takes 60 to 90 minutes to get a 78 percent conversion at room temperature (Hossain and Boyce, 2009).

2.12.4. Reaction time

When trans esterifying oils or fatty acid esters, temperature and duration play crucial roles. Fatty acid conversion speeds up over time since it initially takes a while for oil to turn into methanol or alcohol due to dispersion and mixing. The conversion is typically faster as the reaction gets closer to the threshold due to faster reaction kinetics. Because a mixed catalyst completes the reaction in only 1-2 hours, the choice of catalyst has a big impact on how long it takes. According to reports, the maximum ester conversion can be achieved in 90 minutes; however, because the reaction is reversible, extending the time did not increase the yield, leading to more glycerol generation (Hossain and Boyce, 2009; Gashaw et al.).

CHAPTER THREE

3. MATERIALS AND METHODS

The method for synthesis of lime based potassium doped CaO nanocatalyst, isolation, culturing, and harvesting of C.vulgaris microalgae, extraction and characterization of microalgal oil, biodiesel production, and transesterification parameters optimization methods were discussed in this chapter along with the materials and tools used and the working procedures followed in this study.

3.1. **Materials**

3.1.1. Chemicals

These chemicals were used to carry out the experimental work: KH_2PO_4, K_2HPO_4, $MgSO_4.7H_2O$, $CaCl_2.6H_2O$, H_3BO_3, $MnCl_2.4H_2O$, $CUSO_4.5H_2O$, Co $(NO_3)_2.6H_2O$, $Na_2MoO_4.2H_2O$, $CoCl_2$, NH_2SO4, $ZnSO_4$. $H2SO_4$, $Fe_2(SO_4)_3$, n-hexane, KOH, Ethanol 99.5%, Methanol 99.5%, diethyl ether, phenolphthalein, $ZnSO_4$. All the chemicals used in the production process were analytical grade.

3.1.2. Equipment

The following equipment were used to conduct the experimental work: GX-65B oven drier, agate mortar equipment, grinder, Erlenmeyer flask, water bath, SX-2.3-10 Muffle Furnace, glass desiccators, crucible, microwave assisted lipid extractor, separator funnel, filter paper, condenser, glass rode, aerator, lux meter, beaker, graduated cylinder, distillation flask, thermometer, pH meter, conductivity meter, inverted microscope, digital balances, viscometer, magnetic stirrer, hot plate, three-necked round bottom flasks, 63 µm sieve, aluminum foil, UV/VIS spectrophotometer, X-Ray Diffraction (XRD), Scanning electron microscope (SEM), and Fourier transform infrared spectroscopy (FTIR).

3.2. Methods

3.2.1. Description of the study area

Samples were taken from the pond in Tuludimttu, in the eastern section of the city of Addis Abeba, at three separate places. At an elevation of 2,355 meters, Addis Ababa is situated in the grassland biome at 9°1′48″N 38°44′24″E. The city is located at the base of Mount Entoto and is a part of the Awash watershed, according to "NGA: Country Files" (2012). Addis Ababa climbs to almost 3,000 meters in the Entoto Mountains to the north, rising from its lowest point, around Bole International Airport, at 2,326 meters above sea level on the southern periphery. Although the research region is close to the equator, it has a temperate Afro-Alpine climate. According to measurements made at Addis Ababa Observatory, the average daily temperature ranges from 9.9 to 24.6 °C, and the average annual rainfall is 1254 mm. There are two main seasonal weather patterns that define the climate. Known locally as Kiremt, the major rainy season runs from June to September and accounts for over 70% of the yearly rainfall. Belg, the local term for the minor rainy season, brings rainfall to the area from mid-February to mid-April. (2006) Molla et al. And the dry season lasts for the remaining months. The pond was not designed to dry up in the Ethiopian summer and fill up with water during the rainy season.

3.2.2. Study design

The work was based on a laboratory experiment that used the right technique to isolate the species of chlorella vulgaris microalgae that could be used to make biodiesel utilizing an improved lime-based heterogeneous Nano-catalyst.

3.2.3. Isolation, purification and culture condition of chlorella species

By streaking dilution method, *Chlorella vulgaris* species was separated from water samples and individual *chlorella vulgaris* colonies was recognized by studying colonial and morphological aspects microscopically (Kawamura et al., 2020). The microalgae was cultivated on both liquid and agar slants using Bold's Basal Medium (BBM), incubated at 24 ^{0}C with a 2500 lux light intensity and appropriate CO_2 supply; and a 12:12 h light and

dark cycle. Repeated plating and regular examination under a microscope ensured the cleanliness of the culture (Kong et al, 2012).

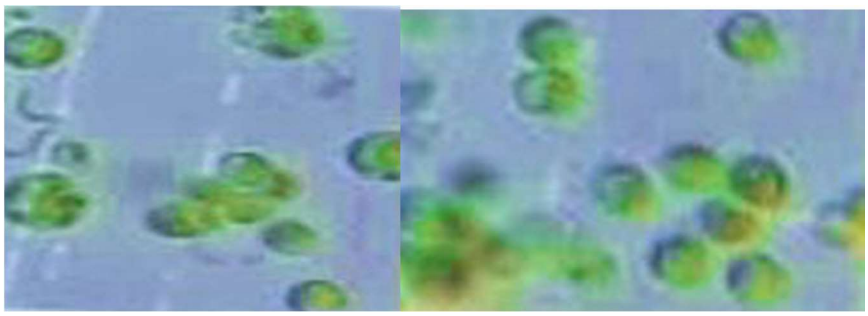

Figure 4: chlorella vulgaris morphological structure

Culturing of *Chlorella vulgaris* was carried out in 500 ml Erlenmeyer flasks containing Bold's Basal Medium (pH 6.5) at 24 ^{0}C with light illumination 2500 lux 12:12 h light: dark cycle),supply of sufficient CO_2 and shaking under 100 rpm in an illumination incubator for 12 days.

The chlorella vulgaris cultures were harvested using flocculation harvesting method. A 0.2 g of $Al_2 (SO_4)_3$ was used for a litter of culture (Matter et al., 2019). The wet cell mass was kept for drying in a laboratory grade hot air oven at 60 °C for 2 days then grinded by pestle and mortar.

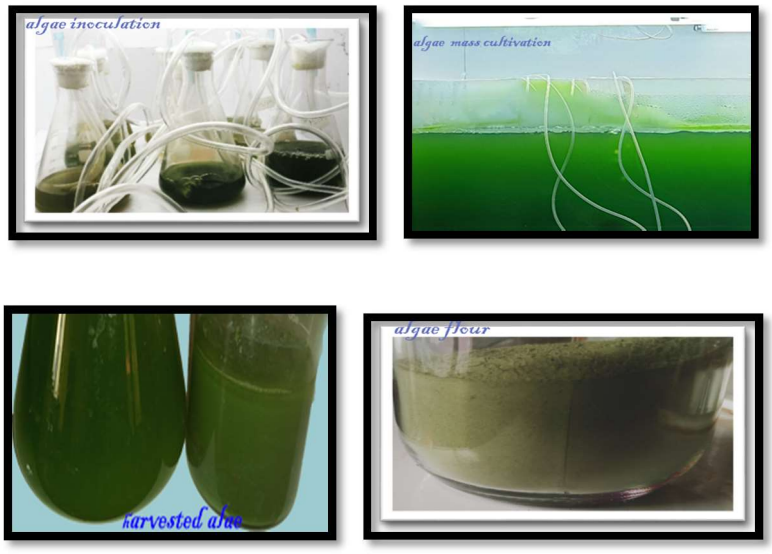

Figure 5: microalgae biomass cultivation, harvesting and drying

3.2.4. Growth evaluation, harvesting and drying of *Chlorella vulgaris*

By measuring the optical density of the algal culture at 750 nm once every 24 hours, the growth of C. vulgaris was spectrophotometrically tracked using a UV/VIS spectrophotometer (Phukan et al., 2011).

Equation 1 was used to compute the microalgae's specific growth rate, which was derived as follows (Krzeminska et al., 2014):

$\mu = \ln (N2/N1)/ (t\ 2 - t\ 1)$……..……Equation (1)

Where: μ is the specific growth rate, N1 and N2 are the biomass at time 1 (t 1) and time 2 (t 2), respectively.

The *Chlorella vulgaris* cultures was harvested by using flocculation harvesting method. A 0.2 gm of $Al_2 (SO4)_3$ was used for a litter of culture (Matter et al., 2019). The wet cell mass was dried in open air at room temperature. The dry weight of the algal biomass was determined gravimetrically and growth was expressed in terms of dry weight (Phukan et al., 2011).

For immediate specific growth rate determination measuring molar concentration or optical density using equation 2 below is best suited.

Dopt= αl

Where, Dopt –optical density, α—absorption coefficient, l—cuvate thickness (1 cm)

$\alpha=2.303*A/l$…….Equation (2)

Where A is absorbance and l is thickness of cuvate or light path length

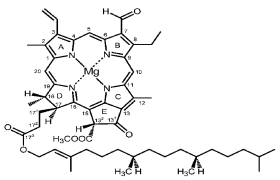

Figure 6 Chlorophyll molecular structure

3.3.Microwave assisted lipid extraction

Microwave assisted extraction was applied to extract oil from the powdered micro algal biomass (Dai et al., 2014). The Ethos One high performance microwave digester was used to extract ten grams of dried *Chlorella vulgaris* microalgae powder using a methanol-hexane solvent mixture of 1:2 ratio at 600 W of microwave power for 30 minutes at 80 ^{o}C. The filtrate and residue were separated from the biomass and solvent combination after extraction.

3.4.Physicochemical properties of extracted oil

The oil was assessed for density, kinematic viscosity, acid value, FFA content, saponification value, ash content, and moisture content, among other physicochemical properties. These elements could impact the biodiesel's quality both directly and indirectly (Ejim and Kamen 2013).

3.4.1. Density

A pyconometer's weight was ascertained by means of an electronic weighing balance. A 25 ml heated sample to 20 0C was put into a pyconometer, and the weight of the oil and bottle was recorded. The following formula was used to compute the density.

Density= (W2-W1)/V…….Equation (3)

3.4.2. Kinematic viscosity

A viscometer was used to measure the oil's viscosity. For thirty minutes, the oil sample in the viscometer cup was maintained in a water bath at a constant temperature of 40 °C. After inserting the viscometer tip into the sample-containing cup, the reading for a predetermined liquid volume was noted. Equation (4) was used to compute kinematic viscosity based on the dynamic viscosity reading obtained from the viscometer.

$N=\mu/\rho$…….Equation (4)

Where; N = kinematic viscosity in mm2/s, μ = dynamic viscosity pa.s, ρ = density in gm/ml

3.4.3. Acid value

Titrating oil or fat in an alcoholic medium directly against a standard solution of potassium hydroxide and sodium hydroxide yields the acid value. The value represents the quantity of fatty acids that have been hydrolyzed out of the glycerides as a result of the action of heat, moisture, and/or the lipolytic enzyme lipase.

0.1N KOH in distilled water was created as a titration solution to measure the acidity of the extracted micro algal oil. The titration beaker containing the sample oil contained two grams of oil, 25 mL of hydrous ethanol (99% w/w), 25 ml of diethyl ether, and 5 drops of phenolphthalein. Finally, until the first color shift (pinkish color appears), add 0.1N of KOH to the titration solution one drop at a time. Following that, the titrant volume was recorded and used to compute the acid value using equation 4:

Acid value (AV) = (N×V×56.1)/W……Equation (5)

Where; V = volume of KOH used in ml, N = normality of KOH, W = mass in gram of oil sample, 56.1 = molar mass of KOH

3.4.4. Free fatty acid value

Using equation (5) and the oil's acid values (AV), the free fatty acid value, or FFA, of the oil samples was determined.

%free fatty acid value (FFA) =AV/2……..…….Equation (6)

Where: %FFA = percentage of free fatty acid, AV = Acid Value of the oil

3.4.5. Saponification value

The saponification value (SV) is the quantity of potassium hydroxide (KOH) in milligrams needed to saponify one gram of oil/fat. One gram of the oil sample, once it has been weighed was added to a 250 ml conical flask along with 25 ml of a potassium hydroxide solution containing 0.5 mol/l of ethanol and 70 ^{0}C for 60 minutes of refluxing. After the heated mixture has cooled, five drops of phenolphthalein was added as an indicator, and the solution was then be titrated with 0.5mol/l hydrochloric acid solution. The titration was stopped when the color shift is visible, and the value was recorded. On a blank sample (on

devoid of oil), the identical method was carried out, and the volume v-b was noted. Before the saponification value is calculated using equation 7.

Saponification Value (SV) = (MW×N (v_b-V_s))/W……..Equation (7)

Where; SV = saponification value, MW = molecular weight of KOH, N = normality of HCl solution, V_b = volume of HCl solute ion used in blank V_s =volume of HCl solution used in the sample and W= weight of oil used.

3.4.6. Ash content

A muffle furnace was used to determine the oil's ash content. The sample was placed in a muffle furnace after being added to a burning cup with 20 gm of sample. Ash content was determined by weighing the leftover sample after burning it in a furnace set at 500 ^{0}C for two hours. The equation 8 was used to determine the amount of ash.

Ash Content (%)=(Mass of oil after burning/Initial mass of oil) ×100……..Equation (8)

3.4.7. Moisture content

A dish was weighed both with and without oil. The oil-covered plate was dried for seven hours at 105 0C in an oven, with the weight being measured every two hours until a steady weight was reached. At that point, the weight was taken and compared to the weight that had been first recorded. Using equation 9, the % weight in the oil was determined.

Moisture Content (%)=(W1-W2)/W1*100 ……..Equation (9)

Where; W1 = Original weight of the sample, W2 = Weight of the sample after drying

3.4.8. Molecular weight determination

The molecular weight of the algal oil was determined according to the equation (10) below:

MW=168300/(SV-AV)…….……Equation (10)

Where; MW= molecular weight of oil, SV = saponification value of oil, AV = acid value of oil.

Figure 7: characterization process

3.5.Catalyst preparation

3.5.1. Sample preparation (calcium oxide catalyst)

To get rid of any contaminants clinging to it, the limestone was rinsed with distilled water after being repeatedly cleaned with tab water. After that, it was dried in an oven at 120 ^{0} C for 24 hours. After being ground into a powder, it was let to go through a 63 µm sieve. In order to preserve it for later use, the powdered lime was lastly calcined for three hours at 900 0 C.

Even though CaO is a well-known catalyst for producing biodiesel, it has been noted that under some reaction circumstances, the single CaO catalyst is unstable. Additionally, the low surface area of CaO limits the amount of active basic sites on the catalyst surface, and during the transesterification process, it occasionally experienced Ca species leaching (N. Mijan, 2015). This had a significant impact on CaO's catalytic effectiveness in generating the highest possible biodiesel output. Thus, a great deal of work has gone into changing the CaO catalyst in an effort to enhance its physicochemical characteristics, such as its basic strength, stability, and catalytic activity (A. P. S. Chouhan and A. K. Sarma, 2011). The production efficiency of biodiesel was greatly impacted by this change in the physicochemical qualities. Doping the catalyst with certain elements like potassium chloride would improve its basicity, surface area, and decrease its particle size.

3.5.2. K substituted calcium oxide nano-catalyst

The KCl/CaO catalyst was made by wet impregnation. Generally, 15 ml of aqueous KCl solution (0.45 mol of KCl/mol of support, lime) was gradually stirred in 2 g of lime-based CaO. The combination was then allowed to sit at room temperature for two hours while

being continuously stirred. The resultant slurry was filtered, and it was then dried at 120°C for six hours. After being dehydrated, the product was calcined for three hours at 800°C to activate it. It was then stored in a desiccator for future usage (Win Win Mar, Ekasith Somsook, 2012).

Figure 8: (a) lime of 63 μmeter dm, (b) calcined CaO, (c) K doping process, (d) K/CaO

3.5.3. Characterization of limestone nano Catalyst

3.5.3.1.XRD analysis of CaO

X-ray diffraction (XRD) was used to investigate the two distinct CaO samples. Using the information obtained from the XRD pattern of the CaO Nanoparticle, the Debye-Scherer equation was used to determine the average crystal size of the catalyst.

Scherer equation=$K\lambda/\beta COS\theta$……..……Equation (11)

The X-ray wavelength, measured in nanometers (nm), is represented by λ, while the peak width of the diffraction peak profile at half maximum height, measured in radians, is represented by β. D is the average crystallite size diameter. Since Cos is the same number as Bragg angle, the Bragg angle can be stated in either degree or radian. K: is a constant associated with crystallite shape and is typically taken to be 0.9.

3.5.3.2.SEM analysis

Scanning Electron Microscope (SEM) used to determine the morphology structure of the CaO Nano catalyst and K/CaO.

3.5.3.3.Fourier transform infrared spectroscopy (FTIR)

The functional groups present on the Nano catalyst surface were identified with the help of Fourier Transform Infrared Spectroscopy (FTIR); the method used was KBr method at room temperature in the IR range of 500–4000 cm−1 (Bharti et al., 2019).

3.6.Optimization of transesterification parameters using Taguchi method

Minitab statistical software was used for the optimization of biodiesel production. Taguchi design of experiment (DOE) in the software makes use of orthogonal arrays to optimize different process influencing parameters and the extent to which they can be varied. The very specialty of this method is that it only investigate few pairs of combinations rather than all the possible parameters combinations. This method make it possible to collate data for the determination of factors that have the most influence on product quality with minimal number of experiments so as conserving valuable time and resources (Sathish Kumar et al., 2015).

In this analysis, only the three levels (L = 3, P = 4, as indicated in Table 3) and the four most influential characteristics were taken into account. The effects of the four chosen parameters, such as A) catalyst loading (% wt.), B) methanol to oil ratio (molar ratio), C) reaction time (min), and D) reaction temperature (oC) at three different levels, were investigated by conducting only twenty-seven experiments (including the replicates) in accordance with L27 OA shown in appendix 3.

Table 3: Parameters and their levels

Level	**Parameter**			
	A	**B**	**C**	**D**
	Catalyst lode (wt %)	**Molar ratio**	**Reaction time(Min.)**	**Temp. (°c)**
1	**1**	**7:1**	**60**	**50**
2	**3**	**9:1**	**120**	**57**
3	**5**	**13:1**	**180**	**65**

3.7.Biodiesel production process and experimental design

The three-necked round bottom flask was submerged in a dish that served as a water bath for controlling the temperature of the hot plate with magnetic stirrer. In order to monitor the reaction temperature, a thermometer was placed in one of the side necks. The other side neck was linked to a water-cooled condenser to minimize methanol loss, and reagents were introduced through the center neck (Chowdhury et al., 2019). The transesterification process was run with different catalyst loadings, alcohol to oil molar ratios, reaction times, and temperatures in order to maximize the biodiesel production. Measured microalgal oil was added to a three-necked flask that had been heated to 50°C. Next, the catalyst was weighed and mixed with the required volume of hot alcohol (methanol). Lastly, the heated oil was mixed with the calcium methoxide solution. Each experiment used 15 milliliters of microalgal oil, and the agitation speed was 600 revolutions per minute.

Figure 9: Transesterification reaction setup

The solution was placed into a separating funnel after the reaction was finished. The product was left hanging for an entire night so that the distinct layers of glycerol, catalyst, and methyl ester could be recognized. The layers of glycerol, catalyst, and crude biodiesel are quite noticeable after a one-night stand. Biodiesel was on top while glycerol was at the bottom due to its density.

3.8.Signal to Noise Ratio (SNR) and statistical analysis of variance (ANOVA)

Using Taguchi DOE in MINITAB software, statistical data analysis of the optimization of biodiesel production data was conducted. Taguchi advised using the log function to calculate the difference between the experimental and desired values of performance attributes. A signal to noise ratio (SNR) was then calculated using the log function value. The optimization problem's objective is the expected outcome's log functions, or SNRs.

The quality function's deviation from the predicted value is then computed using SNR. The Taguchi technique uses three different sorts of SNRs, depending on the goal of the task. It is possible to use Larger-the-Better (LTB) for maximizing problems, Smaller-the-Better (STB) for minimization problems, and Nominal-the-Better (NTB) for normalization problems. The SNR (dB) can be computed for NTB, STB, and LTB models as follows (Saravanakumar et al., 2016; Satish Kumar et al., 2015).

$$\text{Nominal the best} - \text{SNR}_i = 10\log\left(\frac{\overline{y}_i^2}{s_i^2}\right)$$

$$\text{Smaller the better} - \text{SNR}_i = -10\log\left(\sum_{j=1}^{n}\frac{y_j^2}{n}\right)$$

$$\text{Larger the better} - \text{SNR}_i = -10\log\frac{1}{n}\left(\sum_{j=1}^{n}\frac{1}{y_j^2}\right)$$

where

$$y_i = \frac{1}{n}\left(\sum_{j=1}^{n} y_{ij}\right) \text{ (mean value of response)}$$

$$s_i^2 = \frac{1}{n-1}\left(\sum_{j=1}^{n} y_{ij} - \overline{y}_i\right) \text{ (variance)}$$

.....Equation 12

Where: i-the experiment number, j- the trial number and n-the number of trials.

The best parameter combinations have been found by evaluating experimental data using signal-to-noise ratio (SNR). Since the goal of the current study is to maximize biodiesel yield, Larger-the-Better (LTB) was chosen from the three possible SNR quality parameters that are accessible based on the nature of variables. As a result, the level of control with the highest SNR was the ideal design parameter. SNR analysis can be used to determine the ideal concentration of each parameter and combination of parameters that would give the maximum amount of biodiesel; however, it is unable to determine which factors have had a major impact on the output or the relative contributions of each element to the output. This could be achieved by using a statistical analysis of variance (ANOVA) on the response

data (Sathish Kumar et al., 2015).For carrying out ANOVA of response data also, calculating the sum of squares is crucial. The following equation was used to calculate the percentage of contribution.

% contribution of factor= (SSF)/ (SST) x 100……Equation (13)

Where SSF-is the sum of the squares for the parameter and SST-is the total sum of the squares of all parameters.

CHAPTER FOUR

4. RESULT AND DISCUSSION

4.1.Catalyst Synthesis

4.1.1. Sample Preparation (Calcium Oxide Catalyst)

The first step in preparing the CaO Nano catalyst was to thoroughly wash and rinsed the obtained limestone in order to remove any impurities. It was then overnight dried at a temperature of 120°C in an oven. The lime was processed through a 63 μm sieve after being grounded into powder using a grinder machine. In order to prepare the powdered lime for usage in the future, it was calcined at 900°C for 3 hours.

4.1.2. Potassium (K) Substituted Calcium Oxide Nano catalyst

The process of wet impregnation was utilized to manufacture a calcium oxide catalyst doped with potassium. 15 milliliters of aqueous KCl solution (0.45 mol of KCl/mol of support, lime) was gradually mixed with two grams of CaO from lime. The combination was then allowed to sit at room temperature for two hours while being constantly stirred. The resultant slurry was filtered, and it was then dried at 120°C for six hours. After being dehydrated, the product was calcined for three hours at 800°C to activate it. It was then stored in a desiccator for future usage (Win Win Mar, Ekasith Somsook, 2012).

Catalytic activity loss can be significantly attributed to sintering, particularly in the case of supported metal catalysts. It modifies the catalyst's surface structure and reduces its surface area. A porous catalytic surface may experience surface area loss as a result of sintering-induced pore collapse (G. Kuczynski, 2012). Higher temperature calcination can lead to sintering, a process in which a material's particles fuse or melt together to form larger grains. These facts indicate that the ideal temperature ought to be selected. For CaO and K-CaO, calcination temperatures of 900 °C and 800 °C, respectively, were used based on the literature that was accessible.

4.2. Characterization of calcium oxide and potassium doped CaO nano-catalyst

4.2.1. X-Ray diffraction (XRD) analysis

For CaO produced by calcining limestone at 900 °C, the XRD examination of the sample was performed on a diffractometer using Ni filtered CuKα radiation at λ = 0.15406 nm in the range of 2 theta = 10-60o angle. The sample's sharp peaks were located at 2-theta at 29.19, 32.4, 33.85, 37.55, and 48.48 degrees. For K/CaO synthesized by calcination wet impregnated of limestone the sharp peaks of the sample was 32.31°,33.76°,37.48°,48.48°, and 54.01° in the range of 2theta 2θ = 10 – 60°this was similar with the main peak values characteristics of calcium oxide produced from limestone reported by (Kumar & Ali, 2013). As it is shown in table 4 below the average crystalline size diameter of CaO Nano catalyst is 26.646 nm.

Table 4: Results of XRD pattern of CaO Nano catalyst prepared by calcination at 900 ^{0}c

2theta Value (θ) (Degree)	***β*-beta FWHM (radians)**	λ (nm)	**Intensity**	**Crystallite size Dp (nm)**	Average Dp (nm)
29.19	0.22195	0.15406	846.4783	38.615	39.4306
32.4	0.21267	0.15406	548.6943	40.613	
33.85	0.27615	0.15406	2287.631	31.395	
37.55	0.232719	0.15406	341.5791	44.954	
48.48	0.300946	0.15406	1239.597	41.576	

K-scherrer constant = 0.94

There were prominent crystalline diffraction peaks in every sample. The XRD patterns of undoped and K doped CaO are displayed in Figures 8 and 9, and the creation of a strong peak denotes the formation of extremely crystalline materials. The XRD patterns showed no signs of impurities, indicating that the produced products were extremely pure.

The crystallite size diameter (D) in nanometer of the undoped CaO and K doped CaO nanoparticle has been calculated using the Debye Scherer equation shown below for each 2θ value and FWHM β size (radian) as shown in Table 4 and 5.

Scherer equation: $D=(K\lambda)/\beta COS\theta$

Where, D: average crystallite size diameter, λ: is the X-ray wavelength which is 0.15406nm for Cu Kα, β: is the peak width of the diffraction peak profile at half maximum height resulting from small crystallite size in radians, FWHM (full-width at half-maximum or half-width) is in radian, K: is a constant related to crystallite shape normally taken as 0.94, θ: Bragg angle can be in degrees or radians.

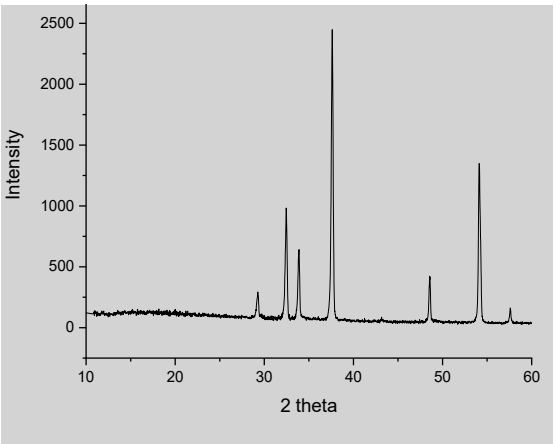

Figure 10: XRD pattern of CaO

Table 5: Results of XRD patterns of K/CaO nanocatalist

2theta Value (θ)	***β*-beta FWHM (radians)**	**K**	**λ (nm)**	**Intensity**	**Dp (nm)**	Dp average (nm)
33.321	0.219	0.94	0.15406	846.4783	39.532	38.1906
35.223	0.2126	0.94	0.15406	548.6943	40.931	
38.861	0.2761	0.94	0.15406	2287.631	31.854	
48.482	0.2287	0.94	0.15406	341.5791	39.773	
54.352	0.2399	0.94	0.15406	1239.597	38.863	

K- Scherrer constant

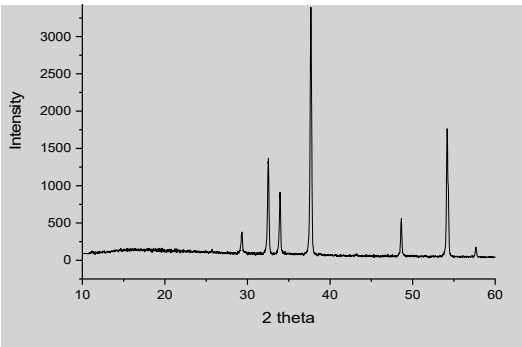

Figure 11 XRD pattern of K/CaO

4.2.2. Scanning electron magnification (SEM)

Large granules and a porous structure characterize the KCl/CaO catalyst, according to the SEM image displayed in Figure 10. Therefore, it may be inferred that improved substrate-methanol interaction on the porous catalyst surface may improve the catalytic reaction's efficiency. At magnifications of 20 μm, 10 μm, and 5 μm, micrographs were captured. Research on the enhancement of structure following calcination and particle size showed comparable outcomes (Fayyazi et al., 2018). Following calcination, a high porosity and high surface area CaO material was produced. This phenomena can be explained by the fact that the CaCO2 complex lost CO2 gas, converting it to CaO and creating a large porosity on the surface. As a result, it was anticipated that this physical attribute would directly lead to a high biodiesel production in addition to being a highly active site.

According to Bharti et al. (2019), a regular and ordered structure confers a benefit of having a more integrated regular pore distribution system. According to the K/CaO characterisation investigations, K doped CaO nanoparticles are predicted to exhibit increased activity toward the transesterification reaction since they are the smallest in size and have a bigger surface area than the other samples (Kumar & Ali, 2013).

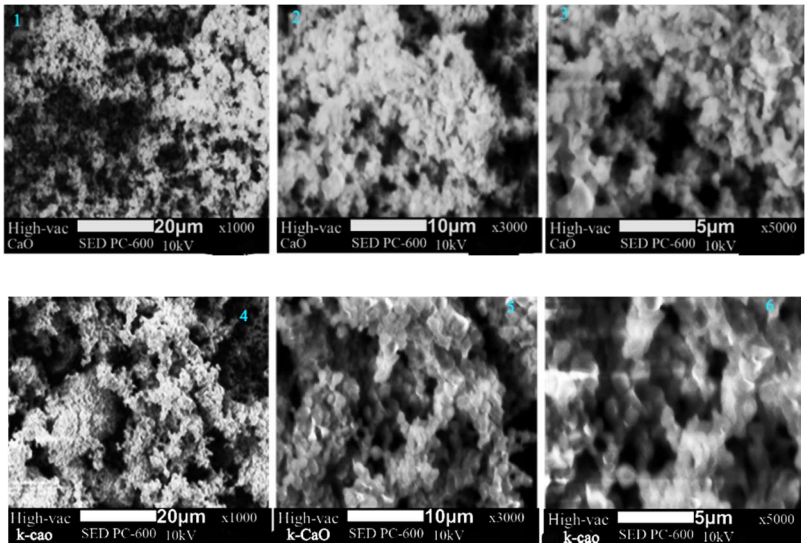

Figure 12: SEM image of lime based CaO and K-CaO

4.2.3. Fourier transform infrared spectroscopy (FTIR)

FTIR, or Fourier Transform Infrared Spectroscopy, was employed to determine which functional groups were on the surface of the nanocatalyst. In the infrared region of 500–4000 cm−1, the KBr technique was employed at room temperature (Bharti et al., 2019). Figure 11 displays the FTIR spectra of K-doped and undoped CaO. Around 552 cm−1, the Ca–O vibrations were apparent. The existence of carbonate species is shown by the absorption at 1448 cm−1, while hydroxide vibrations are visible at 3643 cm−1. Since Ca–O is known to be hygroscopic, the emergence of the OH functional group makes sense; the absorption of CO2 from the atmosphere is what causes CaCO3 to exist. The distinctive signals at 552 cm−1 indicated that Ca–O synthesis was possible. This experiment yielded results similar to those of (Bharti et al., 2019). For CO2 ions in solid catalysts, the broad band at 1459 cm-1 and absorption bands at 713, 877 were ascribed to asymmetric stretch,

out of plane bend, and in plane bend vibration modes, respectively.

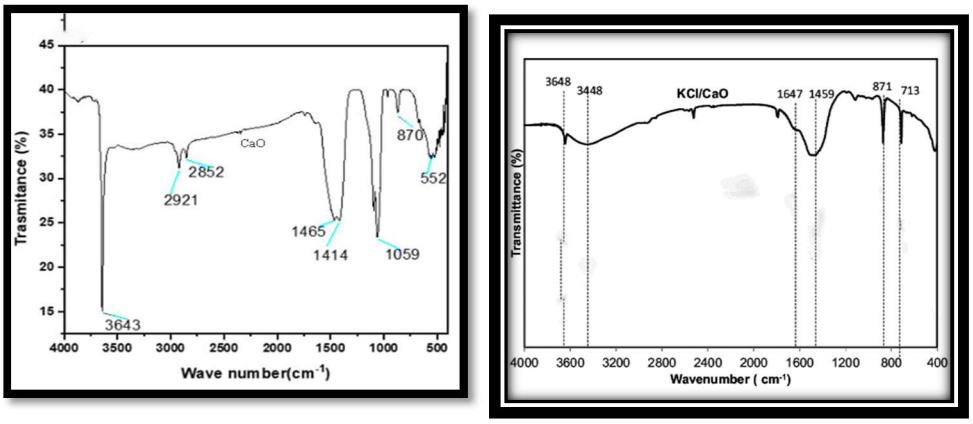

Figure 13: FTIR spectrum pattern of CaO and K/CaO

4.3. *Chlorella Vulgaris* Species Isolation and Culture

Chlorella vulgaris species was isolated using the streaking dilution method from water samples obtained from an artificial pond at tuludimtu in Addis Abeba, and individual chlorella vulgaris colonies were identified by examining colonial and morphological characteristics under a microscope (Kawamura et al., 2020).

Using Bold's Basal Medium (BBM), the microalgae was grown on liquid and agar slants with a 12:12 h light/dark cycle, 2500 lux light intensity, and the proper CO_2 supply.

The purity of the culture was guaranteed through repeated plating and routine microscopical inspection (Kong et al., 2012).

Cultivation of *Chlorella vulgaris* was carried out in 500 ml Erlenmeyer flasks containing Bold's Basal Medium (pH 6.5) at 24 ºC with light illumination 2500 lux 12:12 h light: dark cycle),supply of sufficient CO_2 and shaking under 100 rpm in an illumination incubator for 12 days.

4.4.*Chlorella vulgaris* harvesting and drying

4.4.1. Optical density measurement

Using a spectrophotometer for 12 days, the optical density of the *Chlorella vulgaris* micro algal culture was calculated in order to track its growth pattern. The optical density (absorbance) growth curve was plotted against the number of days as growth indicators (Figures 18 and 19). Microalgae grew in a normal pattern with four phases, including adaption, logarithmic, stationary, and mortality phases, in BBM media with an inoculum. Day 0–1 marked the beginning of the adaptation phase, which was characterized by minimal growth as the microalgae adjusted to their new surroundings for the first time. The growth of *Chlorella vulgaris* significantly increased during the logarithmic phase, which occurred between days 1 and 11.

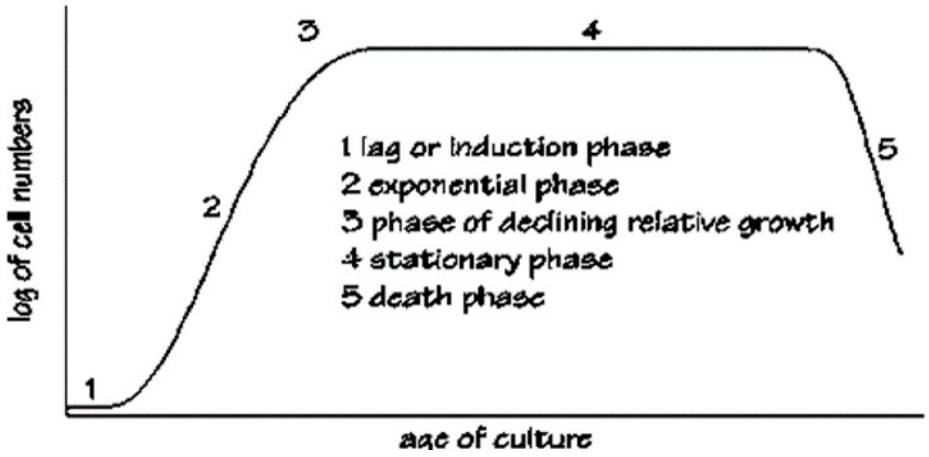

Figure 14: Schematic diagram of algal growth stage

During this period, the micro algal cells absorbed an excessive amount of nutrients from the medium to sustain their growth and produce energy, which caused a logarithmic increase in the number of cells. The microalgae growth entered the stationary phase, which is characterized by slow growth, around day 11. In this stage, the number of cells undergoing development and degeneration is equal (Purkan et al., 2019).

Dopt= αl

Where, Dopt –optical density, α—absorption coefficient, l—cuvate thickness (1 cm)

α=2.303*A/l……..……..equation (13)

Where A is absorbance and l is thickness of cuvate or light path length

Table 6: Optical density based on average absorbance

Days	Absorbance measurement			Average absorbance	absorption coefficient(α)	optical density
	1st	2nd	3rd			
1	0.341	0.322	0.33	0.331	0.762	0.762
2	0.365	0.332	0.336	0.344	0.80	0.80
3	0.504	0.52	0.428	0.484	1.11	1.11
4	0.522	0.533	0.46	0.505	1.16	1.16
5	0.522	0.542	0.478	0.514	1.18	1.18
6	0.609	0.62	0.599	0.609	1.40	1.40
7	0.632	0.631	0.6095	0.624	1.43	1.43
8	0.664	0.66	0.676	0.667	1.54	1.54
9	0.71	0.72	0.7205	0.72	1.66	1.66
10	0.72	0.723	0.771	0.738	1.69	1.69
11	0.745	0.746	0.828	0.773	1.78	1.78
12	0.815	0.82	0.9055	0.8468	1.80	1.80

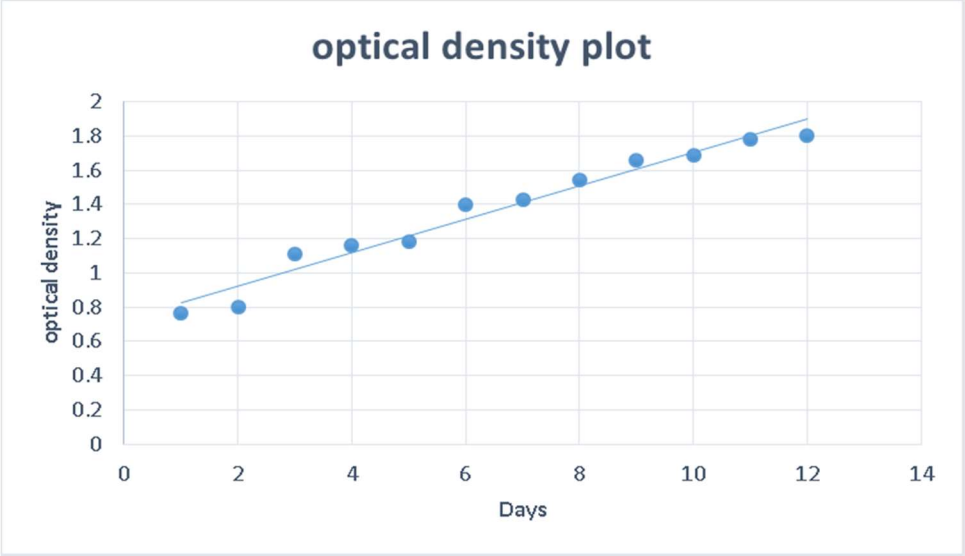

Figure 15: Optical Density Plot Based on Average Absorbance

4.4.2. Harvesting of *Chlorella vulgaris* microalgae

The process covered in section 3.5 was used to plot an absorbance against culture day graph at 750 nm in order to determine the harvesting day. As can be observed, after the 12 day, algal development stops being substantial, and prolonging the culture after this point just serves to waste time and cause the algae to begin dying. Thus, the 12 days of culture will be the harvesting day for this experiment. Equation 1 is also used to determine the culture's specific development. And the result is 0.42 d-1. Xin, Hong-ying, and Yu-ping (2011); Bakuei et al. (2015); Chandra Dev Goswami et al. (2011) reported results that ranged from 0.12 to 0.76 d-1, and it is thought that the results from this experiment are equivalent to those results.

4.5.Oil extraction and characterization

4.5.1. Microwave assisted oil extraction

In comparison to conventional extraction techniques, the modified solvent mixture (n-hexane/methanol) used in the microwave extraction processes produced a greater yield. N-hexane may dissolve and draw oil content from the algal matrix cells since it is a non-polar solvent with high oil solubility. The rate of mass transfer and cell rupture during microwave extraction also increases since methanol is the polar solvent and enables microalgae to absorb more microwave energy. The extractable yield from microalgae using the mixing solvent was considerably higher than the yield with hexane (Kalsum et al., 2019).

Table 7: Extracted oil yield

Algae powder (gm)	Solvent (ml)	Extraction time (min)	Oil yield (ml)	Oil content (%)
10	100 (n-hexane) 50 (methanol)	30	3	30

The total lipid content of *Chlorella vulgaris* biomass was determined to be 30 % in this study; as a result, the lipid content is comparable to the result discovered in the previous works. According to a previous study on *Chlorella vulgaris*, the biomass output was 0.3 g L^{-1} and 0.185 L^{-1} of oil with a 30% lipid content.

4.5.2. Characterization of the extracted oil

Physicochemical properties of micro algal oil were determined after distillation and evaporation.

4.5.2.1.Density of the oil

A pycnometer's weight was calculated using an electronic weighing balance. The weight of the bottle and oil were measured after a 25 ml sample that had been heated to 20 ºC was added to the pycnometer. The density was calculated using equation 3 below.

Density= (W2-W1)/V

Where; W1= weight of pycnometer, W2= weight of pycnometer with oil, V= volume of oil

4.5.2.2.Kinematic viscosity of the oil

The viscosity of the oil was determined using a viscometer. For thirty minutes, the viscometer cup holding the oil sample was kept in a water bath with a steady temperature of 40 °C. The sample cup was inserted into the viscometer's tip, and the reading for a fixed volume of liquid was noted. Equation (4) was used to compute kinematic viscosity based on the dynamic viscosity reading obtained from the viscometer.

$N=\mu/\rho$

Where; N = kinematic viscosity in mm^2/s, μ = dynamic viscosity, ρ = density in gm/mm^3

4.5.2.3.Acid value of the oil

The oil's acid value was calculated using the method described in section 3.4.3. As indicated in table 10, the findings were recorded.

0.7 milliliters was the average titration volume at which the first color shift was seen. Equation 5 was used to determine the oil's acid value, and the result was 1.97 mg KOH/g of oil.

4.5.2.4.Free Fatty Acid of the Oil

Free fatty acids make up half of the acid value of the oil. The free fatty acid is computed using equation 5 and comes out to be 0.98 percent. It is not necessary to pretreat the oil to lower its free fatty acid content in order to stop soap formation during the transesterification process.

4.5.2.5.Saponification Value of Oil

The procedure used to determine saponification value of oil was stated in section 3.4.5. And the result is recorded in table 10.

4.5.2.6.Moisture Content of the Oil

The oil's moisture content was calculated using equation (9) using the procedure stated in section 3.4.7 and found to be 0.55 percent. The results reveal that more moisture must be removed in order to avoid soap production during the transesterification procedure.

4.5.2.7.Ash content of the Oil

Equation 8 was used to calculate the oil's ash concentration, which came out to be 0.042%. Table 10 below provides a summary of the oil's physicochemical characteristics.

Table 8: Physicochemical properties of the extracted oil

No.	physicochemical Properties	Unit	Measured Value
1	Color	-	Light green yellow
2	Ph	-	6.01
3	Density@20 ℃	g/ml	0.81
4	Kinematic viscosity @40 ℃	mm^2/s	35.11
5	Acid value	mg KOH/g oil	1.52
6	Free fatty acid value	%	0.795
7	Saponification value	mg KOH/g oil	212.78
8	Molecular weight	g/mol	791
9	Moisture content	%	0.98
10	Ash Content	%	1.86

4.6. Biodiesel production and analysis of effects of the parameters

4.6.1. Transesterification process

The methods described in section 3.10 were followed in the transesterification process. Appendix 3 displays the yields from the 27 runs (including the replicates), which were utilized to examine the variance.

The twenty seven tests show that an oil to methanol ratio of 1:13, a catalyst loading of 5 wt (percent) at a stirring speed of 600 rpm, a reaction temperature of 50 ^oC for 120 minutes, and a yield of 94 percent biodiesel were the optimal parameters for creating biodiesel yield. This experiment's results are quite good when compared to earlier similar attempts. (Ahmed et al. 2012) discovered that the best biodiesel yield from macro algae using shaker, hot plate, and autoclave processes were 46, 92, and 81 percent, respectively. Another study (Rahman et al. 2017) discovered that Spirulina maxima microalgae could produce a maximum of 86.1 percent biodiesel through second step alkaline transesterification at methanol under the following conditions: oil ratio 9:1, temperature 65 ^0C, mixing intensity 600 rpm, and catalyst concentration 0.75 wt percent KOH.

The current study, in contrast to earlier ones, used lime as a catalyst, had a tolerable reaction time, required less energy, and produced more biodiesel while maintaining a good Alcohol to Oil molar ratio.

4.6.2. Effects of Individual Process Variables on Biodiesel Yield

Specific process factors have a substantial impact on the transesterification process. Below is a discussion of how each process variable affects the yield of biodiesel.

4.6.2.1.Effects of Catalyst loading on Biodiesel Yield

With a 5 weight percent catalyst loading, the biodiesel output from K/CaO catalyst increased to 94.42%, which is equivalent to 54% on a 1 weight percent catalyst. Because of the new active species (KCaCl3), which is most likely a potent Lewis acid, the K/CaO catalyst has a greater catalytic activity than CaO. Its concentration and propensity to generate methoxide ions determine the catalytic activity. Moreover, the K/CaO catalyst's activity was increased by the nucleophilic assault of the ensuing more populated methoxide ions on the triglyceride's carbonyl group, not the CaO system.

Precursors for impregnation are alkali metal ions (Li+, K+, and Na+) in varying concentrations. In comparison to plain CaO, (Kumar & Ali, 2013) found that Nano CaO impregnated with alkali metal ions has a high specific surface area. As ion size increases, so does the fundamental potency of the alkali metal ion impregnating CaO and the catalytic activity toward transesterification. The best amount of impregnated K+ ions is 3.5% (related to carrier amount) (Mahesh et al., 2015), i.e., 3% (related to oil amount) (Kataria et al., 2017), whereas the best amount of impregnated Li+ ions is 1.5% (Kumar & Ali, 2010) or 1.75% (related to carrier amount) (Kumar & Ali, 2012).

4.6.2.2.Effects of Methanol to oil ratio on Biodiesel Yield

Stoichiometry states that in order for the transesterification reaction to produce 3 mol of fatty acid ester and 1 mol of glycerol, 3 mol of methanol and 1 mol of triglyceride are required. In actuality, though, a larger molar ratio is needed to finish the reaction and yield more FAME as products. The findings of this study demonstrate that, as seen in Figure 18,

the methanol to oil ratio positively impacted the methyl ester production; but, as the ratio increased, the yield began to decrease. A separation problem brought on by an increase in glycerol solubility because too much methanol was employed in a higher ratio might potentially be the reason of the drop in yield despite an increase in molar ratio minimize the contact of triglyceride molecules on the catalysts active sites which could decrease the catalyst activity.

4.7.Optimization of biodiesel production

4.7.1. Determination of optimal experimental condition by Taguchi method

The Taguchi Orthogonal array L27 approach is the foundation of the experiment. In the current work, each parameter is defined at three levels, and a L27 orthogonal array scheme is adapted. The process took nine base experiments and two replications of each to complete. The experiment is designed in MINITAB 18 using four variables, namely (A) catalyst concentration range of 1-5%, (B) molar ratio of alcohol to oil range of 7:1-13:1, (C) reaction temperature range of 50-65^0 C, and (D) reaction duration range of 60-180 min at three levels. With the best configuration of the process parameters, the Taguchi technique based on Orthogonal Array lowers variance for the experiment. The results are then transformed into S/N (signal to noise) ratio data. The S/N ratio of the Taguchi technique is used for data analysis and parameter prediction to determine the combined influence of the parameters on yield. S/N ratio is utilized in this method to quantify the quality traits that deviate from the target value. Since high aspect methyl ester conversion rates are necessary, the larger-the-better rates are taken into account when calculating the S/N ratio. By breaking down the variance, a process known as analysis of variance, it is possible to have a better understanding of the relative impact of the various process parameters on the methyl ester conversion ratio. The impacts of process factors on FAME production are investigated using the ANOVA (Saravanakumar et al., 2016).

Personal error is indicated in the summary table in appendix 3 last column by CV (coefficient of variation) values which is almost 0.36% on average. CV value is used to show personal error while carrying out the experiment. We can also use standard error measurement to evaluate personal error. As a rule of thumb If CV≤ 10%, the sample

estimate is considered as a very good approximation of the parametric value. This is usually achieved in experimental research.

4.7.2. Statistical design analysis of signal to noise ratio (SNR)

Table in appendix 3 summarizes the overall mean SNR, the SNRs for the nine intended base tests, and the percentage yield of methyl ester from micro algal oil. The goal of this study is to maximize biodiesel yield; as a result, the larger the better (LTB) SNR model has been used. In accordance with the findings, base experiment number 9 had the highest mean yield (94.21%), whereas experiment number 1 had the lowest mean yield (54.42%).The highest mean yield as indicated in appendix 3 in base experiment number 9 is attributed to parameter combination effect. Our target is to find optimum parameter and their combination for maximum yield. So 50^{o} C temperature and 60 minute time which are both at level 1 in the DOE are the optimum to combine with the other parameter for the production of maximum yield. Both molar ratio and catalyst load are at level 3 in the DOE (design of experiment). The algebraic mean of all the SNRs of a particular control, known as the level mean signal to noise ratio (SNRL), has been found to be significant. For each of the four parameters, the SNR, DSNR (difference between maximum and minimum SNRL of given parameter), and rank have been determined. The DSNR value (designated as delta in table 10) was used to calculate the rank. Greater DSNR values were given rank 1. The molar ratio of methanol to oil has been found as the factor that most significantly affects the yield of FAME based on the rank as indicated in tables 9 and 10. The second and third affecting parameters are catalyst concentration and reaction temperature, followed by reaction time. Figures 18 and 20 display the effects of each parameter on the FAME yield at three distinct levels in terms of mean and SNR. A higher SNR number suggests that the given parameter has more of an impact at that level. The greatest value in each graph indicates the parameter's ideal value for increasing FAME yield. The optimal levels of each parameter, then, for the highest yield of FAME were A: (catalyst concentration) at level 3 (5%), B: (molar ratio of methanol to oil) at level 3 (13:1), C: (reaction time) at level 1 (60 min), and D: (reaction temperature) at level 3 (65 ^{o}c), where the stirrer's agitation speed was maintained constant at 600 rpm throughout the reaction.

Table 9: Response table for means

Level	Catalyst	Molar ratio	Time	Temperature
1	65.43	65.19	79.60	67.94
2	73.51	70.65	77.31	75.93
3	84.80	87.90	66.82	79.87
Delta	19.37	22.71	12.78	11.93
Rank	2	1	3	4

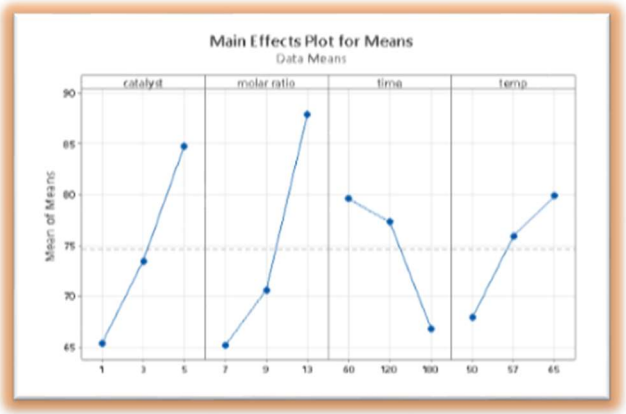

Figure 16: Main Effect Plot for Mean

Table 10: Response table for signal to noise ratios

Level	Catalyst	Molar ratio	Time	Temperature
1	36.23	36.22	37.77	36.34
2	37.13	36.79	37.66	37.50
3	38.49	38.84	36.42	38.00
Delta	2.25	2.62	1.35	1.66
Rank	2	1	4	3

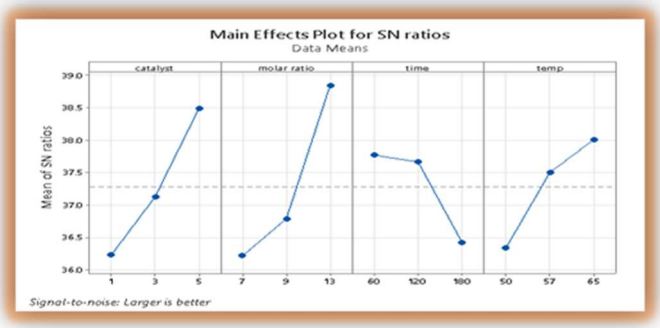

Figure 18: Main Effect Plot for SN

Table 11: Response table for standard deviation

Level	Catalyst	Molar ratio	Time	Temperature
1	0.3089	0.3597	0.3156	0.3071
2	0.1985	0.1594	0.2414	0.3300
3	0.3301	0.3184	0.2804	0.2004
Delta	0.1316	0.2003	0.0742	0.1296
Rank	2	1	4	3

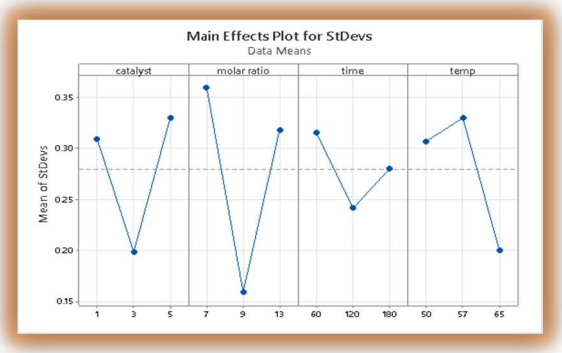

Figure 16: Main effect plot for sd

4.7.3. Analysis of variance (ANOVA)

ANOVA was used to determine the response magnitude for each parameter in the L27 orthogonal array experiment. The most important factor that contributes to the highest yield was found and measured using an ANOVA analysis (Saravanakumar et al., 2016; Sathish Kumar et al., 2015). Using Eq. (14) and Table 16, it establishes the correlation between every biodiesel production parameter. The most crucial factor was also identified by calculating the percentage impact of each process parameter on the production of biodiesel. The concentration of catalyst, which contributed 25.22%, was determined to be the most significant parameter from the contribution table, with a 46.1% contribution to the biodiesel yield from Chlorella vulgaris microalgal oil. The molar ratio of methanol to oil was shown to be the most significant parameter. The temperature and reaction time have a respective contribution to the biodiesel production of 15.41% and 13.27%.

Regression Equation

%yield =4.843 catalyst + 3.860 molar ratio + 0.7882 temp-9.68

……..……..equation (14)

Equation 14 demonstrates a regression equation, a statistical model used to identify the precise relationship between input and output characteristics. It provides the result with a negligible margin of error.

Table 12: Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Regression	4	5556.89	1389.22	170.68	0.005
Catalyst	1	1688.77	1688.77	207.49	0.004
molar ratio	1	2502.98	2502.98	307.52	0.004
Time	1	735.11	735.11	90.32	0.011
Temp	1	630.03	630.03	77.41	0.012
Error	22	179.06	8.14		
Lack-of-fit	4	177.39	44.35		0.000
Pure error	18	1.67	0.09		
Total	26	5735.95			

The ANOVA given in Table 12 above revealed a significant ($p<0.05$) interactive effect of the four factors that have been investigated (Priyadarshi & Paul, 2019).

Table 13: Percentage contribution of process parameters

Source	DF	Seq SS (sum of squares)	% Contribution
Catalyst (wt %)	2	6.8460	25.22
Methanol/oil (Molar ratio)	2	12.5101	46.1
Time (min)	2	4.1821	15.41
Temperature (oC)	2	3.6025	13.27
Total	8	27.1407	100

Table 14: Model summary

S	R-sq	R-sq(adj)	R-sq(pred)
2.85293	96.88%	96.31%	95.53%

R square and adjusted R square were used to determine the Taguchi model's significance. For transesterification processes, coefficients of determination (R square) of 96.88% and R square (adjusted) of 96.31% were found. As a result, the R square value gives an indication of how well the model fits the real data. The generated model is suited to the data in this case, as evidenced by the R square value of 96.88%.

4.7.4. Normal Probability plot of Residuals for %YIELD

The normal probability plot is a graphical tool used to compare a data set with the normal distribution. This graphic displays a straight line that indicates the data fit a normal probability distribution. All of the residuals collected are nearly along the line, and the residual values are very low.

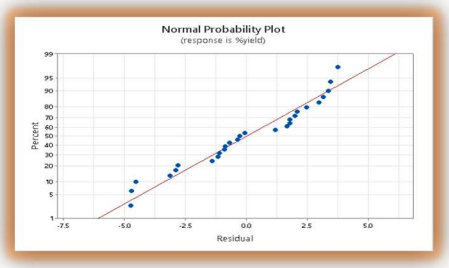

Figure 17: normal probability plot

4.7.5. Effect of Operational Parameters on the Biodiesel Production Process

The simultaneous impact of the independent factors on the biodiesel conversion (%) was investigated using contour plots. They are topographical maps created using data in three dimensions. One variable is displayed on the horizontal axis, and a second variable is represented on the vertical axis. The third variable is represented by an isoline (a line with constant value) and a color gradient. When analyzing data, these plots are frequently helpful, particularly when looking for minimums and maximums in a set of trivariate data. The contour plot related to the impact of two variables on the biodiesel yield (response) was shown in Figures 17–22. The methanol/oil molar ratio with reaction time, reaction temperature, methanol/oil molar ratio with catalyst amount, and curvatures nature all interacted with one another, reaction time with reaction temperature, reaction time with catalyst loading, and, reaction temperature with catalyst loading, respectively.

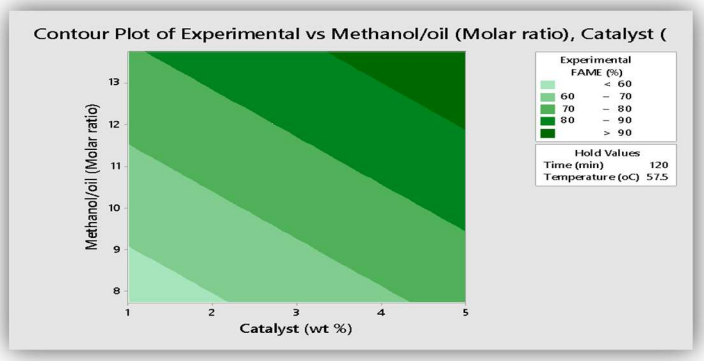

Figure 18: Contour Plot of % Yield vs Molar Ratio, Catalyst

Figure 22 shows the contour plot of the combined effect of methanol to oil ratio and catalyst loading. The graphic shows how the methanol to oil ratio and catalyst concentrations both dramatically improve the output of methyl esters. The yield of methyl ester on catalyst loading 1 wt% was 54.246 (run 1) in the range of methanol to oil ratio 7:1. However, the yield improved to 94.213% (run 9) on catalyst loading 5 wt% in the range of methanol to oil ratio 13:1. The biodiesel conversion rises as the amount of catalyst loading increases, possibly because the catalyst's surface active sites become more numerous. The methanol to oil ratio also causes an increase in conversion. The explanation for this is that the production of biodiesel will be favored as the equilibrium shifts to the product side due to an increase in methanol concentration. Moreover, employing an excess of methanol not only quickens the transesterification process but also clears the catalyst's surface of product molecules to restore the active sites (Borah et al., 2019). Research indicates that there was no significant difference in the FAME yield with a greater catalyst loading (≥5% wt%). This could be because the reaction mixture has a greater catalyst loading and is more viscous, which could prevent mass transfer in the liquid-liquid-solid system (Kumar & Ali, 2013).

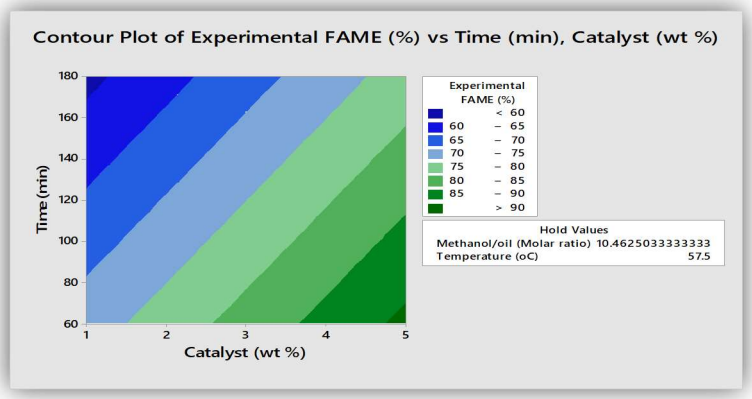

Figure 19: Contour Plot of % Yield vs Catalyst, Time

Figure 21 illustrates how catalyst loading and reaction time interact to affect the conversion of biodiesel. Biodiesel conversion increases with increasing catalyst concentrations and reaction periods. This is because longer reaction times result in more reactant interaction,

which further enhances conversion (Borah et al., 2019). In this study, with an increasing catalyst loading of the reaction, the yield of biodiesel increased quickly from 65.87 % (run 2) to 94.213% (run 9) of yield in the range catalyst loading from 1 wt% with respect to 120min up to 5 wt% catalyst loading with respect to 120min time respectively. The steeper catalyst loading slope indicates that catalyst loading has a greater influence on conversion than reaction time, while the relationship between reaction time and conversion is not very strong. There may be more catalyst active sites when catalyst loading increases, which could explain the rise in conversion.

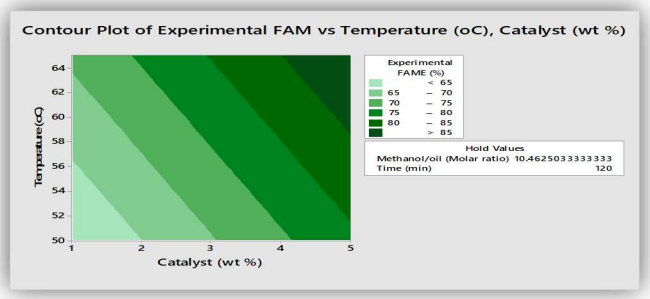

Figure 20: Contour Plot of % Yield vs Catalyst .Temperature

Figure 22 shows the impact of catalyst loading and reaction temperature interaction on biodiesel conversion. When catalyst loading is increased, biodiesel conversion increases as well. This could be because the catalyst has more surface active sites. Additionally, conversion increases when temperature rises (Borah et al., 2019). The FAME yield in the current study increased from 54.246% (run 1) to 91.51% (run 8) of yield in the range of catalyst loading 1 wt% with regard to 50oC and 5 wt% with respect to 65oC, respectively, when the reaction temperature and catalyst loading were raised. The graph unequivocally demonstrates that temperature and catalyst loading both enhance biodiesel conversion. However, the most important factor in this case is catalyst loading, as shown by the

extremely steep increase in conversion that occurs as catalyst loading increases.

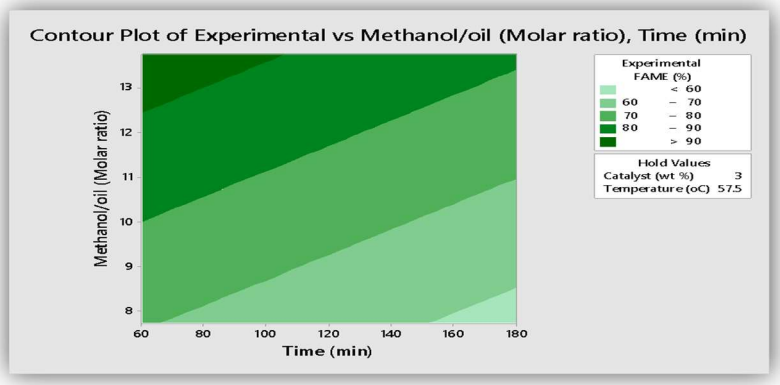

Figure 21: Contour Plot of % Yield vs Molar Ratio, Time

The contour plot of the interaction between reaction time and the methanol to oil ratio is displayed in Figure 25. Biodiesel conversion rises with an increase in the methanol to oil ratio and a decrease in reaction time. One of the most important variables that influences FAME yield efficiency and biodiesel synthesis is the molar ratio of methanol to oil. Therefore, an excess of methanol is used to drive the reversible reaction (transesterification) to the right side in order to produce more biodiesel and improve the equilibrium conversion of reversible reaction-based reactions mechanism (i.e., transesterification reaction) (Çakırca et al., 2019).

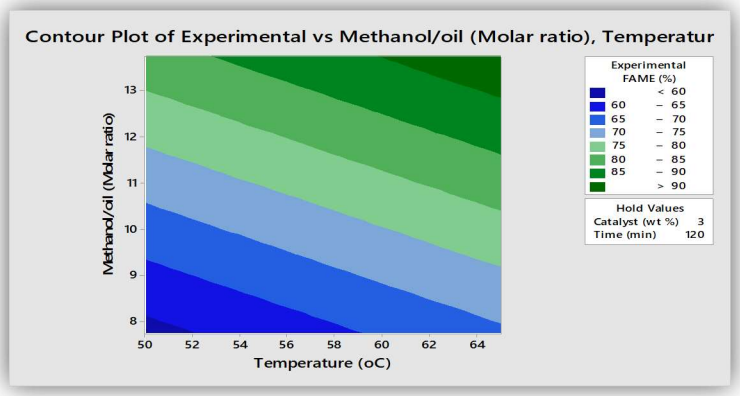

Figure 22: Contour Plot of % Yield vs Molar Ratio, Temperature

Figure 25 shows the contour plot of the combined influence of temperature and the percentage of methanol to oil. Reaction temperature is another key factor to consider when

trying to increase yield. The kinetics and pace of reaction both increase with temperature (Gashaw et al., 2015). The trans-esterification process is typically carried out within an ideal temperature range of 50–60° C, depending on the type of oil employed, and below the boiling point of alcohol. At low temperatures, the relatively limited conversion to methyl ester is noticeable because of the subcritical condition of methanol. Most alcohol used evaporates in high temperatures (Bano et al., 2020). When the ratio of methanol to oil rises, conversion also rises. The rationale is that as methanol concentration rises, the equilibrium will move to the product side, favoring the generation of biodiesel (Borah et al., 2019). Consequently, the yield of biodiesel grew rapidly from 54.246 % (run 1) to 93.196% (run 6) as the reaction's temperature rose.

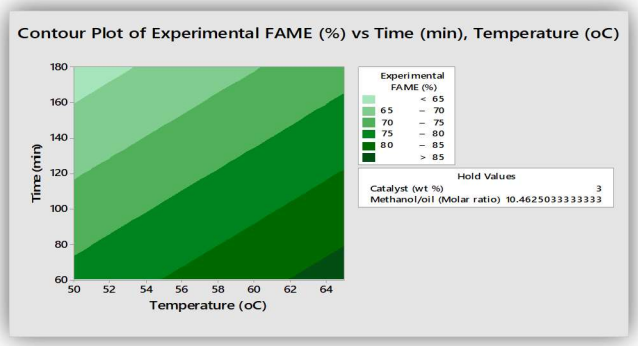

Figure 23 Contour Plot of % Yield vs Time, Temperature

Figure 26 shows the contour plot of the combined influence of temperature and time. Reaction temperature and duration are important factors to consider in order to maximize yield. According to Gashaw et al. (2015), a temperature increase accelerates the rate of reaction and kinetics, which in turn shortens the reaction time. The trans-esterification process is typically carried out within an ideal temperature range of 50–60° C, depending on the type of oil employed, and below the boiling point of alcohol. High temperatures mostly cause the utilized alcohol to evaporate. Therefore, figure shows that with an increasing temperature and time of the reaction, the yield of biodiesel increased quickly from 54.246 % (run 1) to 93.196% (run 6) of yield in the range of time 60min with respect to 50 ^{0}C temperature up to 57.5 ^{0}C temperature respectively. As reported by many groups,

at normal temperature, a higher conversion is easily achieved within a 60–90 min time period (Hossain and Boyce, 2009) (Bano et al., 2020).

4.8.Characteristics of Produced Biodiesel

When using the product in a diesel engine, the fuel qualities of the biodiesel are crucial. The primary characteristics of biodiesel fuels in this instance are density and dynamic viscosity, kinematic viscosity, acid vale, free fatty acid value, saponification value, cloud point, pour point and flash point were measured to evaluate following the ASTM standard methods as summarized in Table 15. The kinematic viscosity (4.3 mm2/s) of the microalgae biodiesel in this study was in the range of ASTM (1.9-6.0 mm2 /s) standard value. The degree of saturation, carbon chain length, and makeup of the main fatty acids are significant determinants of viscosity. For example, viscosity falls with increasing unsaturation and rises with the number of CH2 moieties in the fatty ester chain. Compared with the literature value, the viscosity of the algae biodiesel in the present work (3.2 mm2/s) is lower than microalgae and castor oil biodiesel which is 6.71 mm2/s and 10.43 mm2/s respectively (Dawit Firemichael et al., 2020).

The acid value, which steadily rises due to the hydrolytic cleavage of ester bonds, indicates the extent of fuel aging during storage. The relationship between corrosion and deposit deposition in engines has been studied in connection to high fuel acidity. To lessen these adverse effects, the EN and ASTM Standards permit a maximum acid value of 0.5 mg of KOH/g. The microalgae biodiesel's acid value of 0.46 KOH/g is within the allowed range, suggesting that the algal biodiesel won't have an adverse effect on the engine's long-term performance. Cloud and pour points are useful tools for measuring cold flow characteristics, which are important biodiesel factors. The reduction of temperature may cause the biodiesel to form visible crystals ($d \geq 0.5$ μm) at a limit known as cloud point. The temperature at which biodiesel ceases to flow is known as the pour point (Prathima & Karthikeyan, 2017). Algal biodiesel has a cloud and pour point of 6 and -9.8 °C, respectively. Comparable to biodiesels derived from other feed stocks (microalgae oil: -3 and -7 °C; olive oil: -2 and -3 °C; rapeseed oil: -2 and-9 °C; soybean oil: 0 and -2 °C; sunflower oil: 2 and -1 °C; tallow: 17 and 15 °C) are the CP and PP values of the algae

biodiesel used in this study. Biodiesel's flashpoint tells us that it's safe to store and won't catch fire at low temperatures (Borah et al., 2019; Firemichael et al., 2020). As a result, the biodiesel oil produced in this work satisfies the ASTM standard standards for biomass automotive fuel, indicating excellent quality qualities.

Table 15: Phisico chemical properties of produced biodiesel

No.	Physicochemical property	Units	Test method ASTM	ASTM limit for B100	Produced biodiesel values
1	Color		Observed		Light green yellow
2	pH				8.01
3	Density @ 20oC	Kg/m3	D4052	880	811
4	Density @ 40oC	Kg/m3	D4052	880	809
5	Dynamic viscosity @ 40oC	mpa.sec			2.5
6	Kinematic viscosity @ 40oC	mm2/s	D445	1.9-6	4.3
7	Acid value	mg KOH/g	D974	0.50	0.46
8	Free fatty acid value	%			0.231
9	Saponification value	mg KOH/g			205.03
10	Cloud point	ºC	D2500	-	6
11	Pour point	ºC	D97	-5 to -10	-9.8
12	Flash point	ºC	D93	100-170	124

CHAPTER FIVE

5. CONCLUSION , RECOMMENDATIONS AND FUTURE WORKS

5.1. Conclusion

Chlorella vulgaris species was successfully isolated in this investigation from freshwater samples taken from Tuludimtu artificial water pond, Addis Ababa, Ethiopia. *Chlorella vulgaris* microalgae were grown in modified BBM medium under 2500 lux light intensity from non-heat emitting white florescence 12:12 hour light and dark cycle, yielding a lipid content of 30%. The oil was extracted from the harvested, dried, and grounded microalgae using a microwave assisted solvent extraction process with 600 watt power, 80 ºC temperature and for 30 minutes extraction time were used. The physicochemical characteristics of the extracted oil were identified. The oil was Trans- esterified using methanol and K-doped CaO Nano catalysts made from lime to produce biodiesel. XRD, SEM, and FTIR analyses of synthesized CaO and K doped CaO Nano catalysts were conducted. The results of the X-ray diffraction (XRD) showed that the CaO crystallized size was 0.32 nm, and potassium doping followed by calcination of the CaO produced the nanocrystaline K/CaO with a particle size of 0.28 nm. The SEM analysis reveals that the particle shape became more regular after calcination and wet impregnation treatment, resembling a termite colony and it shows high porosity with large surface area.

The transesterification of Chlorella vulgaris oil using K-doped CaO nanocatalyst was optimized through the application of the Taguchi technique. The results of the investigation show that a 13:1 methanol to oil molar ratio, a 5 weight percent catalyst loading, a 120-minute reaction period, and a 50°C reaction temperature were the most favorable parameters for attaining the maximum FAME yield (94%). The fact that the outcome closely matched the expected value proved the applicability of the model. The relevance of the built models and the conformity of their predictions with the experimental results were shown by the Taguchi method of optimization outcomes. The methanol to oil molar ratio and catalyst loading both had a significant effect on the percentage of oil converted to biodiesel, according to the statistical analysis. When compared to regular diesel, the fuel

characteristics of the created biodiesel met ASTM D6751 requirements. Therefore, it can be concluded that using lime with a small amount of modification as a catalyst makes commercializing biodiesel feasible and affordable. Additionally, the *Chlorella vulgaris* microalgae examined in this study have also proven to be suitable as raw materials for the production of biodiesel and can be thought of as potential feed stock for the production of biodiesel to combat the coming energy crisis.

5.2.Recommendations and future work

5.2.1. Recommendations

This study demonstrates that microalgae are excellent candidates for producing biodiesel to replace petroleum fossil fuels, to reduce environmental pollution brought on by the use of fossil fuels. Microalgae are also potential resources for the third generation of biofuel. To evaluate the species and strain type, more study is needed. Hydrocarbon composition of this specific *Chlorella vulgaris* isolate also needs to be investigated.

More investigation is required into the productivity of isolated strains and the identification of favorable environmental conditions (such as growth media or nutrient requirements, ideal temperature, and light intensity) for strains with higher fatty acid yield per unit of land in order to grow microalgae on a large scale in an outdoor open pond system.

Further and deep study on Enhancement of lipid production from *Chlorella vulgaris* is crucial to use this species for biodiesel production. The conversion of biodiesel from microalgae in to gas to use it for gas engine should also be considered in future research.

Additionally, more research on fuel characteristics such mixing conditions, engine performance and emission tests should be carried out in future studies.

5.2.2. Future work

When producing biodiesel on a commercial scale from a lab scale, heat and mass transfer constraints might provide a serious obstacle. Conventional jacketed reactors, which have trouble keeping heat inside the reactor, are often used for heat transfer. However, at higher sizes, heating or cooling may become challenging to achieve. The absence of power input

necessary for turbulent or multiphase flow might impede mass transfer or mixing of substrates, reactants, and products on a wider scale. Future studies should concentrate on increasing the cost-effectiveness of large-scale biodiesel production from a commercial standpoint. This would involve investigating methods to reduce waste streams, accelerate reaction times, develop better catalysts, and produce alcohol; using methods like micro channeling to enhance heat and mass transfer efficiency; and figuring out how to include renewable energy sources like solar energy in the process reference. Limitations on heat and mass transmission may pose serious problems when scaling up lab-based biodiesel manufacturing. To better understand the environmental effects of producing biodiesel, life cycle assessment, or LCA, might be a useful technique. From the extraction of raw materials to the disposal of finished products, life cycle assessment (LCA) assesses major environmental impacts such energy use, greenhouse gas emissions, and other environmental contaminants. Future studies could look at the complete process life cycle to determine all effects and provide creative solutions for reducing emissions.

Reducing smoke emissions and maybe improving engine performance are two benefits of blending renewable resources with diesel. Subsequent studies have to concentrate on investigating diverse techniques for processing in order to optimize the blending efficiency, in addition to scrutinizing the information gathered from trials carried out on combinations of biodiesel and sustainable resources. Additionally, methods for raising production yields while preserving ideal combustion efficiency ought to be the focus of research. To find the best combinations for long-term sustainability, more research may be done to examine the characteristics of fuel in various conditions and temperatures.

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APPENDICES

Appendix 1: Bold basal medium

	Components	Stoke solution (Gm L^{-1} dH_2O)	Quantity used	Concentration in final medium
1	Macronutrients			
1.1	$-NH_2Cl$	15.74	10 ml or	$2.94x10^{-3}$
	$-NaNo_3$	25.00	10 ml	
1.2	$CaCl_2*2H_2O$	2.50	10 ml	$1.70x10^{-4}$
1.3	$MgSO_4*7H_2O$	7.50	10 ml	$3.04x10^{-4}$

1.4	K_2HPO_4	7.50	10 ml	$4.31x10^{-4}$
1.5	KH2PO4	17.50	10 ml	$1.29x10^{-3}$
1.6	Na Cl	2.50	10 ml	$4.38x10^{-4}$
2	Alkaline EDTA solution		1.00 ml	
2.1	EDTA	50.00		$1.71x10^{-4}$
2.2	KOH	31.00		$5.53x10^{-4}$
3	Acidified Iron solution		1.00 ml	
3.1	$FeSO_{4*7}H_2O$	4.98		$1.79x10^{-5}$
3.2	H_2SO_4			
4	Boron Solution		1.00 ml	
4.1	H_3BO_3	1ml		$1.85x10^{-4}$
5	Trace Metal Solution			
5.1	$ZnSO_{4*}7H_2O$	8.82	2.00 ml	$3.07x10^{-5}$
5.2	$MnCl_2*2H_2O$	1.18	2.00 ml	$7.28x10^{-6}$
5.3	MoO_3	0.36	4.00 ml	$4.93x10^{-6}$
5.4	$CuSO_4*5H_2O$	1.57	2.00 ml	$6.29x10^{-6}$
5.5	$Co(No_3)*6H_2O$	0.49	2.00 ml	$1.68x10^{-6}$

Appendix 2: Experimental design with four parameters for optimization in transesterification reaction using taguchi method

Experiment no.	Concentration of catalyst (wt%)	Methanol/oil (Molar ratio)	Time for reaction (min)	Reaction temperature (0C)
1	1	1	1	1
2	1	1	1	1
3	1	1	1	1
4	1	2	2	2
5	1	2	2	2
6	1	2	2	2
7	1	3	3	3
8	1	3	3	3

9	1	3	3	3
10	2	1	2	3
11	2	1	2	3
12	2	1	2	3
13	2	2	3	1
14	2	2	3	1
15	2	2	3	1
16	2	3	1	2
17	2	3	1	2
18	2	3	1	2
19	3	1	3	2
20	3	1	3	2
21	3	1	3	2
22	3	2	1	3
23	3	2	1	3
24	3	2	1	3
25	3	3	2	1
26	3	3	2	1
27	3	3	2	1

Remarks= the number 1, 2, 3 written under each parameter represents level 1, level 2 and level 3

Appendix 3: Experimental design for optimization of various reaction parameters in transesterification reaction using Taguchi methodology

Test no	Catalyst	molar ratio	Time	Temp	%yield	SNRA1	STDE1	MEAN1	CV1
1	1	7	60	50	54.92	34.7151	0.453909	54.246	0.008340 3
R_{1-1}	1	7	60	50	54.32	*	*	*	*
R_{1-2}	1	7	60	50	53.50	*	*	*	*
2	1	9	120	57	65.81	36.3353	0.202237	65.87	0.003083 8
R_{2-1}	1	9	120	57	66.50	*	*	*	*
R_{2-2}	1	9	120	57	65.30	*	*	*	*

3	1	13	180	65	76.54	37.6481	0.270555	75.94	0.0035469
R_{3-1}	1	13	180	65	76.00	*	*	*	*
R_{3-2}	1	13	180	65	75.30	*	*	*	*
4	3	7	120	65	72.00	37.1639	0.162583	72.60	0.0022536
R_{4-1}	3	7	120	65	73.32	*	*	*	*
R_{4-2}	3	7	120	65	72.50	*	*	*	*
5	3	9	180	50	54.10	34.8351	0.107858	55.12	0.0019548
R_{5-1}	3	9	180	50	55.30	*	*	*	*
R_{5-2}	3	9	180	50	55.96	*	*	*	*
6	3	13	60	57	93.52	39.3879	0.325013	93.1967	0.0034874
R_{6-1}	3	13	60	57	92.87	*	*	*	*
R_{6-2}	3	13	60	57	93.20	*	*	*	*
7	5	7	180	57	69.41	36.7770	0.462637	69.0033	0.0067046
R_{7-1}	5	7	180	57	69.10	*	*	*	*
R_{7-2}	5	7	180	57	68.50	*	*	*	*
8	5	9	60	65	91.22	39.1983	0.168028	91.51	0.0018427
R_{8-1}	5	9	60	65	92.00	*	*	*	*
R_{8-2}	5	9	60	65	91.33	*	*	*	*
9	5	13	120	50	94.33	39.4821	0.359490	94.2133	0.0038157
R_{9-1}	5	13	120	50	94.50	*	*	*	*
R_{9-2}	5	13	120	50	93.81	*	*	*	*

R=replicates