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EVALUATION OF MUNICIPAL SEWAGE SLUDGE AS AN ALTERNATIVE FEEDSTOCK FOR BIODIESEL PRODUCTION

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A dissertation submitted to the faculty of Engineering and the Built Environment, University of the Witwatersrand, in fulfillment of the requirements for the degree of Master of Science in Engineering

Supervisor: Prof Jean Mulopo

March 2022

DECLARATION

I Karabo Zulu declare that this dissertation is my own unaided work. It is being submitted for the degree of Master of Science in Engineering to the University of the Witwatersrand, Johannesburg. It has not been submitted before for any other degree or examination in any other University.

Karabo Zulu

Signature: 

24th day of March 2022

ABSTRACT

Urbanization and industrialization have resulted in an increase in the world's primary energy consumption over the previous 40 years. Transportation and oil are the primary contributors, with non-renewable resources such as coal and petroleum providing the majority of the energy required to meet the world's population's energy needs. Combustion of these materials increases greenhouse gas emissions, which are pollutants that have a negative impact on the environment and human health. Biodiesel has received the most attention in the last two decades as a source of alternative energy. Biodiesel is a viable alternative to petroleum-based diesel fuels since it emits less pollutants into the atmosphere and hence has a lower environmental impact when utilized. The high cost of raw materials, on the other hand, is a key disadvantage to biodiesel production. Frequently, feedstock accounts for between 70% and 85% of the cost of producing biodiesel. Numerous different feedstocks have been investigated in order to reduce the unit cost of biodiesel, increase its energy efficiency, and secure its long-term production.

Non-edible oils, edible oils (soybeans, sunflower, etc.), algae, and waste material are all examples. Sewage sludge has been reported as a suitable feedstock for biodiesel production, containing at least 7% lipids in the dry sludge. One notable advantage is that sewage sludge is readily available in wastewater treatment plants, and a steady supply is provided by urbanization and industrialization. On the other hand, sewage sludge continues to be a financial strain for many South African local municipalities due to the high cost of disposal. In this project, biodiesel was produced from sewage sludge from Johannesburg's municipal wastewater treatment plant (WWTP). Primary sludge produced the most lipids and biodiesel, followed by blended sludge and finally secondary sludge, according to the findings of this study. Pretreatment of sludge samples with hydrochloric acid improved lipid extraction from all types of sludge investigated. Although ionic liquids were shown to be capable of extracting lipids from a variety of sludge samples, the Bligh and Dyer process extracted substantially more lipids. Additionally, the gas chromatography analysis of the various sewage sludge FAMEs indicates that the fatty acid content of all analyzed sludge samples is comparable. The fatty acid content of sludge samples was unaffected by various extraction procedures or acidification. All sludge samples contained myristic (C14:0), pentadecyclic (C15:0), palmitic (C16:0), stearic (C18:0), oleic (C18:1), and linoleic (C18:2) acids, which are typical fatty acids for biodiesel production. The fatty acid composition of primary sludge FAMEs was compared to that of typical biodiesel feedstocks to assess whether primary sludge can be used to produce biodiesel.

Keywords: Sewage sludge, Biodiesel Synthesis and Characterization, lipids, ionic liquid, lipid extraction, pre-treatment

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LIST OF ABBREVIATIONS

ASTM	American Standard for Testing Materials
ATR	Attenuated Total Reflection
B&D	Bligh & Dyer
CEIPPPP	Clean Energy Independent Power Producer
CFPP	Cold Filter Plugging Point
CSP	Concentrated Solar Photovoltaic
DALRRD	Department of Agriculture, Land Reform and Rural Development
DMRE	Department of Mineral Resources and Energy
FAME	Fatty Acid Methyl Esters
FFA	Free Fatty Acid
GHG	Greenhouse Gas
IPPO	Independent Power Producer Office
IRP	Integrated Resource Plan
MSS	Municipal Sewage Sludge
NBIS	National Biofuels Industrial Strategy
NBTT	National Biofuels Task Team
PV	Photovoltaic
SSEG	Small scale energy generation
TS	Total solids
UNFCCC	United Nations Framework Convention on Climate Change
VS	Volatile solids
WWTP	Wastewater Treatment Plant
WRC	Water Research Commission

Chapter 1: INTRODUCTION

Due to the vast number of countries that adopted academic programs on sustainable energy technology, the global growth of renewable energy has strengthened and expanded over the last decade (Kandpal & Broman, 2014). One of the most pressing issues confronting the global energy market are ensuring demand stability while avoiding climate change as a result of outdated or inefficient energy generation technologies (Winkler et al., 2007). As a result, it was important that countries invest in energy-efficient and renewable technology to ensure that emissions were lowered as much as possible (REN21 2015a). The primary sources of greenhouse gas (GHG) emissions, which began with the industrial revolution, are practices related to crude oil and natural gas (Abdul-Wahab et al., 2015).

GHGs were also identified as a significant contributor to the life cycle of agricultural processes such as crop production and other activities (Kramer et al., 1999). Since the early stages of industrialization, the burning of fossil fuels for electricity supply has been described as a source of global warming (Khondaker, 2012). In 1995, the United Nations Framework Convention on Climate Change (UNFCCC) established and convened the Kyoto Protocol and Copenhagen Accord as a measure to tackle climate change (Lau et al., 2012). Members of the Kyoto Protocol agreed to make concerted attempts to reduce pollution using Clean Development Mechanism technology that use renewable energies (Lau et al., 2012). The 13th Conference of the Parties (COP13), which was held in Bali, Indonesia, also backed efforts by the members of the Kyoto Protocol to reduce pollution (Boston, 2008). Because of this, South Africa and other willing countries backed the Kyoto Protocol and signed the agreement to reduce greenhouse gas emissions (Luiz & Muller, 2008). As a result of the pledge, there were noticeable improvements in the energy market, which affected the planning of some South African industries (Winkler et al., 2007).

The Department of Mineral Resources and Energy (DMRE) defined the energy specifications for South Africans in the Electricity Regulation Act 4 of 2006 (Winkler et al., 2007). The Integrated Resource Plan (IRP), which stipulated South Africa's energy requirements as well as the means to satisfy those needs by various clean

energy technologies, presented the DMRE with a mechanism to use (Lawrence, 2020). This was done to efficiently align supply and demand. South Africa has had comparatively strong electricity demand from a fossil fuel-dominated generation for the past decade or so (Winkler et al., 2007). This will enable clean energy technology to be willingly integrated into the energy generation mix.

The most recent IRP was issued by the DMRE in October of this year, and is referred to as the IRP 2019 (Wright et al., 2019). According to the IRP, the energy capacity was just over 52 000MW (IRP 2019). Due to the end of life of the coal plants, capacity was projected to be decommissioned by 2050 (Ireland & Burton, 2018). When coal mining deposits challenged supply and the pledge to eliminate electricity produced by burning fossil fuels, it was widely anticipated that more renewable sources of energy production would be included (Mathu, 2011). Most countries have increased their investments in green energies over the last decade as they make the transition to sustainable energy sources that are less harmful to the atmosphere (Oppong et al., 2020).

Climate change, energy security, and natural gas supplies have all reduced our reliance on fossil fuels, necessitating research into alternative fuels (Rahimnejad et al., 2011). This transformation was also aided by a greater understanding of the negative impacts of climate change in many nations, as well as the development of newer and more cost-effective electricity production technologies (Yu, Xue et al. 2020). This was also seen as a potential alternative for electricity supply in places like Sub-Saharan Africa, where new energy generation technologies were previously unavailable (Sokona et al., 2012). A research by Campbell and colleagues (2003) found that even the richest families in Zimbabwe used a combination of energy generation sources, including wood and electricity (Campbell et al., 2003).

Due to energy scarcity in the South African electricity grid, some households, businesses, and industrial facilities produced their own electricity, referred to as small scale energy generation (SSEG) (Kessides et al., 2007). It is projected that it would have a gross generation capability of 1 MW (Filipova & Morris, 2018). Furthermore, the Clean Energy Independent Power Producer Procurement Programme (CEIPPPP), which was launched by the government in 2011 and operated by the Independent Power Producer Office (IPPO), augmented the procurement of renewable energy for the national grid (Department of Energy 2015). Such a system (a renewable energy

auction/bidding process) has attracted and encouraged expertise and investment in renewable energy projects in South Africa (Kruger & Eberhard, 2018). Renewable energy is certainly advancing, with some mainstream renewable energy and emerging technologies maturing (or matured) whilst others are still under development (McDonald & Schrattenholzer, 2001). The mainstream comprising of the wind, geothermal, solar, hydro, biomass and biofuels (Hussain et al., 2017).

The renewable energy technologies that were viewed as becoming more reliable and matured due to their competitiveness and interest, included the solar photovoltaic (PV), wind technology, bioenergy, marine energy, concentrated solar power (CSP) and geothermal (Hussain et al., 2017). Due to its cleaner processes and less greenhouse gas emitted, the renewable energy leads to better community health conditions and reduced domestic air pollution (Abanda et al., 2012). It can also provide low cost electricity to poorer communities with far less detrimental health effects (Sanoh et al., 2014). Remote areas of sub-Saharan African countries would have a better chance to access electricity over the next 10 years (Bazilian et al., 2012). Figure 1 below depicts progressive projections of electricity access in the world focusing on two decades as of the year 2010.

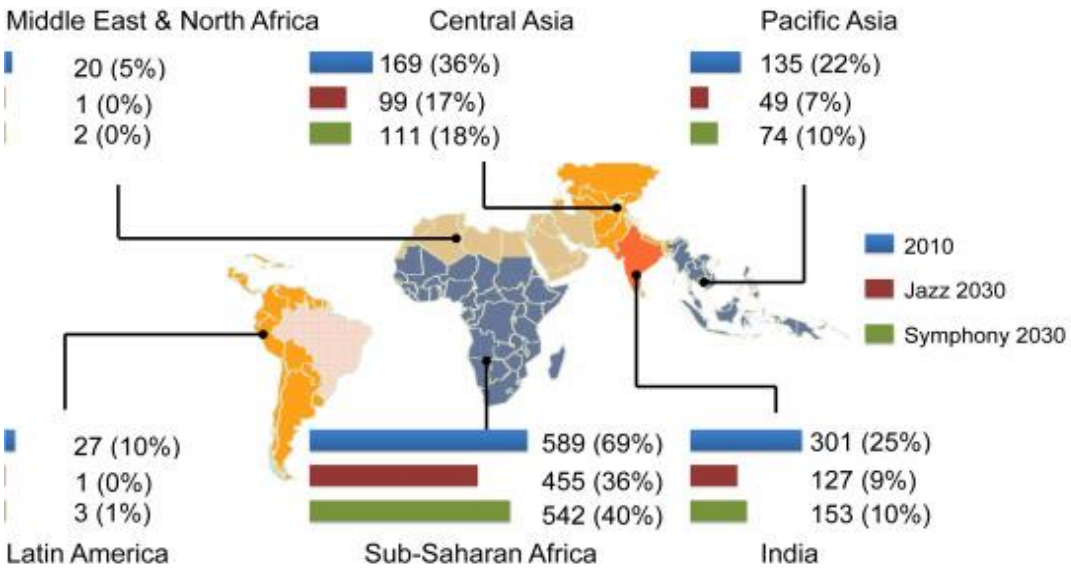


Figure 1. Projections of electricity in Sub-Saharan countries (Densing et al. 2016).

According to United Nations, Department of Economic and Social Affairs, Population Division, it is estimated that the world population will reach 9.8 billion by 2050 and 11.2 billion by 2100 (*World Population Prospects*, 2017). This growth will lead to a growing demand for energy from fossil fuels for the transportation sector. Globally, the transportation sector accounts for 30% of the world's total energy and is the second largest energy consuming sector after the industrial sector (Atabani et al., 2013). However, due to fossil fuel depletion, the rising demand of energy, the increase in crude oil prices and the increase in greenhouse gas emissions, there has been growing interest globally in the research and development of sustainable and environmentally friendly alternative sources of energy (Avinash et al., 2014). Biodiesel has been viewed as a promising renewable alternative to petroleum diesel since it is compatible with current diesel engine and refuelling technology (Revellame et al., 2010). Furthermore, it is biodegradable, less toxic, safe to use, and has low emission profiles (Olkiewicz et al., 2012).

1.1 Problem Statement

The challenge with commercialisation of biodiesel is the cost of common feedstocks such as sunflower, canola, soybean, palm and rapeseed, which make up about 70-85% of the cost of production of biodiesel (Kech et al., 2018). In addition, shortage of agricultural land for growing these feedstocks has limited the growth of the biodiesel market, it has also led to high food prices which raised concerns of food shortage against fuel demand issues (Kwon et al., 2012). Therefore, there is a need to find alternative feedstocks that are non-edible, easily available, inexpensive and do not compete with the food market. Alternative feedstocks such as waste cooking oil, jathropa, karanja, and microalgae have been investigated over the years (Gong & Jiang, 2011; Sahoo & Das, 2009; Wang et al., 2006).

Municipal sewage sludge is gaining interest as a lipid feedstock for biodiesel production due to its high lipid content with dry sludge containing up to 30wt % of lipids (Pastore et al., 2013). Wastewater treatment plants (WWTP) produce large amounts of sewage sludge annually and these need to be treated before they can be disposed or used for other purposes. This treatment contributes about 50% of the total operating costs of the WWTP (Boocock et al., 1992). The standard practice of sludge disposal namely; incineration, land application and landfill disposal of sewage sludge

may release toxins and heavy metals that are harmful for the environment (Pokoo-Aikins et al., 2010). It is for the above reasons that alternative, cost-effective and beneficial ways of using the sludge are investigated as part of the sewage sludge management.

1.2 Research Questions

1. What is the influence of sludge type on the lipid extraction and biodiesel yield?
2. How much lipids can be extracted from the different sludge samples?
3. Does the pre-treatment of the sludge samples have any effect on the amount of lipids produced?
4. What type of FAMEs are produced from the different sludge samples?
5. How do the FAMEs profiles compare to those of the common feedstocks for biodiesel production?
6. Which extraction method is more effective in the extraction of lipids?
7. Do the properties of the biodiesel produced from sludge meet the ASTM D6751 standards for biodiesel?

1.3 Project Aim

Research into the use of MSS as a feedstock for biodiesel production is gaining interest due to the significant amount of lipids found in the sludge (Dufreche et al., 2007; Revellame et al., 2010; Siddiquee & Rohani, 2011). Other factors that make attractive the use of sludge for biodiesel production include the fact that it is abundant, readily available, inexpensive and may assist in the challenge of sludge management and disposal. Several studies have been conducted on the use of sewage sludge for biodiesel production. Most of them are based on the use of dried sludge. Production of the dry sludge involves the expensive dewatering and drying step. The purpose of this study is to evaluate the direct use of wet sludge for biodiesel production and thus eliminate the expensive drying stage in order to save production costs.

1.4 Project Objectives

The objectives of the study are:

1. To determine which sludge type (primary, secondary or blended) produces the highest lipid yield.

2. To determine which extraction method (Bligh & Dyer or ionic liquid) produces the highest lipid yield.
3. To determine the effect of pre-treatment of sludge on the lipid yield.
4. To determine the FAMEs composition using GC-MS profile.
5. To determine whether the FAMEs produced are comparable to those of common feedstocks for biodiesel production using FT-IR.
6. To measure biodiesel properties such as density and viscosity and to determine whether they meet the ASTM D6751 standards for biodiesel.

Chapter 2: LITERATURE REVIEW

2.1 Biofuels

2.1.1 Global Biofuels production trends

According to United Nations, Department of Economic and Social Affairs, Population Division, it is estimated that the world population will reach 9.8 billion by 2050 and 11.2 billion by 2100 (*World Population Prospects*, 2017). Global oil consumption was reported to have increased by 0.9 million barrels per day while there was a decline in global oil production of 60 000 barrels per day in 2019. Furthermore, it was stated that the global oil reserves were 1734 billion barrels at the end of 2019, which is a decline of 2 billion barrels compared to 2018, that would account for 50 years of current oil production (Looney, 2020). This increase in world population may lead to an increase in demand for fuels in the transportation sector. The decrease in oil production and oil reserves will mean that there will be no sufficient fuels to service the transportation sector. The transportation sector is one of the main consumer of petroleum-based fuels such as diesel, petrol and natural gas.

Therefore, the depletion of petroleum-based fuels, the increasing crude oil prices and greenhouse gas emissions from petroleum fuels have led to a growing need to find alternative, renewable energy sources (Kralova & Sjöblom, 2010). Biofuels have drawn the attention of many researchers as potential replacements to petroleum-based fuels since they are easily produced from biomass, they are biodegradable, they offer enhanced energy security, they reduce greenhouse gas emissions, they help in job creation and they enhance agricultural development and investment (Pradhan & Mbohwa, 2014). Biofuels are produced from biomass. They may be liquid, solid or gas (Guo et al., 2015). The cost of production of biofuels depend on the feedstock, production technique, magnitude of production and location.

The most common liquid biofuels are bioethanol and biodiesel. Bioethanol is produced by the fermentation of sugar and starch found in crops such as maize and sugarcane using yeast. Ethanol can be used to fuel a car or be blended with petrol to improve the octane number. Biodiesel is made up of mono-alkyl esters of long chain fatty acids. They are produced when triglycerides in edible or non-edible oils or animal fats react with alcohol in the presence of a catalyst (Knothe & Razon, 2017). The world's biofuels production has increased from 2206 million barrels/day in 2014 to 2690 million barrels/day in 2019. The global ethanol production increased from 1615 million barrels/day in 2014 to 1886 million barrels/day in 2019, while the biodiesel production increased from 591 million barrels/day in 2014 to 805 million barrels/day in 2019 (*Annual Energy Outlook 2021*). Several developed and developing countries have adopted biofuels policies in order to promote their use and improve their competitiveness in the market (Demirbas, 2008b).

2.1.2 **Biofuels production in South Africa**

The use of biofuels in South African dates back to the 1920s when sugar cane was used to produce bioethanol. It was not until the 1960s that this production was halted due to low international crude oil prices. There has been renewed interest in the research and development of biofuels due to recent crude oil price increases and climate change issues. Due to continuing interest in the development of biofuels as alternative energy source in South Africa, The National Biofuels Task Team (NBTT) was put together in December 2005 to carry out a study to investigate the feasibility of establishing a National Biofuels Industrial Strategy (NBIS) (Biofuels Task Team, 2006). The draft National Biofuels Strategy was submitted to Cabinet in November 2006 and submitted for public remarks until May 2007.

Based on their findings and recommendations, the National Biofuels Industrial Strategy was approved in South Africa in December 2007 (Department of Minerals and Energy, 2007). Worldwide the implementation of biofuels strategy is driven by factors such as the promotion of the use of renewable resources, improvement in energy security, reduction of greenhouse gas emissions, support

for rural agricultural development, promotion of investment in rural areas and increased job opportunities. However, in South Africa the main driver of the biofuels strategy was to bridge the gap between the country's first and second economies (Department of Minerals and Energy, 2007).

Therefore, the objective of the NBIS was to address issues such as the alleviation of poverty through job creation in previously disadvantaged communities. Furthermore, to promote agricultural development and investment in these areas. The NBIS proposed a short term, five-year pilot to achieve a 2% penetration of biofuels by 2013, which is equivalent to 400 million litres per annum. It was estimated that the penetration would result in 25000 jobs being created, with a reduction of unemployment by 0.6% and an increase in economic growth of 0.05% (Department of Minerals and Energy, 2007). The strategy also estimated a contribution of up to 30% to the set target of 10 000 GWh of final energy production from renewable sources by 2013 (Brent et al., 2010). The NBIS proposed the use of the following crops for biofuels production in South Africa; sugar cane and sugar beet for bioethanol production and sunflower, canola and soya beans for biodiesel production. Maize and Jathropa were excluded due to concerns over food security, price increases and environmental concerns.

The strategy also mentioned the importance of incentive schemes to ensure that biofuels investors and farmers receive reasonable returns. The NBIS further recommends blending requirements of 2% for biodiesel and 8% for bioethanol. The strategy focuses its attention more on poverty stricken farmers especially in the former homelands in order to promote economic and social development through agricultural development (Blanchard et al., 2011). The strategy proposes that farmers be supported through existing agricultural support programmes. These programmes are intended to support farmers in crop selection, hedging, agricultural methods, logistics, research and development and contract negotiations with biofuels manufacturers. Although National Biofuels Industrial Strategy was approved in 2007, there have been delays in its implementation due to the lack of a formal regulatory framework and policy uncertainties. This has hindered progress in the research and development of biofuels in South Africa. Cabinet approved the Biofuels Regulatory Framework on 13 December 2019 (Department of Minerals

and Energy, 2020). The purpose of the framework is the support and development of emerging biofuels industry in South Africa. There are five features of the Biofuels Regulatory Framework:

The Feedstock Protocol - The feedstock protocol was developed by the Department of Agriculture, Land Reform and Rural Development (DALRRD) to ensure that the feedstock used in the production of biofuels fulfils its objective of creating jobs to disadvantaged farmers in the agricultural sector but does not give rise to food security crisis and environmental issues.

The mandatory blending regulations - The regulations regarding the mandatory blending of biofuels with petrol and diesel were gazetted on 23 August 2012 and came into effect on 1 October 2015. The purpose of the regulations was to create a market for biofuels by invoking interest in licensed manufacturers and wholesalers of petroleum fuels to purchase and blend bioethanol and biodiesel that have been manufactured locally at a minimum of 2% (v/v) with their petrol and 5% (v/v) with their diesel.

The cost recovery mechanism for blending of biofuels – For the first phase of the programme, blending of biofuels will only occur at the depots or refineries to ensure product quality and to evaluate logistics costs. The petroleum fuel manufacturers who will also be the blenders will remain cost neutral. Costs incurred by blenders during the blending operation will be recovered through mechanisms decided upon by the Department of Mineral Resources and Energy (DMRE).

The Biofuels Subsidy Mechanism – The two components of the biofuels subsidy mechanism are the biofuels farmers support and the biofuels manufacturers support. Biofuels farmers support will include financial assistance, technical support and research and development of feedstock that result in high biofuels yields. Support that will be given to biofuels manufacturers include financial support for new investments, infrastructure and industrial projects.

The selection criteria for biofuels projects requiring a subsidy – These projects will be chosen based on the socio-economic benefits they present compared to the amount of subsidy required. The Minister of Finance, Pravin

Gordhan, in the 2013 Budget Review, confirmed that the biofuels industry will be subsidized by increasing the Fuel Levy in terms of the Customs and Excise Act. No. 91 of 1964. He stated that the biofuels levy would be in the region of 3.5- 4 cents per litre on all petrol and diesel sold in South Africa and this was based on the cost estimations of biofuels production at the time.

2.2 Biodiesel

2.2.1 Biodiesel Definition

Biodiesel is an alternative to petroleum-based diesel fuel and it is made from renewable sources such as edible and non-edible oils, animal fats and waste cooking oils (Demirbas et al., 2016a). According to the American Society for Testing and Materials (ASTM), biodiesel can be defined as a fuel that consists of a mixture of mono-alkyl esters of long chain fatty acids acquired from renewable sources and meeting the biodiesel specifications of ASTM D6751.

2.2.2 Advantages of biodiesel

The use of biodiesel has the following advantages (Yusuf et al., 2011; Demirbas, 2008a; Feofilova et al., 2010):

- Biodiesel contains low or no sulphur which leads to an improvement in engine lubrication and increase in engine life
- It is a renewable energy source
- Biodiesel produces less greenhouse gas emissions
- Biodiesel is safe to handle, store and transport due to its high flashpoint
- Biodiesel produces less toxic pollutants which makes it environmentally friendly
- Since it is compatible with the current fuel distribution system, no engine modification is required
- Biodiesel increases fuel lubricity thereby reducing wear in the engine and increasing engine efficiency
- Biodiesel is biodegradable
- Biodiesel can be used on its own (B100) or as a blend with petroleum-based diesel
- Biodiesel has better fuel economy than petroleum-based diesel

- Biodiesel is safe and easy to handle and transport since it is less toxic than petroleum-based diesel
- Biodiesel has a high cetane number and therefore better ignition properties

2.2.3 **Disadvantages of biodiesel**

Disadvantages of biodiesel include the following (Firoz, 2017; Mishra & Goswami, 2018; Feofilova et al., 2010):

- Biodiesel has a high viscosity and therefore need high injector pressure.
- Biodiesel may cause engine clogging when used in low temperatures due to gel formation.
- Biodiesel is expensive compared to petroleum-based diesel.
- Biodiesel use may lead to an increase in nitrogen oxide (NOx) emissions which when dissolved in the atmospheric moisture, may cause acid rain.
- Biodiesel use may result in food shortage if edible oils are used as feedstock in their production.
- Biodiesel has low oxidation stability and this can cause corrosion of fuel tanks, pipes and injectors.
- When biodiesel cleans the dirt in the engine, the dirt can get collected in the fuel filter and clog it.

2.3 Biodiesel fuel properties and quality

2.3.1 **Fuel Properties**

The chemical and physical properties of biodiesel are dependent on the fatty acid distribution of the different feedstocks (Mittelbach & Remschmidt, 2005). Table 1 shows the chemical structures of the most common fatty acids.

Table 1: Chemical structure of common fatty acids

Fatty Acids	Carbons	Double bonds	structure
Acetic	2	0	2:0
Propionic	3	0	3:0
Butyric	4	0	4:0
Caproic	6	0	6:0
Caprylic	8	0	8:0
Capric	10	0	10:0
Lauric	12	0	12:0
Myristic	14	0	14:0
Palmitic	16	0	16:0
Palmitoleic	16	1	16:1
Stearic	18	0	18:0
Oleic	18	1	18:1
Linoleic	18	2	18:2
Linolenic	18	3	18:3
Arachidonic	20	4	20:4

Table 2 shows the fatty acid distribution of some of the common feedstocks used in biodiesel production. Table 3 shows a comparison between the properties of petroleum-based diesel and biodiesel produced from different feedstocks (Gunstone, 2012).

Table 2. Fatty Acid composition of various feedstocks (Lin et al., 2011).

Fatty acid (%w/w)	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3
Feedstock							
Sunflower	-	-	6.08	3.26	16.93	73.73	-
Rapeseed	-	-	3.49	0.85	64.40	22.30	8.23
Soybean	-	-	10.58	4.76	22.52	52.34	8.19
Palm	-	1	42.8	4.5	40.5	10.1	0.2
Peanut	-	0.3	12.3	4.6	53.6	29	0.1
Coconut	46.5	19.2	9.8	3	6.9	2.2	-
Soybean soapstock	-	-	17.2	4.4	15.7	55.6	7.1
Used frying oil	-	-	12	-	53	33	1
Tallow	-	3-6	24-32	20-25	37-43	2-3	-
Lard	-	1-2	28-30	12-18	4-50	7-13	-

Table 3. Properties of petroleum-based diesel and biodiesel (FAME) produced from various feedstocks (Lin et al., 2011).

Properties	K.Viscosity (mm^2/s, @ 40ºC)	Density (g/cm^3, @ 21ºC)	Cetane Number	Flash point (ºC)	Cloud point (ºC)	Pour point (ºC)
Fuel						
Diesel	2.0-4.5	0.820-0.860	51.0	55	-18	-25
Soybean	4.08	0.884	50.9	131	-0.5	-4
Rapeseed	4.83	0.882	52.9	155	-4	-10.8
Palm	4.71	0.864	57.3	135	16	12
Sunflower	4.60	0.880	49.0	183	1	-7
Jathropa	4.4	0.875	57.1	163	4	-
Tallow	5.00	0.877	58.8	150	12	9
Soapstock	4.30	0.885	51.3	169	6	-

Cetane number indicates the ignition properties of a fuel. A higher cetane number result in better ignition properties of the fuel (Meher et al., 2006). The higher the degree of saturation and the longer the fatty acid chain, the higher the cetane number (Bajpai & Tyagi, 2006). In a study conducted by (Knothe et al., 2003), esters with saturated fatty acids such as palmitic acid (C16:0) and stearic acid (C18:0) gave high cetane numbers. (Van Gerpen, 1996) reported that there was an increase in the cetane number of a blend when the percentage of palmitic acid methyl ester was increased in the blend. In addition, he observed that peanut,

sunflower and corn biodiesel showed cetane numbers that were lower due to the presence of high percentage of unsaturated ester of linoleic acid (C18:2).

The presence of polyunsaturated methyl esters affects the oxidation stability of biodiesel (Knothe, 2006). The oxidation stability is one of the most important property of biodiesel that indicates the stability of the fuel over extended period of storage. Oxidation stability of biodiesel fuels derived from soybean, sunflower, almond and corn were found to be low (Ramos et al., 2009). Antioxidants can be used to improve the oxidation stability of biodiesel (Rodríguez et al., 2006). The higher the concentration of polyunsaturated methyl esters the lower the oxidation stability. Therefore, biodiesel derived from soybean, sunflower and rapeseed oil were found to have poor oxidation stability due to the presence of high concentrations of linoleic (C18:2) and linolenic (C18:3) acids (Ramos et al., 2009). The oxidation stability is influenced by the presence of double bonds (Bajpai & Tyagi, 2006).

Cold flow properties of biodiesel indicate the behaviour of the fuel under low temperatures. It is an important property because when the temperatures are low, the fuel form wax and plugs filters and fuel lines (Dunn & Bagby, 1997). Cold filter plugging point (CFPP) is a measure of the lowest temperature at which a fuel will flow freely. Biodiesel produced from feedstocks with a high percentage of saturated fatty acids show high CFPP values. This is because when the fuel is cooled, the saturated fatty acids are the first to precipitate and since they are present in high concentrations, they will clog the biodiesel fuel filters (Mittelbach & Remschmidt, 2005). When the CFPP of peanut biodiesel was tested, it gave poor low-temperature properties, i.e. high CFPP values because of the presence of high amounts of methyl esters of long chain saturated fatty acids (M. Wu et al., 2005).

Biodiesel possess better lubricating properties than conventional diesel (Atadashi et al., 2010). It has been observed that there is a correlation between the fatty acid composition of FAMEs and their ability to improve the lubricity of biodiesel fuels. Some studies have shown that oils which contained high amounts of hydroxylated fatty acids produced FAME mixtures with higher lubricity that those of the non-hydroxylated fatty acids (Knothe & Steidley, 2005). In another

study, it was found that as the degree of unsaturation increased lubricity improving properties of the fuel also increased (Geller & Goodrum, 2004).

Viscosity of biodiesel is a measure of resistance of the fuel to flow. Viscosity increases at low temperatures and this affects the ease with which the fuel flows. This may tamper with the proper operation of the fuel injection equipment. The kinematic viscosity of biodiesel is much higher than that of petroleum diesel and this is attributed to the fact that biodiesel has a large molecular mass and chemical structure (Atabani et al., 2012). Factors such as fatty acid chain length, degree of unsaturation, position of the double bond affects the kinematic viscosity of biodiesel. The longer the chain length the higher the kinematic viscosity. When the degree of unsaturation is increased, the kinematic viscosity decreases. Position of the double bond also affects kinematic viscosity (Moser, 2011).

2.3.2 **Biodiesel quality**

For commercial use of biodiesel, quality standards need to be put in place in order to give consumers the confidence that the biodiesel they are getting is of high quality and meet stringent biodiesel standard specifications. Some countries have developed their own biodiesel standards. The United States uses the American Standard for testing Materials (ASTM D6751), the European Union uses EN 14214 Standards for biodiesel fuel and South Africa is using the SANS 1935 Automotive diesel fuel standard. Table 4 below shows the biodiesel standards developed in the different countries (Mbohwa & Mudiwakure, 2013).

Table 4. Biodiesel Standards for different countries (Mbohwa & Mudiwakure, 2013)

Specification	Units	Europe (EN 14214)	USA (ASTM D6751	South Africa SANS 1935
Density @ 15C	kg/m^3	0.86-0.90	0.86-0.90	0.86-0.90
Viscosity @ 40C	mm^2/s	3.5-5.0	1.9-6.0	3.5-5.0
Distillation	C	n/a	360	n/a
Flashpoint	C	120 min	130/93 min	120 min
Sulphur	mg/kg	10 max	15 max	10 max
Sulphated Ash	% mass	0.02 max	0.02 max	0.02 max
Water and sediments	mg/kg	500 max	500 max	500 max
Total contamination	mg/kg	24 max	n/a	24 max
Oxidation Stability	hr @ 110C	6 min	3 min	6 min
Cetane number		51.0 min	47 min	51 min
Acid value	mgKOH/g	0.50 max	0.50 max	0.50 max
Iodine number	$gI_2/100g$	120 max	n/a	140 max

2.4 FEEDSTOCKS FOR BIODIESEL PRODUCTION

Developing alternative energy source to replace traditional fossil fuels has recently become more and more attractive due to the high-energy demand, the limited resource of fossil fuel and environmental concerns. Biodiesel fuel derived from vegetable oils or animal fats is one of the promising possible sources to be substituted for conventional diesel fuel and produces favourable effects on the environment. It can be used directly or mixed with conventional fuel for diesel engines or as a heating fuel.

However, in spite of the favourable impact, the economic aspect of biodiesel production is still a barrier for its development, mainly due to the lower price of fossil fuels. The costs of biodiesel production are highly dependent on the costs of feedstock which affect the cost of the finished product up to 70–85%. Currently, partially or fully refined and edible-grade vegetable oils, such as soybean, rapeseed and sunflower, are the predominant feedstocks for biodiesel production. Consequently, this is the main reason for the high price of biodiesel. In this regard, one of the most important steps in biodiesel production process is the selection of feedstock. Different areas employ different feedstocks; their choice being mainly depended on climate, local soil conditions and availability.

Biodiesel production feedstocks can be primarily classified into; edible and non-edible oils, animal fats and waste cooking oils (Karmakar et al., 2010). Biodiesel is mainly produced from edible oils such as rapeseed oil, sunflower oil, palm oil, soybean oil and coconut oil (Zahan & Kano, 2018). However, commercialization of biodiesel has been hindered by the high cost of these feedstocks, which account for about 70-85% of the overall biodiesel production cost (Gnanaprakasam et al., 2013), In addition, the use of these edible oils have raised concerns of food supply shortage (Chhetri et al., 2008). If more edible oils are converted to biodiesel, there may be less available for food and this may cause an increase in food prices and subsequently, starvation.

What makes non-edible oils attractive as alternatives to edible oils is the fact that they can be grown in wastelands that are not suitable for food production and the cost of cultivation is much lower due to low maintenance needed (Fatah et al., 2012). Furthermore, they are suitable for arid or semi-arid conditions and they require less moisture to grow (Atabani et al., 2013). Examples of the non-edible oils suitable for biodiesel production are jatropha, castor bean seed, neem, karanja, rubber seed tree, tobacco seed, etc. (Demirbas et al., 2016b). However there are certain problems associated with the use of these non-edible oils; the presence of high concentrations of free fatty acids result in an increase in the cost of biodiesel production (Banković-Ilić et al., 2012), excessive cultivation of these oils may cause deforestation which may result in soil erosion and damage to natural habitats (Zahan & Kano, 2018). Some of the animal fats that can be converted to biodiesel

are tallow, lard, chicken fat and yellow grease. They are relatively cheap compared to edible oils which make them economically viable, however the amount of animal fats available are not enough to meet the worldwide biodiesel fuel production demand (Wen & Johnson, 2009). In addition, they may pose challenges during biodiesel production since they solidify at room temperature (Canakci, 2007). Waste cooking oil, although a promising feedstock for biodiesel production, requires pre-treatment steps to eliminate impurities, reduce free fatty acids (FFAs) and water contents, which makes the conversion to biodiesel complicated (Yaakob et al., 2013). The search for other renewable sources that are cheap, readily available and non-edible is still ongoing. One such feedstock that has drawn attention as a potential lipid feedstock for biodiesel production is municipal sewage sludge.

2.5 Municipal sewage sludge

2.5.1 Municipal Sewage sludge production in South Africa

A number of wastewater sludge management research has been conducted by the Water Research Commission (WRC) since 1984. Although the management of wastewater sludge is the responsibility of local authorities, the Department of Health took over the management and disposal of sludge in the 1970s. They published some guidelines into the use of sewage sludge (Van der Waal et al., 2008). These guidelines stated that all types of sludge may be used, only limited and important requirements and conditions are put in place, quality control to be carried out on a few parameters, care should be taken to ensure minimal unsatisfactory impact on human health and the environment. In 1989 the Sludge Management Division of the Water Institute of Southern Africa was formed and the document "Sewage sludge utilisation and disposal information document" was put together (Van der Waal et al., 2008).

In 1991, the Department of Health published the "Permissible utilisation and disposal of sewage sludge" which defined the acceptable use of sludge with respect to land. In 1997, the "Permissible utilisation and disposal of sewage sludge, Edition 1" was published in order to promote safe sewage sludge management. However,

these guidelines were found to be too strict mainly on the permissible metal concentration in the sludge (Snyman et al., 2000). This led to non-compliance to the sludge management practices by wastewater treatment plants. It was for this reason that in 2002 an addendum was published to develop suitable guidelines to encourage safe sewage sludge management (Snyman et al., 2006).

However, with sludge production increasing each year, it was evident that the current sludge disposal and management practices were unsustainable and sludge management was continuing to become a challenge for many municipalities in South Africa. Therefore, it was necessary to find innovative solutions to sludge management. The South African Water Research Commission (WRC) initiated research programmes to further improve knowledge for the management of sewage sludge in South Africa (Snyman, 2018). From their findings they were able to develop new set of guidelines for the management of wastewater sludge in South Africa. The South African guidelines consist of a set of 5 volumes " Guidelines for the Utilisation and Disposal of Wastewater Sludge, Volume 1-5" (Van der Waal et al., 2008). These guidelines encourage the beneficial use of sewage sludge while controlling sludge disposal by adhering to strict disposal requirements.

2.5.2 **Municipal Sewage Sludge as feedstock for biodiesel production**

Municipal Sewage Sludge (MSS) is a product formed during the municipal wastewater treatment process. Wastewater treatment plants (WWTP) produce large amounts of sewage sludge annually, and with the world population expected to increase (*World Population Prospects*, 2017), the amount of sludge is expected to increase further in the future. MSS generated requires further treatment before it can be disposed or used for other purposes and this treatment contributes about 50% of the total operating costs of the WWTP (Boocock et al., 1992). The standard practice of sludge disposal namely; incineration, land application and landfill disposal of sewage sludge may release toxins and heavy metals that are harmful for the environment (Pokoo-Aikins et al., 2010). It is for these reasons that alternative, cost effective and beneficial ways of using the sludge are investigated as part of the sewage sludge management.

Research into the use of MSS as a feedstock for biodiesel production has gained interest due to the significant amount of lipids found in the sludge (Dufreche et al., 2007; Revellame et al., 2010; Siddiquee & Rohani, 2011). These lipids include triglycerides, diglycerides, monoglycerides, phospholipids and free fatty acids (Kargbo, 2010). It is indicated that dry sludge can contain up to 30wt% of lipids (Kwon et al., 2012). Other factors that make the use of sludge for biodiesel production attractive are that it is abundant, readily available, inexpensive and may assist in the challenge of sludge management and disposal. Table 5 shows the constituents of municipal sewage sludge.

Table 5. Sewage sludge components (Bharathiraja et al., 2014)

Sewage sludge components	
Microorganisms	Pathogenic bacteria, virus and worms eggs
Biodegradable organic materials	Oxygen depletion in rivers, lakes and fjords
Other organic materials	Detergents, pesticides, fat, oil and grease, colourings, solvents, phenols, cyanide
Nutrients	Nitrogen, phosphorus, ammonium
Metals	Hg, Pb, Cd, Cr, Cu, Ni
Other inorganic materials	Acids, for example, hydrogen sulphide, bases

2.5.3 **Wastewater Treatment Plant Process**

The wastewater treatment plant consists of two main types of sludge; primary and secondary sludge. The primary sludge is composed of floating greases and solids and this is collected at the bottom of the primary clarifier after screening and grit removal. Secondary sludge consist of microbial cells and suspended solids that are produced during the aerobic biological treatment and is collected in the secondary settler (Mondala et al., 2009). Secondary sludge contains a variety of microorganisms that use the organic and inorganic compounds in the water as a source of energy, carbon and nutrients (Dufreche et al., 2007). After thickening, the remainder of the secondary sludge is mixed with thickened primary sludge and a blend of the two is formed (Olkiewicz et al., 2012). Blended sludge is then

directed into the anaerobic digester to reduce the level of pathogens, odours and solids. After this process, stabilized sludge is then formed (Demirbas, 2017). Figure 2 shows a schematic diagram of a typical municipal wastewater treatment plant.

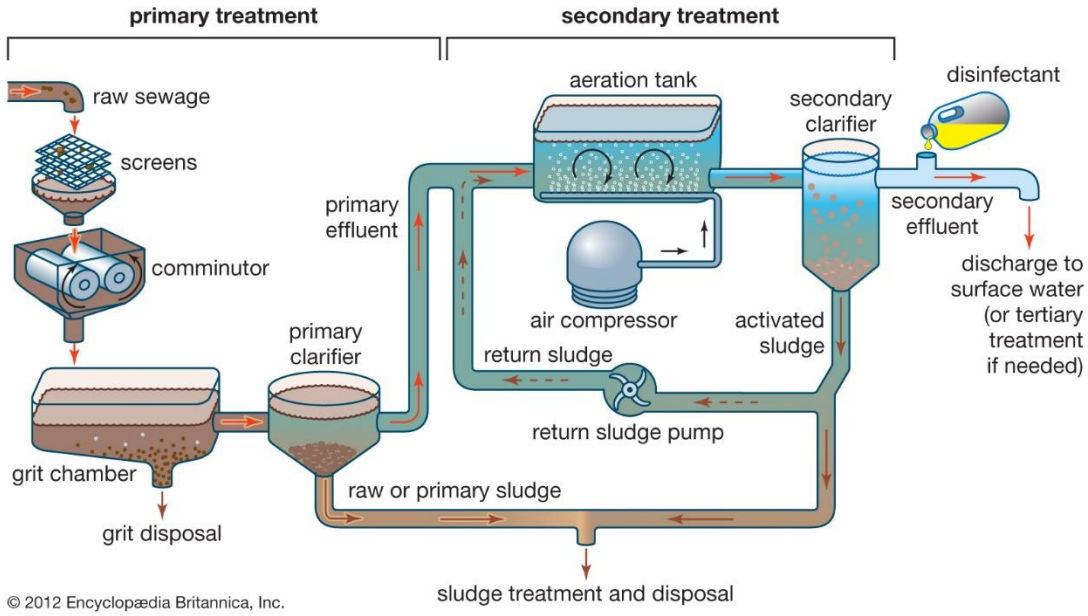


Figure 2. A Schematic diagram of a typical wastewater treatment plant.

2.5.4 **Pre-treatment of sludge for efficient lipid extraction**

Some studies have demonstrated that pre-treatment methods such as ultrasonic, mechanical disintegration and acid hydrolysis can be used to increase the lipid extraction yield from biological samples (Metherel et al., 2009; Pernet & Tremblay, 2003). These pre-treatment methods work by releasing the lipids from the other macromolecules which are not available to solvent when bonded with the molecules. In sludge pre-treatment, the most common methods are ultrasonication and mechanical disintegration (Baier & Schmidheiny, 1997; Kopp et al., 1997). Ultrasonic energy disintegrates sludge flocs and disrupts large organic particles, breaking down bacterial cell wall and releasing intracellular and extracellular substances into the aqueous phase (Appels et al., 2008). In mechanical

disintegration, the size of the sludge particles is reduced, the cells are disintegrated and organic components are released into the sludge (Kopp et al., 1997).

In acid hydrolysis, the acid assists in improving the solubility of the organic matter that is encapsulated within the sludge. This makes available more lipids that can be extracted (Neyens et al., 2003). A study carried out by Olkiewicz et al., 2014, concluded that acidification of sludge subjected to prior drying methods decreased the yield of lipids thus reducing biodiesel yield. In direct liquid-liquid lipid extraction using hexane where no prior drying on the sludge was carried out, acidification improved the lipids extracted and subsequently biodiesel yield. This was also proven by Babayigit et al., 2018 in their study to explore lipid extraction from municipal and petrochemical industry WWTP sludge by using the direct liquid-liquid lipid extraction method, which does not require the expensive dewatering or drying steps and comparing it to the standard drying method.

The results demonstrated that with acid pre-treatment, the lipid yield increased with direct liquid-liquid lipid extraction as compared to the standard drying method. The work done by Olkiewicz et al., 2015 demonstrated that pre-treatment of dried primary, secondary and blended sludge by ultrasonic, mechanical disintegration and acid hydrolysis did not significantly increase the amount of lipids extracted and the biodiesel yield. In this case, the pre-treatment methods were not suitable for biodiesel production from municipal sewage sludge. This is in agreement with the findings by Olkiewicz et al., 2014 that drying methods affect the amount of lipids extracted from pre-treated sludge thus reducing biodiesel yield.

2.5.5 **The lipid extraction method**

A number of techniques such as the Bligh & Dyer, Folch, and Soxhlet have be used to extract lipids from sewage sludge. The most common way of extracting lipids from the sludge is the use of organic solvents, either on their own or in mixtures. The extraction can be carried out using dry or wet sludge. The Soxhlet extraction uses solvents such as hexane, methanol or ethanol at temperatures between 70ºC and 80ºC. This method requires that the sludge be dried before use.

Zhu et al., 2014 compared three different lipid extraction methods. Soxhlet, acid hydrolysis and water bath shaking extraction methods were evaluated. The Soxhlet extraction achieved the highest lipid (10.3%) and biodiesel (6.35%) yields as compared to the other two methods. Mustapha et al., 2017 studied the extraction of lipids from primary sewage sludge by Soxhlet using methanol as a solvent. A lipid yield of 40.21% was obtained. Extraction with wet sludge has the advantage that no expensive drying methods are required. The cost of drying contributes more than 50% of the total cost of biodiesel production (Dufreche et al., 2007). Additionally, removing the water by thermal drying will result in the loss of beneficial organic materials which may reduce the lipid yield.

Olkiewicz et al., 2014 investigated extraction of lipids from wet sludge using hexane as a solvent. The extraction was carried out at room temperature for 1hr at a 2:1 sludge:hexane ratio. The sludge was acidified using hydrochloric acid. A lipid yield of 26.7% and a biodiesel yield of 19.2% were achieved and this was higher than yields obtained when standard drying methods were applied (25% lipid and 17.6% biodiesel yields). Kech et al., 2018 carried out the liquid-liquid extraction using a mixture of solvents cyclohexane/isopropyl alcohol/water (Smedes mixture). This method gave an extraction yield of 34.5% compared to 32.8% when hexane was used. The advantage of this method is that it is inexpensive and does not have any negative effect on the environment.

Babayigit et al., 2018 studied the extraction of lipids from municipal and petrochemical industry sludges by direct liquid-liquid extraction method and this was compared to the standard drying method. Acid pre-treatment effect on lipid yield was also investigated. The results showed a lipid yield of 31.2% from the non-acidified petrochemical industry sludge with the liquid-liquid lipid extraction method. It was found that the direct liquid-liquid lipid extraction method achieved a lipid yield that was 53% higher and a biodiesel yield that was 56% higher compared to the standard drying methods for the petrochemical industry sludge.

Ionic liquids such Tetrakis (hydroxymethyl) phosphonium chloride $[P(CH_2OH)_4]Cl$ and 1-butyl-3-methylimidazolium methyl sulphate $[C_4mim][MeSO_4]$ can also be used for the extraction of lipids from sewage sludge.

Olkiewicz et al., 2015 studied the effect of $[P(CH_2OH)_4]Cl$ and $[C_4mim][MeSO_4]$ in the extraction of lipids from raw and dried sludge and compared the results to those obtained when using the Soxhlet method. The $[C_4mim][MeSO_4]$ ionic liquid produced 18.5% and 26.9% lipid yields and 14.1% and 18.4% biodiesel yields for dried and raw sludge respectively. The $[P(CH_2OH)_4]Cl$ ionic liquid produced lipid yields of 23.4% and 27.6% and biodiesel yields of 17.0% and 19.8% from dried and raw sludge respectively. These results were comparable to those obtained from the Soxhlet method where the lipid and biodiesel yields were 27.2 % and 19.4% respectively. They also concluded that the ionic liquid method is efficient in extracting lipids from wet sludge, eliminating the expensive drying methods.

2.5.6 **Biodiesel production by transesterification**

Four methods have been identified for the production of biodiesel, namely; pyrolysis, dilution, micro-emulsion and transesterification. Transesterification has been identified as the best method for biodiesel production as compared to the other three methods since it is inexpensive and simple to use.

Transesterification is the chemical reaction that takes place between a triglyceride and an alcohol in the presence of a catalyst to form esters and glycerol. The general transesterification process is shown in figure 3 (Mishra & Goswami, 2018).The alcohols that can be used in the transesterification are methanol, ethanol, propanol, butanol and amyl alcohol. Methanol is the most preferred alcohol due to its low cost, high reactivity, does not absorb water that can interfere with the transesterification process, prevents soap formation and is easily recovered (Chongkhong et al., 2009; Demirbas, 2008a). Biodiesel consists of fatty acid alkyl esters (FAAEs) that are produced by the catalysed transesterification of edible or non-edible oils, animal fats and waste cooking oils in the presence of an alcohol. When methanol is used in the transesterification, the biodiesel produced is termed fatty acid methyl esters (FAMEs) (Kargbo, 2010).

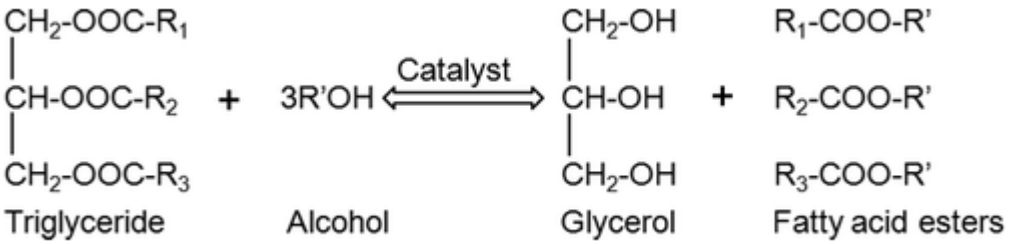


Figure 3. Transesterification process.

It can be observed from Figure 3, for the reaction to be in equilibrium, 3 moles of alcohol for 1 mole of triglyceride are required to produce 1 mole of glycerol and 3 moles of fatty acid esters. In practice, ratios higher than the 3:1 molar ratio of alcohol to triglycerides are used in order to achieve higher yields of esters (Fukuda et al., 2001).

The type of catalyst used in the transesterification reaction plays an important role in the conversion of triglycerides to biodiesel. Catalysts used in the transesterification of triglycerides can be classified as acid or alkali, (Fukuda et al., 2001; Meher et al., 2006) and lipase enzymes, (Sharma et al., 2001). Examples of alkali catalysts used in the production of biodiesel are sodium hydroxide (NaOH), potassium hydroxide (KOH), sodium methoxide (CH_3ONa) and potassium methoxide (CH_3OK) (Sharma et al., 2008). NaOH and KOH are the most commonly used alkali catalyst due to their low cost (Fukuda et al., 2001). Transesterification reaction rates of alkali catalysed reactions are 4000 times faster than acid catalysed reactions (Fukuda et al., 2001). A number of researchers have explored the effectiveness of alkali based catalysts in the transesterification of different raw materials to produce biodiesel. Their results have indicated ester yields of above 90%.

One study indicated ester yield of 97.1% wt. at 60 ^oC when an alkali catalyst was used in the production of sunflower oil methyl esters (Rashid et al., 2008). Another study conducted by Chung, 2010 in the transesterification of two different types of seed oils with methanol using the alkali catalysts KOH, NaOH and CH_3OH under the same reaction conditions showed that KOH was the most effective

catalyst giving biodiesel yields of above 96%. The use of alkali catalysts during transesterification where the raw materials contain high FFAs is undesirable since this may lead to soap formation through a saponification reaction. This affects the purity of the biodiesel formed and thus a decrease in the biodiesel conversion and yield (Marchetti et al., 2007).

In addition, the presence of high water content in the raw materials may affect the biodiesel yield. This is because the water can hydrolyse trigylcerides to diglycerides to form free fatty acids. Therefore, in the presence of a base catalyst, the FFAs will react to form soaps. These soaps may solidify and form a semi-solid mass that is difficult to recover (Felizardo et al., 2006). Figure 4 shows the reaction mechanism of a transesterification reaction of triglycerides in the presence of a base catalyst.

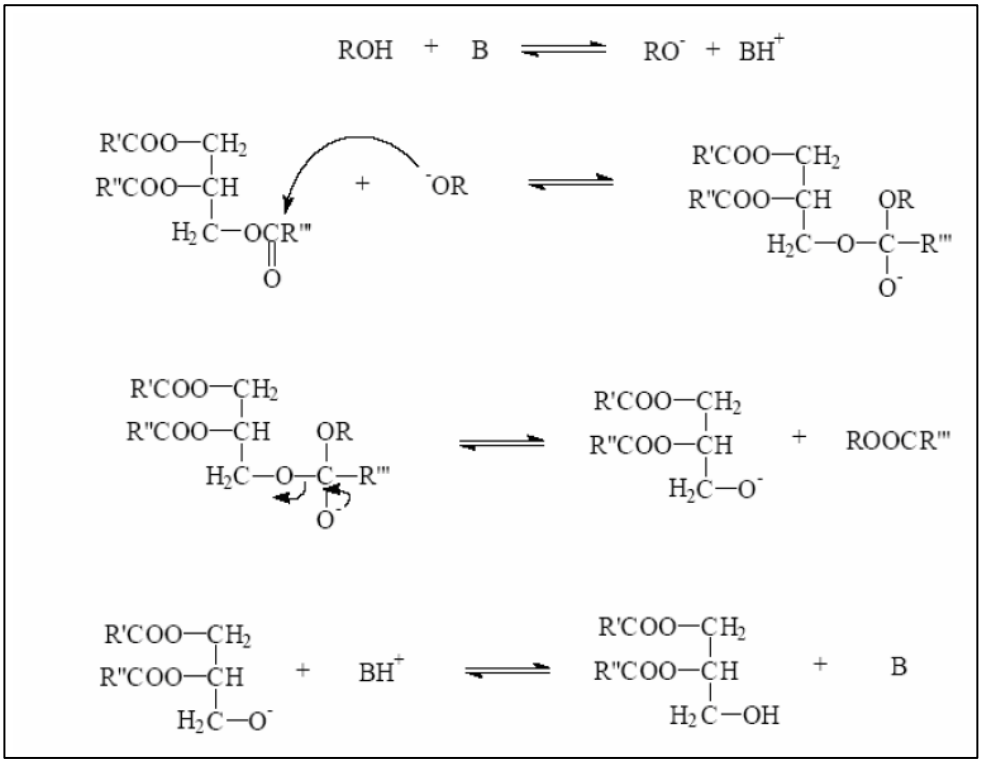


Figure 4. Mechanism of base catalysed transesterification reaction (Sivasamy et al., 2009).

The most commonly used acid catalysts in the production of biodiesel by transesterification are sulphuric acid (H_2SO_4) (Induri et al., 2010) and hydrochloric acid (HCl) (Demirbas, 2009). Due to problems posed by base catalysts in the

presence of high amounts of FFAs and water, acid catalysts have been investigated to overcome these challenges. Acid catalysts in contrast to base catalysts are insensitive to the presence of FFAs in the raw materials (Kulkarni & Dalai, 2006). Additionally, catalysis of the esterification and transesterification can occur simultaneously (Jacobson et al., 2008). It has been reported that the use of acid catalysts is more cost effective than that of base catalysts which require an additional step to convert FFAs to methyl esters (Zhang et al., 2003).

Given that acid catalysed transesterification reactions are much slower than alkali catalysed reactions, the reaction is influenced by alcohol to oil molar ratio, reaction time, temperature and catalyst amount(Atadashi et al., 2013). In one study carried out by Wang et al., 2006 in the transesterification of waste cooking oil using H_2SO_4 as a catalyst, it was noted that FAME yield increased with increased methanol to oil ratio, longer reaction time and increased catalyst concentration. The reaction time was 10hrs, methanol to oil ratio was 20:1 and 4wt% H_2SO_4. A yield of more than 90% was achieved. In another study, 99% FAME yield was reported when 1mol% of H_2SO_4 was used, methanol to oil ratio of 30:1 at a reaction time of 69hrs. Figure 5 shows the reaction mechanism of the transesterification of triglycerides using an acid catalyst.

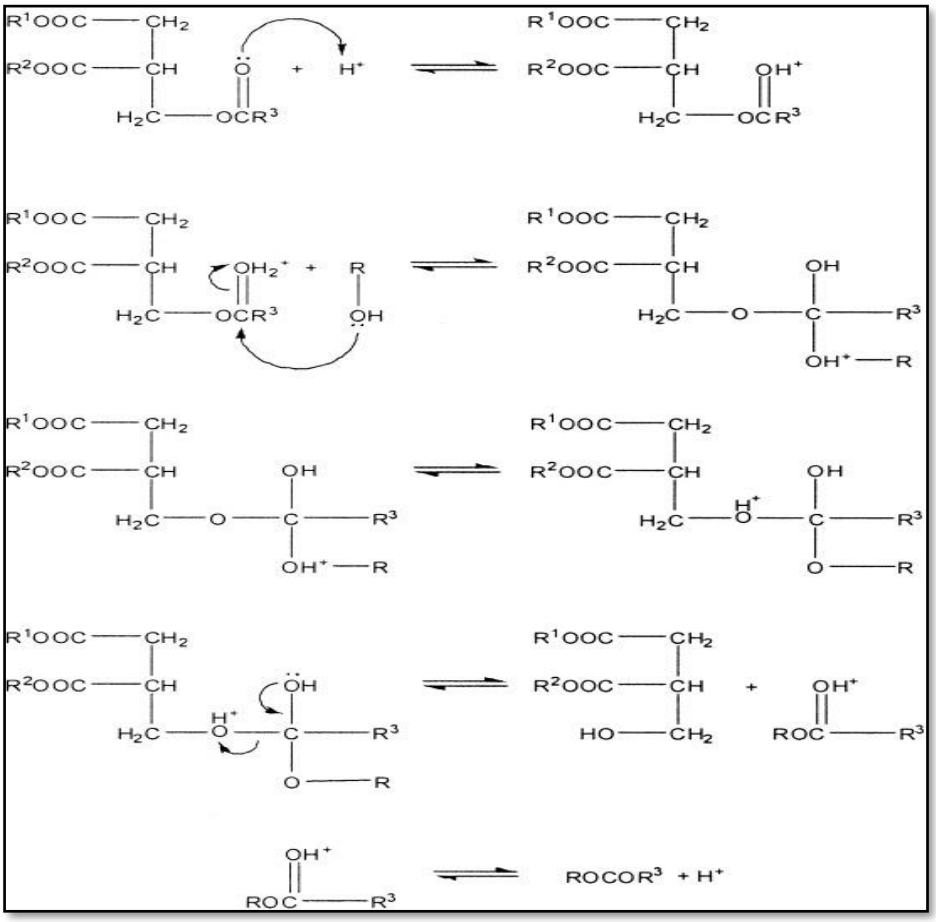


Figure 5. Mechanism of acid catalysed transesterification reaction (Sivasamy et al., 2009).

During the acid or base catalysed transesterification reactions, challenges such as pre-treatment of feedstock, difficulty in the recovery of glycerol, removal of catalyst and high energy demand that increase the costs of production of biodiesel are experienced (Wu et al., 1999). This has led to the research in the use of enzymatic catalysts in the transesterification process. In enzymatic catalysis, there are no by-products formed, product recovery is uncomplicated, catalyst performance is unaffected by high amounts of FFAs, mild reaction conditions are required and the catalyst can be reused (Kulkarni & Dalai, 2006). Some of the disadvantages of enzymatic catalyst are that the catalyst is expensive, the transesterification reaction is slow, there is a possibility of the enzyme being deactivated.

Although the use of enzymatic catalysts for biodiesel production looks attractive, there are still some obstacles to be overcome such as the high cost of the enzyme, longer time required to complete the reaction, low product yield, high energy consumption, the need for organic solvents to increase the solubility between methanol and glycerol and enzyme deactivation (Bajaj et al., 2010). Some studies have been carried out using different types of enzymes to show their effectiveness to catalyse transesterification reactions using different feedstock to produce biodiesel. Halim & Kamaruddin, 2008 studied the transesterification of tallow with primary alcohol in the presence of an enzyme, Lipozym IM 60. They reported biodiesel yields of above 93%. In another study, Nelson et al., 1996, used *Pseudomonas cepacia* in the transesterification of tallow to biodiesel. Low biodiesel yields of 13.9-28.8% were obtained. This may mean that the amount of biodiesel produced is affected by the type of enzyme used in the transesterification process.

Chapter 3: MATERIALS AND METHODS

3.1 Samples collection & analyses

Experiments were conducted using chloroform, methanol, hydrochloric acid, hexane, sulphuric acid, sodium chloride, sodium bicarbonate and anhydrous sodium sulphate. Ionic liquid solution, Tetrakis (hydromethyl) phosphonium chloride was supplied by Sigma-Aldrich. Standard used for the identification and quantification of fatty acid methyl esters (FAMEs) was the Supelco 37 component FAMEs mix.

Primary and secondary sludge samples were collected from a municipal wastewater treatment plant (WWTP) in Johannesburg. Primary sludge was collected before partial dewatering, and secondary sludge was collected after partial thickening by flotation. Blended sludge was prepared by mixing primary and secondary sludge at a ratio of 65:35 v/v (Olkiewicz, et al., 2015).

Figure 6 shows an aerial view of the wastewater treatment plant where the primary and secondary sludge samples were collected.



Figure 6. Aerial View of the wastewater treatment plant used for this research work.

The plant consists of four activated sludge modules. The wastewater flow is split between Modules 1 to 3 and Module 4. Approximately 257 Mega litres (Ml) of wastewater is treated per day. Approximately 177Ml/d flows through Module 1 to 3 and 80 Ml/d flows through Module 4. The plant produces about 40 tons per day of primary sludge and 20 tons per day of secondary sludge. Once the sludge has been dewatered and dried, it is supplied to farmers for use as fertilizer. Table 6 shows some of the processing units found in this plant.

Table 6. Process units of the wastewater treatment plant

Unit Process	Name	Function
	Manual screens	Manual screens are used to remove large solid particles before the wastewater enters the plant. A hand rake is used.
	Mechanical screens	Mechanical screens are used to remove solid particles from the wastewater before it enters the plant. Cleaning is done mechanically.

	Screen Conveyer	The screen conveyer is used to convey the screenings and discard them in a waste bin.
	Degritter	A degritter separates grit and inorganic solids from wastewater
	Primary Settlement Tank	The tank is designed to reduce the wastewater flow velocity allowing the raw primary sludge to settle. This is the first stage of wastewater treatment after grit removal.
	Primary Sludge Pumps	Primary sludge pumps transfer primary sludge to the primary anaerobic digesters.

	Anaerobic mixers	Anaerobic mixers assist in maintaining the quality of the decomposition process by improving circulation and minimizing grit layers.
	Secondary Settling Tank	Secondary Settling Tanks are used to remove organic matter and reduce nutrients such as nitrogen and phosphorus. The function of this tank is to thicken the sludge.
	Dewatered Sludge Conveyer Belt	The conveyer belt conveys the sludge to the storage area.

	Sludge Drying Area	This is the final drying area of the sludge and this sludge is supplied to farmers to use as fertilizer.

Each sample of sludge was characterized in order to determine total solids (TS) and volatile solids (VS) according to the following methods. The pH of all the sludge samples was also measured.

3.1.1 **Total Solids**

The initial weight of the crucible was noted. 20ml of sample was poured in the crucible. The crucible was placed in an oven and heated at 105ºC. After drying in the oven, the sample was cooled to room temperature in a desiccator. The final dry weight of the crucible was then noted. Total solids were calculated according to the formula:

$$Total\ Solids = \frac{(w_2 - w_1)}{vol.of\ sample(ml)} \times 1000$$

where w_1 = Initial weight of crucible

w_2= Final weight of crucible after drying at 105ºC

3.1.2 **Volatile Solids**

The initial weight of the crucible was noted(w_1). 20ml of sample was poured in the crucible. The crucible was placed in an oven and heated at 105ºC. After drying in the oven, the sample was cooled to room temperature in a desiccator. The final dry weight of the crucible was then noted(w_2). The oven dried sample was then placed in a furnace and ignited at 600ºC for 15 minutes. After ignition, the sample was cooled to room temperature in the desiccator. The final dry weight of the

crucible was then noted(w_3). Volatile solids were then calculated according to the formula:

$$Volatile\ Solids = \frac{w_2 - w_3}{vol.of\ sample\ (ml)} \times 1000$$

where w_1= Initial weight of crucible

w_2= Final weight of crucible after drying at 105ºC

w_3= Final weight of crucible after drying at 600ºC

3.1.3 **Acidification of sludge samples**

Before the extraction, primary, secondary and blended sludge samples were acidified with concentrated hydrochloric acid until pH 2 was obtained for all samples. These samples were used directly in the lipid extraction experiment.

3.2 EXTRACTION

3.2.1 **Extraction of lipids using the Bligh & Dyer Method**

Primary, secondary and blended sludge samples were used as is without prior drying. Lipids were extracted from the samples using a modified version of the Bligh & Dyer (B&D) method (Bligh & Dyer, 1959). The lipids were extracted by shaking 6ml of the sludge sample with a solvent mixture of 7.5ml chloroform and 15 ml methanol (1: 2 v/v) in a separating funnel. Then 7.5ml chloroform and 7.5ml distilled water were added to form a two-phase system. The bottom chloroform layer, containing the lipids, was separated. The extraction was repeated once more by adding a further 7.5ml of chloroform to the remaining solution of water and methanol system. The chloroform layers were combined, washed with distilled water, dried over anhydrous sodium sulphate, and the solvent removed in a rotary evaporator. The extracted lipids were stored in a desiccator overnight, and weighed the next day.

3.2.2 **Extraction of lipids using the ionic liquid, Tetrakis (hydromethyl) phosphonium chloride [P(CH2OH)4]Cl**

Primary, secondary and blended sludge samples were used as is without prior drying. Each sludge sample was mixed with Tetrakis (hydroxymethyl) phosphonium chloride $[P(CH_2OH)_4]Cl$ ionic liquid in a ratio 1g TS equivalent to $10cm^3$ ionic liquid in a round bottomed flask fitted with a condenser. The mixture was heated at 100°C in an oil bath for 24 hours under magnetic stirring (Olkiewicz et al., 2015). The mixture was then cooled to room temperature and then 10ml of methanol was added. 5ml of hexane was added to the mixture to extract the lipids. The extraction was repeated three times. The upper hexane phase containing the lipids was separated by centrifugation and then washed with 10ml of distilled water to remove traces of polar compounds. The hexane layer was dried over anhydrous sodium sulphate and evaporated in a rotary evaporator. The lipids were stored in a desiccator overnight and weighed the next day (Olkiewicz et al., 2015).

3.3 TRANSESTERIFICATION OF LIPIDS

Lipids extracted by both the B&D method and the ionic liquid method were converted into fatty acid methyl esters (FAMEs) by acid catalysed transesterification of sulphuric acid (H_2SO_4) in methanol using a modified version of Christie's method (Christie & Han, 2003). Figure 7 shows the setup used for the transesterification process.



Figure 7. Transesterification process

200 mg of lipids were dissolved in 10ml of hexane and transferred into a round bottom flask fitted with a condenser to minimize the loss of methanol due to evaporation. 20ml of 2% H_2SO_4 in methanol was added to the flask. The mixture was heated at 60°C for 24 hours and mixed with a magnetic stirrer. The resulting mixture was allowed to cool and then the reaction and the flask contents were transferred to a separating funnel. 10ml aliquot of 5% sodium chloride in water was added to the funnel. Then 20ml of hexane was added to the separating funnel and shaken. The extraction procedure with hexane was repeated three times. The hexane layer was washed with 10ml of 2% sodium bicarbonate in water followed by 10ml of warm water. This was dried by using anhydrous sodium sulphate and collected into a glass bottle. The hexane phase was transferred into a round bottom flask and the solvent removed using a rotary evaporator at 40°C to obtain FAMEs. Figure 8 shows the rotary evaporator used to separate the FAMEs from the hexane.



Figure 8. Rotary evaporator used to separate the FAMEs from the hexane

In the rotary evaporator, the hexane phase which is a mixture of hexane and FAMEs is transferred into a round bottom flask that is pre-weighed. The flask is then connected to the rotary evaporator using a plastic clip. The flask is then lowered into the water bath with the water temperature set at 40 ºC. The vacuum source is then turned on. The flask is rotated at a moderate speed. The solution is then allowed to evaporate and the solvent collected in the other round bottom flask connected to the rotary evaporator. Evaporation is continued until all the solvent has been removed and a residue is left in the flask. Once the evaporation is complete, the rotation is stopped and the vacuum is turned off. The flask is then lifted from the water bath and removed. The residue is then placed in a dessicator to dry overnight.

3.4 Characterization of fatty acid methyl esters (FAMEs)

3.4.1 Gas chromatograph-mass spectrometer (GC-MS) analysis of FAME

The FAMEs produced were analysed by GC-MS from Shimadzu. The injection volume of a sample was 1µl. The column temperature was at 140ºC and the injector temperature was at 220ºC. Helium was used as the carrier gas. Figure 9 below shows the GC-MS instrument used in this study.



Figure 9. GC-MS instrument used to analyse Fatty Acid Methyl Esters.

3.4.2 **Fourier Transform Infrared Spectroscopy (FT-IR) analysis of FAME**

Lipids and FAME (biodiesel) produced from primary sludge were used for this analysis. The instrument used to analyse the sludge samples is spectrum two FT-IR from Perkin Elmer. The instrument uses Spectrum 10^{tm} software for data generation and analysis. For the analysis the attenuated total reflection (ATR) accessory was used and the detection of the analysis achieved with MIR (Lithium tantalite) detector. The powder sample is pressed on a diamond prism of the ATR and the infrared spectrum is measured using infrared light that is totally internally reflected in the prism. Figure 10 shows the FT-IR instrument used for the analyses.

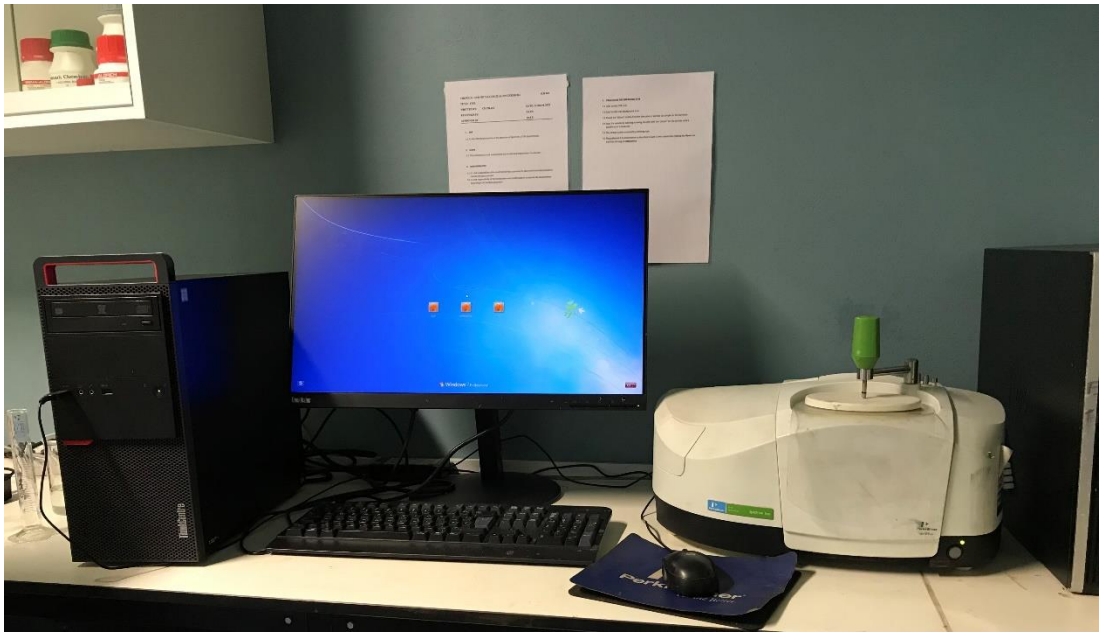


Figure 10. FT-IR instrument used to analyse lipids and FAME from primary sludge.

3.4.3 **Viscosity and Density Measurements**

The SVM 3001 instrument from Anton Paar was used to measure the density and kinematic viscosity of the primary biodiesel. This instrument consists of a viscosity and a density cell and therefore can measure both the viscosity and the density. The instrument covers the full viscosity, density and temperature range with a single measurement cell. Only 1.5ml sample is required for the analysis. The viscosity measuring cell contains a tube which rotates at a constant speed and is filled with sample. A measuring rotor with a built in magnet floats freely in the sample. The sample's shear forces drive the rotor while magnetic forces delay its rotation. After the measurements begin, the rotor reaches equilibrium speed which translates into the fluid's viscosity. The kinematic viscosity is then automatically calculated from the dynamic viscosity and density of the sample. The instrument is ASTM compliant. Figure 11 shows the instrument used to measure the viscosity and density of the primary diesel.



Figure 11. SVM instrument for viscosity and density measurements.

Chapter 4: RESULTS AND DISCUSSIONS

4.1.1 Characterization of Municipal Sewage Sludge

Table 7 below shows total solids, volatile solids and pH of the primary, secondary and blended sludge used in this study. The tests were performed in triplicates. Total solid and volatile solid contents of primary sludge was the highest, followed by blended sludge then secondary sludge. Total solids for primary, secondary and blended sludge were 5.3%, 3.1% and 4.6% respectively. Volatile solids for the primary, secondary and blended sludge were 3.7%, 2.6% and 2.9% respectively.

Table 7. Characteristics of the different sludge samples used in the study

Sludge Type	Total Solids (%)	Volatile Solids (%)	pH
Primary Sludge	5.3±0.0	3.7±0.0	5.12±0.0
Secondary Sludge	3.1±0.2	2.6±0.2	6.62±0.1
Blended Sludge	4.6±0.1	2.9±0.2	5.40±0.0

4.1.2 Percentage lipids and biodiesel yields from non-acidified and acidified sludge samples using the Bligh &Dyer extraction method.

Table 8 shows the amount of lipid extracted and the biodiesel yield from the acidified and non-acidified sludge samples using the Bligh & Dyer method.

Table 8. % Lipid and biodiesel yields from non-acidified and acidified sludge samples on the basis of dry sludge.

Sludge Type	Lipid Yield (%)	Biodiesel yield (%)
Non-Acidified		
Primary	14.75 ± 0.1	6.73 ± 0.7
Secondary	5.02 ± 0.1	2.35 ± 0.6
Blended	12.46 ± 0.1	4.71 ± 0.8
Acidified		
Primary	15.12 ± 0.0	8.02 ± 0.5
Secondary	5.73 ± 0.4	1.47 ± 0.2
Blended	13.86 ± 0.0	7.63 ± 0.7

The tests were performed in triplicated

The tests were performed in triplicates. Non-acidified primary, secondary and blended sludge achieved lipid yields of 14.75%, 5.02% and 12.46% respectively and biodiesel yields of 6.73%, 2.35% and 4.71% respectively. From the results it can be seen that primary sludge produced the most lipids and the highest biodiesel yield, followed by blended sludge then secondary sludge. This is expected since primary sludge is untreated raw sludge that consists of organic matter (a combination of solids and grease) that is screened to remove grit. Since no biological treatment is applied to the sludge, the organic compounds, which include lipids are stored in the sludge in primary sedimentation.

Secondary sludge is made up of microbial cells and suspended solids produced during the aerobic biological treatment of primary sludge. During this treatment, organic matter including lipids are reduced due to digestion by microorganisms (Zhu et al., 2017). The lipid content of blended sludge is intermediate, slightly lower than the lipid content from primary sludge, since blended sludge is a

mixture of both the primary and secondary sludge with a higher fraction of the primary sludge. Lipid yields from acidified primary, secondary and blended sludge were 15.12%, 5.73% and 13.86% respectively and biodiesel yields were 8.02%, 1.47% and 7.63% respectively. Primary sludge produced the highest lipid and biodiesel yields, followed by blended sludge then secondary sludge. This is the same trend as observed in the non-acidified sludge samples. It can also be noted that the lipid and biodiesel yields of the acidified sludge samples were slightly higher than those of the non-acidified sludge samples. This is because the presence of the acid in the sludge improves the solubility of the organic matter encapsulated within the sludge, thus making available more lipids to be extracted (Neyens et al., 2003).

These findings are in agreement with those obtained by Olkiewicz et al., 2014. They tested the primary, secondary and blended sludge without prior drying. Among the samples tested, they found that primary sludge generated the highest yield, followed by blended sludge and then secondary sludge for both acidified and non-acidified samples. The lipid yields were slightly higher for acidified sludge samples.

4.1.3 **Percentage lipids and biodiesel yields from the different sludge samples using the ionic liquid extraction method.**

Table 9 shows lipid and biodiesel yields obtained when the ionic extraction method was used.

Table 9. % Lipid and biodiesel yields from the different sludge samples using the ionic extraction method on the basis of dry sludge.

Sludge Type	**Lipid Yield (%)**	**Biodiesel yield (%)**
Primary	9.30 ± 0.6	4.92 ± 0.9
Secondary	1.84 ± 0.6	0.83 ± 0.2
Blended	6.16 ± 0.7	3.17 ± 0.5

The tests were performed in triplicated

The ionic liquid used for this study is Tetrakis (hydromethyl) phosphonium chloride $[P(CH_2OH)_4]Cl$. From the results it can be observed that the lipid yields for primary, secondary and blended sludge were 9.30%, 1.84% and 6.16% respectively and the biodiesel yields were 3.92%, 0.84% and 3.17% respectively. The lipid and biodiesel yields for primary sludge were the highest, followed by blended sludge then secondary sludge. This indicates that the ionic liquid, $[P(CH_2OH)_4]Cl]$ successfully extracted lipids from the different sludge samples. Municipal sewage sludge, apart from lipids, contains proteins and high amounts of cellulose which originate from waste paper (Honda et al., 2002).

The ionic liquid which is hydrophilic and miscible in water, will dissolve protein and cellulose which are also hydrophilic. Lipids which are hydrophobic will be left undissolved and separated by centrifugation (Kim et al., 2012). This is in agreement with the work done by (Olkiewicz et al., 2015) which demonstrated the ability of ionic liquids to extract lipids from biomass. (Olkiewicz et al., 2015) studied the extraction of lipids from dried and raw primary sludge using ionic liquids. The results obtained showed that the lipids extracted using $[P(CH_2OH)_4]Cl]$ were 23.4% and 27.6% and the biodiesel yield 17.0% and 19.8%

for dried and raw sludge respectively. In another study using microalgae, (Olkiewicz et al., 2015) evaluated the extraction of lipids from two different types of microalgae using $[P(CH_2OH)_4]Cl]$. The results obtained lipid yields of 8.1 % and 12.8% and biodiesel yields of 4.5% and 8.1% for wet and dry biomass respectively. Table 10 shows the lipid yields obtained when lipids are extracted with ionic liquid $[P(CH_2OH)_4]Cl]$ from different biomass.

Table 10. Lipid yield of different biomass extracted using ionic liquid $[P(CH_2OH)_4]Cl]$.

Biomass	**Lipid Yield (%)**	**Reference**
Primary Sludge (wet)	9.30 ± 0.6	This study
	27.6 ± 0.6	(Olkiewicz et al., 2015)
Primary Sludge (wet)	23.4 ± 0.5	(Olkiewicz et al., 2015)
Primary Sludge (dry)	8.1 ± 0.3	(Olkiewicz et al., 2015)
Microalgae *(C. vulgaris)*		

4.1.4 **Characterization of biodiesel produced from the different sludge types by Gas Chromatography-Mass Spectrometry.**

It is important to determine the fatty acid composition of the lipids used for biodiesel production as their composition will directly affect the properties of the biodiesel produced (Knothe, 2005). The fatty acid compositions of the different sludge samples were evaluated using the GC-MS and the results are presented in Tables 11, 12, 13 and figures 12, 13 and 14.

Table 11 shows the fatty acid composition of biodiesel produced from primary, secondary and blended sludge samples. The biodiesel was produced from lipids extracted by the Bligh and Dyer method. Figure 12. shows the characteristic peaks of fatty acid methyl esters from each sludge type. From Fig. 12 and Table 11 it can

be seen that all the sludge types are characterized by similar fatty acid distribution with the main peaks at C14:0, C15:0, C16:0, C18:0, and C18:1. For primary sludge, C16:0, C18:0, C14:0 and C18:1 composition were 42.85%, 21.88%, 10.17% and 7.69% respectively. For secondary sludge, C16:0, C18:0, C14:0 and C18:1 composition were 20.95%, 21.38%, 10.93% and 9.23% respectively. For blended sludge, C16:0, C18:0, C14:0 and C18:1 composition were 43.99%, 21.37%, 10.79% and 7.34% respectively. While all three sludge types followed the same sequence of fatty acid distribution of the main peaks, primary and blended sludge showed similar percentage distribution compared to secondary sludge. The percentage of unsaturated fatty acid methyl esters for primary, secondary and blended sludge were 10.17, 13.68 and 9.65 respectively.

Table 11. Fatty Acid composition of biodiesel produced from non-acidified sludge samples.

Fatty Acid	Compound	Primary	Secondary	Blended
C6:0	Hexanoic Acid Methyl Ester	1.10		
C9:0	Octanoic Acid, 2-Methoxy-, Methyl Ester		0.16	
C12:0	Dodecanoic Acid Methyl Ester	4.52	4.42	5.47
C13:0	Tridecanoic Acid Methyl Ester		0.88	
C14:0	Tetradecanoic Acid Methyl Ester	10.17	10.93	10.79
C15:0	Tetradecanoic Acid, 12-Methyl-, Methyl Ester		3.55	
C15:0	Pentadecanoic Acid Methyl Ester	2.63	6.08	3.02
C16:0	Hexadecanoic Acid Methyl Ester	42.66	20.52	43.94
C16:0	Hexadecanoic Acid, 3-hydroxy-, Methyl Ester	0.19	0.43	0.05
C16:1	9-Hexadecenoic Acid Methyl Ester (Z)	0.43	3.46	0.51
C16:1	11-Hexadecenoic Acid Methyl Ester (Z)	0.36		0.39
C16:1	2-Hexadecenoic acid, 2,3-dimethyl, Methyl Ester			0.04
C17:0	Hexadecanoic Acid, 15-Methyl, Methyl Ester	0.48		0.54
C17:0	Heptadecanoic Acid Methyl Ester	0.47	1.41	0.49
C17:1	10-Heptadecenoic Acid Methyl Ester		0.23	
C18:0	Octadecanoic Acid Methyl Ester	21.14	20.40	20.63
C18:0	Octadecanoic Acid, 3-hydroxy-, Methyl Ester	0.15	0.33	0.09
C18:0	Octadecanoic Acid, 10-oxo-, Methyl Ester	0.59	0.65	0.65
C18:1	9-Octadecenoic Acid Methyl Ester (Z)	3.87	9.23	3.44
C18:1	13-Octadecenoic Acid Methyl Ester (Z)	3.82		3.90
C18:2	9,12-Octadecadienoic Acid Methyl Ester (Z,Z)	1.62	0.80	1.31
C19:0	Nonadecanoic Acid Methyl Ester	0.05		0.04
C20:0	Eicosanoic Acid Methyl Ester	1.61	1.55	1.20
C20:1	11-Eicosenoic Acid Methyl Acid (Z)	0.07		
C21:0	Eicosanoic Acid, 18-Methyl-, Methyl Ester	2.02		2.16
C22:0	Docosanoic Acid Methyl Ester	0.36	0.66	0.31
C22:1	13-Docosenoic Acid Methyl Ester (Z)		0.71	0.06
C23:0	Tricosanoic Acid Methyl Ester			0.04
C24:0	Tetracosanoic Acid Methyl Ester	0.22	0.48	0.19
C30:0	Triacontanoic Acid Methyl Ester		0.93	
Total FAMEs		**98.53**	**87.81**	**99.26**
Others		**1.47**	**12.19**	**0.74**
Total Saturation		**88.36**	**74.18**	**89.61**

Total Unsaturation		**10.17**	**13.63**	**9.65**

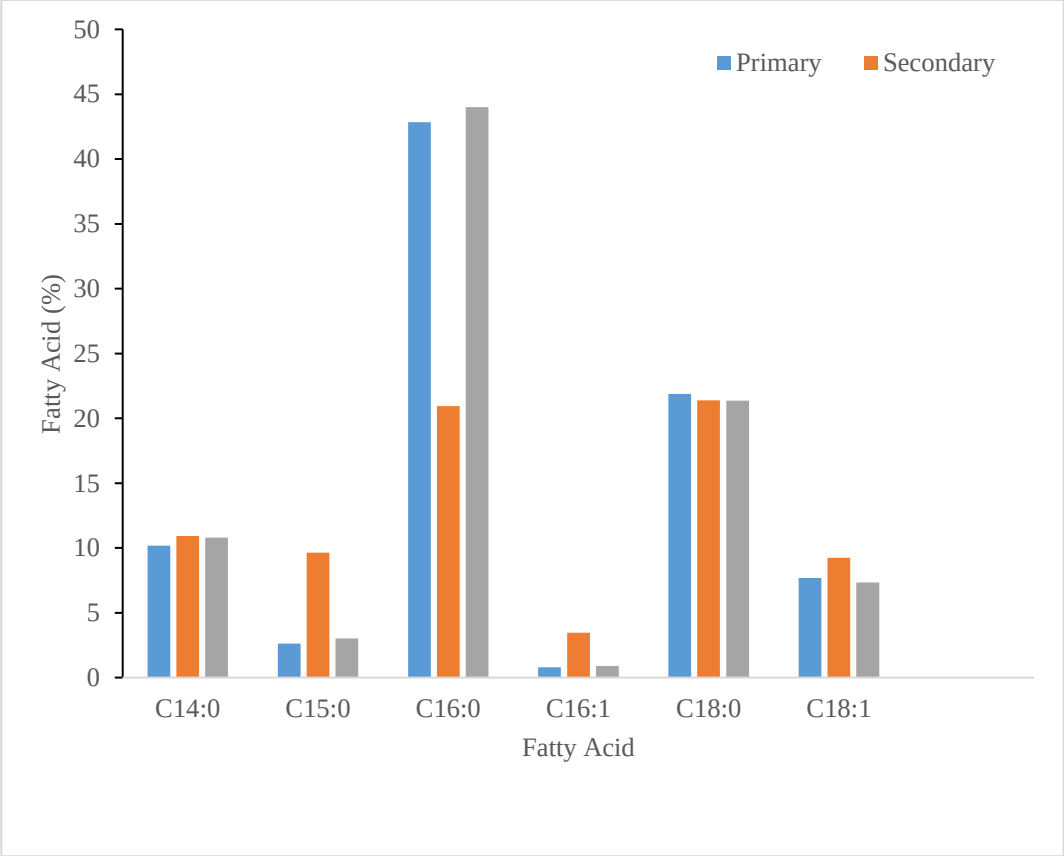


Figure 12. Fatty Acid Methyl Ester Composition of Biodiesel from Non-Acidified Sludge Samples.

Table 12. Fatty Acid composition of biodiesel produced from acidified sludge samples.

Fatty Acid	Compound	Primary	Secondary	Blended
C12:0	Dodecanoic Acid Methyl Ester	4.54	1.60	10.65
C12:0	Undecanoic Acid, 10-Methyl-, Methyl Ester		0.13	
C13:0	Tridecanoic Acid Methyl Ester	0.43	1.92	1.17
C13:1	9-Undecenoic Acid, 2,6,10-trimethyl-, Methyl Ester	0.08		
C14:0	Tridecanoic Acid, 12-Methyl-, Methyl Ester		1.98	
C14:0	Tetradecanoic Acid Methyl Ester	11.46	4.88	15.69
C15:0	Tetradecanoic Acid, 12-Methyl-, Methyl Ester	1.87	3.60	2.45
C15:0	Tetradecanoic Acid, 3-Methoxy-, Methyl Ester		0.18	
C15:0	Pentadecanoic Acid Methyl Ester	3.09	8.92	4.53
C16:0	Hexadecanoic Acid Methyl Ester	14.96	20.50	12.69
C16:0	Hexadecanoic Acid, 2-hydroxy-, Methyl Ester	0.12	0.41	
C16:1	9-Hexadecenoic Acid Methyl Ester (Z)	2.10	11.15	4.22
C17:0	Heptadecanoic Acid Methyl Ester	1.1	1.02	1.07
C17:1	9-Heptadecenoic Acid Methyl Ester		0.31	
C17:1	10-Heptadecenoic Acid Methyl Ester			0.14
C18:0	Octadecanoic Acid Methyl Ester	22.59	4.39	9.27
C18:0	Octadecanoic Acid, 3-hydroxy-, Methyl Ester		1.18	0.40
C18:0	Octadecanoic Acid, 10-hydroxy-, Methyl Ester	3.87		3.20
C18:0	Octadecanoic Acid, 10-oxo-, Methyl Ester	1.05	0.13	0.84
C18:1	6-Octadecenoic Acid Methyl Ester		0.68	
C18:1	8-Octadecenoic Acid Methyl Ester			5.65
C18:1	9-Octadecenoic Acid Methyl Ester (Z)	6.32		7.00
C18:1	11-Octadecenoic Acid Methyl Ester	7.22	11.51	
C18:2	9,12-Octadecadienoic Acid Methyl Ester (Z,Z)	0.09	0.12	0.11
C20:0	Eicosanoic Acid Methyl Ester		0.47	
C22:0	Docosanoic Acid Methyl Ester	0.40	0.27	0.35
C22:1	13-Docosenoic Acid Methyl Ester (Z)		0.22	
C24:0	Tetracosanoic Acid Methyl Ester	0.25	0.23	0.25
C27:0	Heptacosanoic Acid Methyl Ester		2.79	0.49
C30:0	Triacontanoic Acid Methyl Ester	0.47		
Total FAMEs		**82.01**	**78.59**	**80.17**

Others		**17.99**	**21.41**	**19.83**
Total Saturation		**66.29**	**65.75**	**63.05**
Total Unsaturation		**15.72**	**12.84**	**17.12**

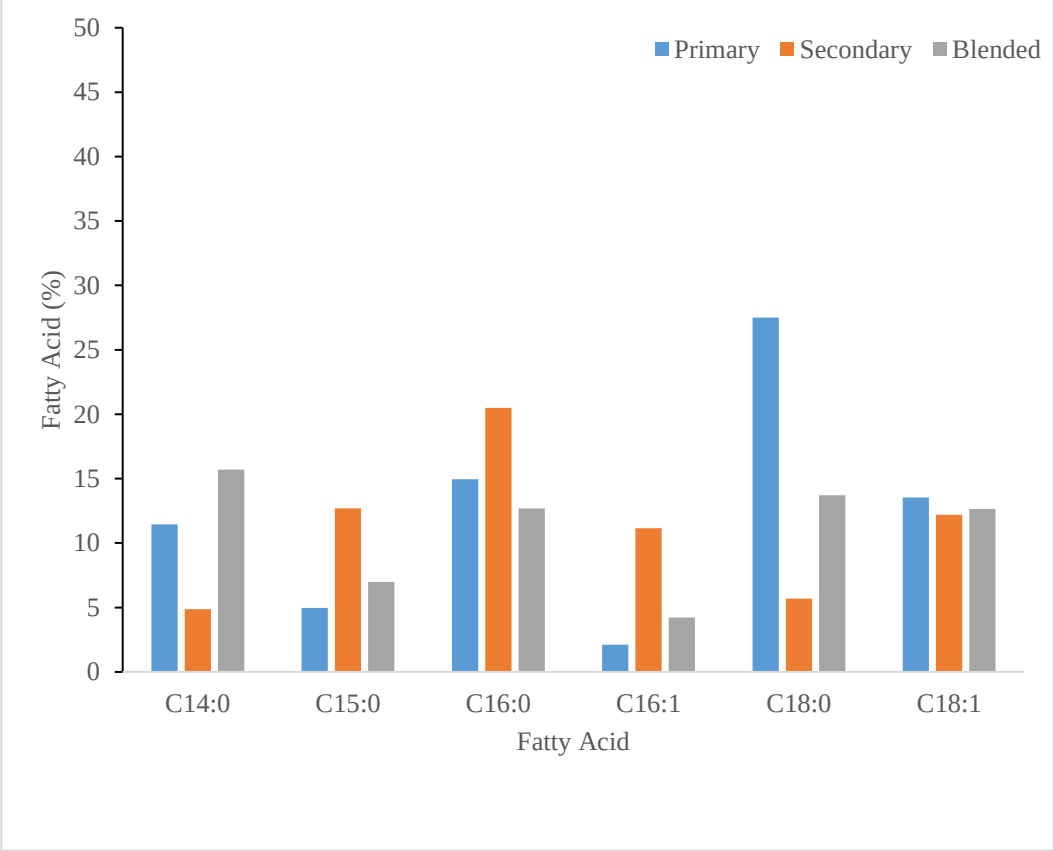


Figure 13. Fatty Acid Methyl Ester Composition of Biodiesel from Acidified Sludge Samples.

Table 12 shows the fatty acid methyl ester composition of biodiesel produced from primary, secondary and blended sludge. The lipids were extracted by the Bligh and Dyer method. The different sludge samples were acidified with hydrochloric acid to bring the pH to 2 before the extraction was performed. Figure 13 shows the distribution of the main characteristic peaks of the fatty acid methyl esters. These are C14:0, C15:0, C16:0, C16:1, C18:0 and C18:1. The main characteristic peaks are the same as those seen in the non-acidified sludge samples. For primary sludge, the composition of C14:0, C15:0, C16:0, C16:1, C18:0 and C18:1 are 11.46%, 4.96%, 14.96%, 2.10%, 27.51% and 13.54% respectively. For secondary sludge,

the composition of C14:0, C15:0, C16:0, C16:1, C18:0 and C18:1 are 4.88%, 12.7%, 20.50%, 11.15%, 5.7% and 12.19% respectively. For blended sludge, the composition of C14:0, C15:0, C16:0, C16:1, C18:0 and C18:1 are 15.69%, 6.98%, 12.69%, 4.22%, 13.71% and 12.65% respectively.

For the primary and blended sludge samples, there is a clear increase in the amount of the following fatty acid C14:0, C15:0, C16:1, C18:0 and C18:1 and a significant decrease in the amount of C16:0 when compared to the non-acidified sludge samples. With the secondary sludge, an increase in the amount of fatty acids can be seen for the C15:0, C16:1 and C18:1, with only a slight change in the composition of the C16:0 and a significant decrease in the composition of C18:0 as compared to the non-acidified secondary sludge. Percentage of unsaturated fatty acid methyl esters for the primary, secondary and blended sludge are 15.72, 12.84 and 17.12 respectively.

Table 13. Characterization of biodiesel produced from the different sludge types using the ionic liquid Method.

Fatty Acid	Compound	Primary	Secondary	Blended
C12:0	Dodecanoic Acid Methyl Ester		1.83	2.10
C13:0	Tridecanoic Acid Methyl Ester		1.00	
C13:0	Tridecanoic Acid, 12-Methyl-, Methyl Ester		0.54	
C14:0	Tetradecanoic Acid Methyl Ester	7.02	10.88	8.94
C15:0	Tetradecanoic Acid, 12-Methyl-, Methyl Ester	1.34		
C15:0	Pentadecanoic Acid Methyl Ester	2.19	6.92	2.33
C16:0	Hexadecanoic Acid Methyl Ester	53.71	41.87	52.13
C16:0	Hexadecanoic Acid, 3-hydroxy-, Methyl Ester	0.15		
C16:1	9-Hexadecenoic Acid Methyl Ester (Z)	0.67	2.02	0.53
C16:2	Hexadecadienoic Acid Methyl Ester		0.30	
C17:0	Hexadecanoic Acid, 15-Methyl, Methyl Ester	0.49		0.41
C17:0	Heptadecanoic Acid Methyl Ester	0.49	0.82	0.44
C18:0	Octadecanoic Acid Methyl Ester	23.54	13.83	21.46
C18:0	Octadecanoic Acid, 10-oxo-, Methyl Ester	0.47	0.39	0.45
C18:0	Cyclopentanetridecanoic Acid, Methyl Ester	1.10		
C18:1	9-Octadecenoic Acid Methyl Ester (Z)	3.35	3.00	2.96
C18:1	11-Octadecenoic Acid Methyl Ester (Z)	3.27	1.72	3.73
C18:1	Methyl Hexadec-11-enoate		0.32	
C18:2	9,12-Octadecadienoic Acid Methyl Ester (Z,Z)	1.34	1.24	1.06
C19:0	Nonadecanoic Acid Methyl Ester		0.97	1.10
C22:0	Docosanoic Acid Methyl Ester	0.30	0.63	0.34
C24:0	Tetracosanoic Acid Methyl Ester	0.19	0.43	0.20
C27:0	Heptacosanoic Acid Methyl Ester	0.35	0.40	
C30:0	Triacontanoic Acid Methyl Ester			0.31
Total FAMEs		**99.97**	**89.11**	**99.77**
Others		**0.03**	**10.89**	**0.23**
Total Saturation		**91.34**	**80.51**	**91.49**
Total Unsaturation		**8.63**	**8.60**	**8.28**

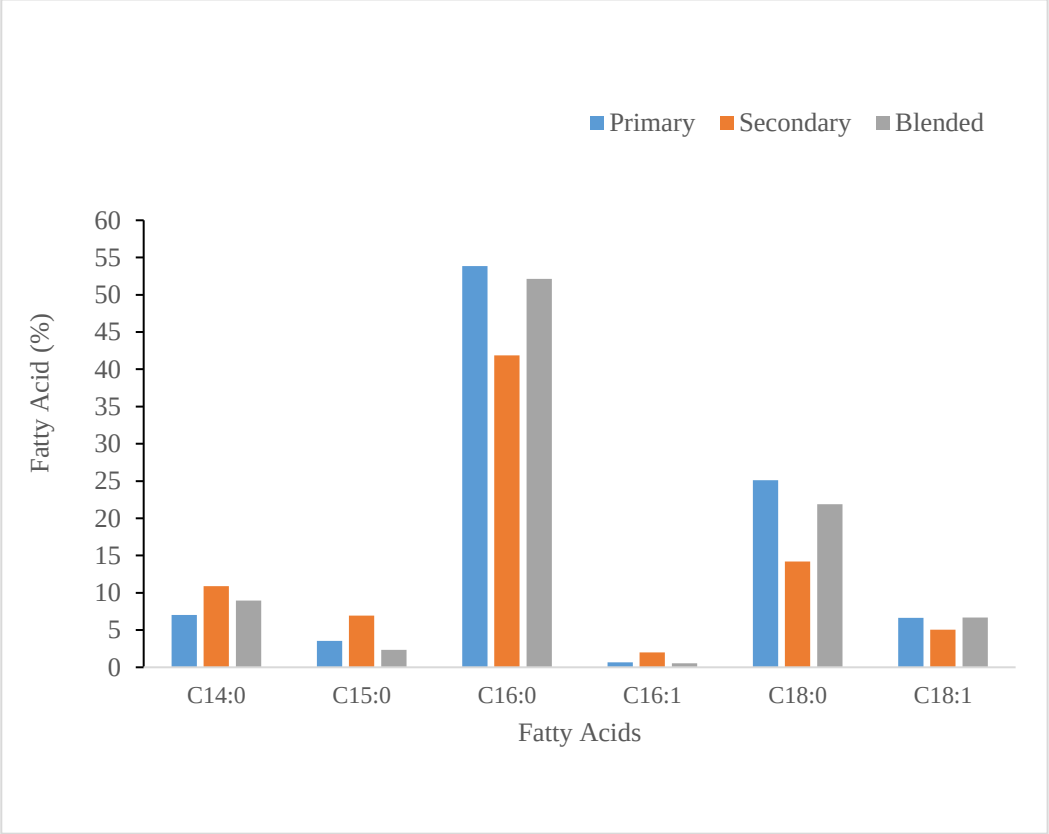


Figure 14. Fatty Acid Methyl Ester Compositions of Biodiesel produced from lipids extracted by ionic liquid, Tetrakis (hydroxymethyl) phosphonium chloride $[P(CH_2OH)_4]Cl$.

Table 13 shows the fatty acid methyl ester composition of biodiesel produced from primary, secondary and blended sludge. The lipids were extracted by the ionic liquid method using the Tetrakis(hydroxymethyl)phosphonium chloride $[P(CH_2OH)_4]Cl$. Figure 14 shows the distribution of the main characteristic peaks of the fatty acid methyl esters. The main fatty acid methyl esters are C14:0, C15:0, C16:0, C16:1, C18:0 and C18:1. This is the same as those found in the biodiesel produced by the Bligh & Dyer extraction method. For primary sludge, the composition of C14:0, C15:0, C16:0, C16:1, C18:0 and C18:1 are 7.02%, 3.53%, 53.86%, 0.67%, 25.11% and 6.62% respectively. For secondary sludge, the composition of C14:0, C15:0, C16:0, C16:1, C18:0 and C18:1 are 10.88%, 6.92%, 41.87%, 2.02%, 14.22% and 5.04% respectively. For blended sludge, the composition of C14:0, C15:0, C16:0, C16:1, C18:0 and C18:1 are 8.94%, 2.33%, 52.13%, 0.53%, 21.91% and 6.69% respectively. The fatty acid methyl ester

distribution is similar to that of the biodiesel produced from the Bligh & Dyer extraction method except for a significant increase in the composition of the C16:0, especially in the case of secondary sludge. Percentage of unsaturated fatty acid methyl esters for primary, secondary and blended sludge are 8.63. 8.60 and 8.28 respectively.

Figures 12, 13 and 14 indicate a similar fatty acid distribution for all types of sludge tested, with (C14:0), (C15:0), (C16:0), (C18:0) and (C18:1) being the most dominant fatty acids. All the different sludge samples tested have large amounts of saturated fatty acids with the ionic liquid samples showing the highest amount, followed by non-acidified and then acidified sludge samples. The quantity of saturated fatty acids is much higher than that of unsaturated fatty acids for all sludge types. Large amounts of saturated fatty acids mean higher cetane numbers and high oxidation stability of the biodiesel (Canakci, 2007), but their presence may negatively impact the cold flow properties of biodiesel in cold weather due to formation of crystals that may result in clogging of filters and fuel lines thus affecting engine performance (Verma et al., 2016).

This problem can be controlled by the use of branched chain alcohols instead of methanol during the transesterification reaction (Canakci & Sanli, 2008). Also, the presence of branched chain and hydroxy fatty acid mono-alkyl esters in the sludge samples may help overcome the problem (Olkiewicz et al., 2014). These compounds can be identified in Tables 11, 12 and 13. There are very low amounts of C18:2 present in all the sludge samples tested, this is valuable because polyunsaturated fatty acids can undergo auto-oxidation reactions due to bis-allylic position of the carbon double bonds and this may affect the oxidation stability of the biodiesel (Ramos et al., 2009). Compounds that are formed during the auto-oxidation reaction may decrease the quality of biodiesel over time (Knothe, 2005).

Since primary sludge attained the highest lipid and biodiesel yields of all the sludge samples tested, its fatty acid profile was compared to that of common biodiesel feedstocks as indicated in Figure 15.

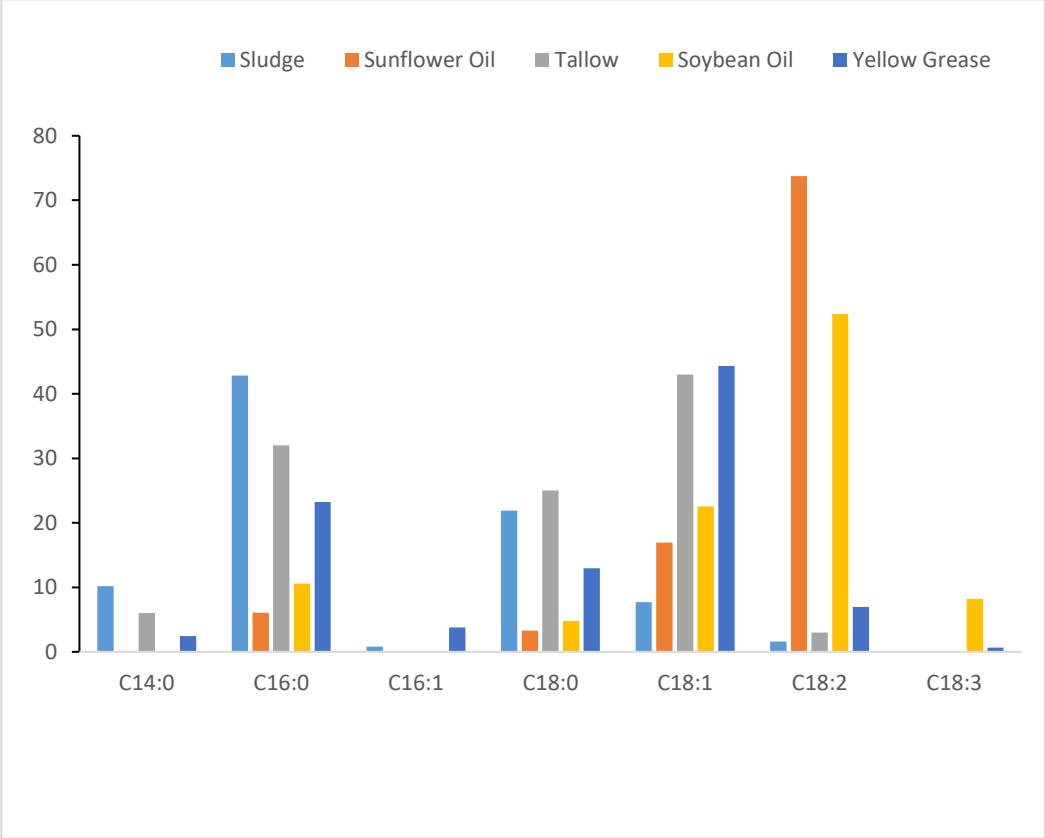


Figure 15. Comparison of fatty acid profiles of primary sludge with the common feedstocks for biodiesel production (Canakci, 2007).

It can be seen that the dominant fatty acid found in the primary sludge are the same as those in the other feedstocks, namely; C16:0, C18:0, C18:1 and C18:2, the difference being the percentage composition. A distinct difference is the absence of C18:3 and presence of small amount of C18:2 in the fatty acid composition of primary sludge. This will prevent the auto-oxidation reaction from taking place due to the presence of these polyunsaturated fatty acids. Sunflower oil and soybean oil show high concentrations of these polyunsaturated fatty acids and this may cause a deterioration in the biodiesel quality produced from these feedstocks due to auto-oxidation reactions. The fatty acid distribution of primary sludge is more comparable to that of tallow which is an animal fat.

4.1.5 **Characterization of Biodiesel using the Fourier Transform Infrared Spectroscopy (FT-IR).**

The presence of FAME was further confirmed by FT-IR analysis of the lipids and biodiesel produced from primary sludge. FT-IR spectra showed the main functional groups present in the lipids and biodiesel produced from primary sludge. Table 14 and 15 show the main characteristic absorption peaks of the sewage sludge lipids and the biodiesel derived from them.

Table 14. FT-IR spectrum of primary lipids.

Group Frequency (cm^{-1})	**Functional Group**
720	$-CH_2$ rocking
1187	C-O stretching
1245	=C-O stretching
1454	CH_2 bending
1700	C=O stretching
2848	$-CH_2$ stretching
2915	$-CH_2$ stretching

Table 15. FT-IR spectrum of primary biodiesel.

Group Frequency (cm^{-1})	**Functional Group**
721	$-CH_2$ rocking
1033	=C-O stretching
1169	$-CH_2$-O stretching
1195	CH_3-O stretching
1245	=C-O stretching
1436	$-CH_3$ bending
1454	$-CH_2$ bending
1743	C=O stretching
2853	$-CH_2$ stretching
2922	$-CH_2$ stretching

Figures 16, 17 and 18 show the FT-IR spectra of the lipids extracted from primary sludge using the Bligh & Dyer method, biodiesel produced from these lipids and a comparison of the profiles from the lipids and biodiesel respectively.

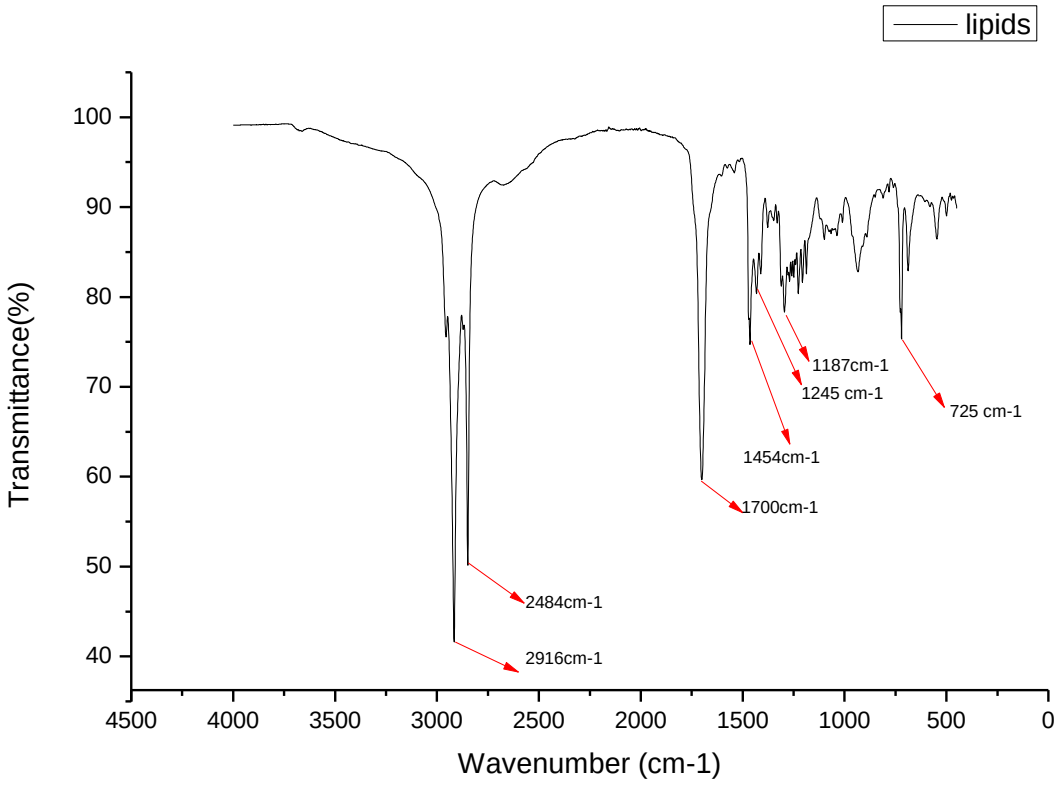


Figure 16. FT-IR spectrum of sewage sludge lipids.

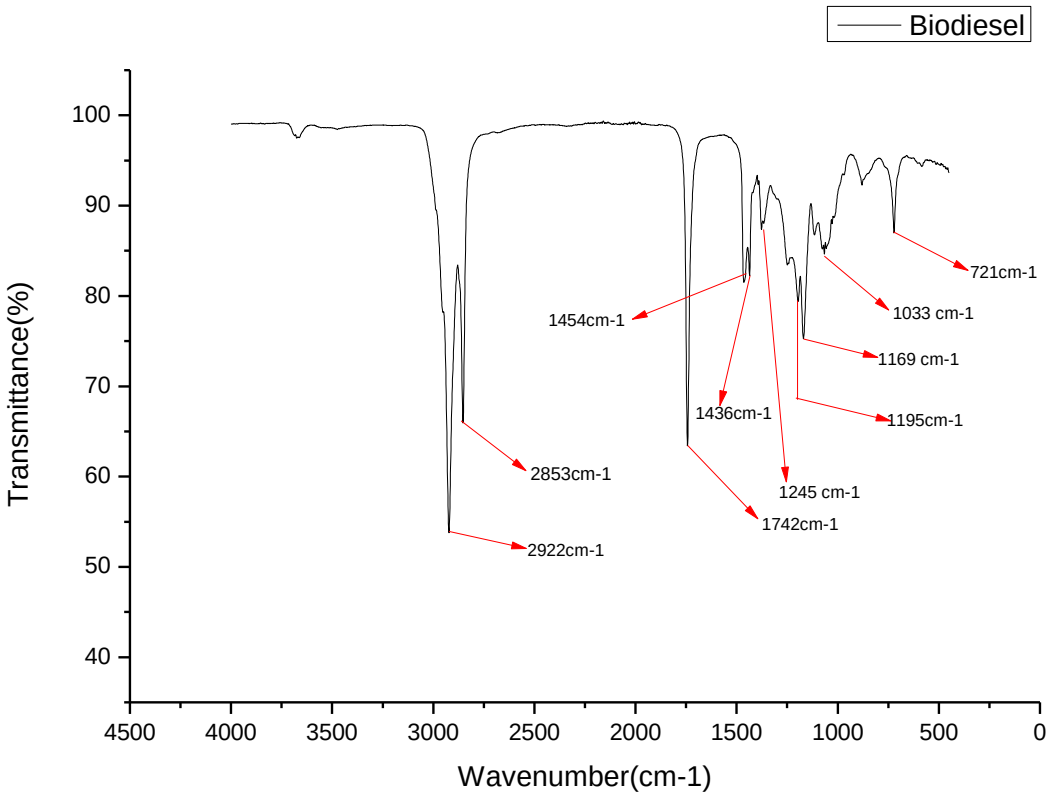


Figure 17. FT-IR spectrum of biodiesel produced from transesterification of sewage sludge lipids.

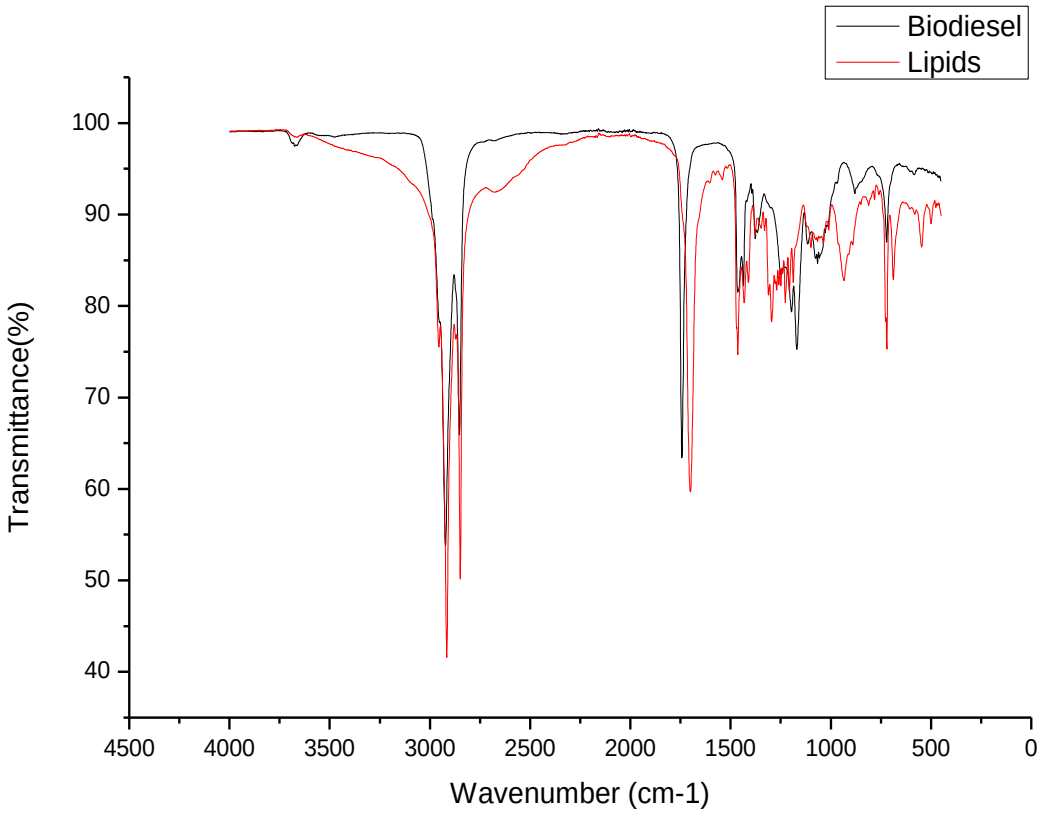


Figure 18. FT-IR spectra of sewage sludge lipids and biodiesel produced by transesterification process.

From Figures 16 and 17, it can be seen that the distribution of the peaks obtained for the primary sewage sludge lipids are similar to the biodiesel derived from these lipids. A sharp peak in the lipids and the biodiesel is observed at 720cm^{-1} and this denotes –CH_2 rocking (Elkady et al., 2015). The peaks at 1033cm-1 correspond to =C-O stretching of polysaccharides and glucose (Bartošová et al., 2013). The peaks at 1187cm^{-1} in the lipids and 1169cm^{-1} and 1195cm^{-1} in the biodiesel corresponds to the stretching -CH_2-O- and CH_3-O groups (Mahamuni & Adewuyi, 2009). The characteristic peak at 1195cm^{-1} corresponds to the CH_3-O stretching which indicates the conversion of the lipids to biodiesel. Vibrations at 1436cm^{-1} in the biodiesel sample indicate the presence of methyl esters (C-O-CH_3). This peak represents the transesterification of lipids in the presence of acid (Karar, 2017). Peaks at 1454cm^{-1} represent bending vibrations of CH_2 (Ngamlerdpokin et al., 2011).

There are high intensity peaks at 1700cm^{-1} of the lipid sample and at 1743cm^{-1} of the biodiesel. The peak at 1743cm^{-1} in the biodiesel indicate the presence of the stretching ester (-C=O). This is due to the presence of oleic (C18:1) and linoleic (C18:2) acids (Omidvarborna et al., 2015). This suggests that the triglycerides in the lipids have been converted to fatty acid methyl esters. The strong vibrations at 2848cm^{-1} and 2915cm^{-1} in the lipids and at 2853cm^{-1} and 2922cm^{-1} in the biodiesel is attributed to the stretching vibrations of the methylene group which are present in lipids and fats (Ngamlerdpokin et al., 2011). The results indicate that the transesterification reaction to convert sewage sludge lipids to biodiesel has successfully occurred.

Figure 19 shows the FT-IR spectra of oil and biodiesel derived from castor oil. When the FT-IR spectrum of lipids and biodiesel from sewage sludge was compared with that of the oil and biodiesel from castor oil it was observed that the absorption peaks obtained were similar.

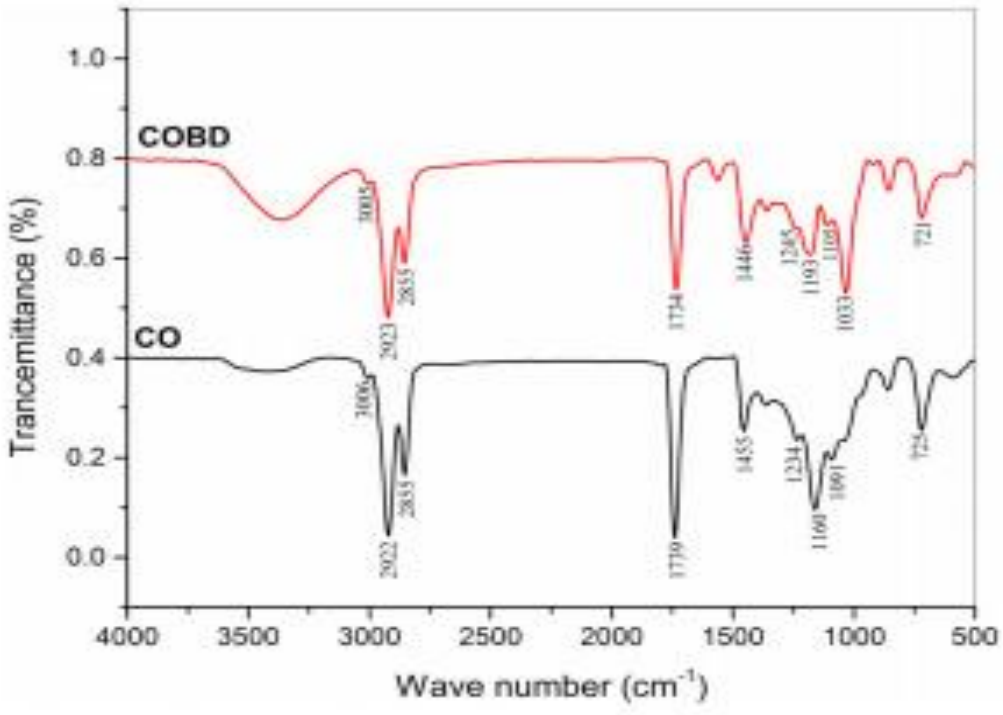


Figure 19. FT-IR spectrum of castor oil and castor oil biodiesel (Elango et al., 2019).

4.1.6 **Properties of primary biodiesel**

The kinematic viscosity of the biodiesel produced from primary sludge was 9.8 mm^2/s at 40ºC. The density was found to be 0.89 g/cm^3 at 20ºC. From the results it can be seen that the density of the primary biodiesel was within the ASTM 6751 standards shown in Table 4 i.e. in the range 0.86-0.90 g/cm^3 at 20ºC. However, it did not meet the specification on the viscosity. The viscosity limit on the ASTM D6751 is in the range 1.9-6.0 mm^2/s at 40ºC.

Chapter 5: CONCLUSIONS

The world's primary energy consumption has expanded significantly during the last 40 years as a result of urbanization and industrialization. Nonrenewable resources such as coal and petroleum are predicted to be the key sources of energy production to meet the world's energy population need, with the shipping and oil industries contributing the most. The combustion of these materials generates greenhouse gases, which are pollutants that have a detrimental effect on the environment and human health. Biodiesel is one of the sustainable energy alternatives that has received the most attention during the last two decades. Due to the fact that biodiesel emits less pollutants into the atmosphere and so has a lower environmental impact when used, it presents itself as a viable alternative to petroleum-based diesel fuels. The high cost of raw ingredients, on the other hand, is a significant disadvantage to biodiesel manufacturing. In some cases, feedstock accounts for between 70% and 85% of the cost of biodiesel manufacturing. A range of alternative feedstocks have been researched in attempt to reduce the cost of biodiesel, increase energy efficiency, and ensure long-term sustainability. Non-edible oils, edible oils (soybeans, sunflower, etc.), algae, and waste products are only a few examples. Sewage sludge has been recognized as a viable feedstock for biodiesel synthesis since it contains a minimum of 7% dry lipids. One significant advantage is that sewage sludge is widely available in wastewater treatment plants, with a consistent supply as a result of urbanization and industrialisation. On the other hand, sewage sludge continues to be a financial burden for a number of South African local governments due to disposal costs. In this study, biodiesel was produced using sewage sludge from Johannesburg's municipal wastewater treatment facility (WWTP). The results of this study indicate that primary sludge produced the most lipids and biodiesel, followed by blended sludge and secondary sludge. The addition of hydrochloric acid to sludge samples enhanced the extraction of lipids from all types of sludge examined. Although ionic liquids revealed the ability to extract lipids from various sludge samples, the Bligh and

Dyer method extracted significantly more lipids in this investigation than the ionic liquid extraction method. The results of the gas chromatography examination of the various sewage sludge FAMEs indicate that the fatty acid content of all the sludge samples analyzed is identical.

Different extraction procedures and acidification of sludge samples had little effect on the sludge samples' fatty acid content. All sludge samples contained a high concentration of myristic (C14:0), pentadecyclic (C15:0), palmitic (C16:0), stearic (C18:0), oleic (C18:1), and linoleic (C18:2) acids, which are all commonly used fatty acids in biodiesel generation. When the fatty acid content of primary sludge FAMEs was compared to that of popular biodiesel feedstocks, it was determined that primary sludge is acceptable for biodiesel production. The presence of large levels of saturated fatty acids in the sludge samples results in biodiesel with improved oxidation stability and a higher cetane number; nevertheless, this may cause engine performance issues during cold weather due to biodiesel crystallisation, which can clog fuel filters and lines. The FT-IR analysis of the lipids and biodiesel from primary sewage sludge demonstrated that the lipids were successfully converted to biodiesel during the acid catalyzed transesterification process. When primary sewage lipids and biodiesel were compared to oil and biodiesel made from castor oil, a typical feedstock for biodiesel production, it became clear that primary sewage sludge is an acceptable feedstock for biodiesel production. These findings indicate that municipal sewage sludges from wastewater treatment plants have the potential to be used as a feedstock for biodiesel synthesis. Additionally, it is a plentiful, low-cost feedstock that may help reduce the cost of producing biodiesel and address sludge management and disposal issues.

As a result of this study's findings, it is clear that sewage sludge from wastewater treatment plants is a good feedstock for the generation of biodiesel. However, a pilot size study is recommended to investigate the synthesis of biodiesel from sewage sludge on a wider scale. This would allow for parameter optimization in order to optimize biodiesel manufacturing conditions. Additionally, it is recommended that wet sludge biodiesel production be compared to dry sludge production, and that a cost study be conducted to determine whether approach is

more cost efficient. Additionally, other strategies for extracting lipids from sludge that are more cost effective and environmentally benign should be studied.

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