IN SITU TRANSESTERIFICATION OF *SPIRULINA*MICROALGAE TO PRODUCE BIODIESEL USING MICROWAVE IRRADIATION

By:

KOECH KIMURGOR ALEX

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Declaration

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Alex Koech..... Date:10th/06/2021

Declaration by Supervisors

This thesis has been submitted for examination with our approval as

University Supervisors.

Prof. Anil Kumar …_______________……… Date:11th/06/2021

MoiUniversity, Eldoret, Kenya

Prof. Zachary Siagi …… …….. Date: 11th/06/2021

MoiUniversity, Eldoret, Kenya

Dedications

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Abstract

The present technology of transesterification of vegetable oils to produce biodiesel that is suited to replace petro-diesel has economic challenges and therefore alternative sources are being explored. Microalgae, a renewable, third-generation biofuel resource have the potential to become a viable feedstock due to their high oil content and environmentally friendly nature. The main objective of this work was to produce fatty acid methyl esters (FAME) from *Spirulina* microalgae by in-situ catalytic transesterification using microwave irradiation. Specific objectives being quantifying and characterizing algae lipid, studying effect of reaction variables on FAME yield in a batch reactor, and FAME characterization. Simultaneous extraction and conversion of oil from algae biomass to biodiesel was studied in a batch reaction system using methanol and sulphuric acid catalyst. The microwave synthesis unit comprised of a 3-necked round bottom flask inside a 1300-watt microwave, fitted with a quick-fit condenser and having an external stirrer. Response Surface methodology (RSM) was used to analyze the influence of process variables; dry algae to methanol ratio $(1:4-1:14\ g/ml)$, algae biomass to catalyst ratio $(1:0.0032-1:0.0368\ wt\ \%)$, and reaction time $(1-11\ min)$ at 500 rpm stirring rate on the FAME conversion. Biodiesel was analyzed for FAME using a gas chromatography (GC) fitted with flame ionization detector. Nitrogen was used as a carrier gas. The column used was a $30m\ by\ 0.25m\ by\ 0.25\mu m$ Zebron ZB-FAME column. Methyl Heptadecanoate $(C17:0)$, was used as an internal standard for analysis. The total lipid content of *Spirulina-Platensis* microalgae biomass was 10.7% by weight. The algae biomass also contained a large amount of proteins at 51.83%, moisture content at 7.8% and ash content 14.30% by weight basis. From Response Surface Methodology (RSM) using Central Composite Design, the optimum process conditions were determined as follows: dry algae biomass feed to methanol (wt/Vol) ratio of 1:9, catalyst concentration of $2\ wt\%$, and reaction time of 7 minutes giving a maximum FAME yield of $83.43\ wt\%$. FAME was analyzed for fatty acid composition and characteristic fuel properties. From GC analysis, palmitic (30.83%), linoleic(43.83%), and linolenic (19.41%), acids were found to be the major fatty acids inferring that *Spirulina-Platensis*is a promising feedstock for biodiesel production. FAME properties obtained according to EN 14214 and ASTM D 6751 standards were: flash point (164^oC) calorific value $(32{,}911\ kJ/kg)$, acid value $(0.475KOH/g)$, viscosity $(4.45\ mm^2/s)$ and specific gravity (0.868). Study showed that *AthrospiraSpirulina-Platensis* microalgae lipid FAME met biodiesel standards (EN 14214 and ASTM D 6751), and has potential to replace petrodiesel. Process variables for optimal FAME yield were identified. Microwave irradiation was found to be a superior heating mode as compared to conventional heating. It increased reaction rate resulting into a reduced reaction time of 7 minutes, as compared to 8 hours for conventional heating. Approaches for making Spirulina microalgae biodiesel be economically competitive in comparison with petrodiesel are discussed. It was found out that *Spirulina*microalgae biomass exhibits good properties for biodiesel production. Further research on the in-situ microwave irradiation transesterification of microalgae for a flow reactor should be carried out to increase FAME production rate.

Keywords: Algae; Biodiesel; Microalgae; *Spirulina-Platensis*; In situ; transesterification

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Nomenclature and abbreviations

ASTM	American Society of Testing and Materials
A.V.	Acid value
CCD	Central Composite Design
COC	Cleveland Open Cup
CPE	Chemical and Process Engineering, Moiuniversity
***C*11:0**	Undecanoic acid
***C*12:0**	Lauric acid
***C*14:0**	Myristic acid
***C*16:0**	Palmitic acid
***C*17:0**	Palmitoleic acid
***C*18:0**	Stearic acid
***C*18:1**	Oleic acid (omega 9)
***C*18:2**	Linoleic acid (omega 6)
***C*18:3**	Linolenic acid (omega 3)
***C*20:0**	Arachidic acid

$\boldsymbol{FFA}$	Free fatty acid
GC	Gas chromatography
$\boldsymbol{H_2SO_4}$	Sulphuric acid
KIRDI	Kenya Industrial Research and development institute
$\boldsymbol{NaOH}$	Sodium hydroxide
$\boldsymbol{pH}$	**(**Potential Hydrogen)- pH expresses the acidity or alkalinity of a solution
ppm	parts per million
RSM	Response Surface Methodology
$\boldsymbol{R'}$	ester
$\boldsymbol{\rho}$	Density

Glossary

Alcohol	Alcohol is any organic compound in which a hydroxyl group $(-OH)$ is bound to a carbon atom of an alkyl or substituted alkyl group.
ArthrospiraSpirulina	*Spirulina-arthrospira*belongs to the group of oxygenic photosynthetic bacteria and are filamentous, non-heterocystous cyanobacteria commonly found in tropical and subtropical regions.
Biodiesel	Refers to a diesel equivalent, processed fuel derived from biological sources (such as vegetable oils or animal fat), consisting of long-chain alkyl (methyl, ethyl, or propyl) esters. Biodiesel is typically made by chemically reacting lipids (e.g., vegetable or animal fat) with alcohol in the presence of a catalyst by a process known as transesterification.
Calorific value	Calorific value is the energy contained in a fuel or food, determined by measuring the heat produced by the complete combustion of a specified quantity of it. Its unit is usually joules per kilogram.
Diesel or diesel fuel	It is a specific fractional distillate of fuel oil (mostly petroleum) that is used as fuel in a diesel engine invented by (German engineer Rudolf Diesel). It refers to fuel processed from petroleum.

Esters	Esters are organic compounds in which an organic group (symbolized by ‘R’ in this article) replaces a hydrogen atom (or more than one) in a hydroxyl group.
Flash point	Flash point of a flammable liquid is the lowest temperature at which it can form an ignitable mixture in air. At this temperature the vapour may cease to burn when the ignitionsource is removed. A slightly higher temperature, the fire point, is defined as the temperature at which the vapour continues to burn after being ignited.
Glycerol	Glycerol, also well known as glycerin and glycerine, and less commonly as propane-1,2,3triol, 1,2,3-propanetriol, 1,2,3 trihydroxy propane, glyceritol, and glycyl alcohol is a colourless, odourless, hygroscopic and sweet-tasting viscous liquid. Glycerol is a sugar alcohol and has three hydrophilic alcoholic hydroxyl groups (OH-) that are responsible for its solubility in water.
In situ	The definition as used here is in its natural or original state.
Methanol	Methanol, also known as methyl alcohol, carbinol, woodalcohol or wood spirits, is a chemical compound with chemical formula CH_3OH. It is the simplest alcohol, and is a light, volatile, colourless, flammable, poisonous liquid with a distinctive odour that is somewhat milder and sweeter than ethanol (ethyl alcohol). It is used as antifreeze, solvent, fuel, and as a denaturant for ethyl alcohol.

pH	pH expresses the acidity or alkalinity of a solution on a logarithmic scale on which 7 is neutral, lower values are more acid and higher values more alkaline. The pH is equal $-\log_{10}c$, where c is the hydrogen ion concentration in moles per litre.
Transesterification	Is the process of exchanging the alkoxy group of an ester compound by another alcohol and is often catalyzed by the addition of an acid or base.
Viscosity	Viscosity is a measure of the resistance of a fluid to deform under shear stress. It is commonly perceived as “thickness” or resistance to flow. Viscosity describes a fluids internal resistance to flow and may be thought of as a measure of fluid friction.

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CHAPTER ONE

1.0 INTRODUCTION

The improvement and prosperity of life in the world are related directly to energy sustainability within a given nation's borders, and the major challenge that existsis trying to satisfy the growing demand for energy in a safe, clean and environmentally responsible manner. In this time of global market uncertainty, one thing we do knowis that the world needs energy—and in increasing quantities—to support economic and social progress and build a better quality of life, and in particular more is required in developing countries(Imperial, 2018).For these reasons, the world is pursuing alternative fuel sources to lessen the dependency on conventional fuels.

In the recent past, there has been intense exploration for non-renewable resources, which supplies most of the energy consumed currently around the world, and it is believed that in 113, 50, and 52 years coal, crude oil and natural gas respectively will be depleted(Dudley, 2016). Additionally, the price of foreign fuels that come from the earth's surface or beneath the ground and take thousands of years to form is ever increasing. On the other hand, renewable energy resources regenerate quickly and can supply a region with its long-term energy to the far reaches of the universe (Brenner, 2018). The dwindling nature of oil fuels due to high energy requirements and high consumption rates, the longevity of its formation which is usually thousands of years makes crude oil, not a viable resource for the future. In light of these speculations and assumptions, it is therefore imperative to consider the benefits of renewable energy sources.

Biodiesel production as a source of renewable energy is in essence one for the future, when the oil reserves have been depleted, or when the international oil prices increase due to future shortages and on the more stringent front whenman finally accepts that it is prudent to use clean/green energy. It is therefore desirable that the world looks at other forms of renewables to produce energy and especially from third generation biofuels. First and second-generation(Radakovits, Jinkerson, Darzins, & Posewitz, 2010) biofuel resources have considerable economic and environmental limitationsand doesn't have the ability to fulfill the current requirement of biodiesel energy as would the third-generation biofuel from microalgae, if the world does decide to go into commercialization.

The most common concern related to the current first-generation biofuels is that as production capacity increases, so does their competition with agriculture for arable land used for food production that may lead to severe food shortages(Giulano et al, 2010). In addition, the intensive use of land with high fertilizer and pesticide applications and water use can cause significant environmental problems (Schenk, et al., 2008).The advent of second-generation biofuels was intended to produce fuels from lignocellulosic biomass, the woody part of plants that do not compete with food production. However, converting the woody biomass into fermentable sugars requires costly technologies involving pretreatment with special enzymes, meaning that second-generation biofuels cannot yet be produced economically on a large scale (Brennan & Owende, 2010).Therefore, third-generation biofuels derived from microalgae according to Nigam & Singh, 2010 are considered a viable alternative energy resource that is devoid of the major drawbacks associated with first and second-generation biofuels.

Algae biofuel production is considered to be in the early stages of experimentation and their cultivation, harvesting, and processing methods for these clean and inexhaustible energy sources are costly and expensive. However, algae give several potential benefits over lignocellulosic biofuel source crops. It has the distinctive ability to produce biodiesel in its natural form and thus avoids the requirements for complex, expensive processing methods/technologies (at the downstream stage), and there is no competition for agricultural land with other food crops as they grow or are cultivated in areas where crops do not grow (Bioethics, 2011).Unlike vegetable oils from various crops that can be extracted by crushing the oil seeds followed by solvent extraction, releasing oil from algae cells is hindered by the rigid cell wall structure. Thus, mechanical crushing seems to be an ineffective way to extract oils from algae biomass (Patil et al, 2012). It is also a fact that using solvents for extraction may involve expensive separation processes (Patil et al, 2012).Another challenge, however, has been the high cost of recovering the oil from microalgae prior to converting it into biodiesel. While there are many extraction methods such as solvent extraction, supercritical fluid extraction, catalytic extraction, ultrasonic extraction, and mechanical pressing, they require longer extraction times, larger volumes of solvent, and are energy –and cost – intensive (Prafulla et al, 2010).

Thus, microwave-assisted extraction or extractive transesterification could be an alternative to address the above concerns as the ability of the microwave to penetrate through the cell wall structure may result in an efficient recovery of lipids. In a microwave-assisted extraction, rapid generation of heat and pressure within the biological system forces out compounds from the biological matrix, producing good quality extracts with better target compound recovery (Hemwimon et al, 2007). The rapid heating leads to localized high temperature and pressure gradients which assist in cellular wall degradation and enhanced mass transfer rates (Kanitkar, 2010).

Among the most costly downstream processing steps in fuel production using microalgae feedstocks are harvesting, dewatering, extraction,and conversion to fuel from biomass. One major solution is to manipulate the process technology to allow for better, efficient, and cost-saving methodologies (Radakovits, Jinkerson, Darzins, & Posewitz, 2010).A production process which provides simultaneous oil extraction and transesterification is thus, worthwhile to develop.

1.1 Problem statement

The ever expanding human population demands for energy security and independence and can’t rely on the ever depleting, non-renewable resources (i.e. coal, crude oil, natural gas). The increasing demand and desire for the renewable resources as a substitute for the non-renewables and the need for reduction of time and energy consumption in algae oil extraction and the conversion of its lipids to biodiesel necessitate optimization of the biodiesel production process. In a nutshell there is need to cut down the cost of production of biodiesel so that the biofuel manufacturers can meet this expanding market.

Microalgae a third generation biofuel is being considered as the major raw material in biodiesel production because of its high oil content. Industry players and researchers have a common knowledge that the bulk of energy costs during biodiesel productionarise from the longer times experienced during algae dewatering, oil extraction, conversion, and high solvent consumption during extraction and reaction to final product (biodiesel). This process occurs in a single stage: there is continuous oil extraction of oil from microalgae biomass and at the same time this oil is converted to biodiesel. Therefore it would be of great economic benefit if a means is found of reducing the time of production and thus the energy consumption. One such method is through the simultaneous algae dewatering, algae oil extraction and conversion to biodiesel (in situ) with the assistance of microwave irradiation.

1.2 Main Objective

Direct production of fatty acid methyl esters from *Spirulina* microalgae by in situ catalytictransesterificationusing microwave irradiation.

1.2.1 Specific Objectives

1. To quantify oil content of *Spirulina* microalgae.
2. To characterize *Spirulina* microalgae oil.
3. To study the effect of reaction operating variables: catalyst concentration, methanol to oil ratio and reaction time on FAME yield, and identify optimal conditions through the use of Response Surface Methodology.
4. To characterize FAME produced.

1.3 Justification

1.3.1 In situ transesterification assisted by microwave irradiation

The conventional methods of biofuel production take longer times for its production. The longer times and energy consumption and high amounts of solvent during algae dewatering, algae oil extraction and during conversion is quite substantial and therefore there would be immediate benefit for researchers and biodiesel producers if the time for its production is greatly reduced and subsequently energy requirement would be reduced. A significant improvement in the biodiesel production process would allow biodiesel to be considered as a substitute to Petro-diesel.Since, the costs of biodiesel from previous research made is about 2.5 times when compared to petro-diesel reducing the time of production would result in significant financial saving for biodiesel producers.

1.4 Hypothesis

The conventional methods (where oil is initially extracted and then transesterification carried out i.e. use of sand baths, oil baths, waterbaths, hot plates,) experience high cost of production, longer extraction time, larger volumes of solvents required and are energy – and cost – intensive. In-situ method of *Spirulina* microalgae transesterification with microwave irradiation eliminates the oil extraction step and reduces the reaction time to produce fatty acid methyl esters. It is hereby hypothesized that adopting the method described herein will have positive attributes (i.e. time saving, less energy consumption and higher yields in comparison with those of conventional methods.

1.5 Scope

The produced biodiesel was not evaluated in a test engine. This studyfocused on the effect/importance of microwave irradiation on biodiesel production from *AthrospiraSpirulinaplatensis* microalgae on the identified variables and its future scope as energy security.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.0.1 Introduction

The world is moving fast and away from the non-biodegradable, non-renewable to pollution-free forms of energy. The increasing rate of crude oil exploration coupled with high consumption rates is slowly leading to its exhaustion. It is imperative, therefore that the world looks at other forms to produce energy, especially from third-generation biofuels. First and second generation biofuel resources doesn’t have the ability to fulfill the current and future requirement of biodiesel without considering biodiesel from algae, as a third-generation fuel (Jones & Mayfieldt, 2012).

The diesel engine was invented byRudolf Diesel in the 1890s and this engine could run on a variety of fuels, including vegetable oil. In the year 1900, one Rudolf’s new diesel engines that featured at the Paris Exposition were powered by peanut oil. However, because cheap petroleum fuels were easily available, few people were interested in alternatives(Gerpen, 2019).

There was interest in separating the fatty acids from the glycerin in vegetable oil as early as the 1930s in order to make a thinner product akin to petroleum diesel. G. Chavanne received a Belgian patent for an ethyl ester of palm oil in 1937 (which today we would call biodiesel).A passenger bus powered by palm oil ethyl ester ran between Brussels and Louvain in 1938 (Knothe, 2005).

Several countries, notably Brazil, Argentina, China, India, and Japan, used vegetable oil as a fuel during World War II (1939–45), when petroleum fuel supplies were disrupted.

However, these vegetable oil fuel, on the other hand, were forgotten when the war ended and petroleum supplies were once again cheap and plentiful (Boakye, 2013).

The petroleum oil embargo in the 1970s prompted many governments to consider vegetable oil as a feasible fuel source.Straight vegetable oil could be used to run diesel engines again, according to scientists in Austria, the United States, South Africa, and many other countries; however, the poor quality of the fuel spray caused by the thickness (viscosity) of the vegetable oil eventually caused damage to the engines. The vegetable oil was subsequently converted into biodiesel in studies done by scientists. The term "biodiesel" was presumably coined about 1984 (Gerpen, 2019).

In 1985, an agricultural institution in Austria constructed the first biodiesel manufacturing unit specially geared to manufacture fuel. Biodiesel has been commercially produced in Europe since 1992, with Germany being the major producer. Biodiesel was first commercially produced in the United States in 1991 in Kansas City, Missouri. Yellowstone National Park employed biodiesel produced by the University of Idaho in a truck that has been driven hundreds of thousands of miles without harm to the engine and is still in service. Other national parks began employing biodiesel in their cars as a result. (Marchetti, Miguel, & Errazu, 2005).

One issue with biodiesel at the time was its inconsistent quality. ASTM (American Standards and Test Methods) International, one of the world's leading voluntary standards development organizations, issued ASTM Standard D6751 for biodiesel in 2001. This gave engine makers and fleet managers assurance that biodiesel would meet their quality standards (Gerpen, 2019).

2.0.2 Controversies Surrounding Biodiesel

The rapid rise of the biodiesel sector has sparked fears that farmers will be encouraged to cultivate more and more fuel crops, leaving less and less land available for food production. As global food prices increased in 2008, the “food vs. fuel” controversy arose and serious debate took off (Jens & Saori, 2016). Although growth of feedstock crops for biofuel is feasible (i.e., sunflower oil, sugarcane, corn, wheat, and barley), its production has raised doubts about possible impacts on food supply and security (Ravanal, et al., 2019). The fuel versus food argument is aggravated by the large-scale cultivation demands and the high levels of resources required. Accordingly, there is an urgent demand for alternative, sustainable fuels and feedstocks in order to replace food-based feedstocks.

Although growth of feedstock crops for biofuel is feasible (i.e., sunflower oil, sugarcane, corn, wheat, and barley), its production has raised doubts about possible impacts on food supply and security (Ravanal, et al., 2019). This are referred to as first-generation biofuel. The fuel versus food argument is aggravated by the large-scale cultivation demands and the high levels of resources required. Accordingly, there is an urgent demand for alternative, sustainable fuels,and feedstocks in order to replace the food-based biofuel sources (Ravanal, et al., 2019).

Scientists are also experimenting with producing fuel from non-oilseed feedstocks such as inexpensive, non-edible biomass sources (i.e. agricultural residue, waste from the wood products industry, and switchgrass and other grasses) that can be converted into a diesel replacement the so called second-generation biofuel. While it is a fairly simple process to convert vegetable oil or animal fat into biodiesel, the conversion of cellulosic feedstocks to fuel is more complicated and more expensive. To produce a hydrocarbon fuel, the biomass is generally first converted into a synthetic gas using high heat. Then the gas can be converted into a liquid diesel fuel (Bera, Inglett, & Wilkie, 2020).

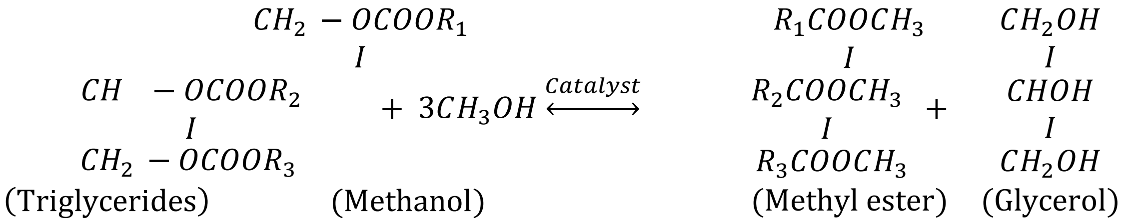
As a result of the negatives associated with these first and second-generation biofuels, a third-generation biofuel came into being (biodiesel is commonly produced from algae, that are single-cell or multicellular organisms). Generally, algae are categorized based on their habitat, such as freshwater algae, marine algae, or wastewater algae. Basing on thelipid content, a specific algalis chosen for biodiesel production (Wilkie et al. 2011).

2.0.3Transesterification process

Most commercial biodiesel is made by a chemical process called transesterification. This involves mixing the feedstock oil with an alcohol, typically methanol or ethanol in the presence of a catalyst, an acid, i.e. H_2SO_4 or base, i.e. $NaOH.$ The reaction produces methyl esters (if methanol is used) or ethyl esters (if ethanol is used) - which comprises biodiesel (fuel) and glycerin by product(Murphy et al, 1995). Methanol is typically used for economic reasons, as the physical and chemical properties between methyl esters and ethyl esters are, according to university of Idaho study, “comparable” (Idaho, 2003).

2.0.4 Biodiesel production basics

Biodiesel as a fuel is made up of mono-alkyl esters of long chain carboxylic acids derived from vegetable oils, and or animal fats. The diesel obtained can be used in diesel engines and for heating purposes (Marchetti et al, 2007).



$$\begin{array}{l} CH_2-OCOOR_1 \\ \qquad I \\ CH\ -OCOOR_2 \\ \qquad I \\ CH_2-OCOOR_3 \end{array} + 3CH_3OH \xleftrightarrow{Catalyst} \begin{array}{c} R_1COOCH_3 \\ I \\ R_2COOCH_3 \\ I \\ R_3COOCH_3 \end{array} + \begin{array}{c} CH_2OH \\ I \\ CHOH \\ I \\ CH_2OH \end{array}$$

(Triglycerides) (Methanol) (Methyl ester) (Glycerol)

The most common method used to produce biodiesel is by transesterification. In this method, triglyceride which is the main component of oil/fats as feedstockcan react with alcohol in the presence of a catalyst to produce biodiesel and glycerol as a by-product.Transesterification is a reversible reaction and is expected to occur in three steps. First is the conversion of long-chain triglycerides to diglycerides, followed by conversion of diglycerides to monoglycerides and finally the conversion of these monoglycerides to glycerol. Each step yields one molecule of an alkyl ester, as given by the reactions below.

$$\text{Triglyceride} + R'OH \xleftrightarrow{\text{catalyst}} \text{diglyceride} + R'COOR_1$$

$$\text{Diglyceride} + R'OH \xleftrightarrow{\text{catalyst}} \text{monoglyceride} + R'COOR_2$$

$$\text{Monoglyceride} + R'OH \xleftrightarrow{\text{catalyst}} \text{glycerol} + R'COOR_3$$

In the above reactions, R' is the alkyl group for the alcohol whereas $R1$, $R2$ and $R3$ are carbon chains of the fatty acids(Kumar A. , Chirchir, Namango, & Kiriamiti, 2016).

2.1 Feedstock

Biodiesel is an attractive fuel for diesel engines that can be produced from any vegetable oil (edible or nonedible), used cooking oils, animal fats as well as microalgae oils (Aullon, 2010). The best choice of a given feedstock for biofuel production should constitute a carbon chain length from the saturated $C14$ to $C22$, the unsaturated free fatty acid groups and the saturation states should be lower for better conversion (Tara, 2014).

The most important aspect considered to be the chain length, whereas the usage of algae biodiesel is negatively affected by high un-saturation levels. The level of un-saturation affects negatively on oxidative stability, cetane number, and heat of combustion (Tara, 2014).

2.2 Algae biomass and its suitability for biodiesel production

The biodegradability, nontoxicity, renewability, safe and green form of alternative fuel and its low environmental impact make*Spirulinaplatensis* algae as a good feedstock for the production of alternative fuel (El-Mashad et al, 2008). Moreover, the use of these algae as suitable alternatives is because some species have high quantities of oil, which can be simultaneously extracted and transesterified into biodiesel and finally refined (El-Shimi et al, 2013). There is extensive literature carried out to explain biodiesel production from vegetable oils(Deng et al, 2010; Phan & Phan, 2008; Patil & Deng, 2009; Brian, 2010). Yet, according to Demibras, (2009)there is little research carried out on the production of biodiesel from microalgae and especially regarding feedstock being *Spirulina*. Microalgae biodiesel production by conventional methods has been studied by (Atiqur & Kamrun, 2016; Nagle & Lemke, 1990; Sayeda et al, 2013; El-Shimi et al, 2013)in which the oil is initially extracted mechanically by using an oil press and the remaining available in the cake extracted by use of the solvent, by the soxhlet apparatus fitted with a condenser. The oil obtained was transesterified to produce biodiesel in the presence of a catalyst (Atiqur & Kamrun, 2016).

2.3 Catalyst (acid and base catalyst)

Rahman et al., (2017)statethe problems that researchers faced using conventional methods in the production of biodiesel, which included low percentage biodiesel production and the longer duration it was required to achieve the objective. Algae contains a higher percentage of free fatty acids (FFA)and when transesterification is carried out in the presence of an alkali catalyst, it may lead to soap formation, increase in catalyst consumption, catalyst fouling and decrease in the yield of biodiesel (El-Shimi et al, 2013; Chiou et al, 2008). AccordingtoRahman et al., (2017)may in effect lead to biodiesel separation problems from glycerol. Furthermore, in an alkali reaction, if an excess of alcohol is used in the experiment, then better conversion of triglycerides is obtained, but recovering glycerol becomes more difficult and that is why the optimal relation between alcohol and raw material should be determined experimentally considering each process as a problem (Marchetti et al, 2007). Yet again,acid catalyst has slower reaction rates than base catalyst reactions(Wang et al, 2007). It is desirable therefore to determine the acid value to esterify or determine the excess number of bases to be added before thetransesterification process is carried out when a base catalyst is required.

The use of the alkaline catalyzed transesterification technology would not be suitable for biodiesel production from microalgae oil, because of the high FFA content of microalgae lipids. This is because the use of alkaline catalysts with high FFA containing oils would result in soap production(Ehimen et al, 2010; Al-Zuhair, 2007)and difficulties in biodiesel separation and purification downstream. The use of sulphuric acid as the reaction catalyst has been considered for the microalgae lipid transesterification, due to the insensitivity to the FFA content of the microalgae feedstock, as the transesterification and esterification reactions in biodiesel production are facilitated via acidic catalysis (Nagle & Lemke, 1990; Haas et al, 2004).

2.4In situ

The in situ process of biofuel productionsimultaneously combines the steps of lipid extraction and transesterification to produce biodiesel. Hincapie & Lopez(2011)suggested in their research that the integration of the two stages minimizes the cost of biodiesel production since the usage of reagents and solvents are greatly reduced and analysis becomes easier and less expensive. Deepalakshimi et al., (2014)carried out an optimization process of biodiesel from waste avocado seeds by in-situ method and they obtained a yield of $94.4\,wt\%$ with respect to the weight of the total oil content of avocado seeds. This suggests that in-situ method optimizesthe production of biodiesel.El-Shimi et al., (2013) carried out research to produce biodiesel from *Spirulinaplatensis*microalgae by in-situ method and stated that 8 hours was the optimum time required for the production of biodiesel when using heat directly from a hot surface (hot plate) to heat the reacting substances in the reaction vessel. The microwave energy was not incorporated in the research. The process took a longer time eight (8) hours to ensure that the reactants proceed in the forward direction.

2.5 Microwave irradiation

In spite of the projected benefits the in-situ biodiesel production methods are associated with, it is imperative to consider a process that achieves transesterification in a shorter time. The conventional methods of biodiesel production usually consume high amount of energy resulting from the requirement of algae dewatering, and extraction of microalgae oil(Patil, et al., 2013). Microwave assisted methods have been developed as a promising way–that is rapid, energy efficient, cost saving, and are environmentally friendly in the production of biodiesel. Numerous tests and optimization studies with different feedstocks have been conducted by researchers (Li, et al., 2013; Azcan & Yilmaz, 2013) in recent years to maximize the advantages of microwaves for biodiesel production. Other than for transesterification reactions, microwave energy is also applied to the oil extraction process to increase the rate of production and produce a greener and better quality biodiesel (Gimbun, et al., 2013).

The shorter reaction times and expanded reaction range that is offered by the microwave-assisted organic synthesis are suited to the increased demand in industry (Veera & Martinez-Guerra, 2018). In general, most organic reactions have been heated using traditional heat transfer equipment such as oil bath, sand baths, hot plates, heating mantles and heating jackets. These heating techniques are, however, rather slow and a temperature gradient (Azcan & Yilmaz, 2013)can develop within the sample. In addition, the local overheating can lead to product, substrate, and reagent decomposition. In contrast, in microwave dielectric heating, the microwave energy is introduced into the chemical reactor remotely and direct access from energy source to the reactor vessel is obtained. Themicrowave radiation passes through the walls of the vessel and heats the reactants and solvents, not the reaction vessel itself (Patil et al, 2012).

Microwave processing according to Saifuddin & Mei, 2017, produces a higher yield with a cleaner profile in comparisson to other methods (i.e. conventional, supercritical, ultrasonication). They reported that microwave is a better heating method than conventional heating methods due to its unique thermal and non thermal effects. They still stated that the best operating for esterification are still inconsistent. They were concerned on only comparing biodiesel from other sources, i.e. vegetable oil, non-edible oil, waste cooking oil to that of algae withoiut considering their efficiencies.

It is therefore prudent to study biodiesel from *Spirulina* algae by simultaneous extraction and transesterification processes with the assistance of microwave irradiation. The method is expected to achieve a high degree of oil/lipid removal from *Spirulina* algae biomass with high efficiency of conversion to biodiesel (Patil et al, 2012).

CHAPTER THREE

3.0 RESEARCH METHODOLOGY

3.0.1 Materials, Equipment and Apparatus

Spirulina microalgae biomass, *Athrospira-Platensis,* was purchased from Masinde Muliro University, Kenya. Methanol, Sulphuric acid, Sodium hydroxide, Hydrochloric acid, Ethanol, and Potassium Hydroxide, all analytical grade, were sourced from Gelsup Kenya. Isopropyl alcohol, analytical grade was from Bevick Kenya. The major equipment was: Microwave oven 1300 watts (Shivaki, Japan), Stirrer $50w$ (Bibby Sterilin Ltd, UK), Rotary Vacuum Evaporator (HAHNVAPOR, Hahnshin Scientific Co. Ltd, Korea), Centrifuge (Itettich Zentrifugen D-7200 Tutlingen $6000\ RPM$, Germany), Hot Plate (Thomas Scientific, CAT no. 984THOCHPEUA), Analytical balance, lab oven, and Soxhlet apparatus (PYREX UK).

3.0.2 Materials

The materials used in this study are summarized in Table 3.1 below.

Table 3.1: The Materials Used and their Sources.

Item NO	Chemicals & Solvents (analytical grade)	Sources
1.	*Spirulina-Platensis* microalgae biomass	MmustPremisses (Kenya)
2.	Methanol	Gelsup(Kenya)
2.	Potassium hydroxide	Gelsup(Kenya)
3.	Sodium Hydroxide	Gelsup(Kenya)
4.	Hexane	Gelsup(Kenya)
5.	Sulphuric Acid (ρ=1.01)	Gelsup(Kenya)
6.	Hydrochloric acid	Gelsup(Kenya)
7.	Biodiesel standard (Methyl Heptadecanoate)	Kobian (Kenya) Ltd.
8.	Phenolphthalein Indicator	Bevick (Kenya)
9.	Ethanol	Gelsup (Kenya)
10.	Isopropyl Alcohol	Bevick (Kenya)

3.0.3 Equipment

The equipment used in this study to produce and analyze biodiesel from*Spirulina-Platensis* microalgae by in-situ is summarized in Table 3.2.

Table 3.2: The Apparatus/Equipment Used and their Sources or Origin.

Item No	**Name of equipment/Apparatus**	**Sources/Origin**
1.	Gas Chromatograph Unit	(Mrc Variant type)
2.	Rotary Vacuum Evaporator	HAHNVAPOR, Hahnshin Scientific Co. ltd, Korea
3.	Microwave oven	Shivaki, Japan
4.	Lab Furnace	DAIHAN LABTECH CO. LTD. –Model LDO-150F
5.	Hot Plates	Thomas Scientific, CAT no. 984THOCHPEUA
6.	pH/ORP meter	HANNA Instruments, Romania. - Model H1 2211
7.	Analytical Balance	Scaltec SBA/SBC, Germany
8.	Stirrer	BibbySterilin ltd, UK - CAT NO. SS10
9.	3-Necked Round Bottom Flasks	Lenz Laborglasinstrumente, SCHOTT-DURAN Germany
10.	Condensers	Pyrex UK
11.	Soxhlet Apparatus	Pyrex United Kingdom
12.	Centrifuge	ItettichZentrifugen D-7200 Tutlingen 6000 rpm, Germany

3.1 Research design

Experiments were done to evaluate the simultaneous extraction and transesterification (in situ) using microwave energy to determine FAME yield.This was done by determinining the influence of the catalyst concentration, volume of methanol to oil ratio, and time of reaction on FAME yield. Additional experiments were carried out to determine yield of oil in algal biomass, carryout characterization of *Spirulinaplatensis*microalgae oil and the produced biodiesel. Most biodiesel production processes operate at a temperature of less than 70°C(Fajardo, Leon, & Vargas, 2010). Response Surface Methodology (RSM), Central Composite Design (CCD) will be used to optimize and maximize production of the three variables under study (index 3.11).

3.2 Choice of microwave

3.2.1 Catalytic Microwave in situ Transesterification Optimization

In situ transesterification of microalgae is a transesterification process that occurs directly (direct transesterification) or simultaneously in producing biodiesel. This process is reported to be superior to two-stage transesterification or conventional processes that are more dependent on extraction results with organic solvents with low separation efficiency because of the incomplete extraction process(Mahfuda, Kalsumb, & Aswiea, 2020). High operational costs and product prices are also caused by ineffective solvent use, the length of time, and the multiple phases of the process. In this study the transesterification of in situ microalgae in producing biodiesel was studied using a microwave as a reactor and heater. Microwave use is intended to replace ineffective conventional heating systems in terms of energy use and time. Energy efficiency of heating by using microwaves is much smaller when compared to conventional heating (Gude et al., 2013). Microwaves, as an energy source, produce heat by their interaction with the materials at molecular level without altering the molecular structure(Refaat, 2010). Microwave heating offers several advantages over conventional heating such as non-contact heating (reduction of overheating of material surfaces), energy transfer instead of heat transfer (penetrative radiation), reduced thermal gradients, material selective and volumetric heating, fast start-up and stopping and reverse thermal effect, i.e. heat starts from the interior of material body. In terms of biodiesel production, the resultant value could include: more effective heating, fast heating of catalysts, reduced equipment size, faster response to process heating control, faster start-up, increased production, and elimination of process steps (Gude, Patil, Martinez-Guerra, Deng, & Nirmalakhandan, 2013).

3.3Extraction and characterization of oil, algae biomass and biodiesel

3.3.1 *Spirulina* microalgae oil extraction by Soxhlet method

Soxhlet apparatusfitted with a quick fit condenser and a 250ml round bottom flask (Fig. 3.1) holding the solvent (a mixture of hexane/isopropyl alcohol, 3:2 to make a total of 120ml) was used to extract oil from the algae biomass held inside a porous thimble. The solvent was heated inside the flask by an external source of heat (hot plate, Thomas Scientific, CAT no. 984THOCHPEUA), provided with temperature control to ensure the boiling point temperature of thesolvent is maintained. Solvent vapours are condensed, fallinginto the Soxhlet chamber where itextracts oil inside a porous media, thimble, containing the solid biomass algae, and the mixture wasfinally allowed back into the round bottom flask. The solvent changes phase from liquid to gaseous state when heated and back to a liquid state when cooled and fills the Soxhlet chamber holding the solid mass inside the porous media extracting oil. The accumulating liquidfills the inner chamber of the Soxhlet apparatus. The oil dissolved in the solvent falls into the lower chamber by a process known as siphoning, to be heated again. This process forms a closed loop that remains continuous until relatively all oil has been extracted.

The termination of the soxhlet extraction process ends the moment colour of the solvent plus oil changed from brown-yellow to clear;colourless liquid, giving an indication that about 100% of the oil has been extracted.The oil was then separated from the solvent by evaporation at reduced pressure using a rotary vacuum evaporator(HAHNVAPOR, Hahnshin Scientific Co. Ltd, Korea). The total lipid content was determined by weighing. Several runs of soxhlet extraction were carried out to obtain enough oil to be used for characterization processes. The acid value of the oil was then determined.

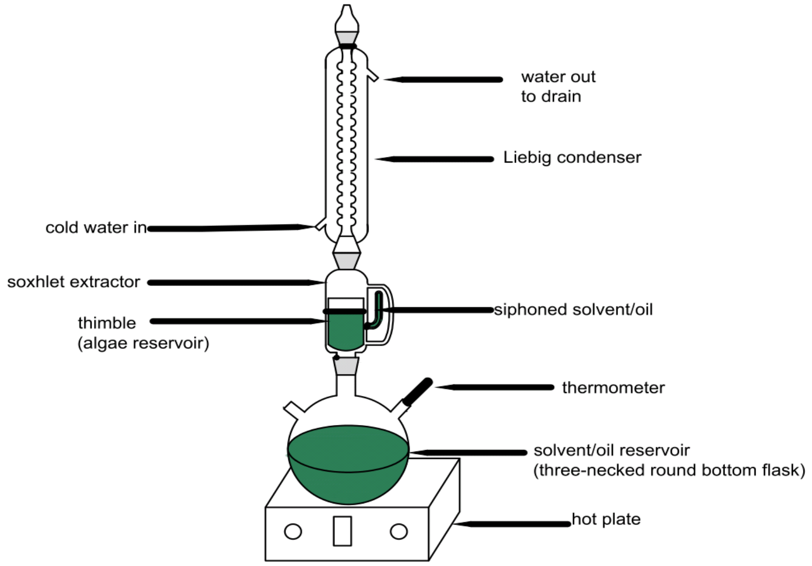


Figure 3.1: Soxhlet Extraction Set-up for the *Spirulina* Algae Oil Extraction Process.

3.3.2 Quantification ofoil content

The quantification of the extracted oil yield of microalgae biomass (%) was calculated according to the following equation:(Elshimi, Attia, & Allah, 2015).

$$Oilyieldofmicroalgaebiomass = \left(\frac{massoftheextractedoil}{massofdriedalgaebiomass}\right) \times 100\% \ldots\ldots..Eq.1.$$

3.3.3Protein content of algae biomass

Protein content of *Spirulina* microalgae was determined in the Chemical and Process Engineering (CPE) laboratories. After extraction of oil/lipids from algae biomass (Index 3.2.1), the defatted powder was air dried and stored at $4^{O}C$.Defatted algae biomass was added to distilled water (8% w/v). The mixture was stirred for 60 minutes and filtered using Whatman filter paper number 1. The crude extract filtrate was then incubated in ice. An optimum concentration of 40% (w/v) ammonium sulphate$(NH_4)SO_4$was used to slowly raise the salt concentration in the algae biomass filtrate to 30% (w/v) to remove proteins and other materials possessing little or no coagulation activity in a process known as salting out. The precipitates are removed by centrifugation at 3000 RPM for 10 minutes and the supernatant is recovered for further careful addition to 40% $(NH_4)SO_4$ (w/v)(Parimi, Singh, Kastner, C., & Fusberg, 2015). The precipitated proteins were finally centrifuged at 3000 RPM for 10minutes. The pellets obtained wereredissolved in distilled water and dialyzed overnight (12 hours) against 2L distilled water at $4^{O}C$ in a beaker. Distilled water was replaced with fresh distilled water after the first 4 hours of dialysis. This was to ensure the concentration gradient was maintained. The total protein as a percentage (%) of total algae biomass was determined.

3.3.4 Ash Content of algae biomass

Two grams of powdered (160µm) microalgae biomass was accurately weighed and placed in an uncovered crucible and kept in a muffle furnace maintained at $550^{o}C$ for 6hrs. Then the sample was cooled to room temperature in a desiccator and weight again. The weight of the residue remaining in the crucible corresponds to the ash content. It is usually expressed as a percentage(Liu, 2019).

$$Ash\ content\ (\%) = \left\{\frac{(W_a - W_c)}{(W_t - W_c)}\right\} \times 100\% \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots . Eq. 2.$$

Where, W_c *- Weight of empty crucible.*
W_t *- Weight of crucible + sample.*
W_a*- Weight of crucible + sample after heating.*

3.4 Free fatty acid *(FFA)*

The acid value and free fatty acid of both microalgae oil and biodiesel were determined at the Chemical and Process Engineering laboratories (CPE Labs), Moi University. The acid value is the milligrams of potassium hydroxide needed to neutralize the free fatty acid in one gram of oil or fat. The free fatty acid in the*Spirulina* oil/biodiesel was analyzed using titration method. 1g of filtered oil/biodiesel was mixed with $25ml$ of ethanol and $2-3$ drops of phenolphthalein indicator. A sample was dissolved with stirring in warm 95% ethanol and titration was performed with a standard solution of $0.1M$ sodium hydroxide using phenolphthalein indicator.

The percentage of FFA was calculated using *Eq.3*.(Sameer Mohammed., 2016).

$$FFA\,\% = \frac{Titre\ volume\ in\ litres\ \times 28.2}{Weight\ of\ the\ sample}$$

$$= \frac{(v-b)x\ N\ x28.2}{W} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq.3.$$

Where:

0.1 = molarity of sodium hydroxide solution used.

v = is the volume in ml of titration solution

b = is the volume in ml of the blank

N = is the normality of the titration solution

28.2 = molecular weight of oleic acid divided by 10 taken as a basis for calculation.

W = weight in (g) of the sample.

Acid Value $(A.V)$ is approximately ½ FFA %.

3.5Acid value

Acid value ($A.V.$) is given by the number of milligrams of potassium hydroxide needed to neutralize the free fatty acid in one gram of oil/fat.

Thus:-

$$A.V. = \frac{V_{catalyst} \times N \times 56.1}{W_{oil/biodiesel}} \left(\frac{mgKOH}{g}\right) \ldots\ldots\ldots\ldots.\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Eq.4.$$

$Where: -V_{catalyst} - Volume of the catalyst used,$

$N =$ molarities of base (sodium hydroxide) used.

$56.1 = Molar\ mass\ of\ KOH solution used,$

$W_{oil/biodiesel} - weight of oil or biodiesel sample\ (g).$

3.6 Properties measurement of*Spirulina* algae biomass, oil, and biodiesel

Specific gravity, kinematic viscosity, calorific value, acid number, flash point, ash content (Index 3.3.4, 3.5, 3.6.2-3.6.8,also followed for algae biodiesel determination) and moisture content properties of *Spirulina* biomass and biodiesel as a product of transesterification was determined using various methods according to American Standard Test Methods (ASTM D 6751).

3.6.1 Moisture content (MC)

Moisture content of dry algae biomass was determined inManufacturing, Industrial and Textile Engineering laboratory (MIT),Moi University. The drying laboratoryoven (DAIHAN LABTECH CO., LTD.,Model LDO-150F) employs a thermo-gravimetric method (loss on drying) in which the sample was dried for an hour and the temperature maintained at$105^{o}C$.The moisture content was determined by weighing 1g of the sample before and after drying and finally determining the difference. Moisture content of *Spirulina* oil and biodiesel products was determined in accordance with ASTM D-2709(Reeb & Milota, 1999).

$$Moisturecontent = \left(\frac{InitialWeight - Oven, dryweight}{InitialWeight}\right) \times 100\% \dots\dots\dots\dots\dots. Eq. 5$$

3.6.2 Specific gravity

Density is the weight of a unit volume of fluid while the specific gravity is the ratio of the weight of the same volume of the oil to the weight of the same volume of water(Sameer Mohammed., 2016). The SI unit for density is Kg/m^3whereas specific gravity is a ratio and therefore has no defining units.Specific gravity is usually measured at$15^O C$,(Teresa M. Mata., 2010)**.**Specific gravity measurements were carried out using a *Pycnometer*(Paul Marienfeld GmbH & Co. KG., Series 27) in accordance with ASTM D 941 standards. Density measurement was carried out at the CPE laboratories.

The specific gravity was calculated using Eq. 6.

$$Sp. gr. = \frac{w_3 - w_1}{w_2 - w_1} \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots.. Eq. 6.$$

Where:-
w_1= weight of empty *pycnometer*.
w_2= weight of the *pycnometer* with water.
w_3 = weight of the*pycnometer*filledwithoil.

3.6.3 Flash point determination of algal oil/biodiesel

The flash point (FP) was carried out at KIRDI laboratories (oils for cosmetic industry methods of test, Part 12). The flash point of a fuel is the lowest temperature at which the fuel will ignite (flash) upon exposure to an ignition source.A liquid is considered to be flammable if its flash point is less than $60^{O}C$. Flash point was determined byPensky-Martens Closed Cup analyzer and in accordance with ASTM D 93for smaller volumes $(1-2mL)$.The test procedure in use here is the Cleveland Closed Cup (CCC). The cup was filled with the sample to be tested to the level indicated by the filling mark. The lid was then placed on the cup and the latter was set on the stove. The sample that was contained in the cup was then heated and at regular intervalsa flame was brought over the surface with the simultaneous interruption of a stirring. The measured flash point will actually vary with the height of the flame above the oil surface, and at sufficient height (spark position is 3 mm above pool)the measured flash point temperature was noted(Ikechukwu, 2013).

3.6.4 Determination of pH

The pH of microalgae oil and biodiesel produced was measured using a pH/ORP meter(HANNA Instruments - Model H1 2211) fitted with temperature and pH probe. This was carried out at the CPE laboratories.

3.6.5Kinematic viscosity

Kinematic viscosity was determined in CPE laboratories. Kinematic viscosity is a measure of a liquid internal resistance to flow due to the internal friction of one part of a fluid moving over another, under gravitational forces(Sheetal & Wagh., 2015).Kinematic viscosity was measured for *Spirulina* oil and biodiesel at $40^{O}C$, by measuring the time in seconds for a fixed volume (2 ml) of liquid to flow a known distance (10 centimetres) under gravity through a capillary of a U-tube (Oswald)Viscometer in accordance with

ASTM D445. Water at$40^{O}C$was also allowed to flow through the apparatus similrly to the above samples and the results obtained were recorded.

3.6.6 Calorific value of fuel

The calorific value of the samplewas carried out at KIRDI laboratories (oils for cosmetic industry methods of the test). This is the heat of combustion of fuel. Referred to as the specific energy of the fuel, and indicates the oil or fuel's energy released when it is burned. Feedstocks whose oil has high specific energy are more likely to produce diesel with high amounts of energy on combustion. This was determined in an oxygen bomb calorimeter (TOSHNIWAL TECHNOLOGIES Bomb Calorimeter, Model: CC01/M2A) and was measured in triplicate by a substitution procedure in which the heat obtained from the sample was compared with the heat obtained from a control material. Calorific value was determined by igniting a small sample of about 1g in an oxygen-filled bomb calorimeter. The bomb was held in a measured quantity of water within a thermal insulating jacket. A thermometer accurate to two decimal places was used to sense the temperature rise due to heat released by the combustion process. The cross heat was calculated from the temperature, fuel, and bomb data using the formulae provided with the calorimeter (equation 7 below). The gross heat of combustion is the heat released by the combustion of a unit of mass of fuel and calculated by multiplying the change in temperature by the instrument heat capacity (IHC) and then deducting the calorific value of the other components that are used to ignite the sample. The adiabatic calorimeter jacket was used in the test in accordance with ASTM D240.

$$\frac{KCal}{g} = \left\{\frac{(\Delta\, in\, temp\ \times IHC) - (Cal.value\ of\ thread + wire\ used)}{Weight\ of\ sample}\right\} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots. Eq.7.$$

Conversion (multiplying by 4,190) was then used to convert $\frac{KCal}{g}$ to $\frac{KJ}{Kg}$.

3.6.7 Carbon residue

When a sample of oil is burned in the absence of air, the oil may leave some carbonaceous residue. This is known as “carbon residue” or carbon content of oil. A sample of biodiesel/oil $(5g)$was weighed and placed in the furnace(DAIHAN LABTECH CO. LTD. –Model LDO-150F)maintained at $550^{O}C$ for 4 hours(ASTM D524)(Karmakar, R. Kendu, K.; Rajor, A.;, 2018).

3.6.8 Sulphur content

This is limited to reduce sulphate and sulphuric acid pollutant emissions and to protect exhaust catalyst systems when they are deployed on diesel engines in the future. Sulphur content of 15 ppmor lower is also required for proper functioning of diesel particle filters. Hence, a good biodiesel generally contains less than15 ppmsulphur.Microalgae sample (2ml - oil and Biodiesel) was initially ashed in a muffle furnace at $500^{o}C.$ Ash obtained was digested by 10ml hydrochloric acid (HCl) at a ratio of 1:1and filtration was then carried out. 10ml of 10% barium chloride was added and ageing allowed for two hours. Filtration of barium sulphate using ashless filter paper was then carried out. The filter paper was put in a platinum crucible and ashedat $500^{o}C.$ The weight of barium sulphate was then determined and finally the percent Sulphur was calculatedusing Equation 8(Tateya, Baikenov, Muratbekova, Nesipbyev, & Nesibbayev).

Sulphur content %= $\left(\frac{S}{BaSO_4}\right) \times 100\%. \ldots \ldots \ldots \ldots \ldots \ldots . \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots ... Eq. 8.$

The sulphur content determination was carried out at KIRDI laboratories.

3.7Biodiesel production process

3.7.1Transesterification reaction

Biodiesel production and analysis were carried out in the CPE laboratories, Moi University. Biodiesel was produced batch-wise, meaning the production process was time staggered through a process known as transesterification. This research tries to optimize the process variables: algae to methanol ratio $(1:4\ to\ 1:14 g/ml.)$, algae biomass to catalyst concentration $(1:0.0032\ to\ 1:0.036818\ wt.\%)$, and reaction time$(1\ to\ 11\ min)$ for the production of biodiesel by in-situmethodwith the assistance of microwave irradiation. These optimal biodiesel production conditions were proposed basing on test in a controlled environment and data obtained from it was used. There was no prior extraction of oil from the microalgae before its conversion by the transesterification process into biodiesel occurred. To ensure maximum dissolution,the catalyst together with methanol was mixed using a stirrer(BibbySterilin ltd, UK - CAT NO. SS10).15 grams (enough to be analyzed), bluish-green, fine uniform dry powder ($160\mu m$ mesh) with an odour and taste that is like seaweed*Spirulinaplatensis* microalgae biomass was then mixed with the catalyst and methanol. Blending of the mixture was carried out for a few minutes to achieve homogeneity before being poured into a reaction vessel (a $250ml, 3$ −necked round bottom flask) fitted with a quick-fit condenser and an external stirrer and the reaction to produce biodiesel occured in a 1300 −watt microwave

(Shivaki, Japan). The microwave was used to provide the microwave energy for the different sample runs. Extraction and transesterification of the bluish-green microalgae biomass in the presence of methanol (solvent) and catalyst and the conversion of its lipids to the desired final product biodiesel and glycerol occurred simultaneously inside the reaction vessel (round bottom flask).The reactants were then heated and maintained for a specified duration under microwave energy.After conversion under the stipulated time, the product formed went through a series of steps(Index 3.7.2) before the pure form of the final desired product (biodiesel) was realized. The experimental setup consisted of a batch reactor (250ml round bottom flask) placed in a microwave oven, as shown in Fig. 3.2.

Microwave Synthesis Unit.

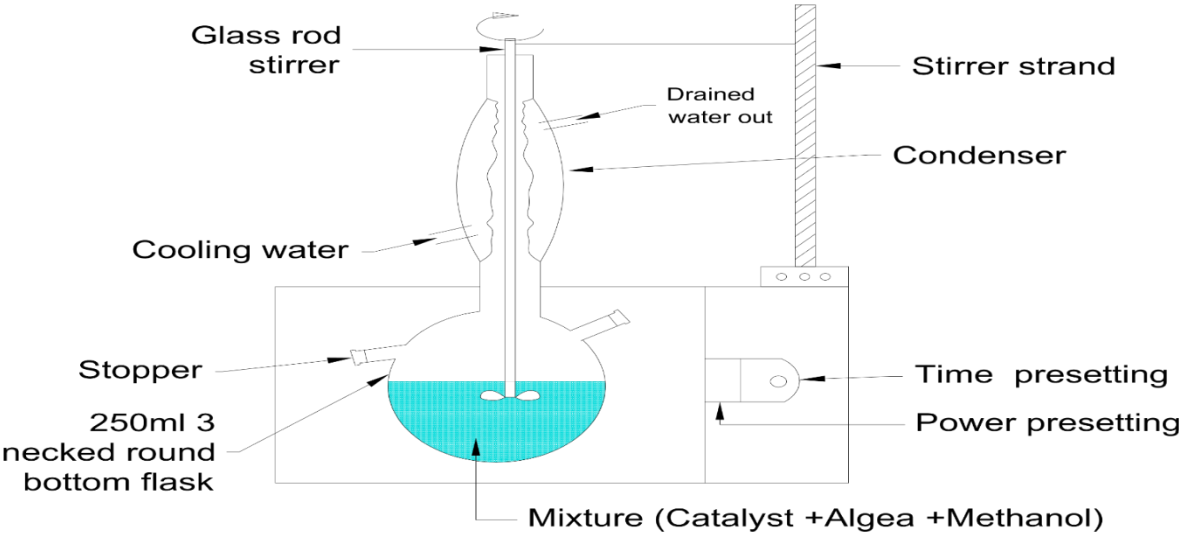


Figure 3.2: Schematic Diagram of a Microwave Synthesis Unit.

3.7.2 Settling, separation and washing processes

After the reaction had taken place, the mixture was cooled with running cold water to stop or arrest the reaction. The mixture was then Decanted to separate the residue (solid phase) from the liquid phase. The solid cake waswashed with 30ml of methanol toensure maximum removal of the remaining liquid phase(methyl esters) in the residue. Filtration was then carried out to separate the remaining solid cake from the flowing liquid phase.

The Rotary Vacuum Evaporator (RVE) (HAHNVAPOR, Hahnshin Scientific Co. ltd, Korea)rotating at 20RPM was used to separate the solvent from biodiesel and glycerol formed. The RVE ensured that the internal temperature of the rotating flask was maintained at a temperature of 22^{o}C while the external temperature of water in the RVE water bath being 50^{o}C. Internal heat was evacuated by the RVE pump ensuring that the separation occurred at lower temperature (22^{o}C)and in the process preventing further reaction.

A centrifuge (Ittettich, ZENTRIFUGEN D-7200 Tuttlingen) rotating at 6,000 RPM for a period of 30 minutes was used to ensure the separation of the biodiesel from the heavier glycerol and suspended solids. Two layers could be observed, dark-brown, and more viscous substance, glycerol settled in the collecting vessel, whereas the biodiesel (light-brown) could be seen to be on top of glycerol. Biodiesel was then separated from glycerol by decantation. The pH of biodiesel was then determined. The remaining layer (mainly biodiesel) was then washed a number of times using warm distilled water to remove any soap, unreacted catalyst, and any solid particles. At about $100^{o}C$the mixture was heated gently to remove excess solvent and water. The substance that is obtained is known as biodiesel.The experimental sequence for single-step (in-situ) microwave assisted extraction and transesterification process is illustrated in Fig. 3.3.

Biodiesel production flow-sheet

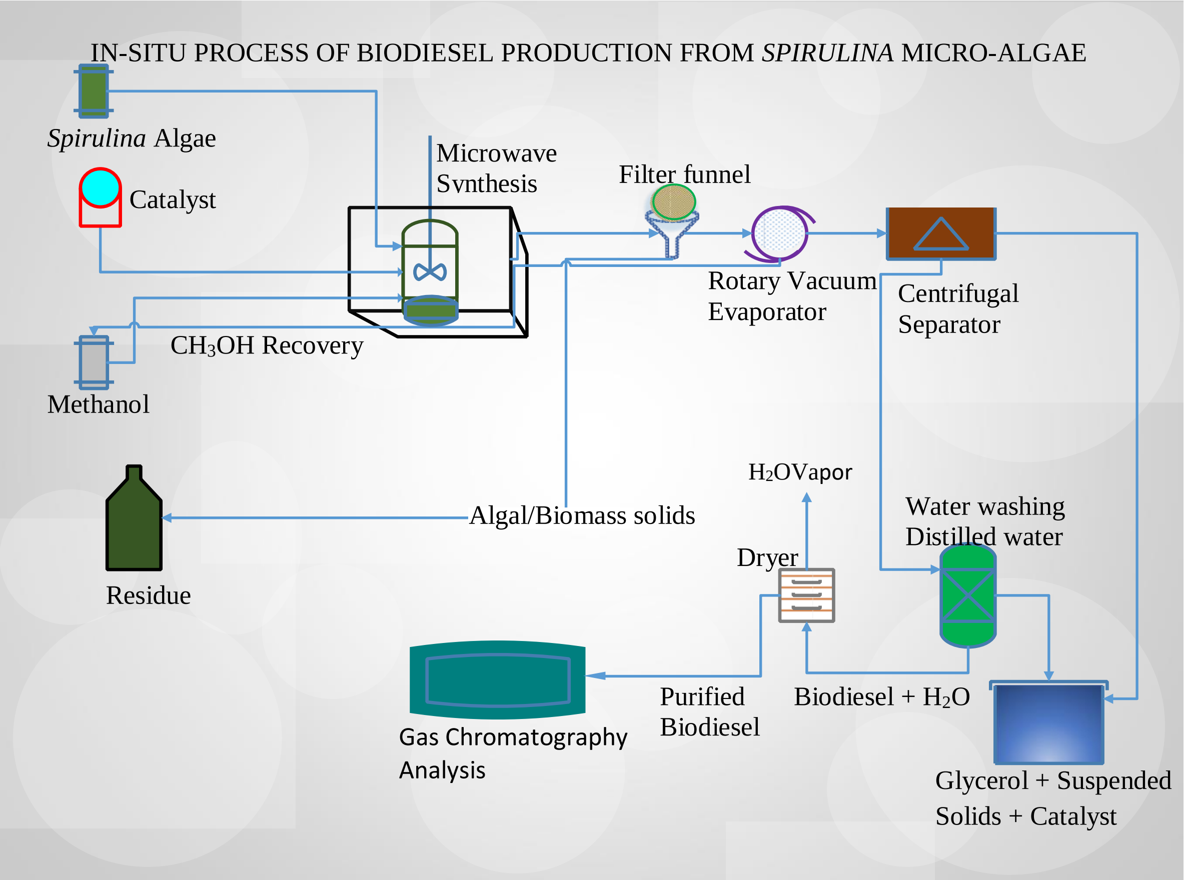


Figure 3.3: Process Flow Diagram of in situ Process of Biodiesel Production.

3.8 Analysis of FAME

Biodiesel was finally analyzed for fatty acid methyl esters (FAME) using a variant gas chromatography(MRC Scientific Instrument: MRC/GC/39621381) fitted with flame ionization detector maintained at$260^{O}C$, the injector port at$240^{O}C$. The oven temperature program was as follows: temperature was held at $100^{O}C$ for $2\ min$, increased to $140^O C$ @$10^O C/min$, then to$190^O C$ @ $3^O C/min$, increased to$260^O C$ @ $30^O C/min$, and finally maintained at $260^O C$ for 2 minutes. Nitrogen gas was used both as carrier and make up gas and allowed to flow inside a$30\ mby\ 0.25\ mby\ 0.25\mu m$ Zebron ZB-FAME column fitted into the injector and detector ports inside the Gas Chromatography. Biodiesel sample $(50mg)$ to be eluded through the capillary column was mixed together with 5ml of Methyl Heptadecanoate ($C17{:}0$, Gelsup 99%)to make a solution of$10mg/ml$. The standard solution (Methyl heptadecanoate,$2mg/ml$) was initially prepared by dissolving in GC grade Hexane (Gelsup, 98.9%). The required injection volume into the capillary column was$1\mu L$, which was used with the split ratio of $1{:}50$ (EN-14214:2003).

3.9 Ester Content (%) (cumulative mass of methyl esters/mass of biodiesel)

It is essential to understand that biodiesel does not mean that it is 100% methyl ester (or pure). It may even have unreacted triglycerides and partially reacted mono-glyceride and di-glycerides. Fatty acid methyl ester content was calculated using the EN-14103 method. According to EN-14103, the minimum ester content should be 96.5%. Internal standard Methyl heptadecanoate $(C17)$ was used.The internal standard (methyl heptadecanoate (C17)) was selected for this analysis as it is highly polar and has been shown to be highly validated and of high purity (Palagano, et al., 2020).When analyzing the results depicted by GC, the area of a peak is proportional to the concentration of that component.

3.9.1 Method

a) A known amount of internal standard $(X\ gram)$ was weighted and mixed with known amount of biodiesel $(Y\ gram)$.

b) GC test/analysis was performed as explained in 3.8.

c) The area corresponding to $C17$ $(Area\ A)$ was determined.

d) The total area corresponding to FAME present$(Area\ B)$ was also determined.

It is important to understand that y gram biodiesel does not contain y gram of methyl ester. It contains‘U’ gram of methyl ester and ‘V’ gram contaminants. The value of ‘U’ and ‘V’ was not known and was to be determined by carrying out the test.

Area A proportional to X gram

Total area corresponds to $x + u$ gram not for $\frac{(x+y)}{(x+u+v)}$

Area B proportional to $x + u$ gram

Area $(B - A)$ proportional to ‘U’ gram

Ester content = $U/_Y$ Where: Y = mass of biodiesel feed.

$$U = \frac{\{(B - A) * X\}}{A}$$

$$Ester\ content = \frac{\{(B - A) \times X\}}{(A \times Y)}$$

$$Ester\ content\ (\%) = \left\{\left(\frac{Calculated\ Area}{Area\ C17{:}O}\right) - 1\right\} \times \left(\frac{Mass\ of\ C17{:}O}{Mass\ of\ biodiesel}\right) \ldots\ldots\ldots.Eq.9.$$

3.10 Determination of FAME content

The determination of the percentage contribution of individual FAME $(fromC11\ toC20)$ present in *Spirulina* microalgae was as follows:

Example:-

Palmitic acid methyl ester content (L), expressed as a mass fraction in percent, was calculated using the following formula(calculation of values in Table 4.4), (Duvekot, 2011).

$$P = \left\{\frac{AP}{(\sum A\,) - AEI}\right\} \times 100\% \ \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \ Eq.\,10$$

$\sum A$ = *totalpeak area from the FAME* $C11{:}0$ *to*$C20{:}0$.
AEI = *peak area of methylheptadecanoate (pure standard).*
AP =*peak area of each of the fatty acids, i.e. Palmitic acid*.

The areas as obtained from the GC used to calculate cumulative mass of methyl esters and FAME content are as displayed in appendices (Report 1-12).

3.11 Design of experiment

Response Surface Methodology (RSM), (Raymond H. Myers., 2009), Central Composite Design (CCD) circumscribed was used to optimize operational factors and maximize production of the three variables under study. The five-level Response Surface Methodology with three independent variables, that is, the concentration of H_2SO_4acid catalyst, volume of solvent(CH_3OH), andreaction time in minutes that were applied in this study (table below), requiring 20 sets of experimental runs consisting of 9 factorial (cubic points), 5 axial (star points), and 6 replicates of centre points. These were used to analyze optimally the influence of various methodology process variables (catalyst concentration, methanol to oil ration, and reaction time on FAME yield) on the fatty acid methyl acid ester conversion and then finally determining its maximum productivity. Subsequent values that were acquired from the runs using the predicted optimal conditions were then used as the validating sets and were compared with the computed optimal values.

The Table 3.3,below indicates the actual levels for independent variables for selected factors designed through CCD approach.A total of 20 experiments with varying feed parameters was performed.

Table 3.3: Levels for Independent Variables for Selected Factors Designed Through CCD.

			Coded Levels				
Factors	**Units**	**Symbols**	**-α(-1.6818)**	**-1**	**0**	**1**	**α(1.6818)**
Catalyst conc.	$wt.\%$	X_1	0.32	1	2	3	3.6818
Methanol(CH_3OH)	$wt./vol$	X_2	4.000	6	9	12	14.00
Reaction Time	Min	X_3	1	3	6	9	11.0

3.11.1 Response Surface Methodology-modeling

To optimize the FAME yield and alsoto investigate the interaction/relationship of the 3 variables under study RSM was employed. Design expert (version 12) was used for regression modeling and data interpretation.

3.11.2 Statistical analysis using RSM

Analysis of variance (ANOVA) and 't' test was performed on the experimental data obtained in the present study using design expert software-12 (student version).

Table 3.4: Actual Values and Coded values obtained from design expert.

STD	Run	Actual Values			Coded Variables		
		Catalyst Conc. (wt%)	**Volume of CH_3OH (wt/vol)**	**Reaction Time (min)**	X_1	X_2	X_3
3	1	1	12	3	-1.000	1.000	-1.000
2	2	3	6	3	1.000	-1.000	-1.000
11	3	2	4	6	0.000	-1.6818	0.000
13	4	2	9	1	0.000	0.000	-1.6818
6	5	3	6	9	1.000	-1.000	1.000
16	6	2	9	6	0.000	0.000	0.000
7	7	1	12	9	-1.000	1.000	1.000
17	8	2	9	6	0.000	0.000	0.000
9	9	0.32	9	6	-1.6818	0.000	0.000
14	10	2	9	11	0.000	1.000	1.6818
20	11	2	9	6	0.000	0.000	0.000
8	12	3	12	9	1.000	1.000	1.000
12	13	2	14	6	0.000	1.6818	0.000
19	14	2	9	6	0.000	0.000	0.000
5	15	1	6	9	-1.000	-1.000	1.000
4	16	3	12	3	1.000	1.000	-1.000
18	17	2	9	6	0.000	0.000	0.000
1	18	1	6	3	-1.000	-1.000	-1.000
10	19	3.6818	9	6	1.6818	0.000	0.000
15	20	2	9	6	0.000	0.000	0.000

Table 3.4 above shows the experimental points with a three-level CCD design with center points replicated six times in experiments 6, 8, 11, 14, 17, and 20. The Response Surface Methodology (CCD, expert 12) method was used to determine the numberof experiments to be evaluated for optimization of the variables and responses. The minimum, intermediate, and maximum values of each variable are labeled as -1.6818, -1, 0, 1, +1.6818 respectively showed by coded values and illustrated by table 3.4.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Introduction

This chapter presents the results and discussions of the impact test experiments. Tables and Response Surface Plots are employed to summarize the information obtained. The first part of this chapter presents the results and the second part presents the analysis and discussions. The analysis of experimental results entailed determining the particle size distribution for the dried algae biomass. The moisture content of microalgae, total lipid content of this algae biomass, ash and protein content were determined as per index 3.4.1, 3.1.2, 3.1.4, and 3.1.3 respectively.The physicochemical characteristics of both the *Spirulina* microalgae biomass oil and subsequent biodiesel were determined in conformity with ASTM D6751 methods, comparison were made to ascertain if within its limits and for their quality. The effect of catalyst, volume of methanol to oil ratio, with the time of exposure of microwaves energy on biodiesel production were also analyzed.

4.1.1Characteristics of algal biomass

4.1.1.1 Total lipid content

Dried,bluish green*Spirulin*a microalgae biomass ($160\mu m$) in powder form had the following key characteristic shown in Table 4.1. The total lipid content of *Spirulinaplatensis* biomass was found to be 10.7%. The species investigated in this study, represents a good raw material for the production of biofuels, due to their high lipid content, which may enhance the environmental cultivation possibilities without any competition with food crops. On the other hand, the lipid level is higher than what was projected by the growers (MMUST) of these microalgae of 6-8%.

Table 4.1: Characterization of *Spirulinaplatensis.*

Compound	**%wt.**
Moisture content	7.8
Total lipid content	10.7
Protein content	51.83
Ash content	14.30
Total dietary fiber (Carbohydrates)	15.37

The total lipid content of microalgae biomassis greatly dependent on the specific growth conditions and the type of microalgae species in use, according toElshimi, Attia& Allah, (2015).While many microalgae strains naturally have high lipid content, it is possible to increase that concentration by optimizing growth determining factors such as the control of nitrogen level, light intensity, temperature, salinity, CO_2 concentration and harvesting procedures(Mendez-Villas & Vicente, 2010). The algae biomass had other parameters i.e. protein content, moisture, ash content that were determined in this research adding up to a toatal of 84.63%. The remainder 15.37 was taken to be total dietary fiber (carbohydrates) as shows similar profile to those studied by other researchers(Saharan & Sudesh, 2017; Sharoba, 2014).

4.2RSM for optimization of the process variables

The central composite design matrix of response surface methodology displayed on the table 4.2 gives information on the constituents’ chosen:-coded and actual limits for variables used in the experimental design, and the actual responses indicating FAME yield. Central composite design (CCD) was proposed for identifying the optimal levels for selected variables; catalyst concentration (wt %), microalgae biomass to methanol ratio (wt/Vol), and reaction time in minutes.

Table 4.2: Experimental Matrix and Results from RSM Central Composite Design.

		Actual Values			**Coded Variables**			**Yield (%)**	
STD	**Run**	**Catalyst Conc.** ($\boldsymbol{wt}$%)	**Volume of CH0$_3$OH** ($\boldsymbol{wt/vol}$)	**Reaction Time** ($\boldsymbol{min}$)	$\boldsymbol{X_1}$	$\boldsymbol{X_2}$	$\boldsymbol{X_3}$	**Fame Yield (Experimental)**	**Activity Yield (Predicted)**
3	1.	1	12	3	-1.000	1.000	-1.000	37.56	38.5363
2	2.	3	6	3	1.000	-1.000	-1.000	42	42.8006
11	3.	2	4	6	0.000	-1.6818	0.000	38.32	37.6164
13	4.	2	9	1	0.000	0.000	-1.6818	46.56	46.9381
6	5.	3	6	9	1.000	-1.000	1.000	63.76	63.9538
16	6.	2	9	6	0.000	0.000	0.000	79.4	80.294
7	7.	1	12	9	-1.000	1.000	1.000	44.4	44.7695
17	8.	2	9	6	0.000	0.000	0.000	80.66	80.294
9	9.	0.32	9	6	-1.6818	0.000	0.000	49	48.0875
14	10.	2	9	11	0.000	1.000	1.6818	72	69.9672
20	11.	2	9	6	0.000	0.000	0.000	83.43	80.294
8	12.	3	12	9	1.000	1.000	1.000	69.96	71.8784
12	13.	2	14	6	0.000	1.6818	0.000	42.62	41.6688
19	14.	2	9	6	0.000	0.000	0.000	78.12	80.294
5	15.	1	6	9	-1.000	-1.000	1.000	48.958	50.8439
4	16.	3	12	3	1.000	1.000	-1.000	54.41	53.6942
18	17.	2	9	6	0.000	0.000	0.000	78.75	80.294
1	18.	1	6	3	-1.000	-1.000	-1.000	42.39	41.6417
10	19.	3.682	9	6	1.6818	0.000	0.000	72.6	71.8578
15	20	2	9	6	0.000	0.000	0.000	81.12	80.294

4.2.1ANOVA for quadratic model

Table 4.3 gives the ANOVA for regression analysis for microwave irradiation for a full quadratic model.

Table 4.3: ANOVA for Response Surface quadratic model.

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	5464.62	9	607.18	170.10	< 0.0001	Significant
$\boldsymbol{X_1}$-Catalyst concentration	682.05	1	682.05	191.07	< 0.0001	
$\boldsymbol{X_2}$-Volume of Methanol	19.82	1	19.82	5.55	0.0402	
$\boldsymbol{X_3}$-Time	640.17	1	640.17	179.34	< 0.0001	
$\boldsymbol{X_1X_2}$	97.99	1	97.99	27.45	0.0004	
$\boldsymbol{X_1X_3}$	71.41	1	71.41	20.01	0.0012	
$\boldsymbol{X_1}^2$	743.91	1	743.91	208.40	< 0.0001	
$\boldsymbol{X_2}^2$	2976.89	1	2976.89	833.96	< 0.0001	
$\boldsymbol{X_3}^2$	859.35	1	859.35	240.74	< 0.0001	
Residual	35.70	10	3.57			
Lack of Fit	17.15	5	3.43	0.9247	0.5332	not significant
Pure Error	18.55	5	3.71			
Cor. Total	5500.32	19				

The Model F-value of 170.10 implies the model was significant. There was only a 0.01% chance that an F-value this large could occur due to noise**.** P-values of “Prob. > F” less than 0.0500 indicatesthe model terms are significant. In this case $X_1, X_2, X_3, X_1X_2, X_1X_3, {X_1}^2, {X_2}^2, {X_3}^2$ were significant model terms. Values greater than 0.1000 indicate the model terms are not significant.

The “Lack of Fit F-value” of 0.92 implies the Lack of Fit was not significant relative to the pure error. There was a 53.32% chance that a “Lack of Fit F-value” this large could occur due to noise. P-value for lack-of-fit was > 0.05 was non-significant which implies that there was no evidence that the model did not fit. The lack of fit was good.

Table 4.2.2: **Fit statistics/Model comparison Statistics.**

Fit Statistics		**Model comparisons statistics**	
Std. Deviation	1.89	R^2	0.9935
Mean	60.30	Adjusted R^2	0.9877
C.V. %	3.13	Predicted R^2	0.9700
		Adeq Precision	31.9451

The “Predicted R^2” of 0.9700 was in reasonable agreement with the “Adjusted R^2”of 0.9877; i.e. the difference is less than 0.2 as seen in Table 4.2.2.“Adequate Precision” measures the signal to noise ratio. A ratio greater than 4 is desirable. A ratio of 31.945 indicated an adequate signal. This model can be used to navigate the design space. The model that predicts the percentage of FAME yield in terms of coded factors is given by the following equation. Yield,

$$Y_{FAME}\% = 80.29 + 7.07X_1 + 1.20X_2 + 6.85X_3 + 3.50X_1X_2 + 2.99X_1X_3 - 7.18468{X_1}^2 - 14.3724{X_2}^2 - 7.72209{X_3}^2 . \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots \dots\dots\dots Eq. 11.$$

Where X_1 refers to the actual catalyst concentration as a percentage of microalgae feed, X_2 refers to the volume of methanol to algae biomass feed, and X_3 refers to the reaction time inside the microwave synthesis unit. The second-order polynomial Eq. 11 above was applied to determine the relationship between variables and responses and regression coefficients were calculated. A flat surface on the three-dimensional (3D) response graph indicates an optimum condition for a given response, as shown in Fig. 4.1, 4.2, and 4.3.

From the RSM model's sum of squares obtained from the Design Expert Software demonstrated that the second-order polynomial regression was suitable to explain the relationship between input variables and output (responses).

Equation 11 showed that the significance of factors affecting FAME yield obtained from RSM were in the following order:$X_1,\ X_3,\ X_1X_2,\ X_1X_3,\ X_2, X_1{}^2,\ X_3{}^2\ and\ X_2{}^2$.

Equation 11was then used to plot response surface and contours of FAME yield asan acid catalyst (H_2SO_4) was taken into consideration. The relationship between yield and the three factors are shown in figures 4.1, 4.2 and 4.3.

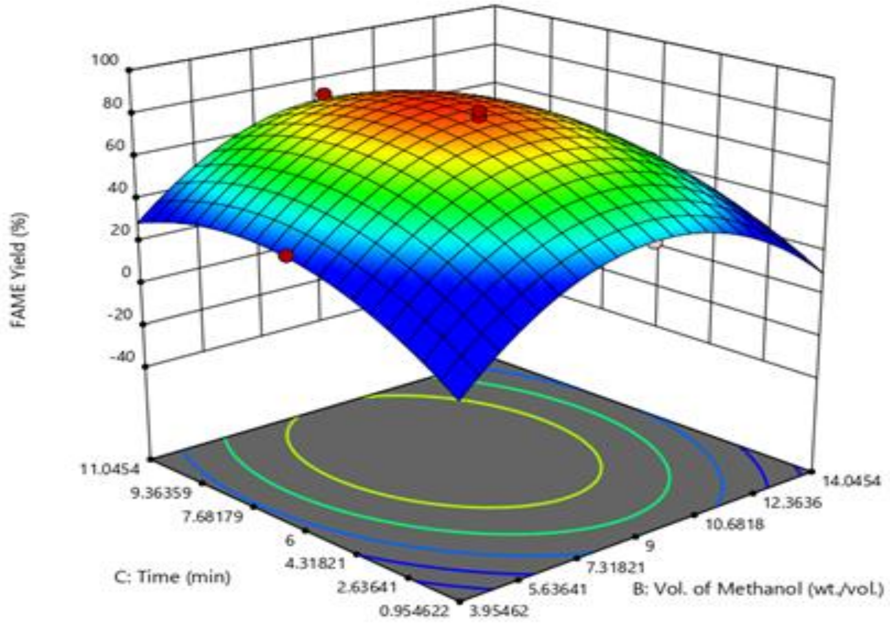


Figure 4.1: RSM plot: Effect of Volume of Methanol *(CH_3OH)* and Time on FAME Yield.

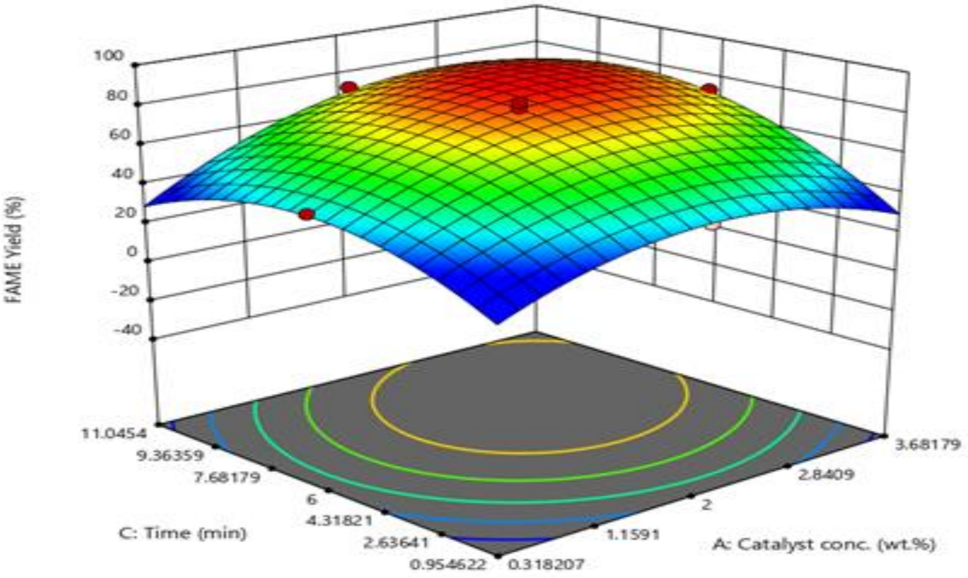


Figure 4.2: RSM plot: Effect of catalyst concentration *(H_2SO_4)* and time on FAME Yield.

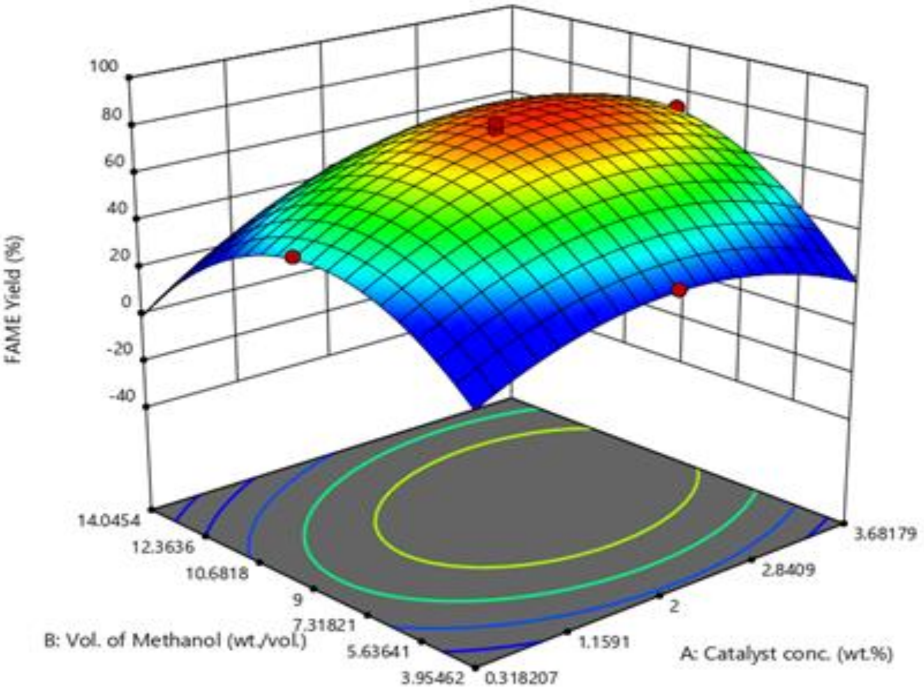


Figure 4.3: RSM plot: Effect of Catalyst Concentration *(H_2SO_4)* and Volume of Methanol *(CH_3OH)* on FAME Yield.

4.3 Fatty acid composition of *SpirulinaPlatensis* lipids and lipid

Table 8 gives the fatty acid composition of *Spirulina* microalgae oil in percentage terms. These results were obtained after detection by Gas Chromatography analysis of the fatty acid feed. The main fatty acid components arelinolenic $(C18{:}3)$, Palmitic $(C14{:}0)$, and linoleic acid $(C18{:}2)$, respectively.

Table 4.4: Fatty Acid Content in Spirulina Microalgae Biodiesel.

No.	Fatty Acid	Structure	Reference Values (%)
1	Undecanoic acid	$C11{:}0$	0.88
2	Lauric acid	$C12{:}0$	0.64
3	Myristic acid	$C14{:}0$	1.15
4	Palmitic acid	$C16{:}0$	30.83
5	Methyl Heptadecanoate	$C17{:}0$	0.43
6	Stearic acid	$C18{:}0$	0.89
7	Oleic acid (omega 9)	$C18{:}1$	0.96
8	linoleic acid (omega 6)	$C18{:}2$	43.83
9	Linolenic acid (omega 3)	$C18{:}3$	19.41
10	Arachidic acid	$C20{:}0$	0.98

The fatty acid profile varied differently as linoleic, palmitic, linolenic, myristic, oleic, and docosahexaenoic acids (in order of abundance) were most prevalent. The percentage of saturated fatty acids present in biodiesel is 35.80 whereas the unsaturated is 64.2. The high fatty acid percentage contribution of the saturated Palmitic $(C16{:}0)$, andboth the unsaturated linoleic $(C18{:}2)$, and linolenic $(C18{:}3)$ fatty acids indicate good properties of *Spirulina* microalgae in the production of biodiesel. Fatty acids detected in trace amounts and deemed insignificant were not included in the analysis.Table 8above was then used to determine the average molecular masscontribution of each of the major fatty acids under investigation of *Spirulina-Platensis*lipids.

Table 4.5: Determination of average Molecular Mass of constituent Fatty acid (MMC_{FA}).

NO	Fatty Acid	Structure	Molecular Mass (g/mol)	Reference Values (%)	Molecular Mass contribution (g/mol)
1	Undecanoic acid	$C11:0$	186	0.88	1.6368
2	Lauric acid	$C12:0$	200	0.64	1.28
3	Myristic acid	$C14:0$	228	1.15	2.622
4	Palmitic acid	$C16:0$	256	30.83	78.9248
5	Methyl Heptadecanoate	$C17:0$	270	0.43	1.161
6	Stearic acid	$C18:0$	284	0.89	2.5276
7	Oleic acid (omega 9)	$C18:1$	282	0.96	2.7072
8	linoleic acid (omega 6)	$C18:2$	280	43.83	122.724
9	Linolenic acid (omega 3)	$C18:3$	278	19.41	53.9598
10	Arachidic acid	$C20:0$	312	0.98	3.0576
	Average Molecular Mass of Constituent Fatty acid ($MM\boldsymbol{C_{FA}}$)				**270.6008**

The average molecular mass of algae oil can be calculated using equation 12 explained in detail by(El-Shimi et al, 2013).

$$MM_{Oil} = \left(3MM_{FA} + MM_{glycerol}\right) - 3MM_{OH,H} \ldots\ldots\ldots\ldots\ldots.\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots. Eq. 12.$$

Where:-MM_{Oil}, $3MM_{FA}$, $MM_{glycerol}$, $3MM_{OH,H}$are molecular masses of oil, fatty acid, glycerol, OH group and hydrogen atom respectively.

The average molecular weight of *Spirulina-platensis*oil was calculated to be 849.80g/Mol.One mole of triglyceride reacts with three moles ofCH_3OH to produce three moles of fatty acid methyl esters and one mole of glycerol. Hence, stoichiometrically a molar ratio 1:3 oil to CH_3OHwould be required for a transesterification process to occur. The FAME molecular mass was therefore calculated based on the stoichiometric chemical reaction equation depicted in the literature review. The FAME chemical formula of constituent fatty acids has increased over that of the average molecular mass of the constituent fatty acids,(El-Shimi et al, 2013) so that the molecular weight of biodiesel is calculated as follows:-

$$MM_{FAME} = MM_{FA} + 15 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \, Eq.13.$$

Where: 15 indicates the molecular mass of the substituted CH_3.

Therefore, the average molecular weight of the biodiesel is 270.6008 + 15 = 285.6 g/mol. The minimum amount of methanol required for transesterification of oil in *Spirulinaplatensis*microalgae biomass is calculated as follows:

$$\text{Methanol required} = \left\{\left(\frac{(\%\text{ lipid content } \times \text{algae biomas (g)}}{\text{molecular weight of biodiesel } (\frac{\text{g}}{\text{mol}})}\right) \times \left(\text{MM}_{CH_3OH} \times \text{molar ratio of } CH_3OH\right)\right\} \ldots\ldots\ldots\ldots\ldots\ldots.\ldots\ldots\ldots\ldots\ldots \; Eq.14.$$

$$CH_3OH\ required = \left\{\frac{\left(\frac{10.7}{100}\right) \times 15\ g}{285.6\ g/mol} \times (32\ \times 3)\right\}$$

= 0.5395

4.4Physicochemical properties of *Spirulinaplatensis* oil

In the evaluation of the quality of the extracted*Spirulina*microalgae oil, tests were carried out as per the procedures stated in “Materials and Methods” in to determine the physicochemical properties and the results are as shown in Table 4.6.These properties are directly related to the yield and quality of the biodiesel produced. Kinematic viscosity and specific gravity were found to be$5.8\ \text{mm}^2/\text{s}$ at$313.15k$and0.877 respectively, which were within the ASTM limits. These values show a good agreement with the information presented in Table 4.6, and along with other properties confirmsthe good characteristics of *Spirulina* oil to produce biodiesel.

Table 4.6: Physicochemical Properties of Spirulina Microalgae Oil.

No.	Property	Test Method	Limits ASTM D6751	Values of algal oil
1	pH	-	7-9	7
2	Specific gravity	ASTM D 941	0.86-0.89	0.877
3	Kinematic viscosity, 40^oC mm^2/s	ASTMD445	1.9-6.0	5.76
4	Acid value KOH/g	ASTMD664	-	0.4
5	Free fatty acid value		-	0.802
6	Moisture content%	ASTM D 2709	0.050 max	0.04
7	Carbon residue %.	ASTMD524	0.050 max	< 0.1
8	Refractive index			1.365
9	Sulphur, wt %	ASTM D5453	0.0-0.0024	Nil

4.5 Parametersthat Influence Biodiesel Production

While there are many factors affecting transesterification reactions the most important variables that influence biodiesel production and its quality are:- reaction time, temperature, type of catalyst in use and its concentration and the ratio of microalgae oil/biomass to alcohol. Although transesterification reactions are well established techniques, it is important that the parameters are always optimized to avoid either incomplete reactions or lower yield.

4.5.1Optimization and the effects of process variables

4.5.1.1Effect of the catalyst loading

The effect of the catalyst to dry algal biomass ($wt\%$) on biodiesel yield is shown in Fig.4. Catalyst concentration varied from0.32 to 3.68 $wt\%$ for the acid catalyst(H_2SO_4) with respect to total algae biomass feed, algae biomass to methanol ratio of $1:4$ to $1:14$ (g/ml) and duration of (1 to 11min) with stirring at 500RPMand microwave energyadopted for all runs. The speed at which a reaction proceeds is greatly dependent on the amount of catalyst fed into a given reaction. Lower catalyst feed indicates slower reaction rate, whereas higher amount until equilibrium gives faster reaction rate and beyond this point the reaction isimpeded and the reaction slows as shown in Fig. 4.1 and Table 4.2.At $2-2.5\%$ catalyst concentration indicating 0.3g: 1g catalyst to dry *Spirulina* algae biomass giveshigher conversion efficiency. For the range below 2% $(0.32\%\ and\ 1\%)$ catalyst loading, the catalyst concentration fed was not enough to ensure that the reaction goes to completion. The FAMEs content was significantly lower than at 2% per weight ratio of catalyst used. According to Nomannbhay & Mei, 2017 and Prafulla et al.,2010, lower concentration of the catalyst may not efficiently advance the reaction as the catalyst effect is hindered by the presence of a variety of organic compounds (lipids, olefins, fatty alcohols, phytols and sterols) which may arise from algae biomass. When the catalyst ratio loading to dry microalgae biomass were increased farther beyond 2.5%, the results obtained indicated a decrease in FAMEs content. This was brought about by the interaction of other molecules/compounds resulting in increased amounts of by-products. The addition of the catalyst in excess gives rise to the formation of an emulsion, which leads to an increase in viscosity and in the process to the formation of gels(Nguyen, Nguyen, & Bui, 2013). Other disadvantages of high catalyst concentrations, in general, are their corrosive nature which hinders the transesterification reaction(Saifuddin & Mei, 2017). Corrosive catalyst such as H_2SO_4 leads to corrosion on the reactor and pipeline.

4.5.1.2 Effectof methanol

The ratio of microalgae to methanol in use in the in-situ transesterification of microalgae to produce FAME remains significant as the ratio increases from 1:4 to 1:12 (g/ml). The methanol used serves two purposes: it acts as an extraction solvent of the microalgaelipids and also as reactant species for transesterification to occur, and in the process, biodiesel and glycerol is produced as stated byPrafulla et al., 2010. However, according toLi P. , Xiaoling, & Jianjiang, 2011, an adequate amount of methanol is required for higher yield of in-situ biodiesel production processes because the methanol in use plays the role of both reactant and substance to submerge algae biomass. The ratio of algal biomass/oil to methanol is one of the important variables that affect the conversion efficiency as well as the production cost of biodiesel. According to the results which were presented in Fig. 4.2 and Table 4.2, 1:9 dry algae biomass to methanol (g/ml) was the most efficient feed ratio of the two variables under study. The theoretical minimum methanol required for transesterificationis a ratio of 1: 0.5395 algae to methanol as calculated from Equation 14. With the algae biomass to methanol (g/ml) increasing from 1:4 to 1:9, the FAMEs yield increased considerably. The FAMEs content achieved is clearly the highest at 1:9 dry algae biomass to methanol (g/ml) ratio. The higher the dry algae to methanol ratios above 1:12 (g/ml) does not favor the extraction and transesterification as much of the microwave energy will be absorbed by the solvent, and in the process having no effect on the algae biomass which may result in inefficient extraction of algae oils(Prafulla et al, 2010). Increased amounts of the solvent may also result in greater losses of the solvent or aggravated rates of solvent recovery. Moreover, excessive methanol amounts may reduce the concentration of the catalyst in the reactant mixture and in the process retard the transesterification reaction(Prafulla et al, 2010).

4.5.1.3Effect of reaction time on yield

Fig.4.3shows the effect homogeneously catalyzed transesterification reaction time has on the FAME yield of biodiesel produced from *Spirulina* microalgae. Reaction time has a significant influence on the transesterification reaction with high yield values (the optimum biodiesel conversion) occurring after 6 minutes. The fatty acid methyl yield (FAME) increased as the reaction time was increased. More specifically, the FAMEscontent in biodiesel increased drastically from 4min to 6min reaction time, after that, the FAME yield rose at a slower rate and then practically at its ultimate (83.43%) at around 6min. The reaction was at its equilibrium and that’s the reason the rate rose slowly. The results indicate that the biodiesel quality (heating value based on FAMEs content) at 6-7minutes reaction time is significantly higher than that in others. The extended reaction times until when the reaction is in equilibrium provided for enhanced exposure of microwave energy to the reaction mixture which results in better yields of extraction and biodiesel conversion(Prafulla et al, 2010). Lower reaction times do not provide sufficient interaction of the reactant mixture.Higher reaction time does not increase the conversion, but favours the backward reaction (hydrolysis of esters and thus the formation of glycerol), which results in a reduction of product yield (Magida, 2013). Also, having higher reaction times above 8 minutes, may not favour sufficient conversion, as it may lead to loss of the solvent, overheating of reaction mixture, by-product formation and energy losses. Therefore, a shorter reaction time is preferred as there would be saving on energy to be used in the production of biodiesel.

The optimal process conditions of the three variables chosen for thein-situ process of biodiesel production as obtained from analysis above are: dry *Spirulina* algae biomass to methanol ratio of $1:9$ (g/ml), catalyst concentration of 2 $(wt\,\%)$ and the reaction time of 6minutes.

4.6Properties of*Spirulina* microalgae biodiesel

The biodiesel sample produced in around 6 minutes by 2% catalyst which gave a maximum yield of 83.43% (According to $Eq.$ 11), was assessed for density, specific gravity, Kinematic viscosity, acid value, water content, carbon content, ash content, sulphur, flash point and calorific value. Table 11 shows the characteristics of the *Spirulina* microalgae biodiesel alongside European standards. The ester content of 83.43% is satisfactory.Transesterification successfully reduced the viscosity of microalgae oil from 5.76 to 4.45 mm^2/s. The fuel had good viscous properties, and a desirable flash point.The values represented in Table 4.7 are within the ASTM D 6751 agreement with the information presented in Table 4.7, and along with these properties the calorific value was similar to that presented by other researchers that include a value of $32{,}911.34 kj/kg$ confirming the good characteristic of *Spirulina*Biodiesel.

Table 4.7: Physicochemical Characteristics of Spirulina Microalgae Biodiesel.

No	Property	Test Method	Limits ASTM D6751	Values of algae Biodiesel
1	pH	-	7-9	7
2	Specific gravity	ASTM D-941	0.86-0.89	0.868
3	Flash point ^oC	ASTM D93	100-170	164
4	Kinematic Viscosity, $40^O C\ mm^2/s$	ASTM D445	1.9-6.0	4.45
5	Acid value KOH/g	ASTM D664	-	0.475
6	Free fatty acid value	-	-	0.95
7	Calorific value kJ/Kg	ASTM D240	-	32911.34
8	Moisture content %	ASTM D 2709	0.050 max	0.001
9	Carbon residue %.	ASTM D524	0.050 max	0.045
10	Ash content mass %.	ASTM D482	0.0001	Nil
11	Sulpur, wt %	ASTM D5453	0.0-0.0024	Nil

CHAPTER FIVE

5.1. Review of the research objectives

All research objectives have been reviewed and adequately covered.

5.2. Key findings as per the objectives

- It is reported in this research that the lipid content is 10.7%,giving an indication that the value is greater than the value given by growers (MMUST) of between 6-8%.
- The oil characteristics analyzed were within the ASTM D 6751 limits as seen in table 4.6.
- The optimum parameter (optimal process conditions) verified were: dry *Spirulina* algae biomass to methanol ratio of $1:\ 9\ (g/ml)$,$2\ (wt\ \%)$ catalyst concentration, and reaction time of $6\ \mathrm{minutes}$ giving a maximum FAME yield of $83.43\ wt\%.$ FAME was analyzed for fatty acid composition and characteristic fuel properties. From GC analysis, palmitic $(30.83\%,$ linoleic (43.83%), and linolenic (19.41%), acids were found to be the major fatty acids inferring that *Spirulina-Platensis*is a promising feedstock for biodiesel production.
- Similarly, the biodiesel characteristics analyzed were also found to be within the ASTM D 6751 limits. After the oil had been converted to biodiesel it was noticed that specific gravity and viscosity decreased whereas transesterificaton led to an increase in acid value.

5.3. Conclusion from the research

Theprimary objectiveofthisinvestigationwastostudy and optimize the production of fatty acid methyl esters from *Spirulina* microalgae by in situ catalytic transesterification using microwave irradiation. The main conclusions to be drawn from the experimental results reported in this research work can be summarized as follows:

1. The*Spirulina* microalgae biomass contains a substantial amount of lipids (10.7%) in their composition and hence can be used as potential for biodiesel production which is an alternative fuel. Depletionof non-renewable source of energy,suchasfossils,demandsthe exploration oflarge-scale non-petroleum-based alternative fuels, such as biodiesel. Biodiesel made from third-generation algae biomass with their high growth and having high lipid content is highly desirable. The development of non-petroleum based fuels using non-food based biomass subjected to environmentally friendly fuel production techniques will go a long way in supplementing the dwindling petroleum oil reserves while easing food versus fuel competition.
2. This work has characterized the microalgae oil, that is determination of pH, specific gravity, Kinematic viscosity @ 40^{o}C, Acid value, Free fatty acid value, Moisture content, Carbon residue, Refractive index and Sulphur content as summarized in Table 4.6. All the parameters studied in the table 4.6 were within the American Standards and Test Methods (ASTM) limits. This gives an implication that the oil could be used for biodiesel production.
3. *AthrospiraSpirulinaplatensis* microalgae biomass that its oil was transesterified showed that 83.43% of it was converted to FAME and only the remaining

16.57%being the unreacted triglycerides, at optimal conditions. These conversion characteristics give an indication that *Spirulina*microalgae have great potential for use or for the production of commercial biodiesel that would be able to compete with petro-diesel.

- Microwave heating has proved to be very efficient in the conversion of fatty acids to esters in the biodiesel synthesis. Just at only 6 minutes, an optimal FAME yield had been obtained meaning shorter processing time than other conventional methods. Microwave technology solves the problems encountered in algae biodiesel production, such as the longer extraction time of algae lipids from biomass, tedious separation and purification process for final product recovery and finally low conversion yield due to process complications. In situ microwave transesterification process for biodiesel production from algae biomass was demonstrated by this research to solve the problems associated with conventional methods of biodiesel production.
- According to our experimental results, the highest FAME yield of 83.43% was obtained with a 9:1 methanol/algae biomass ratio, with 2wt% sulphuric acid catalyst and a reaction time of 6 minutes. Both the experimental and the RSM results show that reaction time and algae biomass to catalyst (wt%) are the most effective parameters on FAME yield. The coefficients of the catalyst concentration and reaction time, respectively, as can be seen in the regression equation, are the highest for catalyst and time of reaction among all variables under study and their effects on FAME are strongest.

- GC analysis showed that there are three main fatty acids. The highest amount of fatty acids determined in *Spirulina*microalgae biodiesel was found to be the unsaturated linoleic acid (C18:2) in most samples, followed by the saturated palmitic acid (C16:0) and the unsaturated linolenic acid (C18:3). The three fatty acids constitute about 94% of FAMEs at optimal conditions.

4. The viscocity and specific gravity of the raw materials used for production of biodiesel are lowered in the process. There was an increase in acid value after the oil had been transesterified as a result of acid catalyst used in the process. This requires neutralization using base catalyst.The characteristics (Table 4.7) analyzed for the species under study give a promising indication that biodiesel obtained is of good quality to be used as a fuel. These quality characteristics of biodiesel obtained in this work were in good agreement with ASTM D 6751 specifications. Therefore, it can be acceptable and suitable for diesel fuel.

5.4. Research contribution to the theory and practice

Recently, it is challenging to find the different alternative for the fossil fuels. Due to presence of various advantages of algal biofuels low land requirement and high productivity it has been considered as best resource to replace the fossil fuels. The alternative fuel source could help reduce overall carbon emission without taking land from foodi.e. exhaust from industries. With locally produced biofuels, many countries have reduced their dependence on fossil fuels. It may not solve all problems in one blow but a nation can save billions by reducing their usage on foreign oil. The biodiesel is capable of bringing revolution in future by replacing the various non-renewable resources.

In addition to biodiesel production, algae can be also used as feedstock to produce different valuable products such as bio-plastics, cosmetics, fertilizers, other energy forms (i.e. biogas, bioethanol, biobutanole.t.c.), nutraceutcals, proteins, animal feeds (Mathimani & Pugazhendhi, 2018). The other significant property is that microalgae can remove some heavy metals, phosphorus, and nitrogen from water during its growth (Abdel-Raouf, al-Homaidan, & Ibraheem, 2012).

5.5. Recommendations

- Spirulina microalgae can be embraced for biodiesel production since it has high oil content and also due to the fact that they are non-food based source biomass and therefore can be used in biodiesel production as an alternative fuel.
- The correlation between different microwave parameters (power intensity, time of exposure and frequency) should be obtained.
- The effect of microwaves, if any, on the chemical composition of the fatty acids, alcohol, and catalysts in use and consequently the effect on biodiesel quality should be investigated.
- Other material parameters, which may affect the heating behaviour and subsequent extraction and transesterification such as particle size should also be determined.
- The performance of the produced biodiesel should be tested in a test engine for quality and comparison made with petro-diesel.

5.6. Future research.

This project showed that microalgae can be utilized for the production of biofuels. However to improve the utilization of this biomass in a more efficient and profitable way further research has to be done. The major suggestions for additional research are reported below:

In this work biodiesel quality parameters (specific gravity, acid value,moistiure content, viscosity, calorific value, flash point, sulphur content, FAMEs content) were studied. But the experimental procedure adopted in present work can be extended. Thus further experiments of fuel analytics could be done to get other fuel properties as cloud point, cetane number, etc. and the further emission testing could be done on different engines using the biodiesel fuel to get results that show the reduction of emissions when using biodiesel fuel.

It is of great significance to note that the results analyzed so far are from laboratory-scale experiments and so the reproducibility of similar outcomes at a large-scale industry level is still doubtful because of the safety aspect of microwave energy. Nevertheless, it seems plausible that the biodiesel used in the future may involve microwaves at some point in their production processes. In essence, more in-depth research is necessary for the scaling-up purpose in terms of process design, and optimization, the reaction kinetics and thermodynamics, and the system protocols of biodiesel analysis. Thus, further research on the in situ microwave irradiation transesterification of microalgae in a flow reactor should be carried out to increase FAME production rate.

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Appendices

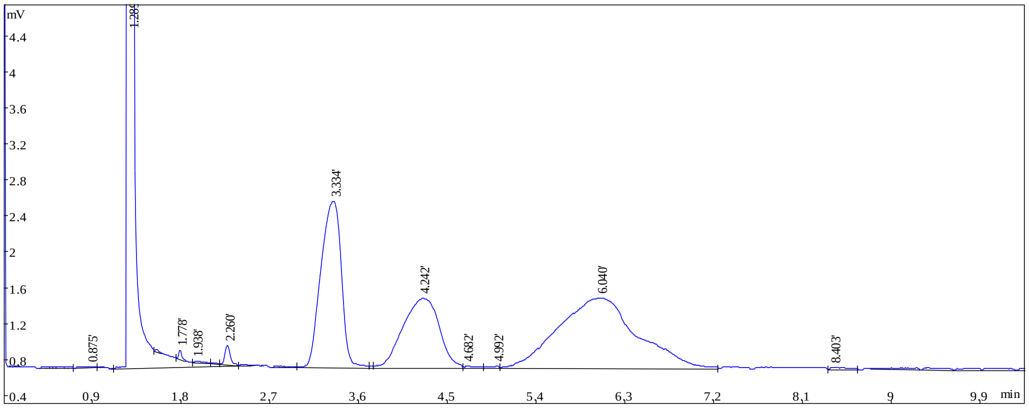
Fame yield determination by calculation

Analysis of biodiesel as obtained from the GC is explained by the examples given below:-

Microalgal biodiesel report 1
(3.682: 9: 6, H_2SO_4, STD 10)

```
Printing time: Fri Dec 13 09:12:49 2019
Injection time: Fri Dec 06 11:05:53 2019

File opened: C:\Users\PC\Desktop\Peak-ABC\program\003(2019:12:06
11:05:53).hw
```



```
-----------------------------------------------
Rank    Time      Name    Area%       Area
-----------------------------------------------
1       1.289             94.36       1833442
2       1.778             0.009534    185
3       1.938             0.006079    118
4       2.260             0.03942     766
5       3.334             1.344       26110
6       4.242             1.01        19624
7       4.682             0.01079     210
8       4.992             0.008378    163
9       6.040             2.23        43337
10      8.403             0.01781     346
-----------------------------------------------
Total                     100         1924301
```

$$EsterContent\,\% = \left\{\left(\frac{Areatotal}{AreaC17{:}0}\right) - 1\right\} \times \left(\frac{Massof\,C17{:}0}{Massofbiodiesel}\right) \times 100\%$$

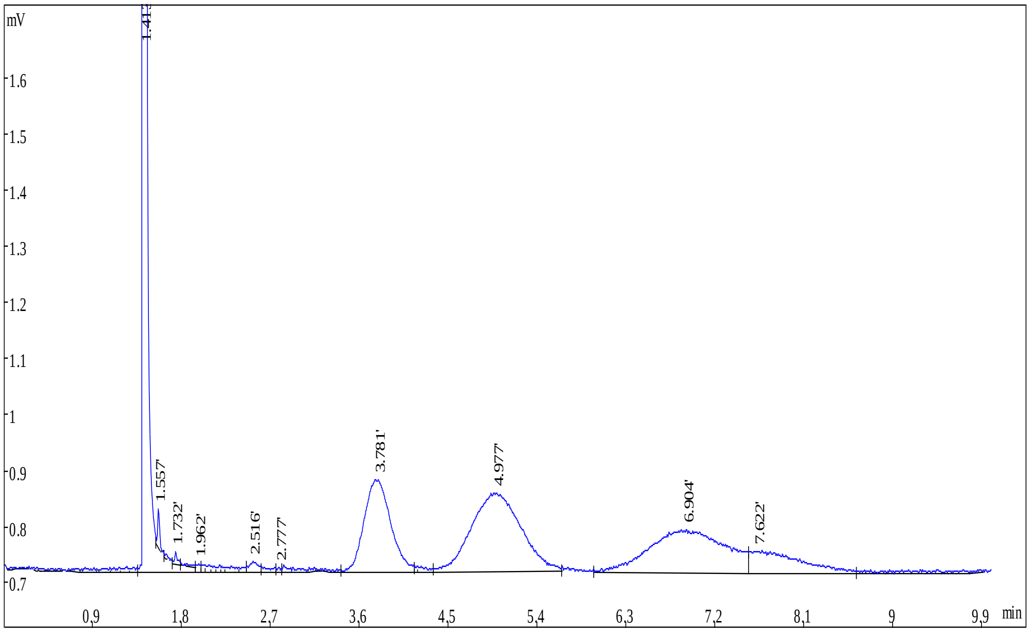
$$EsterContent\,\% = \left\{\left(\frac{90859}{19624}\right) - 1\right\} \times \left(\frac{2\text{mg/ml}}{10\text{mg/ml}}\right) \times 100\%$$

=72.60%

Microalgal biodiesel report 2
(1: 12: 3, H_2SO_4)

```
Printing time: Fri Dec 13 12:36:34 2019
Injection time: Mon Dec 09 09:39:05 2019

File opened: C:\Users\PC\Desktop\Peak-ABC\program\005393(2019:12:09
09:39:05).hw
```



```
-----------------------------------------------
Rank    Time      Name    Area%       Area       393
-----------------------------------------------
1       1.413             98.62       993462
2       1.557             0.181       146
3       1.732             0.01300     125
4       1.962             0.00731     77
5       2.516             0.01213     122
6       3.781             0.3056      3078
7       4.977             0.4903      4939
8       6.904             0.4222      4253
9       7.622             0.1463      1474
-----------------------------------------------
Total                     100         1007676
```

$$Ester\ Content\ \% \ =\left\{\left(\frac{Areatotal}{AreaC17:0}\right)-1\right\}\times\left(\frac{Mass of C17:0}{Mass of biodiesel}\right)\times\ 100\%$$

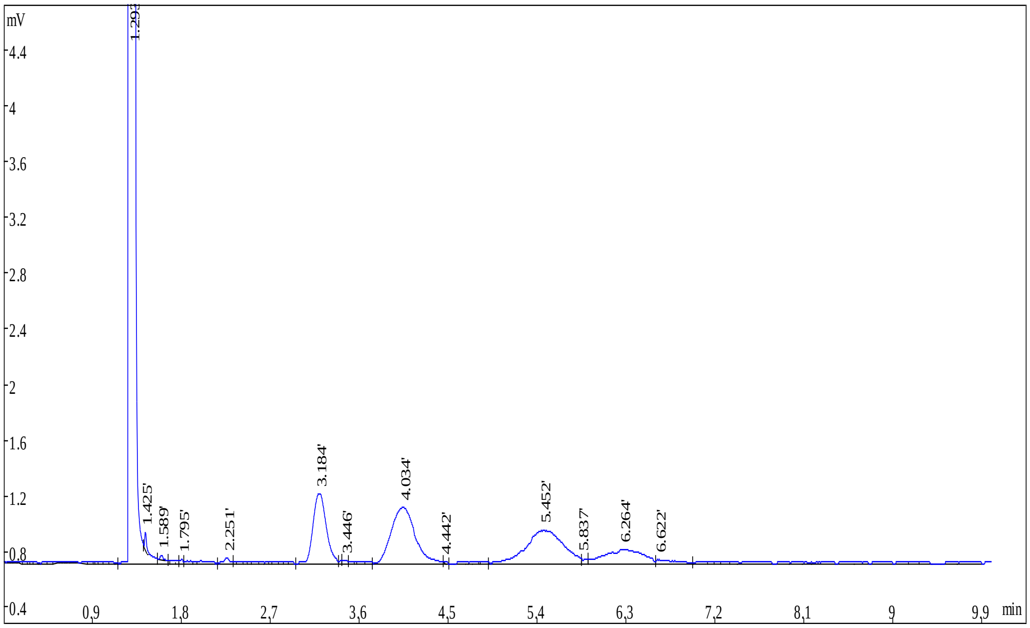
$$Ester\ Content\ \% \ =\left\{\left(\frac{14214}{4939}\right)-1\right\}\times\left(\frac{2\text{mg/ml}}{10\text{mg/ml}}\right)\times\ 100\%$$

$$=37.56\%$$

Microalgal biodiesel report 3 (1: 12: 9,H_2SO_4 Catalyst)

Printing time: Sat Dec 14 09:12:04 2019
Injection time: Sun Dec 08 16:46:19 2019

```
File opened: C:\Users\PC\Desktop\Peak-ABC\program\393 half dilute
004(2019:12:08 16:46:19).hw
```



```
----------------------------------------------
Rank    Time      Name    Area%        Area
----------------------------------------------
1       1.293             98.25        1470439
2       1.425             0.02132      387
3       1.589             0.0132       197
4       1.795             0.007533     113
5       2.251             0.01671      250
6       3.184             0.3156       4724
7       3.511             0.006781     102
8       4.034             0.4761       7126
9       5.452             0.4588       6867
10      6.264             0.2124       3179
----------------------------------------------
Total                     100          1496693
```

$$Ester\ Content\ \% = \left\{\left(\frac{Areatotal}{AreaC17:0}\right) - 1\right\} \times \left(\frac{Mass of C17:0}{Mass of biodiesel}\right) \times\ 100\%$$

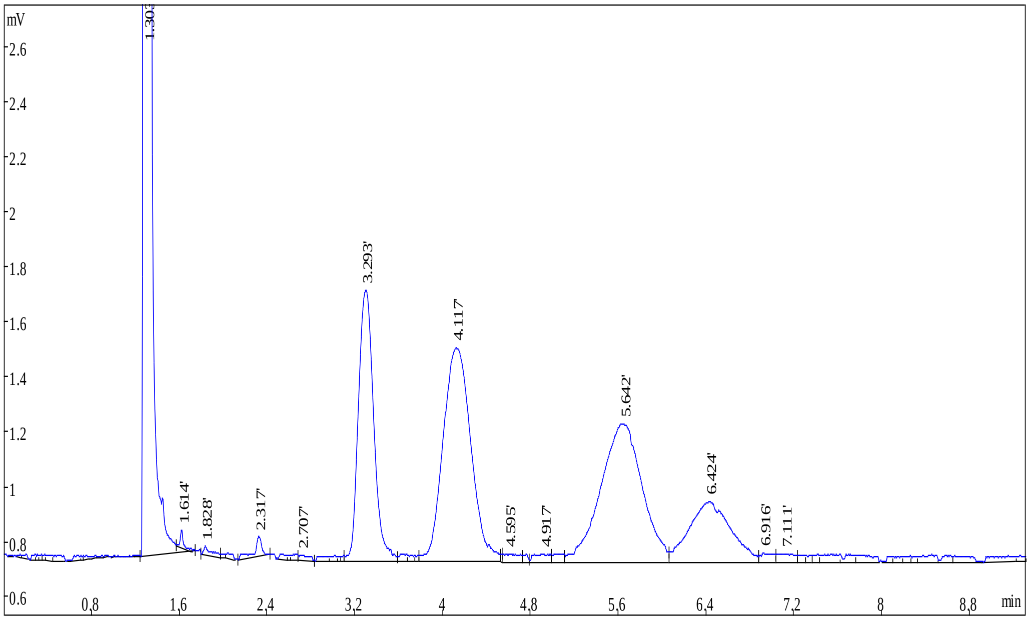
$$Ester\ Content\ \% = \left\{\left(\frac{22945}{7126}\right) - 1\right\} \times \left(\frac{2\text{mg/ml}}{10\text{mg/ml}}\right) \times\ 100\%$$

$$= 44.40\%$$

Microalgal biodiesel report 4
(2: 9: 6, STD 17; H_2SO_4)

```
Printing time: Fri Dec 13 09:49:43 2019
Injection time: Sun Dec 08 17:43:16 2019

File opened: C:\Users\PC\Desktop\Peak-ABC\program\3 9 3 microalgae
opt.(2019:12:08 17:43:16).hw
```



```
-----------------------------------------------
Rank    Time       Name    Area%        Area
-----------------------------------------------
1       1.303              97.35        1613663
2       1.614              0.016        269
3       1.828              0.01152      191
4       2.317              0.02099      348
5       2.707              0.007662     127
6       3.293              0.5631       9335
7       4.117              0.8233       13648
8       4.595              0.01635      271
9       4.917              0.0174       289
10      5.642              0.8004       13268
11      6.424              0.3545       5877
12      6.916              0.01792      297
-----------------------------------------------
Total                      100          1657583
```

$$Ester\ Content\ \% = \left\{\left(\frac{Areatotal}{AreaC17:0}\right) - 1\right\} \times \left(\frac{Mass of C17:0}{Mass of biodiesel}\right) \times 100\%$$

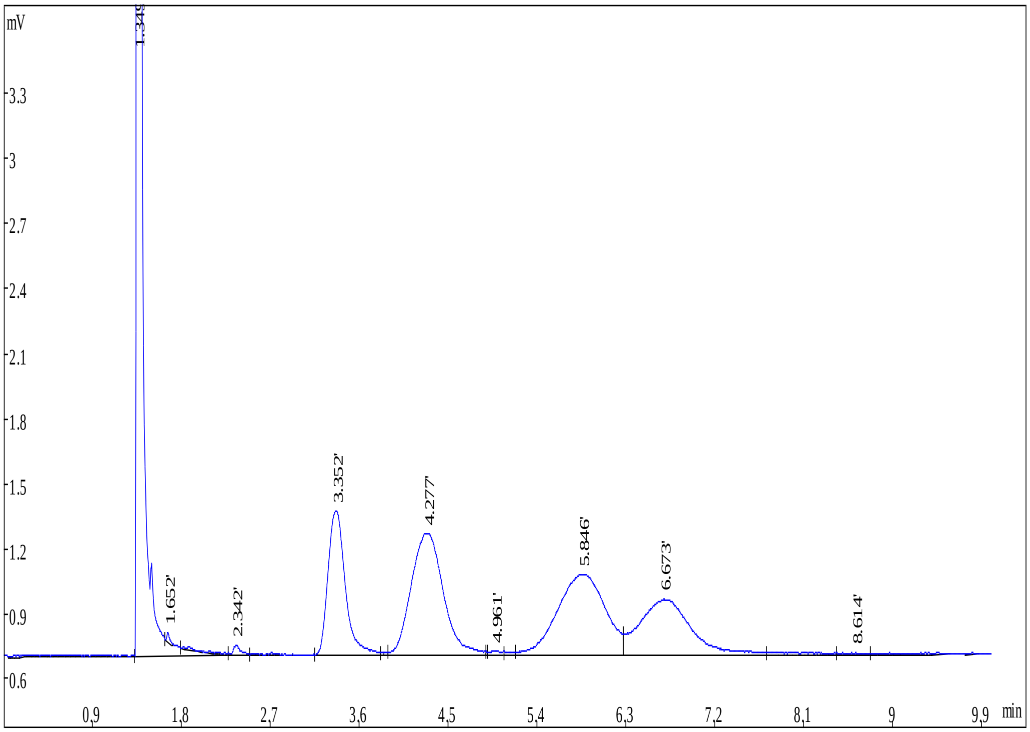
$$Ester\ Content\ \% = \left\{\left(\frac{43920}{13648}\right) - 1\right\} \times \left(\frac{2\text{mg/ml}}{5.5\text{mg/ml}}\right) \times 100\%$$

$$= 80.66\%$$

Biodiesel microalgae Report 5
(2: 9: 6,STD 20; H_2SO_4)

```
Printing time: Thu Dec 12 18:42:35 2019
Injection time: Mon Dec 09 12:47:24 2019

File opened: C:\Users\PC\Desktop\Peak-ABC\program\2 9 6 H2SO4.hw
```



```
-----------------------------------------------
Rank    Time      Name    Area%        Area
-----------------------------------------------
1       1.349             96.04        1047644
2       1.652             0.0203       221
3       2.342             0.02585      282
4       3.352             0.7354       8022
5       4.277             1.162        13307
6       4.961             0.01655      181
7       5.846             1.1462       12503
8       6.673             0.8417       9182
9       8.614             0.01269      138
-----------------------------------------------
Total                     100          1090852
```

$$Ester\ Content\ \% = \left\{\left(\frac{Areatotal}{AreaC17:0}\right) - 1\right\} \times \left(\frac{Mass of C17:0}{Mass of biodiesel}\right) \times 100\%$$

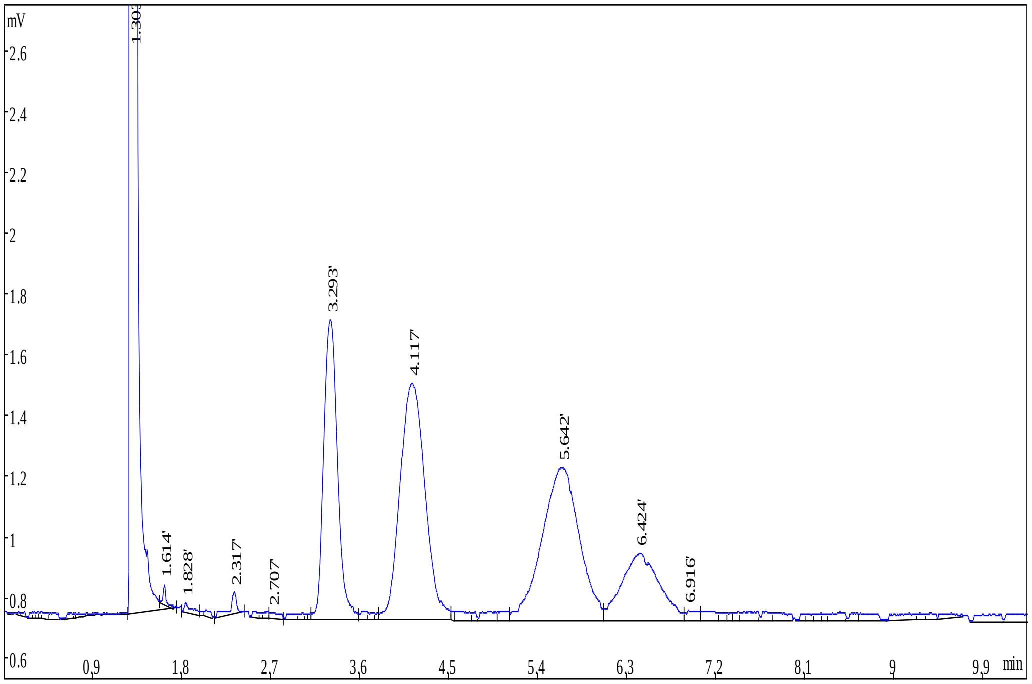
$$Ester\ Content\ \% = \left\{\left(\frac{43836}{13307}\right) - 1\right\} \times \left(\frac{2\mathrm{mg/ml}}{5.5\mathrm{mg/ml}}\right) \times 100\%$$

$$= 83.43\%$$

Spirulina Microalgae biodiesel report 6
(2: 9: 6, STD 18; H2SO4 Catalyst)

```
Printing time: Sat Dec 14 14:29:34 2019
Injection time: Sun Dec 08 17:43:16 2019

File opened: C:\Users\PC\Desktop\Peak-ABC\program\3 9 3
microalgae(2019:12:08 17:43:16).hw
```



```
-----------------------------------------------
Rank    Time      Name    Area%        Area
-----------------------------------------------
1       1.303             97.39        1613663
2       1.614             0.1213       201
3       1.828             0.01153      191
4       2.317             0.02100      348
5       2.707             0.007665     127
6       3.293             0.5634       9335
7       4.117             0.8237       13648
8       5.081             0.01267      210
9       5.642             0.8008       13268
10      6.424             0.3554       5877
-----------------------------------------------
Total                     100          1656868
```

$$Ester\ Content\ \% \ = \left\{\left(\frac{Areatotal}{AreaC17{:}0}\right) - 1\right\} \times \left(\frac{Mass of C17{:}0}{Mass of biodiesel}\right) \times \ 100\%$$

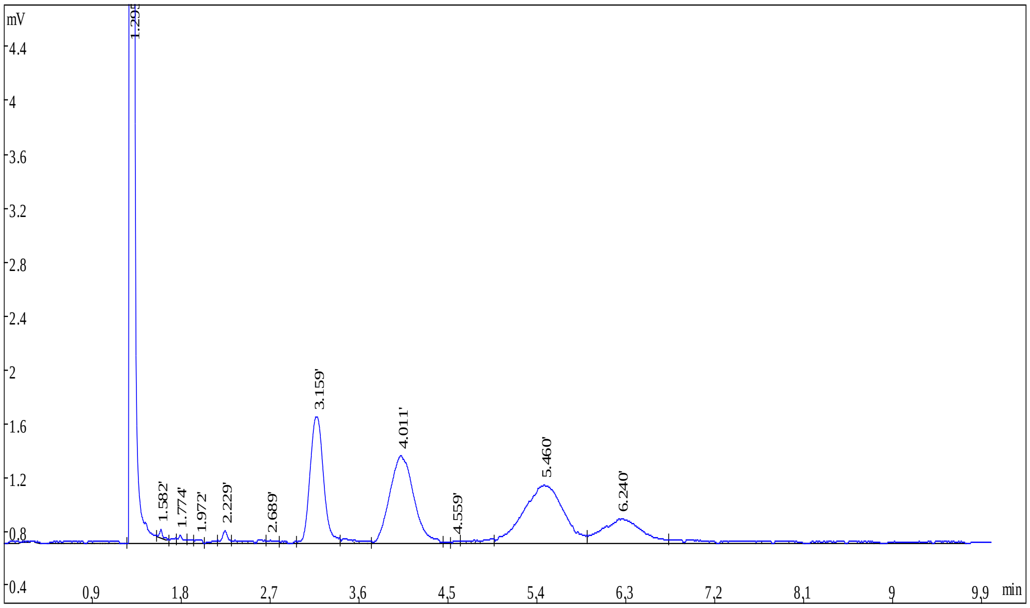
$$Ester\ Content\ \% \ = \left\{\left(\frac{43205}{13648}\right) - 1\right\} \times \left(\frac{2\text{mg/ml}}{5.5\text{mg/ml}}\right) \times \ 100\%$$

$$= \ 78.75\%$$

Spirulina Microalgae Biodiesel Report 7 (2: 9: 11, H_2SO_4)

```
Printing time: Sat Dec 14 12:18:58 2019
Injection time: Sat Dec 07 18:23:00 2019

File opened: C:\Users\PC\Desktop\Peak-ABC\program\microalgae
biodiesel (2019:12:07 18:23:00).hw
```



```
-----------------------------------------------
Rank    Time      Name    Area%       Area
-----------------------------------------------
1       1.295             97.22       1372329
2       1.582             0.007793    110
3       1.774             0.01842     260
4       1.972             0.01204     170
5       2.229             0.02862     404
6       2.689             0.01396     197
7       3.159             0.6179      8721
8       4.011             0.7856      14020
9       4.559             0.01048     148
10      5.460             0.8860      12506
11      6.240             0.3805      5371
12      6.814             0.0143      202
-----------------------------------------------
Total                     100         1414438
```

$$Ester\ Content\ \% = \left\{\left(\frac{Areatotal}{AreaC17:0}\right) - 1\right\} \times \left(\frac{Mass of C17:0}{Mass of biodiesel}\right) \times\ 100\%$$

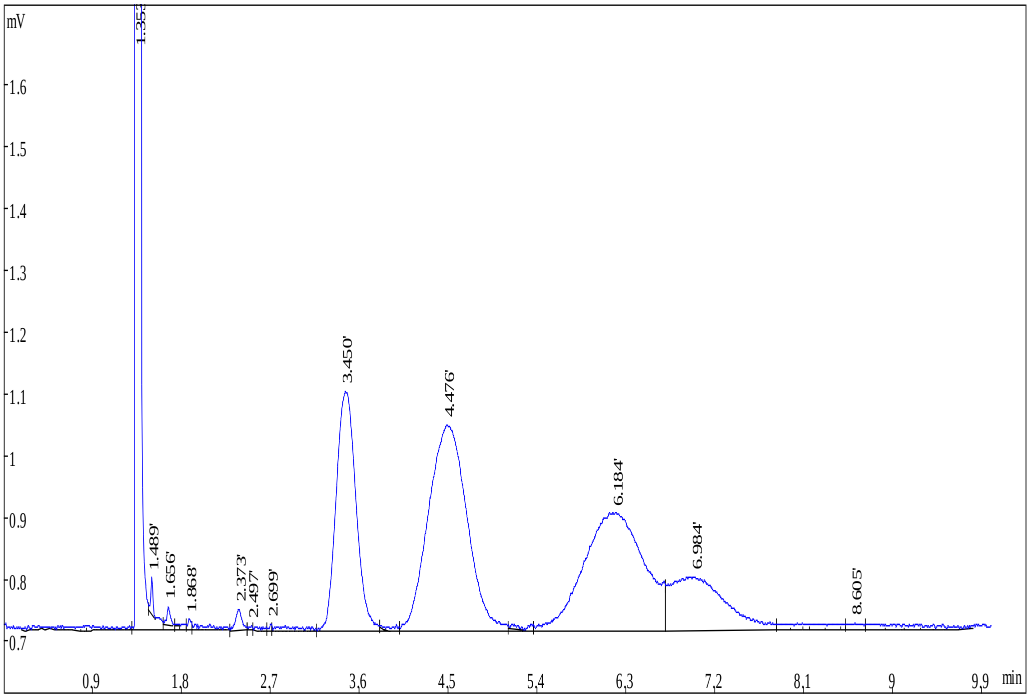
$$Ester\ Content\ \% = \left\{\left(\frac{42109}{14020}\right) - 1\right\} \times \left(\frac{2\text{mg/ml}}{5.5\text{mg/ml}}\right) \times\ 100\%$$

$$= 72.85\%$$

Microalgae biodiesel Report 8
(3: 12: 9, STD 8; H_2SO_4)

```
Printing time: Fri Dec 13 13:46:08 2019
Injection time: Mon Dec 09 10:03:42 2019

File opened: C:\Users\PC\Desktop\Peak-ABC\program\006 (2019:12:09
10:03:42).hw
```



```
-----------------------------------------------
Rank    Time       Name    Area%        Area
-----------------------------------------------
1       1.353              97.75        1130938
2       1.489              0.01321      143
3       1.656              0.01210      111
4       1.868              0.00723      74
2       2.373              0.01203      139
3       3.450              0.4584       5304
4       4.476              0.7804       9030
5       6.184              0.704        8146
6       6.984              0.2894       3349
7       8.605              0.00923      107
-----------------------------------------------
Total                      100          1157013
```

$$Ester\ Content\ \% = \left\{\left(\frac{Areatotal}{AreaC17:0}\right) - 1\right\} \times \left(\frac{Mass of C17:0}{Mass of biodiesel}\right) \times 100\%$$

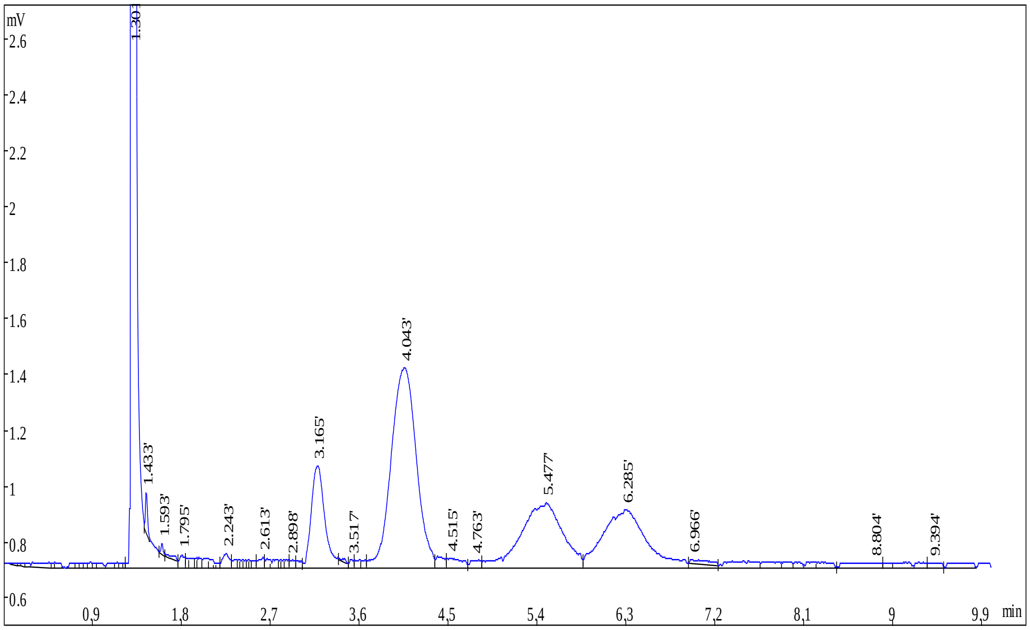
$$Ester\ Content\ \% = \left\{\left(\frac{26403}{9030}\right) - 1\right\} \times \left(\frac{2\text{mg/ml}}{5.5\text{mg/ml}}\right) \times 100\%$$

$$= 69.96\%$$

Microalgae biodiesel Report 9
(3: 12: 3, H_2SO_4;STD 4)

```
Printing time: Thu Dec 12 18:28:50 2019
Injection time: Sat Dec 07 17:38:16 2019

File opened: C:\Users\PC\Desktop\Peak-ABC\program\2 9 6 H2SO4
microalgae biodiesel 296 (20191207 17:38:16).hw
```



```
-----------------------------------------------
Rank    Time      Name    Area%       Area
-----------------------------------------------
1       1.301             97.77       1365376
2       1.433             0.01009     141
3       1.795             0.01325     185
4       2.243             0.01961     275
5       2.743             0.01035     145
6       3.165             0.2514      3511
7       4.043             0.8925      12464
8       4.515             0.02651     372
9       4.763             0.01546     217
10      5.477             0.4950      6913
11      6.285             0.4723      6595
12      6.966             0.02114     296
-----------------------------------------------
Total                     100         1396490
```

$$EsterContent\,\% \ = \left\{\left(\frac{Areatotal}{AreaC17{:}\,0}\right) - 1\right\} \times \left(\frac{Massof\,C17{:}\,0}{Massofbiodiesel}\right) \times\ 100\%$$

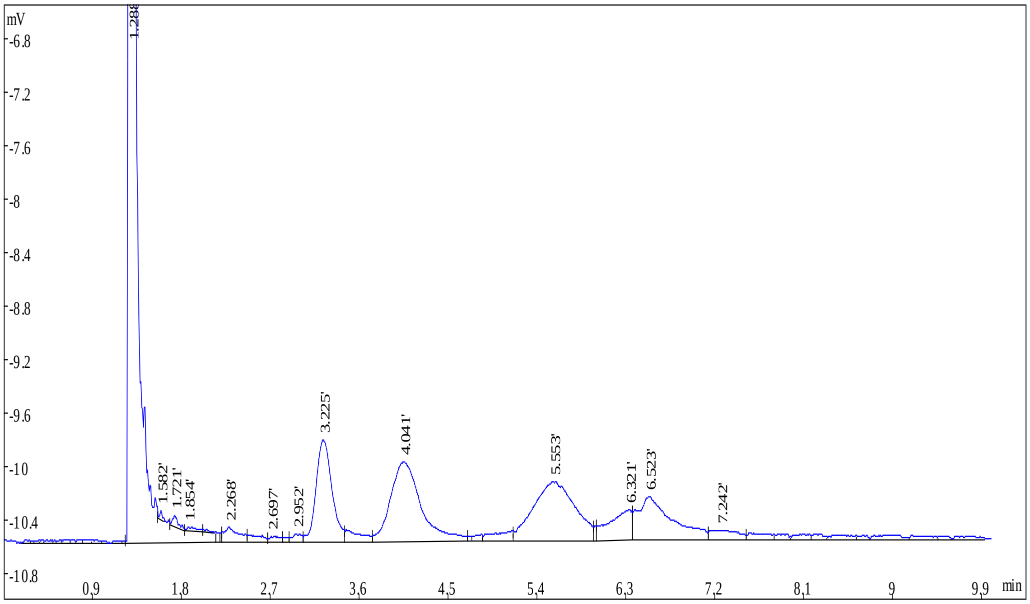
$$EsterContent\,\% \ = \left\{\left(\frac{32691}{12464}\right) - 1\right\} \times \left(\frac{2\text{mg/ml}}{5.5\text{mg/ml}}\right) \times\ 100\%$$

$$=\ 54.41\%$$

Microalgae biodiesel report 10
(2: 9: 11,H_2SO_4 ;STD 14)

Printing time: Fri Dec 13 17:56:07 2019
Injection time: Sun Dec 08 16:33:33 2019

File opened: C:\Users\PC\Desktop\Peak-ABC\program\296 H_2SO_4 004(2019:12:08 16:33:33).hw



```
-----------------------------------------------
Rank    Time      Name    Area%       Area
-----------------------------------------------
1       1.288             95.89       1400313
2       1.721             0.01608     235
3       1.854             0.01194     174
4       2.268             0.08695     1270
5       2.697             0.02691     393
6       3.225             0.5773      8431
7       3.454             0.07614     1112
8       4.041             0.8928      13037
9       5.553             0.8993      13132
10      5.985             0.01281     187
11      6.321             0.2644      3861
12      6.523             0.554       8089
-----------------------------------------------
Total                     100         1460281
```

$$\mathit{Ester\ Content}\ \% = \left\{\left(\frac{Areatotal}{AreaC17{:}0}\right) - 1\right\} \times \left(\frac{Mass of C17{:}0}{Mass of biodiesel}\right) \times\ 100\%$$

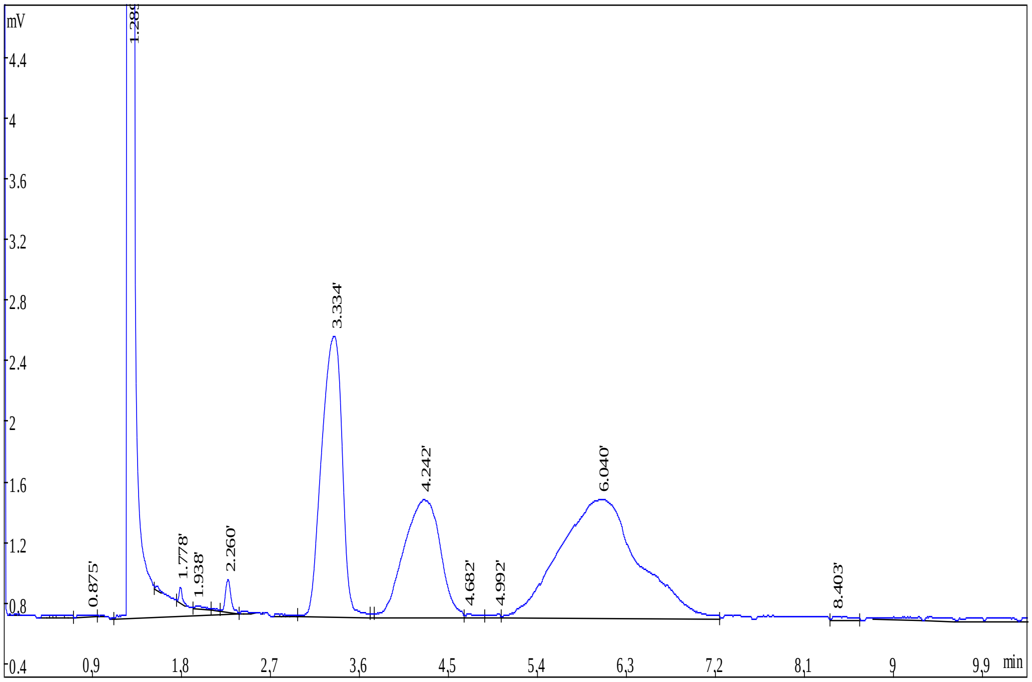
$$\mathit{Ester\ Content}\ \% = \left\{\left(\frac{59968}{13037}\right) - 1\right\} \times \left(\frac{2\text{mg/ml}}{10\text{mg/ml}}\right) \times\ 100\%$$

$$= 72.00\%$$

Microalgae biodiesel report 11 (3.682: 9: 6, H_2SO_4; STD 10)

```
Printing time: Fri Dec 13 09:12:49 2019
Injection time: Fri Dec 06 11:05:53 2019

File opened: C:\Users\PC\Desktop\Peak-ABC\program\003(20191206
11:05:53).hw
```



```
-----------------------------------------------
Rank    Time      Name    Area%       Area
-----------------------------------------------
1       1.289             94.36       1833442
2       1.778             0.009534    185
3       1.938             0.006079    118
4       2.260             0.03942     766
5       3.334             1.344       26110
6       4.242             1.01        19624
7       4.682             0.01079     210
8       4.992             0.008378    163
9       6.040             2.23        43337
10      8.403             0.01781     346
-----------------------------------------------
Total                     100         1924301
```

$$Ester\ Content\ \% = \left\{\left(\frac{Areatotal}{AreaC17:0}\right) - 1\right\} \times \left(\frac{Massof C17:0}{Massofbiodiesel}\right) \times\ 100\%$$

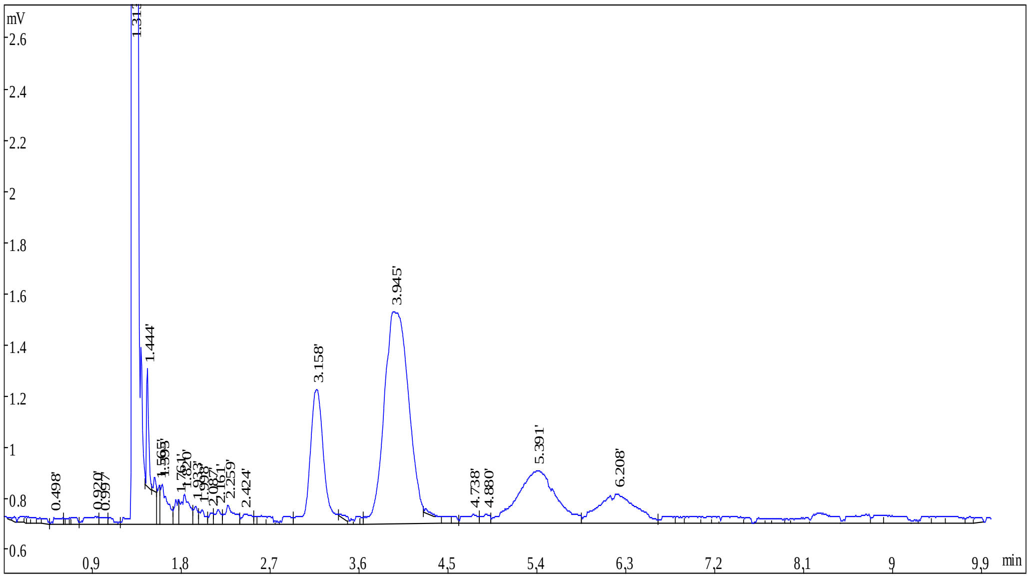
$$Ester\ Content\ \% = \left\{\left(\frac{90859}{19624}\right) - 1\right\} \times \left(\frac{2\mathrm{mg/ml}}{10\mathrm{mg/ml}}\right) \times\ 100\%$$

$$= 72.60\%$$

Microalgae biodiesel report 12
(1: 6: 3, H_2SO_4)

```
Printing time: Sat Dec 14 11:14:44 2019
Injection time: Sat Dec 07 13:42:29 2019

File opened: C:\Users\PC\Desktop\Peak-ABC\program\best biodiesel
microalgae (2019:12:07 13:42:29).hw
```



```
-----------------------------------------------
Rank    Time       Name    Area%        Area
-----------------------------------------------
1       1.313              97.24        1109362
2       1.444              0.04102      468
3       1.565              0.02147      245
4       1.595              0.06539      746
5       1.761              0.02638      301
6       1.820              0.05952      679
7       2.259              0.04365      498
8       2.838              0.01639      187
9       3.158              0.4379       4996
10      3.945              1.275        14550
11      5.391              0.5001       5706
12      6.208              0.2747       3134
-----------------------------------------------
Total                      100          1140872
```

$$Ester\ Content\ \% \ = \left\{\left(\frac{Areatotal}{AreaC17{:}0}\right) - 1\right\} \times \left(\frac{Mass of C17{:}0}{Mass of biodiesel}\right) \times\ 100\%$$

$$Ester\ Content\ \% \ = \left\{\left(\frac{31510}{14550}\right) - 1\right\} \times \left(\frac{2\text{mg/ml}}{5.5\text{mg/ml}}\right) \times\ 100\%$$

$$= \ 42.39\%$$