In situ Transesterification of Rapeseed for Production of Biodiesel and Secondary Products

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A Thesis Submitted for the Degree of Doctor of Philosophy

At Newcastle University, United Kingdom

School of Chemical Engineering and Advanced Materials

July, 2018

Abstract

In situ transesterification (IST) can potentially reduce the cost of biodiesel production by avoiding the oil extraction and refining stages of conventional transesterification, through the direct reaction of the oilseed with alcohol in the presence of a catalyst. However, a large excess of alcohol is currently required in IST to achieve comparable yields to conventional transesterification. Hence, in this study, methods for improving *in situ* transesterification of rapeseed for biodiesel production have been investigated. The focal point of this study is to reduce or utilize the excess alcohol. Pre-soaking seeds in methanol and reactive coupling were subsequently attempted. The respective rationales are to reduce the excess methanol requirement, and convert/use the excess methanol in a secondary process. Pre-soaking involved a chemical pre-treatment of the oilseed prior to the transesterification reaction.

Pre-soaking was performed with methanol to oil molar ratio (MOMR) of 360:1 at 60°C using a catalyst (NaOH) concentration of 0.1M. A two-level factorial design was used to determine the optimum conditions for pre-soaking. It was found that a biodiesel yield of 85% was obtained for pre-soaking at 360:1 MOMR while the 'un-soaked' biodiesel yield was 75% at 475:1 MOMR. The higher biodiesel yield with 24% reduction in methanol requirement could potentially translate to energy savings in the downstream separation of biodiesel from excess methanol.

Reactive coupling (transesterification + a glycerol polymerisation reaction) should increase the equilibrium conversion of biodiesel, whilst generating valuable secondary products. It was carried out in a pressure vessel at 10 bar, 140°C in an inert atmosphere. Polyglycerol was identified in the reaction mixture using FTIR and ^{1}H-NMR. Using a MOMR of 375:1 with catalyst concentration (H_2SO4) of 4.8 v/v% at 140°C, a biodiesel yield of 90% and polyglycerol (PG) yield of 10% were observed after 4 hours of reaction. Overall, the material balance indicated that at the end of the reaction, 19% of the unused methanol had been converted to dimethyl ether (DME). This would lead to energy savings in the separation of product. The Central Composite design of experiment for reactive coupling indicated that catalyst concentration was the most significant variable in biodiesel production, whilst molar ratio is significant for both polyglycerol and DME production. Moreover, the study demonstrates the practicality of FTIR online monitoring of IST, which could be valuable for on-line monitoring at industrial scales, where

traditional off-line GC analysis is time-consuming and ineffective to correct immediate production problems. Furthermore, the online monitoring could be used for “fast IST” of rapeseed to biodiesel to detect the onset of saponification.

This work has demonstrated co-production of valuable chemicals with biodiesel production via reactive coupling for the first time. This could be the initial step toward an integrated biodiesel-based bio-refinery.

Acknowledgments

Glory be to Allah (God) for successful completion of the work without His support and help nothing can be achieved. I wish to express my sincere appreciation to my supervisors Prof Adam Harvey and Dr Jon Lee for their invaluable contribution, encouragement and criticism during the course of the programme. I will like to acknowledge the support and assistance I received from friends and colleagues at Newcastle University particularly Auwal Aliyu, Dr Kamoru Salam, Malik Musthofa, Dr Val Eze, Jonathan McDonough, Ahmed Althroosi, Ibrahim Mohammed and the entire members of Process Intensification Group.

I appreciate the technical support I enjoyed from the CEAM technical staff, of particular note is the help I received from Mr Iain Strong in the construction of my reactive coupling rig. I will also like to thank Mr Latimer Steward, Rob Dixon and Simon Daley for providing solution whenever the equipment breaks down. The CEAM administrative staff members were also very helpful in sorting out administrative issues as they arouse worthy of mention are Justine McGruther, Gayle Thompson and Aimee Leech. The IT team are very wonderful in prompt resolution of computer software and hardware challenges.

I will want to appreciate the tremendous support and prayers I received from friends, families and elders outside the University. First among them is the Sanni family whose motivation and encouragement and prayers culminated in studying in the UK. The assistance and guidance from Ameer Sheriff is thankfully appreciated. Also, the warm embrace and support I received from Madina masjid muslim community in Newcastle ably led by the Imam, Abdullah Kolla is really appreciated. I appreciate Sheikh Saleem Dhorat for his spiritual guidance and mentorship. The moral support I received from Dr Edidi and Engr. Olabisi's families are very well appreciated. I also thank Mr and Mrs Coker, for their assistance and support.

This acknowledgement will be incomplete without mentioning the backbone of the support, prayers and goodwill I received from my immediate family particularly my lovely wife Fatima and my boys; Faruq, Harith and Abdhakim Jr. I thank them for their patience and sacrifice during the period of the course. I also thank my parent, siblings and in-laws for their prayers that have taken me this far in life. I am so much proud of you all as I hope you will be of me.

Finally, I wish to express my gratitude to my sponsor Tertiary Education Trust Fund (Tetfund), Nigeria for providing funding for the programme. I also received a Newcastle Nigeria postgraduate scholarship, so I thank Newcastle University for this gesture. To so many others I cannot mention because of space I say a big thank you and God bless you all.

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Abbreviations

ANOVA	-	Analysis of variance
CC	-	Catalyst concentration
CFPP	-	Cold filter plugging point
DG	-	Diglyceride
DiG	-	Diglycerol
DME	-	Dimethyl ether
FAME	-	Fatty acid methyl ester
FFA	-	Free Fatty acid
FTIR	-	Fourier transform infra red
GC	-	Gas chromatogram
GRP	-	Glycerol-rich phase
IST	-	*In situ* transesterification
LC-MS	-	Liquid chromatogram mass spectrometry
LNG	-	Liquified natural gas
MOMR/MR	-	Methanol to oil molar ratio
MUFA	-	Mono unsaturated fatty acid
^{1}H-NMR	-	Proton nuclear magnetic resonance
PG	-	Polyglycerol
PUFA	-	Poly unsaturated fatty acid
RC	-	Reactive Coupling
RI	-	Refractive index
TEMP/TE	-	Temperature
TG	-	Triglyceride
TrG	-	Triglycerol
TtG	-	Tetraglycerol

Chapter 1. Introduction

1.1 The Study Background

The challenge of a continuously increasing world population and associated economic activity, together with finite fossil fuel energy reserves, with their negative environmental impacts, necessitate the development of clean sustainable alternative sources of energy. At present, all world economies still run primarily on liquid fossil fuels for transport. However, renewable sources of energy are now on the increase mainly due to legislative drivers such as clean environmental policy and taxes. The British Petroleum (BP) energy outlook 2017 in Figure 1.1 shows a decline of oil and coal energy sources from about 42% and 39% in 1965 to 29 and 25% respectively by 2035. In contrast, the report reveals a gradual increase in renewable energy sources from 0% in 1965 to 10% by 2035 (BP, 2017).

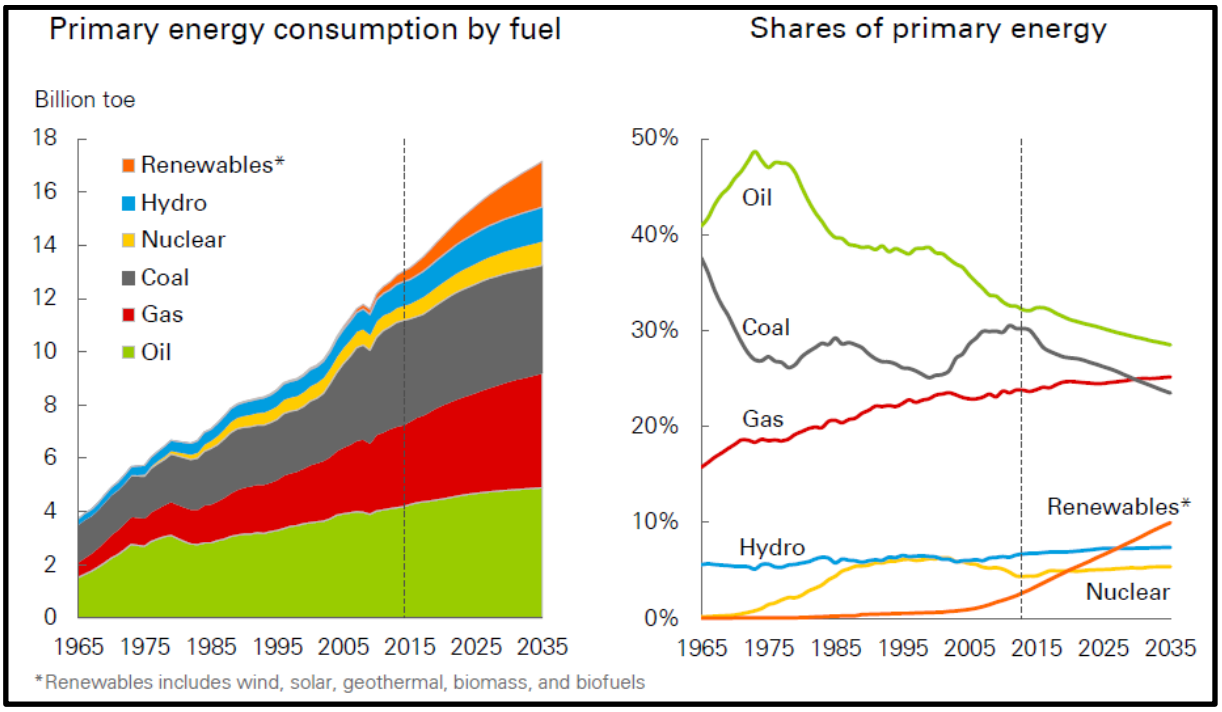


Figure 1.1 Global primary energy resources consumption from 1965 to 2035 (BP, 2017)

Incidentally, the number of renewable sources used as transport fuels in the UK from 2010 to 2016 have increased tremendously. For example, Fig. 1.2 shows an increase in bioethanol from 355,000 metric tons of oil equivalent (Mtoe) in 2010 to 428,000 Mtoe in 2016. However, biodiesel declined from 862 Mtoe in 2010 to 582 Mtoe in 2016. This may be due to rise in volumes of various types of lignocellulosic feedstocks (e.g wheat and corn) that can be used for bioethanol by fermentation compared to only oil-bearing biomass for biodiesel. The UK interim renewable energy consumption target of 5.4% over 2013 and 2014 was reported to have been met (BEIS, 2017). However, there is doubt of meeting the 2020 and 2030 targets of 15% and 27% respectively unless government policies promote effective cooperation across departments to maintain existing support for renewable electricity projects, and increasing the use of renewables in transport and heat sectors (BEIS, 2017).

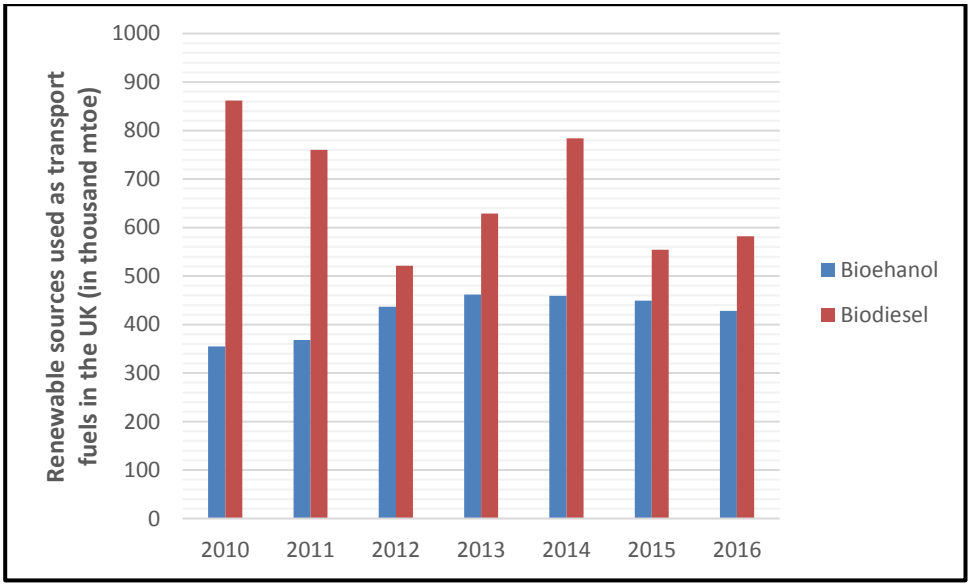


Figure 1.2 Renewable energy sources used as transport fuels in the UK (UK Department for Business, 2017)

In Nigeria, though blessed with abundant sources of renewable energy, is yet to harness these resources to tackle the basic challenge of electricity power. This is a result of lack of necessary facilities and good government policy and implementation (Okedu *et al.*, 2015). However, with new government in place, there is new energy policy shift from zero renewable energy outlook to 1-2% of the nation's energy mix in the next ten years (Akuru *et al.*, 2017). This new policy direction is being pursued using solar, wind, geothermal, hydropower and biomass renewable energy potentials of the country. Individuals are encouraged to champion the course by privately embraced the new initiatives.

1.2 Biofuel Alternatives

Biodiesel is a sustainable clean-burning liquid fuel similar to conventional diesel. It has found application as an energy source in many areas, particularly in the transport sector, where it can be used as the sole fuel in diesel engines or as a blend with petrodiesel without any major modification to the motor system (Gutiérrez *et al.*, 2009). There is now an increasing demand for biodiesel because of its environmental benefits as a result of its lower lifecycle level of greenhouse emissions than fossil fuel (Demirbas, 2008). Fig. 1.2 shows the world-leading producers of biodiesel in 2016. The U.S. led with a production volume of 5.5billion litres. Apart from the environmental benefits, availability is an additional advantage of biodiesel because it can be produced from different feedstocks ranging from oil-bearing plant materials to animal fat. One of the major challenges to petrodiesel use is the rapid depletion of petroleum reserves from which it is produced. It is predicted that at the current consumption rate, reserve depletion times for oil, gas, and coal are approximately 35, 37 and 107 years respectively (Shafiee and Topal, 2009). Therefore, there is a need to replace fossil fuel with sustainable biofuels to avoid an energy crisis.

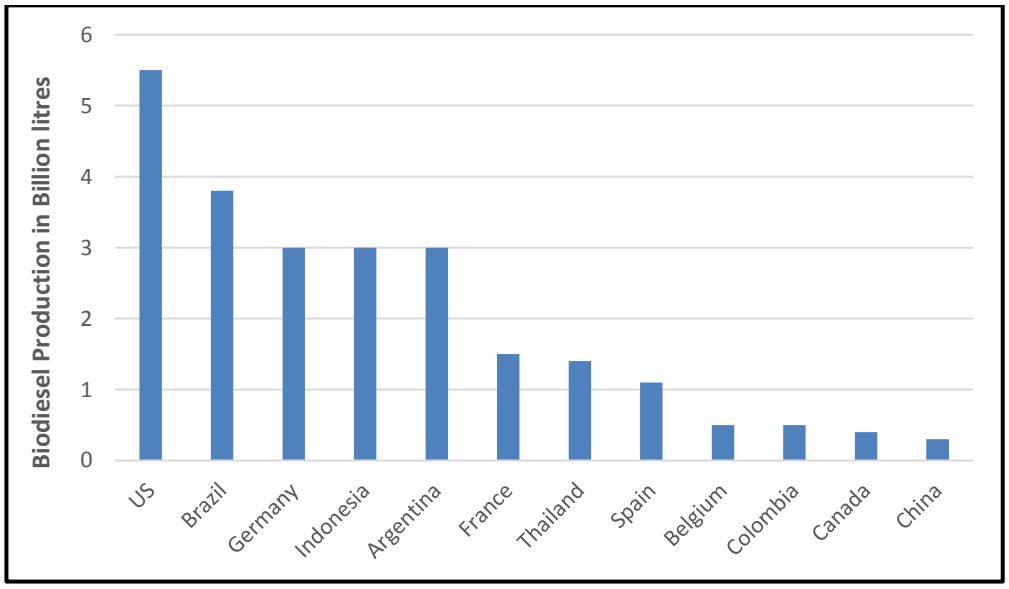


Figure 1.3 Global Biodiesel Production by Country in 2016 (statista, 2016)

1.3 The Problem Statement

In situ transesterification (IST) or reactive extraction is a form of process intensification by "process integration" or "hybridization" (Harvey and Lee, 2012). In this type of approach, intensification is achieved by reducing the number of process steps by developing multifunctional steps. The process combines a chemical (transesterification)

reaction and extraction from the oilseed in one unit, thereby reducing the process steps in the production. This potentially reduces the overall cost of production.

IST is the direct reaction of oil-bearing materials with alcohol in the presence of a catalyst to produce fatty acid methyl ester (FAME), known as "biodiesel" and glycerol. This should be contrasted against conventional transesterification in which oil is extracted and purified prior to the reaction step. A significant advantage of IST is that it removes the process steps involved in extracting of oil such as mechanical pressing, solvent extraction and refining the oil, thereby simplifying the method, reducing production time, cost and environmental impact of solvent (hexane) use in the extraction (Özgül-Yücel and Türkay, 2003).

Many studies have shown that for high yields of FAME, IST requires a high solvent to oil molar ratio. The high alcohol usage makes the cost of IST higher than conventional transesterification because of the high cost of energy required to recover excess or unused alcohol, in the condensation and distillation steps (Haas and Wagner, 2011).

The focus of this study, therefore, is to find ways of reducing/utilizing the high volume of alcohol (methanol) in IST of rapeseed for the production of biodiesel, which can potentially reduce the cost of production. This study investigates pre-soaking and reactive coupling as means of achieving this objective. Pre-soaking is chemical pre-treatment method of soaking the crushed seed in methanol before the actual transesterification reaction. The method was reported to reduce the methanol requirement in IST of microalgae (Salam, 2015).

Reactive coupling is 'pulling through' the IST reaction by coupling it to another reaction. In this case the reaction chosen was the polymerisation of the glycerol by-product to obtain polyglycerol in addition to the biodiesel. Polyglycerol is a value-added platform chemical that can be used to produce further useful chemicals (e.g polyglycerol ester) with pharmaceutical, food and cosmetic applications. Excess alcohol is required in IST to push the equilibrium to the product side because the reaction is reversible. If however, one of the products is removed as it is being formed, the alcohol requirement may be reduced, following Le Chatellier's principle. The hypothesis being tested is that the FAME yield can be increased by *in situ* glycerol product conversion/removal. Also, dimethyl ether (DME) is co-produced produced by dehydration of the excess methanol used in IST. The DME is a potential fuel gas that can be used to replace propane in liquefied petroleum gas (LPG). The coproduction of polyglycerol and rapeseed meal from

biodiesel production could be the basis of an integrated oilseed-based biorefinery. Fig 1.3 gives a simplified pictorial representation of the production cycle where biodiesel, polyglycerol, DME and rapeseed meal are produced in biorefinery from edible energy crop (rapeseed) on the farm. The plant obtains the energy via photosynthesis and stores much of it in the form of oil, the carbon of which comes from CO_2. The spent rapeseed is used as a meal for livestock after purification. The glycerol byproduct is further converted to polyglycerol by polymerisation whilst part of the excess methanol is converted to DME. The biodiesel is majorly used as liquid fuel to power the transport sector with low carbon (CO_2) emission which is later taken up by the plant to produce energy by photosynthesis in a cycle.

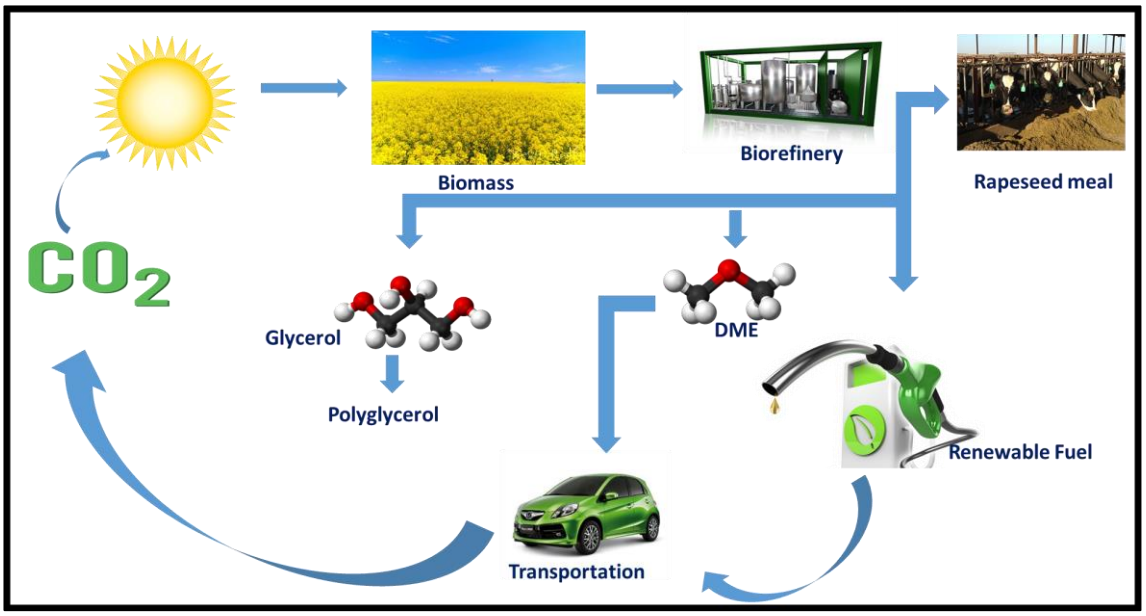


Figure 1.4 Production Cycle: Biodiesel, Polyglycerol Dimethyl ether and rapeseed meal

1.4 The myth of food versus fuel debate

The belief that use of edible energy crop such as rapeseed in the production of biodiesel will negatively impact on the food supply is probably incorrect. Biodiesel production is from excess oil that is not used in the food supply. The carbohydrate and protein in rapeseed meal are still intact after the reactive extraction, so can be a significant source of feed for livestock. The consumption of all the rapeseed oil that is produced with rapeseed protein meal is not feasible. The abundant solar energy that is provided by nature is converted by plants and stored in energy crops known as biomass. It is these plants that provide energy for animals, while the oil can be sustainably converted to biodiesel. Biodiesel production plays a significant positive role in agriculture absorbing about 3

million hectares of arable land in the EU which could have been idle thereby contributing to economic growth of the sector (EBB, 2015). This can produce an estimated 4.5 million tonnes of biofuel at 1.5 tonnes per hectare (UFOP, 2016/17).

On the other, there have been reports on the negative impact of using edible food for biofuel production. For example, it was reported by the head of Nestlé food company, Peter Brabeck-Letmathe that the use of food grains in biofuel to meet 20% of the growing demand for oil products will lead to starvation of the populace (Tenenbaum, 2008). Another online African news blamed the food riot in Haiti and other parts of the world to the diversion of food crops to biofuel production. A report from African Biodiversity Network claimed that production of biofuels will reduce the land that will be available to grow food crops. All these reports are a genuine concern because the priority is food, however, there are enormous opportunities in biofuel. The solution should be improved agricultural productivity to cater for abundance food and biofuel production. Therefore, the debate should shift from food vs fuel to food and fuel debate (Rosillo-Calle, 2012).

1.5 Aim and Objectives of the Study

The overall aim of the project is to seek ways of reducing/utilizing the excess alcohol requirement of IST. This was achieved through the following objectives:

(i) Characterise the rapeseed for moisture, free fatty acid (FFA) and lipid content.
(ii) Investigate the effect and interactions of methanol to oil molar ratio, sodium hydroxide catalyst concentration and pre-soaking time on FAME yields using the design of experiments.
(iii) Find a method of online monitoring of IST, to aid in optimising and understanding reaction conditions
(iv) Complete a systematic screening study to determine the various glycerol conversion processes that are compatible with IST of rapeseed for biodiesel production, to find a viable “reactive coupling”
(v) Proof-of-concept of reactive coupling to demonstrate the feasibility of the process
(vi) Investigate the effect and interaction of various process conditions on reactive coupling products

Chapter 2. Literature Review

This chapter covers the importance of biodiesel as an alternative renewable energy vector; it also discusses general methods of biodiesel production with emphasis on the *in situ* transesterification process. Variables or parameters that influence the yield of biodiesel are reviewed. The work highlights several ways of increasing *in situ* transesterification yield, focusing on the reactions for reactive coupling and different means of polyglycerol production. Furthermore, the basis of the various analytical techniques for product analysis is discussed.

2.1 Biodiesel

Biodiesel is a word obtained from Greek where bio, translates to life, and diesel from Rudolf Diesel, the innovator of the diesel engine. It can be produced using oil extracted from plant or animal sources. Biodiesel is defined chemically as the monoalkyl esters of long-chain fatty acid made from sustainable lipids. (Demirbas, 2008). The transesterification reaction of a plant oil or animal fat using alcohol (methanol) with acid, base or enzyme catalyst to yield fatty acid methyl esters (biodiesel) and glycerol is shown in equation (2.1). Currently, biodiesel is usually made from soybean, rapeseed and palm seed.

$$C_3H_5(OOCR)_3 + 3CH_3OH \longrightarrow 3RCOOCH_3 + C_3H_5(OH)_3 \qquad 2.1$$

Triglyceride Methanol Methyl ester Glycerol

The advantages of biodiesel as a fuel are its portability, availability, renewability and lower emissions than petrodiesel as a result of lower sulphur and aromatic content (Ma and Hanna, 1999). Moreover, the higher heating value (HHV) or gross heating is relatively high for liquid fuel as compared to the Lower heating value (LHV) or net heating value of the solid fuel. For instance, the HHV of biodiesel is 39-41MJ/kg while those of coal is 32-37MJ/kg (Demirbas and Demirbas, 2011). The main difference in the HHV and LHV is that the former include the heat of vaporization (Hv) of the water produced during the combustion of the fuel while this is excluded in the latter (Sivaramarkrishnan, 2011) Some of the disadvantages of biodiesel over petrodiesel reported in the literature include cold flow properties as a result of high cloud point, lower energy content compared to petrodiesel and higher price (Dermirbas, 2008). Cloud point and energy content can be improved by using fuel additives such as glycerol ethers (Noureddini *et al.*, 1998).

2.2 Methods of reducing high viscosity in oils

Diesel engines can be powered by vegetable oil as fuel except that the viscosity of the oil is higher than petrodiesel. This can have a negative impact (carbon build up) when used for a long time in a diesel engine (Agarwal *et al.*, 2008). Reduction in the viscosity of vegetable oil can be achieved by pyrolysis, dilution, microemulsion, esterification, and transesterification. The first three methods are not commonly used as esterification and transesterification due to complex procedures and environmental safety issues involved. The Transesterification of triglycerides can be divided into conventional and *in situ* transesterification methods. The Fig. 2.1 shows the main methods of reducing viscosity in lipids for biofuel production.

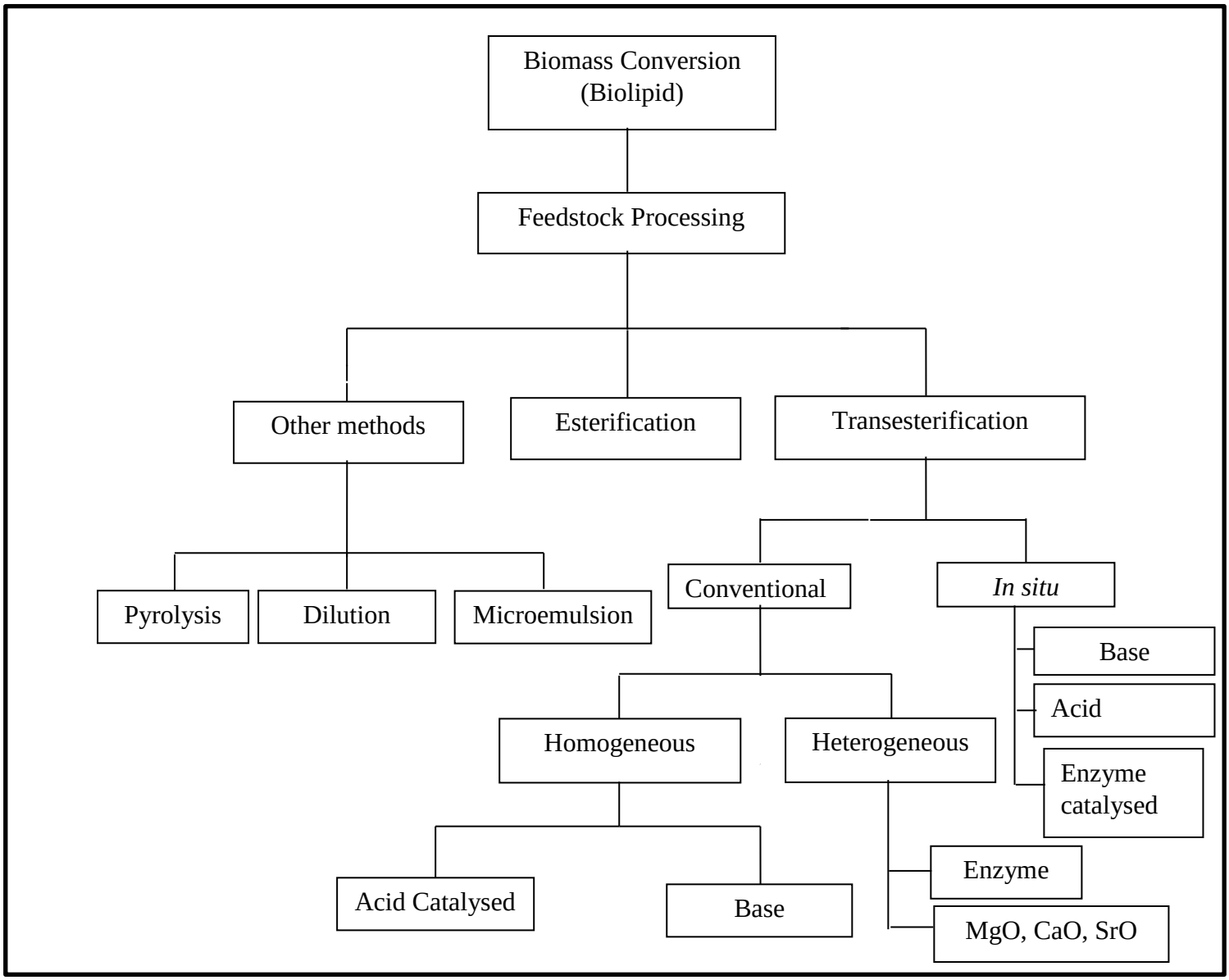


Figure 2.1 Biomass conversion methods

2.2.1 Other methods

The feasibility of using vegetable oils as a fuel has been known since the first development of diesel engine in 1893 (Dermirbas, 2008). The other challenges apart from high viscoscity of vegetable oil include inadequate fuel atomization as a result of coking formation on the engine injector, sticking of oil on the engine ring and gel contamination of the lubricating oil by the vegetable oil (Ma and Hanna, 1999). There are different ways of reducing the viscosity of vegetable oil so that it can be used as fuel oil. They are blending or dilution, microemulsion, pyrolysis or cracking, esterification, and transesterification. Esterification and transesterification are the methods of choice in the industry because they are simple and cost-effective compared to other ways (Ma and Hanna, 1999). The first three methods are briefly discussed below.

Blending or Dilution

The blend of vegetable oil and diesel fuel can be used to power diesel engine. Many researchers have successfully blended vegetable oils with diesel fuels. Anon (1982) powered a diesel fleet with a mix of 95 mass % used filtered cooking oil and 5 mass % diesel. The effect of cooler ambient temperatures can be reduced by blending or preheating of the fuel. There was no reported problem of coking or carbon build-up. This may have been due to filtering of the used cooking oil. The only problem reported was contamination of the engine oil was the only problem reported which resulted in changing the lubricating oil every 4,000-4,500 miles (Ma and Hanna, 1999). However, using vegetable oil mixture may be uneconomical in the long run because of the additional cost of changing the oil. In another related study, Ziejewski *et al.* (1986) blended 25 parts by volume of sunflower oil and 75 parts by volume of diesel as diesel fuel. The viscosity at 313K was 4.88 cSt, while the maximum value stated by ASTM at 313K is 4.0 cSt. This mixture was, therefore, not appropriate for extended period use in an injection engine. Furthermore, Karaosmonoglu (1999) conducted a study using dilution technique on frying oil. The torque, brake thermal efficiency and power were increased, but specific fuel consumption was reduced. Using blends of vegetable oils directly as fuel engines is doubtful. The little success reported in the literature are only for short-term use mainly because of high viscosity they pose potential mechanical problems in vehicles for long-term usage (Harwood, 1984).

Microemulsion

Microemulsion is a colloidal distribution of visible isotropic liquid microstructures with the length of 1-150nm produced immediately from two immiscible liquids and ionic or non- ionic

amphiphiles (Schwab *et al.*, 1988). A microemulsion can be made of plant oils using an ester and dispersant or of vegetable oils, an alcohol and a surfactant with or without diesel fuels. The volumetric heating values of microemulsion are lower than that of diesel fuel due to their alcohol content. However, latent heat of vaporisation of the alcohol cools the combustion chamber and reduces nozzle coking (Yusuf *et al.*, 2011). Srivasta and Prasad (2000) demonstrated the use of 2-octanol as an amphophile in the micellar solubilisation of methanol in triolein and soyabean oil. The viscosity was reduced to 11.2 cSt at 25^{o}C but the reported engine test showed the deposit of carbon near the orifices of the injector nozzles and on exhaust valves.

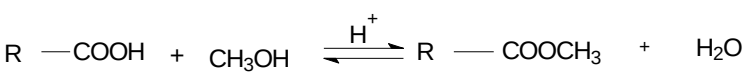
Pyrolysis

The conversion of organic material into another by use of heat in the absence of oxygen with or without a catalyst is known as pyrolysis. Pyrolysis and catalytic cracking of oils and fats result in the production of different hydrocarbons and gaseous products. Pyrolysis is a complex process because of the different reaction paths and products that can be obtained. Many different materials can be pyrolysed such as natural fatty acids, vegetable oils, animal fats and methyl esters of fatty acids. Schwab et al. (1988) thermally decomposed and cracked soybean oil and safflower oil respectively using distillation with air or sparge of nitrogen. The amount of alkanes and alkene produced from the distillation of soybean and safflower oils were 73-77% and 80-88% respectively. The pyrolysis of rapeseed oil was conducted at 500˚C and 850˚C in a tubular reactor to obtain a combination of methyl esters (Billaud *et al.*, 1995).

The main limitation of pyrolysis as a method of biofuel production is that equipment for thermal cracking and pyrolysis are expensive because of high temperatures involve in the processing (Ma and Hanna, 1999). Moreover, pyrolysis of oil requires upgrading before it can be used as engine fuel. This may be expensive and uneconomical.

2.2.2 Esterification

Fatty alkyl ester and water are produced from the esterification of free fatty acid (FFA) and alcohol (methanol) with acid (e.g., sulphuric acid) as the catalyst. Esterification is usually used as a pre-treatment method in base catalysed transesterification to reduce the free fatty acid of the lipid feedstock. An example of the reaction is as shown in Equation 2.2



2.2

Fatty acid Methanol Methyl ester water

The increased cost of biodiesel is as a result of the cost of feedstock because 60-70% total production expense is of the feedstock cost (Haas *et al.*, 2006). It is therefore essential to use cheap raw materials such as non-edible oils, waste food oil or animal fat. However, these oils contain high free fatty acids which are difficult to convert by homogeneous base catalyst transesterification due to the formation of undesirable soap from the free fatty acid and the catalyst by saponification. The soap formation significantly reduces the yield of biodiesel. When an alkali such as sodium hydroxide is added to high FFA feedstock, the FFA reacts with them to produce soap and water as shown by Equation 2.3

2.3

Fatty acid Sodium hydroxide Sodium soap Water

The esterification reactions are reversible, and equilibrium conversion is limited by the accumulation of the by-product water. The presence of water limits the conversion to a methyl ester that can be achieved in acid catalysed reactions, and its continuous removal has been shown to increase yields (Lucena *et al.*, 2011).

The acid esterification of fatty acids mechanism is as given in Fig. 2.2. In the first step, the FFA is the protonated to produce oxonium ion (1). An intermediate is then obtained as a result of the conversion of the oxonium ion with the alcohol (2). In the last step, an ester is formed, by the loss of a proton, from the intermediate complex (3). Each of the process steps is reversible, but the use of an excess of alcohol, ensure that the reaction equilibrium is displaced so that esterification proceeds almost to the end.

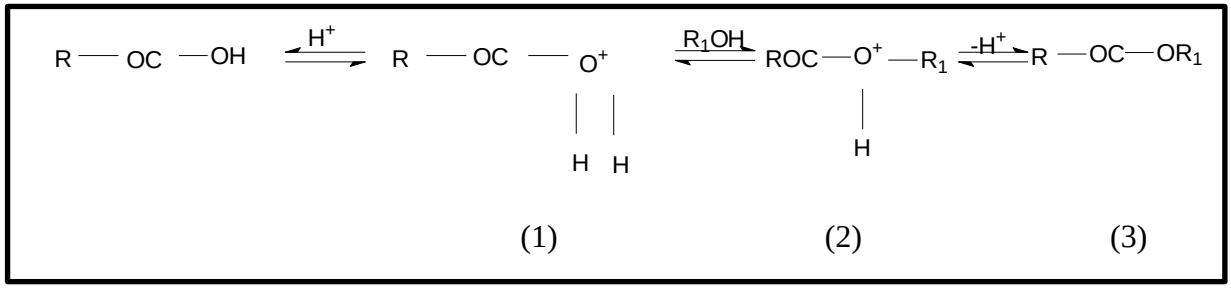


Figure 2.2 Mechanism of acid catalyst esterification of fatty acid (Dermirbas, 2008)

A significant disadvantage of acid catalysed esterification is that the reactions are usually slower than base catalysed. The reaction constants K for esterification (acid catalysed) and transesterification (base catalysed) of rapeseed oil at 60^{o}C are computed to be 0.0031min^{-1} and 0.0078 min^{-1} respectively (Jain *et al.*, 2011). Also acid catalysed esterification requires excess

alcohol and no water. There are, however, several ways to increase the reaction rates. These include increasing the temperature, increasing catalyst concentration and removing the by-product (Stacy *et al.*, 2014). These can increase the conversion to 95%.

The combination of esterification and transesterification reaction can only allow the FFA content up to 3% in the oil without adverse base-catalysed alcoholysis (Gerpen and Knothe, 2005). In the work of Fattah et al. (2014) laurel oil was investigated as a potential feedstock for biodiesel production using a two-step esterification-transesterification process. The study showed an improvement in overall evaluation characteristics of biodiesel from the blend of 10 and 20% volume of the oil and diesel compared to ordinary diesel.

In another work, Stacy *et al.* (2014), studied the performance of an acid catalysed bubble column reactor conducted at 120^{o}C and atmospheric pressure for esterification of free fatty acids to fatty acid alkyl esters. The work indicated that the FFA reacts with the methanol that is bubbled through the reactor and removes the by-product water. Conversions of 98% of the FFA were achieved in less than 2h. Similarly, Liu et al. (2014) produced biodiesel from esterification ethanol and oleic acid catalysed by PA/NaY. The result showed that oleic acid conversion was 79.5 $\pm$ 0.7%. The molar ratio of alcohol to oleic acid is 7:1 with 1.7g PA/NaY catalyst at 105^{o}C for 7h.

Kombe *et al.* (2013) in their work reviewed reverse esterification as a likely approach for the reduction of high FFA feedstock for biodiesel production utilizing glycerol instead of methanol for the process. The advantage of the method is the use of glycerol byproduct to lower the FFA of feedstock instead of methanol which reduces the cost of production of biodiesel. However, there is a need for further research to make the process kinetics and optimization more economical. Furthermore, the high temperature (200^{o}C) of the process is a drawback to adoption of the technology for large-scale biodiesel production.

The recent work of Veillette *et al.* (2017) investigated esterification of oil (oleic acid + canola oil) and microalgae lipid with methanol to produce biodiesel utilizing a heterogeneous catalyst. The results showed that Amberlyst-15 catalyst gave the maximum FFA conversion of 84% at 60mins reaction time and temperature of 120^{o}C. However, alkali transesterification was suggested for the conversion of the remaining lipids present in the microalgae. The disadvantage of this 2-stage transformation is the increase processing cost that is associated with the method.

2.2.3 Transesterification

Among all the alternatives for reducing the viscosity of the oil, transesterification appears to be the best choice because fatty acid esters (biodiesel) physical characteristics are similar to diesel fuel. Furthermore, the process is cost effective and simple. Transesterification is the term used to describe the class of organic reactions where an interchange of alkoxy moiety, i.e., the triglyceride ester is transformed into fatty acid methyl ester (Dermirbas, 2008). The reaction proceeds with short-chain alcohols in the presence of a catalyst as shown in Figure 2.3

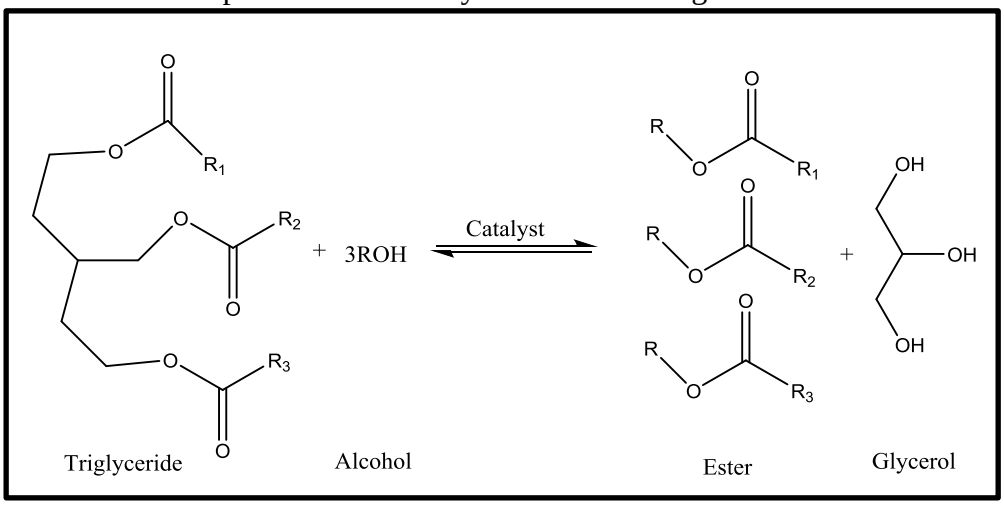


Figure 2.3 Structural equation of transesterification reaction

Transesterification can be catalysed by an alkali (alkoxide ion), acid (H_2SO_4) or enzyme (lipase). However, biodiesel can be produced by non-catalytic transesterification of vegetable oil with supercritical methanol (Dermirbas, 2008). To shift the reaction equilibrium to the product side, it is necessary to use an excess of the alcohol or remove one of the products from the reaction. The methyl or ethyl esters of fatty acids formed from transesterification reaction can be used to power diesel engines without any modification with low carbon deposit formation of 0.05% maximum (EN 14214 standard).

Transesterification can be classified into conventional and *in situ* transesterification. For traditional transesterification, the oil is extracted before the primary reaction with an alcohol in the presence of the catalyst. For *in situ* transesterifications the extraction of the oil and reaction are combined in a single operation.

Conventional Transesterification

Conventional transesterification is commonly used biodiesel production method. In this method, triglycerides are converted into fatty acid esters using short-chain alcohol (methanol, ethanol or isopropanol) and acid, base or enzyme catalyst. The material of conventional transesterification is

extracted oil obtained from vegetable and animal sources. The oil-bearing seeds are crushed and oil is extracted by mechanical pressing and solvent extraction with hexane. The extracted oil is then purified by degumming before transesterification. The cost of pre-extraction and purification of oil increases the cost of production of biodiesel by about 20% (Kasim *et al.*, 2010a).

During transesterification, the triglycerides react to form diglycerides, which are afterward converted to monoglycerides and fatty acid ester and glycerol as shown in the consecutive and reversible reactions given by equations 2.5-2.7

$$R\text{-}OOC\text{-}R_1 + ROH \rightleftharpoons Diglyceride + RCOOR_1 \quad (2.5)$$

$$R\text{-}OOC\text{-}R_2 + ROH \rightleftharpoons Monoglyceride + RCOOR_2 \quad (2.6)$$

$$R\text{-}OOC\text{-}R_3 + ROH \rightleftharpoons Glycerol + RCOOR_3 \quad (2.7)$$

R denotes carbon chain of the alcohol used and R_1, R_2 and R_3 are fatty acid chains.

The rate determining step is the formation of alkyl ester from monoglycerides because it is the most stable of all the intermediates compound (Ma and Hanna, 1999). The reaction rate constants, K at 60^{o}C from triglyceride to diglyceride, diglyceride to monoglyceride and monoglyceride to glycerol are 0.0208, 0.0610 and 0.1528 wt. %.min^{-1} respectively (Krishnan and Dass, 2012).

Free fatty acid and water should be minimised to avoid the adverse effect of soap formation in conventional transesterification (Kusdiana and Saka, 2004). Several aspects, including catalyst type and concentration, alcohol/oil molar ratio, and temperature are reported to influence the yield of biodiesel. Others are moisture content and mixing speed (Freeman *et al.*, 1984; Fukuda *et al.*, 2001; Meher *et al.*, 2006; Hincapié *et al.*, 2011). These aspects are discussed in the following sections:

Catalyst type and concentration

The catalyst used for conventional transesterification can be base, acid or enzyme and homogeneous or heterogeneous. Examples of base catalysts are sodium hydroxide, sodium methoxide, potassium hydroxide and potassium methoxide. The acid-catalysts could be sulphuric acid, hydrochloric acid or organic sulfonic acid.

Ma *et al.* (1998) studied transesterification of beef tallow using sodium hydroxide and sodium methoxide. It was found that sodium hydroxide is better than sodium methoxide because they

reached their maximum activity 0.3% and 0.5% w/w of the beef tallow respectively. Also, using sodium methoxide caused the formation of many side products mainly sodium salts which constitute to waste product. Furthermore, high-quality refined oil is required for NaOMe, which increases the cost of production (Ma *et al.*, 1998). This contradicted the study of Freedman *et al.* (1986) where ester yield at 6:1 alcohol to oil molar ratio of 1% NaOH and 0.5% NaOMe were same after 60 min. The contradiction may be due to the difference in process conditions and quality of the oil used in their studies.

Mohammed and Ali (2002) studied acid-catalyzed transesterification of a waste vegetable oil with four different catalyst concentrations, 0.5, 1.0, 1.5, and 2.25 M HCl using of 100% excess alcohol and compared their findings with 2.25 M H_2SO_4. The reduction in viscosity was used to measure the extent of reaction. H_2SO_4 showed a higher catalytic activity of 1.5-2.25 M.

Alkaline catalysis produces high yields and is a relatively simple process. However, there is a number of downsides: difficulty in recovery of the glycerol by-product, catalyst removal from product and treatment of waste are some of the drawbacks of the process. These impediments can be removed by the replacement of alkaline catalyst with the enzyme. However, the high cost of enzymes (e.g., lipase) has hindered its adoption for commercial production of biodiesel (Meher *et al.*, 2006). The glycerol by-product can be easily separated without any complicated process and also the free fatty acids contained in the waste oils. Moreover, the fats can be entirely converted to alkyl esters.

The molar ratio of alcohol to oil and type of alcohol

The alcohol to oil molar ratio can affect the yield of ester in transesterification reaction for biodiesel. According to the reaction equation, the ratio of alcohol to oil is 3:1. However, excess alcohol is required to shift the equilibrium to the product side (Meher *et al.*, 2006). For maximum conversion molar ratios of alcohol to oil vary from 6:1 to 40:1 depending on the process being used (Dermirbas, 2008). The excess alcohol can be recovered and reused.

Encimer *et al.* (2002) used alcohol to a molar ratio of between 3:1 and 15:1 to study the transesterification of Cynara oil and ethanol. Higher yield of ester was observed as the molar ratio increases up to the value of 12:1. Maximum ester yields were obtained between 9:1 and 12:1, incomplete reaction were observed at below 6:1 and for ratios higher than 15:1 the separation of glycerol by-product was difficult and ester yield reduced.

For conventional transesterification, methanol and ethanol are most commonly used, however, the formation of ethyl ester from ethanol is more difficult than the formation of methyl esters from methanol because of the formation of the stable emulsion during ethanolysis (Meher *et al.*, 2006). In the case of methanolysis, the emulsion formed breaks down to produce a lower and upper glycerol-rich phase and methyl ester rich phase respectively (Zhou *et al.*, 2003). The formation of the monoglycerides and diglycerides intermediates is partly due to the emulsion with polar hydrocarbon chains.

Reaction time

The conversion of triglycerides to FAME by conventional transesterification increases with time until equilibrium is attained. Transesterification of cottonseed, peanut, sunflower and soybean oil to biodiesel were investigated by Freeman *et al.* (1984). The reactions were conducted using methanol to oil molar ratio of 6:1, 0.5 mass % sodium methoxide catalyst and 60^{o}C. After 1 min, ester yield for both soybean and sunflower oils was about 80%. The yield was later increased to 93 – 98% for all the oils after 1 h. The kinetics of the process could not be measured because of the single data point at 1 h. In another study, transesterification of cottonseed oil and sodium methoxide was conducted by Goswami and Usmani (2014) at 60^{o}C for 2-3 h. The highest biodiesel yield of 75% was observed after 2 h of the reaction. The variation in the yields may be due to the difference in process condition particularly the catalyst concentration which was 0.5% and 0.2% in the two studies.

Reaction temperature

Transesterification reaction can proceed at various temperatures depending on the catalyst and oil used. Usually, the optimum temperature is between 30-60^{o}C (Ma and Hanna, 1999). Freedman et al. (1984) studied the effect of temperature on the rate of reaction and yield of ester. Transesterification of refined oil with methanol at 1 wt/wt % was investigated at different temperatures. The ester yield after 0.1 h were 94%, 87% and 64% for 60, 45 and 32^{o}C respectively. Temperature higher than 60^{o}C should be avoided because it is the boiling point of methanol except where pressure vessel is used as reaction vessel which can keep the alcohol in the liquid state.

Mixing intensity

The immiscibility of oil/fat and alkaline alcohol phase in transesterification makes mixing an essential parameter for effective formation of methyl ester. Mixing will ensure that the phases are brought together for the reaction to commence after the reaction is initiated stirring is no longer

required as further stirring does not have any significant effect on rate or yield of FAME (Meher *et al.*, 2006). To proof the importance of mixing, Ma *et al.* (1999) studied the transesterification of beef tallow. The results showed no reaction without mixing. On the other hand with stirring, the formation of ester occurred and yield was significantly increased. In a more comprehensive study, on mixing effect on the kinetics of transesterification of soyabean oil using methanol conducted by Noureddini and Zhu (1997), showed that the initial slow rate of transesterification was reduced as mixing intensities were increased which confirm the theory of mass transfer controlled during the early stage of the reaction. However, the effect of mixing became insignificant at a later stage of the reaction when the reaction is kinetically controlled as a result of the complete dispersion of the phases. It has been observed that increasing the contact area between two immiscible phases (e.g methanol and oil) increases the FAME yield (Sun *et al.*, 2009). This is because the mass transfer between the boundaries of the immiscible liquid is enhanced. A micro-droplet can be created by using a microfluidic system (microreactors and capillary reactors) which increases the interfacial area of the phases because they have a large surface-to-volume ratio and therefore enhance FAME yield by transesterification (Madhawan *et al.*, 2017). The adequate intermixing of immiscible liquid within a microchannels can be used in many chemical engineering applications. For effective mixing, turbulence must be developed in the fluid created by the impeller to provide shear force. Usually, electrical power is needed to drive impellers in mixing tanks. The power number (N_p) of fluids depends on the stirrer speed, the impeller diameter and geometry, and properties of the fluid such as density and viscosity. The relationship between these terms is expressed in dimensionless number N_p and given in equation 2.8 (Paul *et al.*, 2004):

$$N_p = \frac{P}{\rho N_i^3 D_i^5} \qquad 2.8$$

Where P = Power requirement of mixer (kW), ρ = density of fluid (kg/m3), N_i = stirrer speed (s^{-1}) and D_i = impeller diameter (m)

Effect of organic co-solvent

The use of organic co-solvents to increase the rate of transesterification was demonstrated in the work of Meher *et al.* (2006). In their study, the methoxide base catalysed methanolysis of soybean oil at 40^{o}C reacts slowly than butanolysis at 30^{o}C. This is as result of 2-phase in which methanolysis occur. Co-solvent like tetrahydrofuran (THF), 1,4-dioxane and diethyl ether are needed to conduct single phase reaction. The rate of methanolysis can be increased by the use of co-solvent of 1.25 volumes of THF per volume of methanol at 6:1 methanol oil ratio. This produces an oil-rich single-

phase system in which methanolysis is as fast as butanolysis. The drawback of the use of co-solvent in biodiesel production is the additional cost of its separation which increases the overall cost of production.

2.2.4 Exergy Balance for Biodiesel Production

Exergy is a quality index for energy which indicates the quantity of mechanical work required to produce a material in its specified state from components common in the natural environment, in a reversible way, heat is being exchanged only with the environment (Szargut, 2005). In contrast, to energy which is a state function, exergy is a function both the state and common components of the environment.

The exergy balance equation for the overall biodiesel production can be written using the method of Ptasinski (2016) as

$$E_{rapeseed\ oil} + E_{methanol,in} + \sum_{in} E_i + \sum_{in} E_{stream} + W_{el} = E_{biodiesel} + E_{glycerol} + E_{methanol,out} + \sum_{out} E_i + \sum_{out} E_{steam} + I_{overall} \quad 2.9$$

Where $E_{rapeseed\ oil}$, $E_{methanol}$, $E_{biodiesel}$, $E_{glycerol}$, and E_i are exergy flowrate of rapeseed oil feedstock, methanol, biodiesel, glycerol, and chemicals respectively, E_{steam} is the exergy flow rate of steam, W_{el} is the exergy of electricity consumed and $I_{overall}$ represents the overall irreversibility (internal exergy loss).

It follows that the overall exergetic efficiency (η) of rapeseed oil-to-biodiesel process can be derived from equation 2.9 as

$$\eta = \frac{E_{biodiesel}}{E_{rapeseed\ oil} + \Delta E_{methanol} + \sum_{in} E_i + \Delta E_{steam} + W_{el}} \quad 2.10$$

Where Δ denotes the difference between the exergy flow rates of entering and leaving methanol and steam streams respectively.

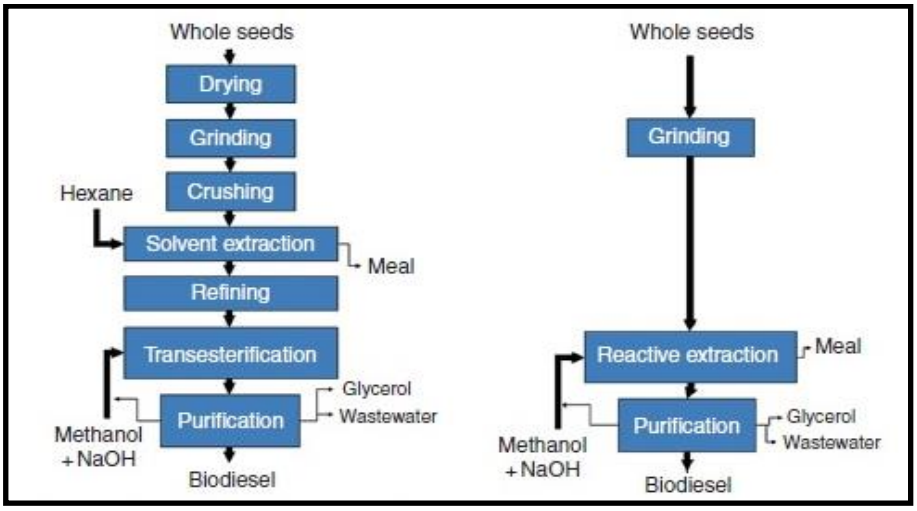
As it can be seen from equation 2.9 and 2.10 if the methanol requirement for the reaction can be reduced the exergy efficiency can be increased.

In situ Transesterification

In situ transesterification (IST) or reactive extraction is another method of biodiesel production where the feedstock is an oil-bearing solid such as rapeseed or microalgae. The ground feedstock is directly contacted with alcohol using acid or base catalyst to form biodiesel and glycerol.

Extraction and reaction proceed in one step. Hence the process is intensified. This can potentially reduce the total expense of production since the cost of pre-extraction and refining the oil are eliminated. The process condition for IST is similar to that of conventional transesterification. However, the alcohol to oil molar ratio is 100-folds in IST as against 10-folds for the conventional transesterification. Another disadvantage of IST over conventional method is that water content of oilseed may be difficult to remove unlike that of extracted oil.

Figure 2.2 below compares the processing steps of conventional transesterification with *in situ* transesterification. Drying may be required for IST to standardize especially where base catalysed IST method is adopted.



(a) (b)

Figure 2.4 Comparison of process steps in conventional (a) and in situ transesterification (b) (Harvey and Lee, 2012)

In situ transesterification of sunflower, oilseed was first published in 1985 by Harington and D'Arcy-Evan (1985). After that many researcher, have investigated the method, focusing on different feedstocks, catalysts and reaction conditions to improve the economic viability of biodiesel production via IST (Haas *et al.*, 2004; Haagenson *et al.*, 2010; Lei *et al.*, 2010; Kasim and Harvey, 2011; Zakari and Harvey, 2012; Salam, 2015). There are some variables that can influence the yield: type of raw materials, temperature, time, catalyst type and concentration, the moisture content of the feedstock and ratio of alcohol to oil. These process variables are discussed in the sections below:

Effect of raw materials

One of the most critical considerations in the biodiesel production via *in situ* transesterification is the selection of the feedstock because this accounts for 60-80% of the total cost of production (Leung *et al.*, 2010). Feedstock/raw material that contain high free fatty acid are not suitable for base catalysed transesterification unless the FFA is reduced by esterification followed by transesterification. The reaction of FFA with the base catalyst can lead to the formation of soap which limits the amount of FAME produced. However, acid catalysed *in situ* transesterification can be used for these kinds of feedstock because soap formation can be avoided. For example, rice bran with high acidity was converted to 97% ester using *in situ* transesterification (Ozgul-Yucel and Turkay, 2002; Ozgul-Yucel and Turkay, 2003).

Apart from FFA content in the feedstock, oil and moisture content in raw material are essential in determining the FAME yield. The higher the oil/lipid content of the feed the higher the lipid that is transesterified into FAME. The availability of different oil bearing materials as feedstocks for *in situ* transesterification is a major advantage of the method over conventional transesterification since the cost and steps associated with the extraction of oil from the feedstock are removed. This will potentially reduce not only the overall cost of production but also save the processing time because there are fewer steps involved. The different oil-bearing feedstock can be converted to biodiesel by *in situ* transesterification. Canola (Haagenson *et al.*, 2010), castor seed (Hincapié *et al.*, 2011), Chlorella Vulgaris microalgae(Velasquez-Orta *et al.*, 2012; Salam *et al.*, 2016c), *Jatropha curcas* (Kaul *et al.*, 2010; Kasim and Harvey, 2011), rice bran (Ozgul-Yucel and Turkay, 2002; Ozgul-Yucel and Turkay, 2003), soybean (Haas *et al.*, 2013), sunflower seed (Georgogianni *et al.*, 2008b), cottonseed (Georgogianni *et al.*, 2008a) and rapeseed (Zakari and Harvey, 2012; Qian *et al.*, 2013) have been investigated for their suitability as feedstock for biodiesel via *in situ* transesterification. All reported feedstock were observed can be used for biodiesel production. None of these studies have addressed the fundamental challenge of excess alcohol used in IST which is a focus of this present study. Table 2.1 gives a summary of selected biomass used for the production of biodiesel by IST and their operating conditions.

Table 2.1: Summary of feedstock, operating conditions and yield by *in situ* transesterification

Feedstock	Solvent	Solvent to feedstock ratio	Temperature (°C)/Time (h)	Catalyst	Stirring rate (rpm)	Performance indicator (%)	Reference
Activated sludge	Methanol	25ml:1	55/24	H_2SO_4	-	4.88 (yield)	(Revellame *et al.*, 2010)
Canola (*Brassica napus*)	Methanol	275:1	60/0.5	KOH	200	80 (conversion)	(Haagenson *et al.*, 2010)
Jatropha curcas L	Methanol	400:1	30/0.5	NaOH	300	82 (yield)	(Kasim and Harvey, 2011)
Jatropha curcas L	Ethanol	140ml:20g	30/2	CH3ONa (2.0 wt. %)	600	99.9 (yield)	(Ginting *et al.*, 2012)
Jatropha curcas L	Ethanol	6.5:1	30/3.5	NaOH	~400	99.2 (yield)	(Hailegiorgis *et al.*, 2011)
Rapeseed	Methanol	475:1	60/1	NaOH (0.1 M)	200	88.8 (yield)	(Zakaria and Harvey, 2012)
Rapeseed	Methanol	720:1	65/1	KOH	-	90 (conversion)	(Abo El-Enin *et al.*, 2013)
Chlorella sp. microalgae	Methanol	315:1	60/8	H_2SO_4	500	88.5 (conversion)	(Ehimen *et al.*, 2012)

Chlorella Vulgaris microalgae	Methanol	600:1	60/1.25	NaOH	380	78 (yield)	(Leung *et al.*, 2010)
Chlorella vulgaris microalgae	Methanol	925:1	60/1	NaOH (0.5N)	450	97 (yield)	(Salam *et al.*, 2016a)
Soybean	Methanol	18 ml:50 g	25/16	NaOH (0.1N)	-	97 (yield)	(Haas and Scott, 2007)
Rice Bran	Methanol	200 ml:50 g	65/5	H_2SO_4	-	86 (yield)	(Ozgul-Yucel and Turkay, 2002)
Sunflower	Methanol	200:1	65/4	H_2SO_4	-	97 (yield)	(Siler-Marinkovic and Tomasevic, 1998)

Moisture content

The moisture content of the feedstock has an impact on the ester yield for *in situ* transesterification as it does for conventional transesterification. Excess water in alkali catalysed transesterification can hydrolyse the triglyceride releasing FFA which can react with the alkaline to form soap. This consumes the catalyst and reduces its efficiency and reduces the biodiesel yield. Also, it makes the separation of glycerol from product difficult due to the formation of a gel (Fukuda *et al.*, 2001). Furthermore, the dissolution of triglycerides in methanol can be reduced due to the polar structure of water which may increase mass transfer resistance for methanol diffusing into the seed (Lim and Lee, 2013). The influence of moisture on milled cottonseed conversion into FAME was investigated by Qian and Yun (2009). They compared different water removal methods before *in situ* transesterifications; the highest conversion of 98% was obtained after methanol was used to wash the seed while yields of 21% and 35% were obtained for untreated and vacuum dried cotton meal respectively. Haas and Wagner (2011) also reported a decrease in methanol requirement when dried soybean flakes were used *in situ* transesterification compared to undried seed. However, Zakaria and Harvey (2012) found that drying rapeseed did not affect the ester yield and methanol requirement. The difference in these findings may be as a result of the difference in the biological morphology of soybean and rapeseed. However, in enzyme catalysed reactive extraction, there is a

need for a small amount of water because enzymes require water medium to be active. However, excess water can cause the reverse reaction (Ycel *et al.*, 2012). In more recent work Salam *et al.* (2016b) investigated the IST of *Nannochloropsis Occulata* using sodium dodecyl sulphate (SDS) in sulphuric acid. A FAME yield of 98.3% was obtained even at 20% moisture of the feedstock. This study suggests a high water tolerant of the biomass used. In contrast, at 30% moisture, the FAME yield declined to 60%. Despite reported high FAME production with wet biomass by IST the cost of drying feedstock remains a barrier to commercializing the technology.

Particle size

To obtain high yields of ester by *in situ* transesterification, there is need to have small particle sizes of the crushed oilseed to facilitate the transfer of the methanol to the seed. Kasim and Harvey (2011) investigated IST of Jatropha curcas seed and the effect on the seed of different particle sizes of 0.5-4 mm were investigated. Using the catalyst concentration 0.1 N, the temperature of 60^{o}C, mixing speed of 400 rpm and methanol to oil molar ratio of 400/1, the highest yields were obtained with <0.5 mm particle size at 86.1%. Moreover, the results showed that increasing the particle size about to 2 mm decreased the ester yield to about 40%. In the same trend, Zakaria and Harvey (2012) studied the effect of particle size on the rate of reactive extraction of rapeseed. They showed that the rate of reaction for the particle size of 300-500 µm was faster than that of 1000-1400 µm. This was probably because the smaller seed particles release more oil at the surface due to the greater fracturing of the oil and greater surface area. However, contrary to Kasim and Harvey's work on Jatropha seed, Kaul *et al.* (2010) investigated the reactive extraction of Jatropha seed using different particle sizes from 0.85 - 2.46 mm and found that the seed size (>2.46 mm) exhibited a higher yield. The different report in their findings may be due to the difference in the feedstock and operating conditions. Most of the works on the effect of biomass particle size for IST may be limited by the challenge in aggregating the average particle size for the entire portion of biomass used for the experiment.

Type of alcohol

The commonly used alcohol in transesterification is methanol because of it is inexpensive, availability and ease of recovery at the purification stage (Dermirbas, 2008). Kildiran *et al.* (1996) investigated the effect of ethanol, methanol, n-propanol, and n-butanol on the IST of soybean. The maximum yield of 84.6% was obtained using n-propanol, with ethanol and n-butanol, 80.8% and 78% yield respectively. Methanol recorded the least yield of 41.5% because of lesser alcohol chain length of methanol compared to ethanol and butanol. Another factor to consider is the solubility of

triglycerides in monohydroxyl alcohol which increases as alcohol chain length increases. In another study by Ozgul and Turkay (1993) studied the *in situ* transesterification of rice bran oil using methanol and ethanol. It was found that ethanol gave a higher yield than methanol which was 98.7% and 85.9% respectively. Recently, Verma and Sharma (2016) conducted a comparative study of using of methanol and ethanol for IST Karanja oilseed for biodiesel. The results showed a higher biodiesel yield of 91.05% when methanol was used compared to ethanol which gave 77.4%. However, a major drawback of this approach is the variability in the process conditions for both processes which limits the general conclusion on the better alcohol to use for IST. In most studies the cost and availability of the alcohol is the overriding factor in their selection for IST while methanol is the cheapest of the monohydroxyl alcohol in most areas, ethanol is mostly used in Brazil because of the abundance of sugarcane from where bioethanol is produced.

Alcohol to Oil Molar ratio

The volume of alcohol used in IST influences the FAME yield. Transesterification is a reversible reaction, thus according to Le Chatelier's principle, the reaction equilibrium will shift to the product side, when one of the reactants is increased resulting in higher FAME yields (Qian and Yun, 2009). Alcohol plays a dual role in reactive extraction; it is used both as solvent (lipid extractor) and reactant (conversion of lipid to ester) (Wahlen *et al.*, 2011). Kasim and Harvey (2011) investigated the influence of alcohol to oil molar ratios of 100 - 600 on the reactive extraction of *Jatropha curcas*. The results showed that there was no reaction at a molar ratio of 100. However, molar ratios of 200, 300, 400, 500 and 600 gave FAME yields of 52.0%, 74.7%, 81.9%, 85.7% and 86.9% respectively. Further increases in alcohol did not increase the yield but will increase the energy cost of removing the excess alcohol which would increase the cost of production. In related work carried out at Newcastle University by Zakaria and Harvey (2012), the effect of molar ratio of alcohol to oil on the reactive extraction of rapeseed for FAME production was investigated. Ester yield increased significantly as the molar ratio increased from 0 to 300. The maximum yield of 82% ester was attained at 600:1 molar ratio, with 2.1 wt % catalyst concentration and 1 h reaction time. However, there was a decrease in ester yield at molar ratios of 900:1 and above.

In the related work of Abo El-Enin *et al.* (2013), alkaline *in situ* transesterification of rapeseed was investigated to determine the effect using different molar ratios of methanol to oil for the reaction. They obtained 90% yield at 720:1 and 96% at 1440:1 ratio, however, the ratio of 720:1 was suggested as optimum because of a higher cost of separation if 1440:1 molar ratio was used. In more recent work, Salam (2015) investigated the effect of methanol to oil molar ratio on FAME

yield from microalgae. FAME yield was observed to increase from 38% to 74% when the ratio was increased from 600:1 to 1275:1. However, excess alcohol used in IST increases the energy cost of separation downstream.

In situ transesterification for biodiesel utilising sunflower, oilseed was investigated by Siler-Marinkovic and Tomasevic (1998). A conversion of 97% was obtained at 200:1 molar ratio after 4 h with 100% sulphuric acid estimated on oil basis (3-19 ml). Most researchers have reported that higher alcohol to oil molar ratios is required in reactive extraction than conventional transesterification. This can potentially erode the gains of reduced process steps in reactive extraction by the high energy cost of recovering the excess alcohol used for *in situ* transesterification. This study seeks to reduce the high cost of recovery of alcohol by converting the excess alcohol to useful dimethyl ether (DME) which can be a potential biofuel gas with higher cetane number than biodiesel.

Temperature

In situ transesterification is an endothermic reaction consequently the increase in temperature increases the FAME-yield (Zakari and Harvey, 2012). The increase in the rate of *in situ* transesterification reaction is noticed only at the initial stage of the extraction, as similar time is needed for lower and higher temperatures. Many researchers have investigated the effect of temperature on reactive extraction. Favourable temperature is specific to each production; both high and low temperatures have been reported in the literature (Haas *et al.*, 2004; Abo El-Enin *et al.*, 2013). For example, the most favourable temperature that is required for reactive extraction of *Jatropha curcas* (Kasim and Harvey, 2011) and rapeseed (Zakari and Harvey, 2012) is 30^{o}C while that of soy flour (Bollin and Viamajala, 2012) is 110^{o}C with other reaction conditions. The solubility of triglyceride in methanol increases with increase in temperature this leads to a faster rate of transesterification reaction with less or no catalyst. However, higher energy cost as result of high energy consumption will increase ester production cost (Go *et al.*, 2016). Minor improvement is gained by increasing the temperature thereby low temperature is usually favoured for process economics of the production.

Mixing

In reactive extraction, adequate mixing is important to the overall success of the method. Good mixing or agitation prevents clumping of seed particles and enhances contact between the biomass, solvent, and catalyst. This will improve the rate and yield of reaction (Pradhan *et al.*, 2012; Tsigie

et al., 2012; Sulaiman *et al.*, 2013). Agitation can be produced by the impeller, magnetic stirrer or incubator shakers depending on the mixing intensity required for the biomass being converted. Tsigie *et al.* (2012) reported FAME yields of 88.5% by *in situ* transesterifications of *Chlorella vulgaris* in 4 h with stirring and 8hrs without stirring to attain 89.1% FAME yield. Kasim and Harvey (2011) observed that mass transfer limiting is not limited by mixing in IST. Their investigation showed that increasing the mixing intensity did have a significant effect on ester yield. Reducing the mixing intensity in an incubator shaker from 300 rpm to 200 decreases the yield from 94.8% to 85.7% and a further decrease in 100 rpm reduced the ester yield to 37.2%. They also found that increasing the mixing intensity above 300 rpm did not affect the yield. In another related study, Pradhan *et al.* (2012) investigated the reactive extraction of castor seed using a response surface method. The maximum mixing speed was found to be 350 rpm, and further increase showed no significant increase in ester yield. Together all these studies provide an insight into the impact of mixing on ester yield. However, there is no study yet that compares the mechanism of mixing of IST to FAME yield to determine most effective mixing device for improved productivity of the biodiesel process.

Quality of Biodiesel

There are established standard through which quality of biodiesel is measured. An example of this is the American Society for Testing and Materials ASTM 6751 which sets out the technical standard methods to use in determining certain properties of biodiesel. This standard identifies the specification the pure biodiesel (B100) must meet before it can be utilised as a neat fuel or mixed with petroleum-based diesel fuel. Another related regulator is the European standard EN 14214, which describes the requirement and test methods for biodiesel. It is an internationally accepted standard that prescribes the minimum requirement for rapeseed methyl esters from its feedstock (Demirbas, 2008). Ramos *et al.* (2009) analysed the quality of methyl ester produced from rapeseed. Table 2.1 shows the quality of methyl ester from IST compared to ASTM D6751 and EN 14214 standard.

Table 2.1 Comparison of Rapeseed methyl ester against ASTM D6751 and EN 14214 (Ramos et al., 2009)

Property	Rapeseed methyl ester from IST	ASTM D6751	EN 14214
Flash point (°C)	170	>130	>101

Water and sediment (vol%)	0	0.05	0.05
Carbon residue (wt%)	<0.010	0.05	-
Sulfated ash (mass %)	0.000	0.020	-
Viscosity (cSt at 40°C)	4.478	1.9-6.0	3.5-5.0
Acid number (mg KOH/g)	0.16	0.80	0.50
Ester content (wt %)	99.5	-	96.5[a]
Free glycerine (wt %)	0.01	0.02	0.02
Total glycerine (wt %)	0.09	0.240	0.25
Cetane number	55	51[a]	51[a]
Cloud point °C	-4.1	-26.0	-5.0
Pour point	-12	-46	-26
Density at 15°C. Kg/m^3	882	882	882
Iodine value (gI_2/100g)	109	-	120[b]
Oxidative stability, 110°C	2.0		6.0[a]
Monoglycerides content (wt.%)	0.41	-	0.80[b]
Diglycerides content (wt.%)	0.08	-	0.20[b]
Triglycerides content (wt.%)	0.03	-	0.20[b]

Minimum limit: [a] Maximum limit: [b]

One of the significant barriers to the current adoption of IST, as a viable biodiesel production method, is the excess solvent or alcohol that is needed to extract and react with the lipids in the biomass. The energy cost of recovery of the remaining alcohol at the downstream increase the overall production cost. Hence, the research community has been actively engaged in finding ways of improving IST for better efficiency.

2.3 Methods of improving in situ transesterification

Since 1985 when Harrington and D'Arcy-Evans discovered IST as a better alternative to conventional transesterification (Harrington and D'Arcy-Evans, 1985), there have been many studies on improving IST as a viable alternative method of biodiesel production. These studies have shown that IST yield can be increased by:

- The use of co-solvent to reduce alcohol requirement and mass transfer limitation.
- Microwave heating aided *in situ* transesterification

- Supercritical fluid assisted *in situ* transesterification
- Ultrasound aided transesterification.
- Physical pretreatment of feedstock.
- Presoaking
- Reactive Coupling

These methods are discussed in the section below.

2.3.1 Use of co-solvent

Co-solvents used in reactive extraction reduce viscosity and increase the penetration/contact of catalyst and alcohol into the biomass particles. Different chemicals have been used as co-solvents for reactive extraction. These include dichloromethane, ethyl acetate, chloroform benzene, n-hexane and tetrahydrofuran (THF). Bollin and Viamajala (2012) used chloroaluminate catalyst and an ionic liquid to study *in situ* transesterification of lipid with soy flour. Dichloromethane was introduced as a co-solvent to decrease the viscosity of the ionic liquid. The results indicated that a FAME yield of >90% could be obtained for 4 h at 110^{o}C and the enhanced FAME yield was as a result of creating a homogeneous reaction medium. In more recent work Suganya *et al.* (2014a) wherein IST of *Enteromorpha compressa* algal biomass was investigated for biodiesel production. The optimization results showed that maximum methyl ester yield of 98.89% was attained at 30% vol of THF, 10 wt% of sulphuric acid catalyst, 5.5:1 volume ratio of methanol to algal biomass and 600 rpm of mixing intensity at 65^{o}C for 90 min reaction time. THF (65.8^{o}C) was selected because of its close boiling point to methanol (64.4^{o}C) so that after the reaction, both methanol and THF is recycled in a single step to be used again, consequently, simplifying the separation process (Mohammed-Dabo *et al.*, 2012). Apart from selecting the right co-solvent (which will not react with the transesterification reagent), it is essential to find the optimum co-solvent volume to maximise conversion to esters. Johnson and Wen (2009) used direct transesterification of microalga *Schizochytrium limacinum* to produce biodiesel. The results indicated that maximum yield of 72.8% ester was obtained when chloroform was used as co-solvent compared to hexane and petroleum ether which gave 10.5% and 11.5% yields respectively. These studies proved that mass transfer limitation and alcohol need of *in situ* transesterifications could be decreased with the addition of co-solvent. However, the additional cost of purification of the products downstream limits the usefulness of co-solvent as a production process for biodiesel because these studies use combinations and amounts of solvent that are unrealistic at industrial scale.

Microwave heating aided transesterification

An energy efficient way of producing biodiesel is using microwave irradiation. The process uses microwave energy to provide internal heat of the transesterification reagents in the reaction mixture thereby improving the reaction rate and reducing the cost of production (Rahmanlar *et al.*, 2012).

Patil *et al.* (2011) produced biodiesel by IST of dry algal using the microwave. The effect of the irradiation on ester yield was investigated via the *in situ* transesterification of dry algal for biodiesel production using a response surface design of experiments to analyse the impact of process variables on FAME conversion. The optimum conditions they found were as follows; dry algae to methanol ratio 1:12, KOH catalyst concentration of about 2 wt % and a reaction time of 4 min. The observed FAME conversion at 4 min was 71%. In related work, Jatropha curcas was utilised to produce fatty acid ethyl ester with a two-step reactive esterification/transesterification using a microwave system (Jaliliannosrati *et al.*, 2013). Using microwave heating in the first step for acid catalysed esterification, the free fatty acid content was decreased from 14 mass% to less than 1mass% in 35 min. The effect of variables on the yield of fatty acid ethyl ester was investigated with response surface design of experiment technique. The optimum conditions for 97.3% conversion are <0.5 mm seed size, 12.2 min irradiation time, 8.15 ml KOH catalyst loading, 331 rpm agitation speed using a mechanical stirrer and 1100 W microwave power.

Ali and Watson (2014) compared the energy requirement of oil extraction and biodiesel production from flax seed using ultrasonic and microwave. The energy difference between the methods indicated that microwave aided extraction is the most energy efficient with a net ratio of 25.2% and it also produced the highest biodiesel yield of 93%. The net energy ratio was calculated based on the amount of energy recovered (energy content of samples) to the amount of energy supplied (energy requirement of the process such as grinding, filtration, mixing/heating etc) to the seed for the oil extraction. The technology is useful regarding the energy required for IST and enhances the rate of transfer of alcohol through the biomass cell walls which in turn improves the reaction rate. However, the process is not mature enough for industrial production mainly because of the limited availability of materials for construction that are transparent to microwaves. This barrier has to be crossed before it can be commercially adopted.

Supercritical fluid aided in situ transesterification

The reaction temperature of IST determines the criticality of the process. A fluid is said to be at supercritical when it is at temperature and pressure above its critical point where there is no distinction between the liquid and gas phases. Supercritical fluids can diffuse into solid at the same

rate as gases and dissolve solids like liquids. Based on the reaction media (solvent methanol) IST can be classified as subcritical (reaction temperature: 120-280^{o}C and pressure: 15-100 bar) and supercritical (above 280^{o}C and > 221 bar) (Go *et al.*, 2016). Supercritical fluids have been used to produce biodiesel by reducing the reaction time, catalyst requirement and excess alcohol but at the expense of high energy consumption. When transesterification occurs at the supercritical state of methanol (300^{o}C and 400 bar) the oil dissolves in methanol to form a homogeneous phase (Demirbas, 2006). The reaction is then completed in few minutes without any catalyst. The method simplifies the purification stage and has the potential for reaction to proceed without a catalyst. Levine *et al.* (2013) produced biodiesel from algal char with a two-step hydrothermal carbonization and supercritical *in situ* transesterification. The result showed a yield of 79% for the fatty acid ethyl ester (FAEE) with 5:1 molar ratio of ethanol: fatty acid (EtOH: FA) and a reaction time of 150 min. The yield increased to 89% when the molar ratio was 20:1 EtOH:FA and the reaction time 180 min. In both cases the reaction temperature was 275^{o}C. Patil *et al.* (2013) demonstrated that *Nannochloropsis saltina* microalgae could be converted into FAEE under microwave-mediated supercritical ethanol condition without catalytic transesterification at a temperature of 265^{o}C and pressure of 80 bar. Tsigie *et al.* (2012) studied FAME production from *Chlorella vulgaris* at 175^{o}C with a reaction time of 4 h using subcritical water as catalyst reacting 5 g biomass with 20 ml of methanol. The technology holds potential for high yield ester production with low alcohol requirement and simple separation of products. However, the energy demand of the process is great due to the high temperatures and pressures which substantially increases the overhead cost of production.

Ultrasound aided in situ transesterification

Ultrasound is a type of mechanical sound energy characterised by vibrating particles within a medium. Low-frequency ultrasonic irradiation (20 – 40 KHz) can be utilised to maintain mixing and provide the potential energy to initiate transesterification reaction. Many researchers have demonstrated the use of ultrasound in the production of biodiesel by *in situ* transesterification (Siatis *et al.*, 2006b; Boey *et al.*, 2011; Chadha *et al.*, 2012; Suganya *et al.*, 2014b). Siatis *et al.* (2006a) used ultrasound to increase the efficiency of FAME production from different oilseeds. The results showed an increase in FAME yield from 46 to 85.5% for cottonseed, 67.2% to 93% for sunflower and 43.2% to 83.5% for sesame. The overall advantages of this method are the elimination of saponification, increased reaction rate, milder condition and higher FAME yields. Chadha *et al.* (2012) Studied production of biodiesel from Jatropha seeds via IST under ultrasonic

irradiation. The maximum ester yield of 94% was produced at seed/methanol (w/v) ratio of 1:10, 80 min reaction time and catalyst concentration of 1% (w/w).

All these studies showed that ultrasound-assisted transesterification provides shorter reaction times, and requires reduced energy and alcohol in comparison to ordinary IST. However, further research is needed before the adoption of this technology can be achieved mainly on economic evaluation of the process. In particular, there is little evidence of ultrasound processing at the large scales of transport fuel.

2.3.2 Physical Pre-treatment

Another method to improve the yield of *in situ* transesterification and reduce the alcohol requirement is physical pre-treatment of feedstock before the primary reaction. Many researchers have found that for high yields of FAME high methanol to oil molar ratios is required. For example, Kasim and Harvey (2011) reported a molar ratio of 400:1 for reactive extraction of *Jatropha curcas*, Zakaria and Harvey (2012) reported a molar ratio of 475:1 for rapeseed and 543:1 molar ratio was used by Haas *et al.* (2004) for soybean. These high molar ratios result in high energy requirements for recovering the excess alcohol at the purification stage, which increases the cost of production for the biodiesel. However, Haas and Wagner (2011) investigated the effect of four physical pre-treatment (dehulling and flaking, dehulling, flaking and passage through twin screw extruder, passage through expander type extruder and conversion to flour-like particles) of soybean on alcohol requirement of soybean during biodiesel production by *in situ* transesterification. The results indicated that the combined pre-treatment of flaking, extrusion, and drying achieved the most significant reduction in methanol requirement compared to other pre-treatment methods. The molar ratio was reduced from 181:1 to 9:1 by these pre-treatment methods. The technique is yet to be demonstrated for other feedstock. The scale-up of this technique need to be investigated first in a pilot plant before the industrial adoption of the method.

2.3.3 Pre-soaking

This method is a chemical pre-treatment of the feedstock before the transesterification reaction. It involves soaking the oil-bearing seed in methanol before the catalyst is added to begin the reaction. The method has been demonstrated to improve FAME yield and reduce alcohol requirement by 40% for the *in situ* transesterification of microalgae (Salam *et al.*, 2014). However, this is achieved by a combination of freeze drying and soaking of the feedstock in methanol before reaction which increases the time and cost of production.

2.3.4 Reactive Coupling

This is a method that combines transesterification and transformation of glycerol by-product of the reaction to useful commodity chemicals. It entails "pulling through" the transesterification reaction by removing the glycerol byproduct thereby forcing the reaction equilibrium to the product side. This is an example of Le Chateliers principle as is the use of excess methanol. Coupling it with chemical conversion of the glycerol by-product to more valuable commodity chemical in one reaction stream. Biodiesel fuel, dimethyl ether and commodity chemical are produced in the process, this to some extent will achieve a "biorefinery" concept of biofuel and co-products (Ren *et al.,* 2010). The incentive for developing this process is the ability to offset part of the cost of biodiesel from the value of chemicals produced from the glycerol. The increase in production of biodiesel has reduced the market price of glycerol due to oversupply from biodiesel plants. For every 10% of biodiesel, about 1% of crude glycerol is produced (Zheng *et al.*, 2008). The need to find new uses for glycerol has arisen due to the market glut of glycerol. A considerable amount of work has been published on glycerol processing for value-added chemicals with applications in cosmetic, pharmaceutical, food and agricultural industries. Significant among them is the work of Pagliaro *et al.* (2007a) which focuses on crucial glycerol chemical and biochemical conversions with process conditions. It highlights how glycerol and biodiesel can be economically and sustainably produced. Behr *et al.* (2008) detailed the process chemistry involved in the transformation of glycerol to useful new products. A more critical assessment of conversion of glycerol as a renewable source of platform chemicals for further chemical production was conducted by Zhou *et al. (2008).* Many vital compounds and their industrial applications are discussed. Compounds identified in work include acrolein, dichloropropanol, epichlorohydrin, dihydroxyacetone, 1,3-propanediol, 1,2-propanediol, glycerol carbonate, polyglycerol, oxygenate fuels and glyceric acid. Many of these products are formed after the separation and refining of the glycerol byproduct from the biodiesel. Therefore, they are not the secondary product of IST but chemical derivatives from glycerol. Secondary products of IST are rarely studied or monitored in literature. Im *et al.* (2015) observed the production of valuable chemicals such as ethyl levulinate (EL), ethyl formate (EF) and diethyl ether (DEE) in addition to biodiesel by sulphuric acid catalysed subcritical ethanol by IST of algal biomass. The investigation revealed that the main route for the synthesis of the EL and EF was by acid hydrolysis of algal cell and esterification with ethanol rather than by reaction of the products from the biodiesel reaction

The aim of this study on the improvement of *in situ* transesterification is not only to produce valuable chemicals from the glycerol byproducts of the biodiesel reaction but also to study the

effect of this on the alcohol requirement of the *in situ* transesterification. Therefore glycerol conversion reactions that are compatible with *in situ* transesterification will be sought for coupling. Potential candidates are polyglycerol, glycerol tert butyl ether (GTBE) and epichlorohydrin. Table 2.2 gives a list of chemicals derivatives from glycerol their applications and reaction conditions. The table also shows the reaction routes for the identified chemicals for compatibility with *in situ* transesterification based on their process conditions.

Table 2.2 List of chemicals derivatives available in the literature from glycerol their applications and reaction conditions.

Chemical Derivatives	Type of reaction /reagent	Method	Reaction conditions	Conversion/ Selectivity (%)	Applications	Comment	Reference
Acetaldehyde	Chemical	Pyrolysis	-H_2O, 240°C Raney-Ni	0.4-31	Intermediate in the production of acetic acid, esters and some chemicals	Not compatible with reactive extraction (NCRE).	(Zhou *et al.*, 2008; Herseczki *et al.*, 2012)
Acetate	Biochemical	Micro-organism (Enterobacteriaceae)	Not Available (NA)	NA	Synthesis of chemicals	Not compatible with reactive extraction.	(Herseczki *et al.*, 2012)
Acetol	Chemical	Dehydration	-H_2O, 200°C, 0.65 bar, Cu-Cr	NA	Intermediate in 1,2-propanediol synthesis	Not compatible with reactive extraction.	(Zheng *et al.*, 2008; Herseczki *et al.*, 2012)
Acrolein	Chemical	Dehydration and pyrolysis	250-340°C H^+	40/84	Preparation of polyester resin, polyurethane, propylene glycol acrylic	Not compatible with reactive extraction.	(Pagliaro and Rossi, 2008b)

					acid and acrylonitrile		
Acrylic acid	Chemical	Oxydehydration	280°C, Zirconia/ W-Sr-V-Cu-Mo Cat.	NA	Plastics, coatings, elastomers, and paints	Not compatible with reactive extraction.	(Pagliaro and Rossi, 2008b; Herseczki *et al.*, 2012)
Alkanes	Chemical	Fisher-Tropsch, pyrolysis	275°C, 5-17 bar Pt-Re/C	NA	Fuels, chemicals, waxes, and bitumen	Not compatible with reactive extraction.	(Zhou *et al.*, 2008)
Alkenes	Chemical	Fischer-Tropsch, pyrolysis	650°C, CuZSM-5	NA	Synthesis of chemicals	Not compatible with reactive extraction.	(Pagliaro and Rossi, 2008b; Herseczki *et al.*, 2012)
1,2-Butanediol	Biochemical	Microorganism (klebsiella pneumoniae)	NA	NA	Resolution of carbonyl compounds in GC	Not compatible with reactive extraction.	(Herseczki *et al.*, 2012)
Butanol	Biochemical	Microorganism (C. Pasteurianum)	NA	NA	As Solvent, intermediate in chemical synthesis and as fuel	Not compatible with reactive extraction.	(Herseczki *et al.*, 2012)

Butyraldehyde	Biochemical	Microorganism (*C. Pasteurianum*)	NA	NA	Chemical intermediate and other applications	Not compatible with reactive extraction.	(Herseczki *et al.*, 2012)
Carbon monoxide	Chemical	Aqueous phase reforming (APR)	200-250^{o}C 15-50 bar Al^{3+}	NA	Chemical Building block (CBB)	Not compatible with reactive extraction.	(Pagliaro and Rossi, 2008b; Herseczki *et al.*, 2012)
Citric acid	Biochemical	Micro-organism (*Yarrowia lipolytica*)	NA	NA	Flavouring, preservatives and water softener	Not compatible with reactive extraction.	(Herseczki *et al.*, 2012)
Diacyl glycerol	Chemical/ Biochemical	Esterification /Enzymes	220-250^{o}C KOH / NaOH	NA	Additives and emulsifier	Not compatible with reactive extraction.	(Zheng *et al.*, 2008; Herseczki *et al.*, 2012)
1,3-dichloropropanol	Chemical	Halogenation	HCl, 80-180^{o}C >5 bar	NA	Intermediate in the synthesis of epichlorohydrin	Not compatible with reactive extraction.	(Pagliaro and Rossi, 2008b; Herseczki *et al.*, 2012)

Dihydroxyacetone	Chemical/ Biochemical	Electrochemical oxidation, mico-organisms enzyme (glycerol dehydrogenase)	Air (0.1MPa) 60^{o}C, Bi-Pt/C, 5 h, pH 2	75/50	Tanning and winemaking	Not compatible with reactive extraction.	(Garcia *et al.*, 1995; Herseczki *et al.*, 2012)
Epichlorohydrin	Chemical/NaOH	Halogenation, dehydrochlorination	50-90^{o}C	NA	Building block in plastics, epoxy resins, phenoxy resin and other polymers.	Compatible with reactive extraction	(Pagliaro and Rossi, 2008b; Herseczki *et al.*, 2012)
Ethanol	Biochemical	Bacteria (*Escherichia coli*)	NA	NA	Solvent, fuel, antiseptic and antidote	Not compatible with reactive extraction.	(Herseczki *et al.*, 2012)
Ethylene glycol	Chemical/H_2	Reduction	200^{o}C >10 bar Cu-Cr	NA	Coolant, hydrate inhibition and geothermal	Not compatible with reactive extraction.	(Pagliaro and Rossi, 2008b; Herseczki *et al.*, 2012)
Formaldehyde	Chemical	Pyrolysis	650-800^{o}C, atm press.	NA	CBB, textiles, disinfectant, and photography	Not compatible with reactive extraction.	(Herseczki *et al.*, 2012)
Formic acid	Chemical/ Biochemical	Oxidation	NA	NA	Preservatives, antibacterial agent and fuel cell	Not compatible with reactive extraction.	(Herseczki *et al.*, 2012)

Fumarate	Biochemical	Anaerobic (Biospirillum succiniciproducens)	NA	NA	Intermediate in succinate synthesis	Not compatible with reactive extraction.	(Herseczki *et al.*, 2012)
Glyceraldehyde	Chemical/H_2O_2	Oxidation	80^oC, Ti-Si co-gel, pH 7	NA	Intermediate in carbohydrate metabolism	Compatible with reactive extraction.	(Sproge *et al.*, 2013)
Glyceric acid	Chemical	Oxidation	From glyceraldehyde	100/92		Not compatible with reactive extraction	(Sproge *et al.*, 2013)
Glycerol carbonate	Chemical/CO_2	Esterification	125^oC, 50 bar Zn^{2+}	80	Solvent, additive and chemical intermediate	Compatible with reactive extraction.	(Pagliaro and Rossi, 2008b; Herseczki *et al.*, 2012)
Glyceroldimethacrylate	Chemical	Esterification	NA	NA	Building block for polymers, medical app.	Compatible with reactive extraction.	(Herseczki *et al.*, 2012)
Glycerol tert butyl ethers (GTBE)	Chemical/isobutylene	Etherification	zeolite, 80-120^oC and 0.6-2.1 MPa	NA	Fuel additives	Not compatible with reactive extraction.	(Noureddini *et al.*, 1998)
Glycidyl nitrate	Chemical/HNO_3, NaOH	Esterification(nitration and causticization)	HNO_3/CH_2Cl_2 NaOH 5^oC	NA	Energetic binder, smoke propellant and explosives	Not compatible with reactive extraction.	(Pagliaro and Rossi, 2008b)
Hydrogen	Biochemical	APR, Pyrolysis, bacteria (*E. Coli*)	NA	NA	Internal-combustion engines,	Not compatible with reactive extraction.	(Herseczki *et al.*, 2012)

					turbines, fuel cells and CBB		
Hydroxypropionaldehyde	Chemical/Bio-chemical	Dehydration, aerobic conversion (Klessiella pneumonia)	NA	NA	Chemical intermediate and precursor to acrolein	Not compatible with reactive extraction.	(Herseczki *et al.*, 2012)
Hydroxybutanone	Biochemical	Microorganism	NA	NA	Food and cigarette additive	Not compatible with reactive extraction.	(Herseczki *et al.*, 2012)
Hydroxyethanoic acid	Chemical	Oxidation	NA	NA	Skin care products and organic synthesis	Not compatible with reactive extraction.	(Herseczki *et al.*, 2012)
Hydroxypyruvic acid	Chemical/O_2	Oxidation	60^{o}C, 0.1MPa Au-Pt/C, pH 12		Intermediate in biological synthesis	Compatible with reactive extraction.	(Herseczki *et al.*, 2012)
Lactate	Biochemical	Micro-organism (*enterobacteriaceae*)	NA	NA	Polymer precursor, plastic and other uses	Not compatible with reactive extraction.	(Herseczki *et al.*, 2012)
Malate	Biochemical	Micro-organism	NA	NA	Intermeidate in fumarate synthesis	Not compatible with reactive extraction.	(Herseczki *et al.*, 2012)
Mesoxalic acid	Chemical	Oxidation	From hydroxypyruvic acid		Chemical intermediate, antidote to	Compatatible with reactive extraction	(Herseczki *et al.*, 2012)

					cyanide poisoning		
Methanol	Chemical/H_2	Hydrogenation	120^{o}C, Pd/FeO_2	NA	Synthesis of chemicals, solvent	Not compatible with reactive extraction	(Pagliaro and Rossi, 2008b)
Monoacyl glycerol	Chemical	Esterification	220-250^{o}C, Na^+ N_2 atm.	NA	Additive, emulsifiers	Not compatible with reactive extraction.	(Pagliaro and Rossi, 2008b)
Oxalic acid	Chemical	Oxidation	From mesoxalic acid	NA	Chemical intermediate	Not compatible with reactive extraction	(Herseczki *et al.*, 2012)
Polyglycerol	Chemical	Etherification	140^{o}C, H^+	25/98	Lubricant, surfactant, cosmetics and additives	Compatible with reactive extraction.	(Salehpour and Dube, 2011)
1,2-propandiol	Chemical /H_2	Hydrogenolysis	200^{o}C, >10 bar, Cu-Cr	54.8/85	Moisturiser, lubricant, food additive, anti-freeze, coolant other uses	Not compatible with reactive extraction.	(Sharma *et al.*, 2011)
1,3-propandiol	Chemical /-H_2O, H_2 Biochemical	Hydrogenolysis-dehydroxylation, acetalisation-detosylation. bacteria (Clostridum)	Ru/C	NA	Polyester fibres, carpet and textiles	Not compatible with reactive extraction	(Pagliaro *et al.*, 2007a)

Propanol	Chemical	Pyrolysis, gasification	Ru/C	NA	Solvent in pharmaceutical industry resins and cellulose esters	Not compatible with reactive extraction.	(Pagliaro *et al.*, 2007a)
1,2-propenol	Chemical	Dehydration, hydrogenation	NA	NA	Pesticide and CBB	Not compatible with reactive extraction	(Zakaria *et al.*, 2013)
Propionate	Biochemical	Micro-organism (*Propionibacterium acidipropionici*)	NA	NA	Food preservative, chemical intermediate pesticide.	Not compatible with reactive extraction.	(Ilham and Saka, 2012)
Pyruvate	Biochemical	Enzyme (Dihydroxy acetone kinase)	NA	NA	Intermediate in biosynthesis	Not compatible with reactive extraction.	(Pagliaro *et al.*, 2007a)
Solketal	Chemical	Condensation	H^+, Acetone	NA	Synthesis of Chemicals	Compatible with reactive extraction.	(Suriyaprapadilok and Kitiyanan, 2011)
Succinic acid	Biochemical	Anaerobic (*Biospirillum succinicproducens*)	NA	NA	Food additives, soldering fluxes, pharmaceutical products,	Not compatible with reactive extraction.	(Pagliaro *et al.*, 2007a)

					surfactants, green solvents biodegradable plastics		
Tartronic acid	Chemical	Oxidation	From glyceric acid		Chemical intermediate in mesoxalic acid	Not compatible with reactive extraction	(Herseczki *et al.*, 2012)

2.4 Methods of Polyglycerol Production

The compatibility of transesterification and polymerisation reactions makes the production of polyglycerol a good candidate for reactive coupling. Another advantage of the process is that it will enhance the yield of biodiesel by pushing equilibrium to the product side by removing the glycerol by-product. The higher value of polyglycerol is also an important factor considered in focusing on its production as a secondary product. It is expected that efficient utilization of the secondary product (e.g. polyglycerol) as an additional source of income should reduce the production cost of biodiesel.

Polyglycerol production has been widely reported in the literature by different researchers with different methods of production and analysis. The classification between oligomers and polymers are often confusing with both of these term being used interchangeably. Polyglycerol with 2-4 units of glycerol are referred to as oligomers (Martin and Richter, 2011). Depending on the degree of glycerol condensation polymerisation, polyglycerol can be diglycerol, triglycerol, tetraglycerol or mixture of all of these.

The Fig. 2.1 below gives the general glycerol condensation polymerisation reaction:

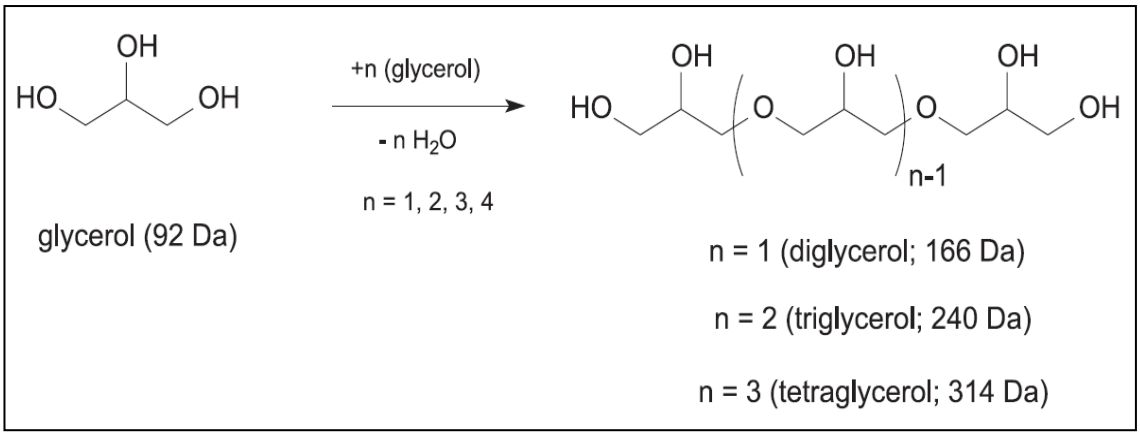


Figure 2.5 Synthesis of polyglycerol by condensation polymerisation of glycerol (Medeiros et al., 2009)

Where n = the degree of the polymerisation

The following section highlights production methods in the literature using thermal and catalytic processes:

2.4.1 Polyglycerol Production by Homogeneous Alkaline Catalyst

The polymerisation of glycerol to produce polyglycerol has been accomplished using either homogeneous acid or base catalyst. Base catalysed polymerisation is the commonly used method in the industry to form polyglycerol and avoid the side reaction which produces acrolein that can occur when an acid catalyst is used. One of the pioneering studies in the homogeneous alkaline polymerisation of glycerol is the work of Garti *et al.* (1981) where pure glycerol was polymerised using sodium hydroxide catalyst. The result showed that the optimum condition for the polymerisation was 260-280^{o}C under an inert gas condition and 2.5 mol% of NaOH catalyst. The water vapour by-product was collected and measured to monitor the progress of the reaction while the polymerised glycerol was characterised by viscosity, refractive index density, and composition. However, it was reported that presence of air or acid leads to the formation of acrolein and other condensation products which darken the final product. Richter *et al.* (2008) studied the cesium hydrogen carbonate catalysed liquid-phase etherification of glycerol to oligomers in a batch reactor at 260^{o}C under normal pressure. Linear diglycerol was the dominant product, and the reaction kinetics were confirmed to be 1st order. In more recent work by Shikhaliev *et al.* (2016), synthesis of polyglycerol was performed by heating and stirring glycerol under inert gas condition using sodium hydroxide catalyst, the initial reaction temperature was 150^{o}C and increased to 240^{o}C over about 2 hours until the attainment of a target refractive index and hydroxyl value. All these studies attempted to synthesize polyglycerol from pure glycerol which is very expensive to refine. Only few studies convert the directly significant amount of crude glycerol from biodiesel plant to polyglycerol.

2.4.2 Polyglycerol Production by Homogeneous Acid Catalyst

The polymerisation of glycerol using sulphuric acid catalyst was investigated by Medeiros *et al.* (2009). In their work 272 mmol of ultrapure glycerol was charged into a 200 ml glass tube, 1% acid catalyst and 3 ml of distilled water were added. The glass tube was connected to a condenser where the liquid was collected. The starting temperature was 150^{o}C with a rate of 30^{o}C/min till 280^{o}C. The product sample was analysed using electrospray ionisation mass spectrometer (ESI MS), FTIR and NMR. Diglycerol, triglycerol, tetraglycerol and pentaglycerol were found in the sample. It is not clear in the study how acrolein formation was prevented since the use of inert gas was not mentioned in the report. In another study by Salehpour and Dube (2011), high weight polyglycerol was synthesised from glycerol using different catalysts. The results showed that sulphuric

acid catalyst and 140^{o}C reaction temperature under an inert gas gave the highest yield of 72% polyglycerol as analysed by GPC, NMR, and FTIR. In a follow up to the work of Salehpour and Dube, the effect of temperature and catalyst concentration on polymerisation of glycerol to polyglycerol was investigated by Ardila-Suárez *et al.* (2015). The experiment was conducted by mixing 20 ml of glycerol with sulphuric acid catalyst between 1.5-5.2% (w/w) under nitrogen condition at a pressure of 80.7 kPa in a 50 ml closed glass reactor. The reactor was connected to a vacuum pump to collect the water formed during the reaction at 130˚C and 170^{o}C. The product was analysed by the functional group by use of FTIR and hydroxyl number to determine the degree of polymerisation. It was found that temperature and catalyst concentration are the main process conditions that can be tuned to produce polyglycerol for a specific application.

2.4.3 Polyglycerol Production by Heterogeneous Alkaline Catalyst

The ease of separation of the reaction product from the catalyst is one of the leading advantages of using heterogeneous catalyst over the homogeneous catalyst. Ruppert *et al.* (2008) reported the polymeristaion of glycerol to di- and triglycerol over highly active alkaline earth metal oxides. It was found that glycerol conversion increases with increasing basicity of the catalyst used, that is MgO<CaO<SrO<BaO. The analysis of the final product was carried out using HPLC. In another related study, García-Sancho *et al.* (2011) demonstrated polymerisation of glycerol to polyglycerols over Mg and Al mixed oxides. The highest conversion (50.7%) was observed when the MgAl mixed oxides catalyst was coprecipitated with NaOH/Na_2CO_3 at 220^{o}C after 24 h of reaction. The reaction products were analysed using a GC equipped with flame ionisation detector. Diglycerol and triglycerol were detected in the sample of 24 h of reaction with a maximum diglycerol yield of 43%. Similar to the study of García-Sancho *et al.* (2011) is the investigation of the effect of a composite solid catalyst on the polymerisation of glycerol to polyglycerol by Gholami *et al.* (2013). The catalyst for the study was synthesised by coprecipitation as was the case in the previous survey but at 560^{o}C for 4.5 h. The glycerol was later polymerised using the synthesised catalyst at 250^{o}C for 8 h and 2 wt. % catalyst loading under a nitrogen atmosphere to avoid oxidation of the glycerol. The resultant product was analysed using GC with pure glycerol (99%), diglycerol (90%) and triglycerol (90%) as standards. The catalyst showed high selectivity to di and tri glycerol at 96.3% conversion. Recently, Ayoub *et al.* (2016) synthesised sodium chloride treated aluminum pillared clay catalyst for the conversion of glycerol to polyglycerol (diglycerol, triglycerol). The effect of temperature (220 - 260^{o}C) and reaction time (2 -

12 h) on the activity of the synthesised catalyst was investigated. The catalyst was found to be stable with high mesoporosity and basicity with 98% glycerol conversion and 80.5% polyglycerol yield.

2.4.4 Polyglycerol Production by Heterogeneous Acid Catalyst

Many researchers have reported polymerisation of glycerol to polyglycerol using a solid acid catalyst. For example, Martin *et al.* (2012) demonstrated the conversion of glycerol in the presence of Nafion® solid acid catalyst, with a glycerol conversion of 83% and 68% yield of diglycerol after 24 h of reaction time in a falling film reactor under a reduced pressure of 4 mbar and temperature of 145-150^{o}C. The isomer distribution of the reaction product was 69% (αα') : 27% (αβ): 4% (ββ') where αα', αβ and ββ' are three constitutional isomeric diglycerol dimers which are based on the position of the oxygen in the OH groups of the glycerol that interact. Other researchers who have used solid acid catalyst for polymerisation of glycerol to polyglycerol under different process conditions are Ayoub and Abdullah (2013) with lithium-modified clay catalyst, Sultana *et al.* (2014) utilised Li-ZeY catalyst and Pérez-Barrado *et al.* (2015) calcined MgAl and CaAl catalysts. All these studies were carried at temperatures between 240 -260^{o}C and reaction times of 12-24 hrs with different analytical procedures to measure the reaction products. They all found their catalysts to be active after 8 h of reaction with over 90% conversion of glycerol to polyglycerol. The high cost of refining crude glycerol from biodiesel before conversion to polyglycerol has a negative impact on the commercial viability of this research work. An economical route is therefore desirable to make the biodiesel production more profitable.

2.5 Infra-red Spectroscopy for Biodiesel Analysis

Spectroscopy is a precise analytical method in quality control analysis of biodiesel. It is a study of the interaction between molecules and radiations. It measures radiation intensity as a function of wavelength. Spectroscopic methods can be classified according to the region of electromagnetic spectrum involved in the measurement (Demshemino *et al.*, 2013). X-ray, Ultraviolet (UV), Visible, Infrared (IR) and Microwave are some of the regions that have been used.

FT-IR is a modern analytical method for detecting the conversion of triglycerides to FAME (biodiesel**)** by monitoring the carbonyl peak. The ester carbonyl group stretching vibration occurs at 1740 cm^{-1} (Demshemino *et al.*, 2013). Another study by Lin-Vien *et*

al. (1991) put the typical methyl ester peak at 1436 cm^{-1} though this is narrow and moves along the raw oil peak.

The Beer-Lambert law (Lampman *et al.*, 2010) which expresses the fact that the greater the number of molecules capable of absorbing the light of a given wavelength, the greater the extent of light absorption, may be formulated in the equation below:

$$A = \in cl \qquad 2.11$$

Where A = Absorbance, $\in$ = molar absorptivity, c = molar concentration of sample and l = length of sample cell.

By plotting the graph of A against known c, $\in l$ can be determined which can be used to calculate the unknown concentration of the sample. However, the linearity of the Beer-Lambert law is limited by the concentration of the sample and instrumental factors (Lampman *et al.*, 2010). Some of the causes of nonlinearity are:

(i) Deviations in absorptivity coefficient at high concentration (>0.01M) due to electrostatic interactions between molecules in close proximity
(ii) Scattering of light due to particulates in the sample
(iii) Changes in refractive index at a high analyte concentration
(iv) Shifts in chemical equilibria as a function of concentration
(v) Stray light

Apart from identifying the FTIR FAME fingerprint, some researchers have quantified FAME with FTIR and validated FAME same using GC. One of such work was carried out by Rabelo *et al.* (2015) where FAME was produced by microwave-assisted transesterification of soybean oil with methanol as an esterifying agent and sodium methoxide as catalyst. FTIR spectroscopy was used as an analytical technique to quantify the ester content using partial least square method base on IR spectra obtained. The method was validated with GC which showed a good agreement. In another related work of Yuan *et al.* (2014) that monitored on-line transesterification of canola oil for biodiesel production using FTIR. The resulting quantification of ester by FTIR was compared with its corresponding GC profile. On-line FTIR analysis was found to be a workable, simple and an improvement over other traditional analytical methods. A more recent work of Forfang *et al.* (2017) where FTIR spectroscopy was used to evaluate and monitor lipid extraction efficiency for oleaginous fungi represented a milestone in demonstrating the possibility of using FTIR technique to estimate the extraction of biomass using Bligh, Folch and Lewis extraction methods against the traditional GC procedure. All this

research apart from that of Forfang and coworkers has been mostly restricted to FTIR monitoring using conventional transesterification no previous study has monitored reactive extraction using FTIR until this present study.

2.6 Nuclear Magnetic Resonance (NMR) Spectroscopy

The basis for NMR is that magnetic properties of atomic nuclei can be used to yield chemical information. NMR spectroscopy is the absorption of radio frequencies radiation by atomic nuclei within a sample placed in magnetic field. The magnetic field is varied, and peaks are recorded by radio frequency detector. Atoms are determined by their peak positions. There is a multiplicity of signals in H-NMR compared to C-NMR because of the higher isotopic abundance of 1H which is more than 99% (stronger H-atom signal) as against 1% ^{13}C (weak C-atom weaker). As a result of this more information is extracted from H-NMR than C-NMR. Proton (1H) NMR is the oldest method applied to organic molecules. The NMR spectroscopy has been applied to both biodiesel and polyglycerol production as an analytical technique. Apart from its use in determining the molecular structure, it can also be used as a tool in purity and quality assessment of the product. The characteristic signals for FAME conversion chosen for integration are those of methoxyl group in methyl ester at 3.7 ppm (singlet) and methylene group occurring in the ester derivatives at 2.3 ppm (triplet) (Pinto *et al.*, 2005). Gelbard *et al.* (1995) used ^{1}HNMR spectroscopy to monitor FAME formation by transesterification of rapeseed oil. The result indicated that the method is simpler than the use of GC. In another related work, Samios *et al.* (2009) used 1H-NMR to determine the percentage conversion of sunflower and linseed oils to FAME by acid and base transesterification of the triglyceride. High conversion of 97% and 85% yields were reported. Recently, Naureen *et al.* (2015) characterized the sunflower oil biodiesel from base catalysed methanolysis using ^{1}HNMR among other analytical techniques. Furthermore, Salehpour and Dubé (2011) characterised synthesized polyglycerol using 1HNMR and 13CNMR. The ^{1}HNMR spectrum of methylene and methine protons of polyether appeared between δ =3.2-4.1 while the OH proton signal was at δ =5. In another study by Ardila-Suárez *et al.* (2015) polyglycerol morphology was analysed using ^{13}CNMR under different synthesis conditions. The spectra region δ = 60-64 ppm indicated –CH2OH carbon of polyglycerol terminal, δ = 68-73 ppm was for OH groups and δ =74-82 ppm revealed the presence of –CH-O carbon related to the beginning of branched chains. However, there are limited studies of the use of the technique to analyse FAME from IST as against conventional transesterification and combined transesterification and polymerisation reactions.

2.7 Liquid Chromatography-Mass Spectrometry (LCMS)

The combination of LC and MS is challenging due to the need to remove large volumes of solvent before the MS analysis. In the past, HPLC peaks were collected, then the solvent was evaporated before samples were injected into MS source. However, improvement in technology by the simultaneous development of micro-bore columns and ionization techniques such as atmospheric-pressure chemical ionization (APCI) and electrospray ionization (ESI) has allowed the integration of the two powerful technologies (Hasenhuettl and Hartel, 2008). Larsen *et al.* (2005) demonstrated that LCMS coupled with ESI could be used to characterise complex phospholipid mixtures by separation whereas mass assisted laser desorption/ionization (MALDI MS) was not. The lipid separation was based on head group class, and the MS was used to determine the molecular weights of a class member. LC has been used to separate polyglycerol esters while MS was used to confirm their structures (De Meulenaer *et al.*, 2000). LCMS is powerful analytical tool, however it is expensive for routine analysis except in large research centres.

2.8 Summary of Literature Review

FAME or biodiesels are produced from natural oils or fat by transesterification using methanol as the solvent and sodium hydroxide as the catalyst. The FAME produced has similar properties to petrodiesel, making it a possible replacement or blend fuel in engines. Apart from the environmental benefit of biodiesel, the flexibility of the feedstock from which it can be produced has drawn the attention of many researchers and industries to it as a sustainable and renewable fuel that would compete with petrodiesel.

There are different methods of reducing viscosity of vegetable oil so that it can be used as fuel in engines. These techniques are pyrolysis, dilution, microemulsion, esterification, and transesterification. The most commonly used of these methods is transesterification because it is simple and cost-effective. Transesterification can further be divided as conventional and *in situ* transesterification.

In-situ transesterification is the direct reaction of oil-bearing materials with alcohol in the presence of catalyst to produce biodiesel and glycerol as against pre-extracting the oil from the seed before the reaction in conventional transesterification. This method has the potential to reduce the cost of production by eliminating the cost of extraction and purification of oil. However, *in situ* transesterification requires higher alcohol to oil

molar ratio than conventional transesterification. The cost of recovery of the excess alcohol increases the cost of production.

Many factors affect the FAME yield during situ transesterification. These are temperature, mixing, alcohol to oil molar ratio, catalyst type, catalyst concentration and the use of co-solvent. These variables have been investigated by researchers to select the optimum conditions for biodiesel. To address the challenges of *in situ* transesterification, a number of improvement methods have been investigated by many researchers. The improvements are targeted at reducing the high alcohol to oil molar ratio and include pre-soaking and reactive coupling which are the subject of this study. Apart from research into process conditions, there is also a focus on synergistically converting the glycerol by product of biodiesel to a useful commodity chemical. This to make biodiesel production plants into biorefineries thereby enhancing cost competitiveness of biodiesel production.

The basis of various analytical techniques used in the product characterization of reactive extraction and coupling were reviewed, significantly highlighting improvement in technology that has enabled scientists use them to identify the products from transesterification and quantify yields.

Chapter 3. Materials and Methods

This chapter describes the experimental methods used to investigate the following.

- Characterisation of rapeseed and the physical properties of its oil.
- Pre-soaking as a means of reducing the large excess of methanol used for IST
- On-line monitoring of IST using FTIR to understand the dynamics of the reaction.
- Reactive coupling to convert the glycerol by-product of biodiesel reaction to a more useful product (polyglycerol) to add value to the biodiesel production process.

The main analytical methods used in this study were gas chromatography (GC), Fourier transforms infrared spectroscopy (FTIR), gas chromatography-mass spectroscopy (GCMS), and liquid column-mass spectroscopy (LCMS).

3.1 Characterisation of Rapeseed

The physicochemical properties tested are moisture content, lipid content, free fatty acid and the lipid fraction. The methods used to measure these properties are described below.

3.1.1 Moisture Content

Moisture content determination was carried out to ensure that the rapeseed is moisture free to the lowest degree possible. 10 g of uncrushed rapeseed was weighed into sample pan and placed in MB45 moisture analyzer (Ohaus, USA) at 105^{o}C for 4 h. The drying was stopped when a constant weight of the sample was attained. The experiment was repeated twice with repeatability of 0.015% as a criterium for error acceptance.

3.1.2 Lipid Content

The rapeseed used was obtained from a local farm in Northumberland and was preserved in an air-tight container kept in the laboratory. The lipid content of the seed was determined using a Soxhlet apparatus with hexane as solvent as shown in Fig 3.1. The hexane (99%) was purchased from Sigma-Aldrich. 20g of the rapeseed was ground using a 75 g capacity coffee grinder for 2-3 min. The ground seed was transferred into a cylindrical thimble covered with cotton wool and placed in the Soxhlet extraction unit. 230ml of hexane was placed in a round bottom flask and heated using the heating mantle. The Soxhlet unit was allowed to run for 3, 6 and 9 hours. The mixture of solvent and oil was then placed in a rotary evaporator (Buchi Switzerland) to remove the hexane. The

experiment was repeated for each of the runs to confirm the time required to achieve maximum oil extraction.

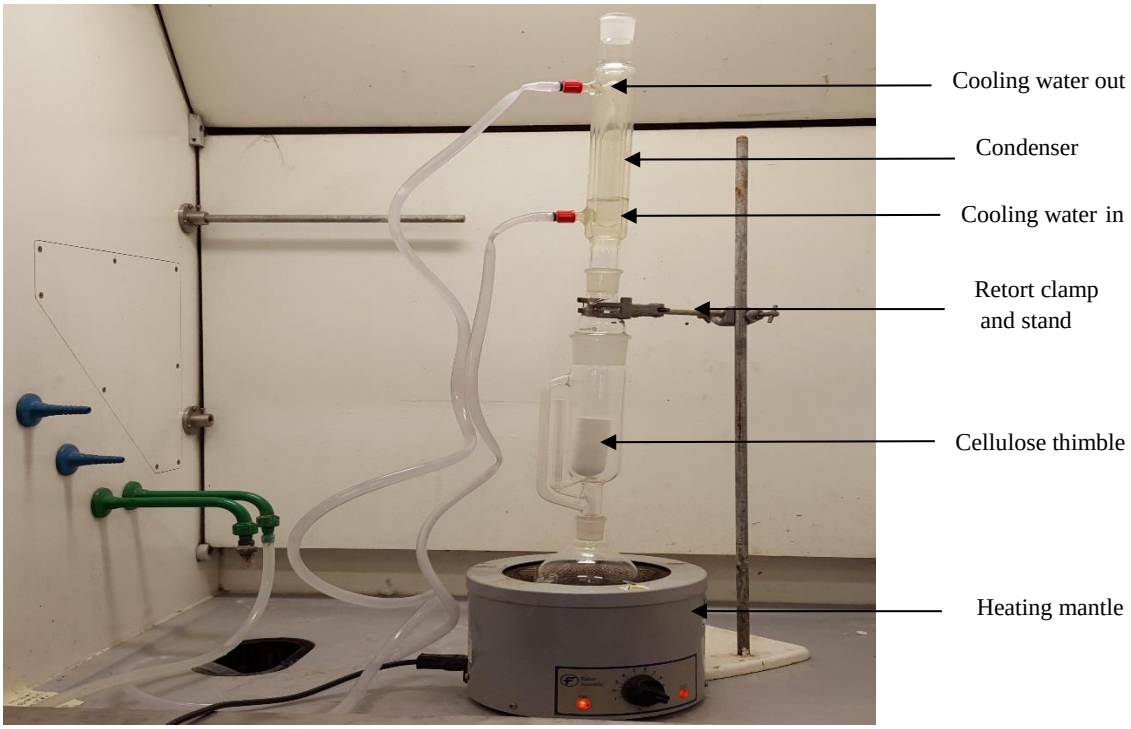


Figure 3.1 Soxhlet extraction apparatus unit

3.1.3 Free Fatty Acid (FFA)

The method used was based on the American Oil Chemists' Society method Ca 5a-40 (Firestone, 2009). 7.04 g of the extracted rapeseed oil was weighed into a 200 ml Erlenmeyer flask. 75 ml of 95% ethanol purchased from Sigma-Aldrich was heated to 40^{o}C and added to the oil along with 2 drops of phenolphthalein indicator. The mixture of the oil, indicator, and ethanol was titrated with 0.25 M of sodium hydroxide solution. The oil mixture was vigorously shaken until the first appearance of a permanent pink colour. The experiment was repeated thrice. The percentage FFA was evaluated as a function of oleic acid because it is the most dominant of all the fatty acids in the oil, using the equation below:

$$FFA\ (as\ oleic\ acid, \%) = \frac{ml\ of\ alkali\ x\ alkali\ molarity\ x\ 28.2}{mass\ (g) of\ oil\ sample}\ x\ 100 \qquad (3.1)$$

3.1.4 Lipid Fractionation

The Soxhlet-extracted rapeseed oils were fractionated to remove the phospholipids using solid phase extraction method of Kaluzny *et al.* (1985). 10 mg of the oil was dissolved in 0.5 ml chloroform (Sigma-Aldrich, UK). This was then fed under vacuum in amino

propyl column (Bont Elut NH_2 Agilent Technology, UK). The columns were first pre-conditioned with hexane (Sigma-Aldrich, UK). The lipid fractions were adsorbed onto the column while the chloroform in the mixture is eluted. Thereafter, lipid fractions were eluted using mixtures of the solvent of different polarities as shown below in Fig 3.2. The fraction of lipids eluted with mixtures of chloroform and propanol (2:1) were neutral lipids. The isolation of free fatty acid was achieved using 2% acetic acid and diethyl ether (Fisher Scientific, UK). Phospholipids were removed using methanol (Sigma-Aldrich, UK). The solvent in the lipid fraction was evaporated and their weights recorded.

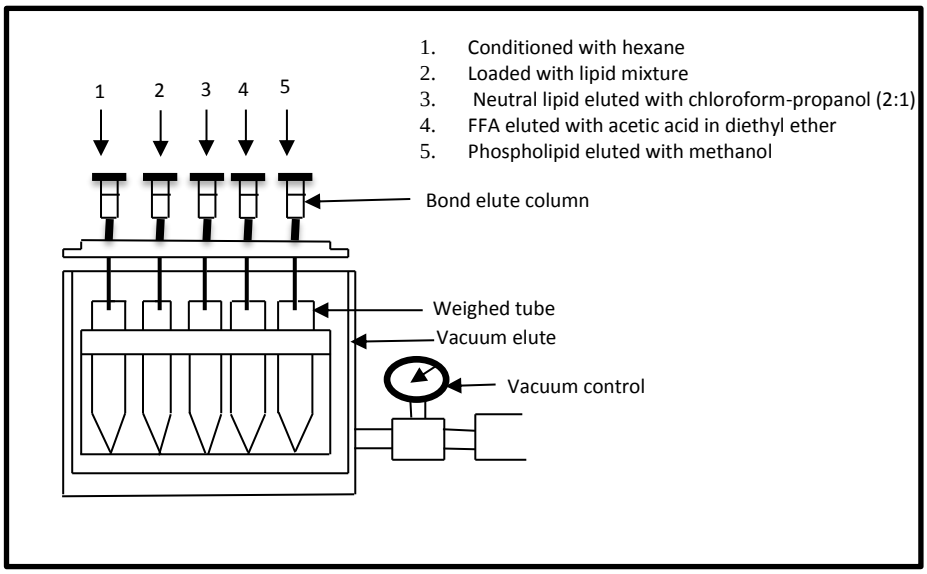


Figure 3.2 Lipid fractionation unit

3.2 *In situ* Transesterification

In situ transesterification was carried out in 2 ml plastic leakproof tubes (Fisher Scientific, UK). 100 mg of ground rapeseed was placed in the tube and 1ml of alkaline methanol (1 M NaOH + MeOH) was mixed with the ground seed in the tube. The tubes were loaded into a programmable IKA KS 4000 incubator shaker (IKA, Germany) as shown in Fig. 3.3. The temperature was kept at 60^{o}C and 500 rpm shaking rate. This was the shaking rate that gave maximum biodiesel yield as claimed by an earlier researcher working in the same field (Zakaria, 2010). After 1 h, the reaction was quenched with acetic acid. The spent rapeseed (solid) and the supernatant liquid in the tubes were subjected to centrifugation at 1300 rpm for 1 h using an accuSpin Micro 17 centrifuge (Fisher Scientific, Germany). The supernatant liquid, which consisted of methanol, biodiesel and glycerol was decanted and stored in pre-weighed tubes and later weighed to determine the weight of the sample. The FAME concentration was determined using gas chromatography as described in section 3.4.1

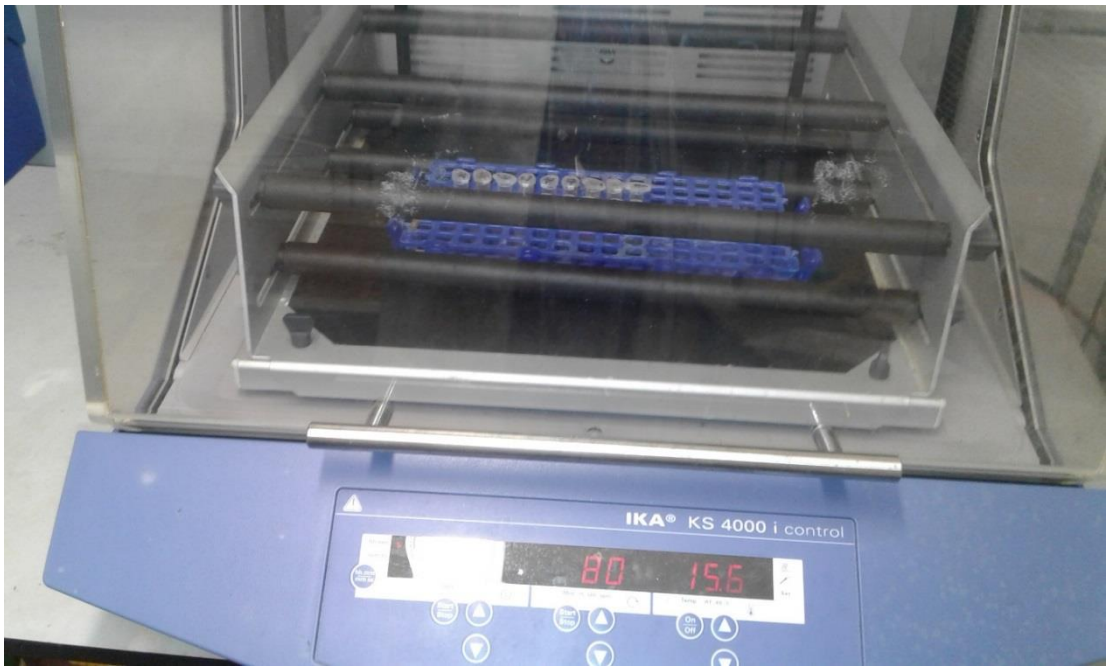


Figure 3.3 Programmable incubator shaker loaded with 2 ml plastic leak proof tube as reaction vessel

3.2.1 Pre-soaking *In situ* Transesterification

Pre-soaking was performed to study the effect of reducing the amount of methanol used during biodiesel production because it has previously been reported to reduce alcohol requirement of IST of microalgae by 40% (Salam, 2015). The experiment described in section 3.2 was modified by pre-soaking the ground seed in methanol for 12 h. Then the transesterification reaction begins when the alkaline methanol is added to the pre-soaked ground seed. The matrix for the experimental program was generated using Minitab 16 software (Minitab, UK) where three factors were considered: pre-soaking time, methanol to oil molar ratio and catalyst concentration. The response of the yield to these variables was investigated.

3.2.2 Design of Experiment for IST

The design of experiments approach was used to create a matrix of experiments to analyse the response to factors such as soaking time, methanol to oil molar ratio and catalyst concentration. A two-level factorial design of experiment was used.

Two-level Factorial Design

The two-level factorial design was used to identify the most important factors and also to demonstrate their interactions. High (+1) and low (-1) levels of each factor were determined based

on Zakaria's optimised condition (Zakaria, 2010). The table below shows the factors involved in the design and their levels.

Table 3.1 Factors investigated, their codes and level

Code	Factor	High (+)	Low (-)
A	Methanol to Oil ratio	475	360
B	Catalyst Conc. (mol/l)	0.1	0.07
C	Pre-soaking time (h)	12	0

Experiments were conducted as described in section 3.2.2 varying the process conditions as generated in the matrix of the experiment. The pre-soaking time of 0h was the control experiment.

3.2.3 On-line Monitoring of *In situ* Transesterification

The on-line monitoring of IST was carried out using a Fourier Transform Infrared (FTIR) spectrometer Mettler Toledo React IR equipped with a diamond probe mercury cadmium telluride (MCT) detector. The range of IR absorption wave numbers was 4000-400 cm^{-1}. The diamond probe absorption wavenumber was 2250-1950 cm^{-1}. The equipment was calibrated by maintaining signal to noise ratio (SNR) of 2500-3500. Air and water vapour backgrounds were collected prior to the start of the experiment to ensure there is no interference with the signal of the data obtained. The equipment was set up as shown in Fig 3.4 below. The spectra collected over 5 scans per sec at 8 cm^{-1} resolution were analysed using the computer software "IC-IR", version 4.2. The FTIR probe was immersed in the alkaline methanol inside a round bottom flask mounted on a US152 hotplate stirrer with a digital temperature controller (Bibby-scientific, UK). The alkaline methanol spectra were collected as a reference spectra, then the experiment was initiated by the addition of a known mass of ground rapeseed at 60^{o}C. 1 ml of liquid samples were collected in a vial at 10 min interval and was rapidly quenched using acetic acid to stop the reaction and later analysed using the gas chromatography method described in section 3.4.1.

Figure 3.4 FTIR On-line Monitoring of IST experimental setup

3.3 Reactive Coupling

A 300 ml bench reactor, model 4560 (Parr Instrument Company, Illinois, USA) was used for simultaneous transesterification of rapeseed and polymerisation of glycerol by product in the presence of methanol and sulphuric acid. The reactor vessel was made from 316 stainless steel. The head assembly of the reactor was equipped with pressure relief valve, thermocouple, sampling and a gas charging port. The gas sample was collected from the top of the reactor via a valve using a gas sample bag. The reactor was mounted on a hotplate stirrer with the temperature probe inserted inside the thermocouple port to measure the reaction temperature inside the reactor. The sampling pipe was coiled inside a condenser to cool the samples collected through the tap. The magnetic stirrer was placed inside the reactor. The reactor vessel was loaded with 6 g of ground rapeseed and 60ml of acidified methanol (H_2SO_4 + MeOH). The vessel and its content were purged of air by passing helium gas (BOC, UK) through the vent for about 5 min. The outer surface of the vessel was insulated. The reaction was carried out under a blanket of helium gas and was initiated when the desired temperature and pressure were attained. Fig 3.4 shows the experimental setup. Product samples were collected at the 30 min, 1 h, 2 h, 3 h and 4 h for each runs of variable catalyst concentration, temperature and methanol to oil ratio. The gas sample was collected after 4 hours at the end of the reaction.

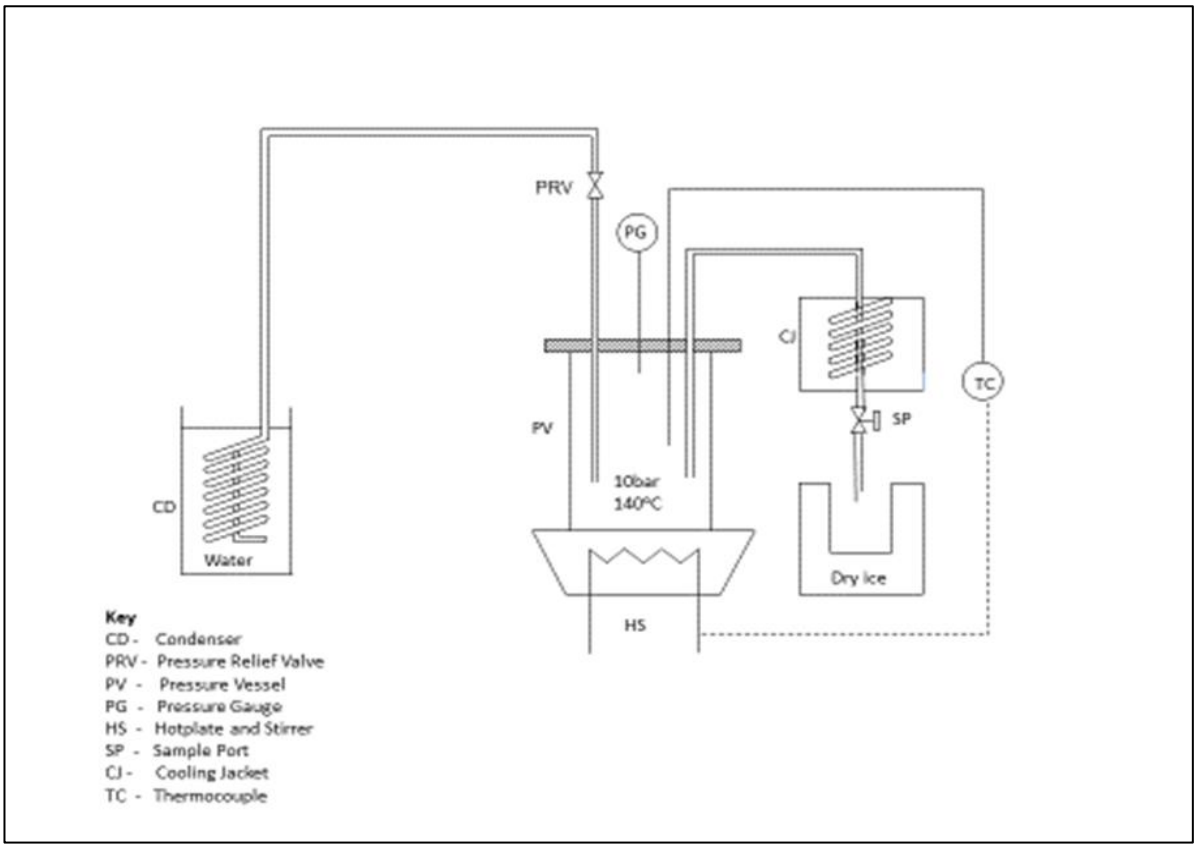


Figure 3.5 Reactive Coupling Experimental design diagram

Figure 3.6 Reactive coupling experimental rig

3.3.1 Central Composite Design (CCD)

The CCD responses surface design of experiment method was used to investigate optimum process conditions for reactive coupling because it is more robust (Oehlert, 2010). The effect and interaction of process variables such as methanol to oil molar ratio, catalyst concentration and temperature on product formation were investigated. A matrix of 20 reactive coupling experiments was generated and performed as explained in section 3.3. The value of methanol to

oil molar ratio is chosen based on the report of Zakaria and Harvey (2012) whilst the catalyst concentration and temperature are based on the report of Salehpour and Dubé (2011). Table 3.2 below gives the matrix of the CCD used for the reactive coupling experiment.

Table 3.2 CCD matrix for the reactive coupling experiment

Code	Factor	High (+)	0	Low (-)
A	Methanol to oil ratio	375	275	175
B	Catalyst con. (%)	4.8	3	1.2
C	Temperature (^{o}C)	140	130	120

3.4 Analytical techniques

The analytical techniques used for this study are gas chromatography equipped with flame ionization detector (GC-FID), Fourier Transform Infrared (FTIR) Spectroscopy, Hydrogen Nuclear Magnetic Resonance (^{1}H NMR), gas chromatography mass spectroscopy (GCMS) and liquid chromatography mass spectroscopy (LCMS).

3.4.1 Gas Chromatography Determination of FAME Weight Content

An autosampler gas chromatograph HP6890 (Hewlett Packard, USA) equipped with a flame ionisation detector was used to measure the FAME content of experimental samples. A BPX10 column, 30 m long x 320 mm ID x 0.25 µm film thickness (SGE, UK) was installed in the oven to separate the methanol, FAME, glycerides and glycerol in the samples. The carrier gas was helium set at 5 ml/min flow rate. The initial oven temperature was held at 150^{o}C for 1 min and then ramped to 210^{o}C at the rate of 10^{o}C/min this temperature was held for another 10 min making a total of 17 min for the run time. A known weight of the sample was mixed with 0.2 ml of a methyl heptadecanoate internal standard (IS) solution of 10 mg IS/ml of methanol in a 2.5 ml leak proof vial. The mixture of sample and standard was homogenised using a Reax top vortex mixer (Heidolph, Germany). The samples were loaded in the autosampler tray of the GC, where 1 µl of the sample was injected using a split injector onto the column from a 10 µl micro-syringe (SGE, UK). The output data from the flame ionisation detector was collected with Clarity version 5.0 software. FAME weight content (C) in the sample was expressed as mass concentration w/w % using the Equation 3.1 below:

$$C = \frac{[\sum A] - A_{std}}{A_{std}} \times \frac{C_{std} V_{std}}{W_{sam}} \times 100\% \qquad 3.1$$

Where:

$\sum A$ = total peak area from C8:0 – C22:1

A_{std} = peak area of methyl heptadecanoate (IS)

C_{std} = mass concentration (mg/ml) of methyl heptadecanoate (IS)

V_{std} = volume (ml) of methyl heptadecanoate (IS)

W_{sam} = weight (mg) of sample

3.4.2 Gas Chromatography for Determination of Total Mass of FAME

The total weight (w_{total}) of the sample was calculated by subtraction of weight of empty vial from the weight of the vacuum-filtered sample and an empty vial. The total mass of FAME was then determined as a product of (w_{total}) and FAME weight content (C) as measured by GC using the method described section 3.4.1 and Equation 3.1 above. The FAME yield was then calculated by Equation 3.3

$$Total\ FAME\ (mg)\ = C\ (\%) \times\ W_{total} \qquad 3.2$$

$$FAME\ Yield\ (\%) = \frac{Total\ FAME(mg)}{Maximum\ Theoretical\ Mass\ of\ FAME\ (mg)} \times 100\% \qquad 3.3$$

Where the Maximum Theoretical Mass of FAME ≡ Oil content (wt) in the seed (with the assumption of 100% conversion of the tri glyceride since there are other types of lipids that were converted to FAME).

3.4.3 Gas Chromatography for Gas Product Analysis

The gas product from reactive coupling was analysed using Variance GC series 450. The GC was equipped with two detectors namely thermal conductivity detector (TCD) and flame ionisation detector (FID) at 175^{o}C and 255^{o}C respectively but FID was used to acquire the chromatogram. The carrier gas was argon flowing at 10 ml/min. The GC was also equipped with four columns namely HayeSep T 0.5 m x 1/8" ultimetal, HayeSep Q 0.5m x 1/8" ultimetal and Molsieve 13 x 1.5m x 1/8" ultimetal and CP-SIL 5CB FS 25X5. Sample gas was injected into the column manually using a 1 ml syringe. A standard gas of known concentration was injected into the GC for comparison with the sample gas using the peak area and the retention time.

3.4.4 Solution Nuclear Magnetic Resonance (NMR) Spectroscopy

The liquid products of reactive coupling experiments were analysed for the presence of polyglycerols. ^{1}H NMR spectra of the degradation solutions were obtained using Bruker 500 Avance III HD NMR spectrometer operating at 500.15 MHz with deuterated chloroform as the chemical shift reference.

3.4.5 Water Content Analysis for Liquid Samples of Reactive Coupling

The rate of reactive coupling was measured by monitoring the formation of water as the reaction progressed. The amount water produced from the side reaction formation of dimethyl ether is assumed to be limited compared to that produced from the polymerization of glycerol to polyglycerol because the former reaction occurs in the gas phase. The water content of the liquid sample collected at different time interval was determined by Karl Fischer titration (Metrohm 915 KF Ti-touch). The titrator consists of a titration cell with a magnetic stirrer, solvent and waste bottles, dosing unit, platinum and weighing balance. The analysis was carried out by injecting a known weight of sample into the titration cell this amount was inputted on the touch screen of the equipment and titrated with dry methanol hydranal (Sigma-Aldrich, UK) in continuous stirring mode until the endpoint was attained. The amount of water as a percentage of the dry solvent was then displayed on the screen.

3.4.6 Gas Chromatography-Mass Spectrometry (GCMS) Analysis

The total soluble extract (TSE) compounds of liquid samples from the reactive coupling experiment were further analysed with GC-MS. The analysis was performed on an Agilent 7890A GC split/splitless injector (280^{o}C) linked to 5975C MS detector (electron voltage 70eV, source temperature 230^{o}C, quad temperature 150^{o}C multiplier voltage 1800V, interface temperature). The acquisition of spectra data was controlled by an HP Compaq computer using Chemstation software in full scan mode. The diluted sample (1 µl) in DCM was injected by an Agilent 7683B auto sampler and the split opened after 1 min. After the solvent peak had passed the GC temperature programme and data acquisition commenced. Separation was performed on an Agilent fused silica capillary column (30 m x 0.25 mm i.d) coated with 0.25 µm dimethyl polysiloxane (HP-5) phase. The GC was temperature programmed from 50-310^{o}C at 5^{o}C/min and held at final temperature for 5 min using helium as the carrier gas. Peaks were identified after comparing their mass spectra with that of the NIST05 library.

3.4.7 Liquid Chromatography-Mass Spectrometry (LCMS) Analysis

The polyglycerol (PG) compounds were not eluted from the column inside the GCMS column due to their polarity and molecular weight, hence, to determine the amount of the PG in the reactive coupling samples, LCMS analysis was performed. The analysis was performed with Waters Assoc. Model 244 LCMS, equipped with a 6000A Model pump, PDA and MS detector. The mobile phase was acetonitrile-water (83:17) with a flow rate of 1.5 ml/min. Waters Carbohydrate column (30 x 3.9 mm I.D., 10µm) was used for the analysis.

Electrospray ionisation (ESI) was used disperse the eluent from the liquid chromatography column prior to it entering the mass spectrometer. A Shimadzu Chromatopak EIA integrator was used to acquire the spectra.

3.4.8 Refractive Index Analysis

The liquid samples of reactive coupling were analysed using a refractometer to quickly determine the reaction endpoint and the degree of polymerisation of the glycerol in the sample. 100 µl each of stored liquid samples taken from different process conditions (temperature, catalyst concentration, and methanol to oil molar ratio) were applied to the measuring surface of the prism of a 30PX Mettler Toledo digital handheld refractometer. Some of the light is transmitted through the solution and lost, while the remaining light is reflected onto a linear array of photodiodes. When the shadow line has been automatically determined by the instrument, the position is correlated to the refractive index by the internal software of the instrument and shown on a liquid crystal display (LCD) screen as a function of the sample's temperature.

Chapter 4. Results and Discussion

4.1 Introduction

The results and discussion of different experiments carried out are presented in this chapter. The results are presented in graphical and tabular forms. The characterisation tests displayed include the lipid content, moisture content, free fatty acid and liquid fractionation. Experimental results of the presoaking and the effect of the process conditions on the yield are highlighted. Furthermore, reactive coupling results as an integrated method of producing biodiesel, polyglycerol, and dimethyl ether are presented.

4.2 Oilseed Characterisation

The rapeseed oil for the experiment was analysed for lipid content, moisture content, free fatty acid and lipid fraction. The aim of this characterization was to determine the physical and chemical composition of the oilseed, which was to be the basis for various calculations of molar ratio etc.

4.2.1 Lipid Content

The lipid content of the rapeseed was determined using Soxhlet apparatus method described in section 3.1.2. Clearly, (Figure 4.1 below) there was no significant increase in the weight of the oil extracted after 3 h. The maximum extracted oil with the experimental error was found to 46 $\pm$ 0.1%. This agrees broadly with the findings of Wang *et al.* (2010) which gave the range of lipid content of rapeseed (*Brassica napus L.*) to be 32-53%. The specific amount of oil in any rapeseed sample depends on the genetics of the plant species, a method of harvesting and storage of the seed (Zhao *et al.*, 2006).

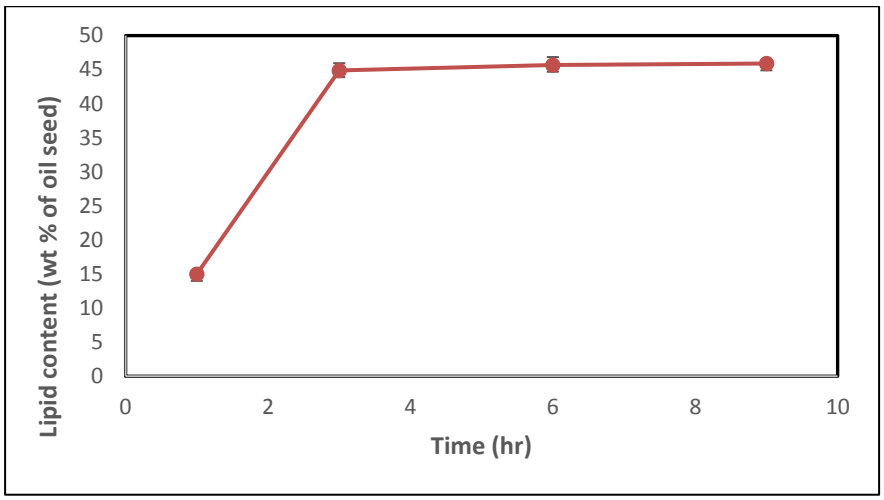


Figure 4.1 Plot of the weight of lipid extracted by Soxhlet apparatus vs. time of extraction

4.2.2 Moisture Content

The moisture content of the seeds should be as low as possible, to prevent the formation of soap. The high moisture can hydrolyse the triglyceride to form free fatty acid which then reacts with the sodium hydroxide to produce soap. Soap formation reduces the ester yield and makes the separation of glycerol by-product difficult. The moisture content of ground rapeseed used for this study was found to be 8.0 ±0.1%. This is within the range expected not to cause saponification effect during transesterification. Haas and Scott (2007) reported that drying flakes of soybean reduced the methanol requirement during transesterification. However, Zakaria and Harvey (2012) reported that reducing the moisture content of rapeseed does not reduce the methanol requirement. These conflicting reports may be due to the difference in the oil content of the seeds and the oilseed type. The oil content of rapeseed (46 wt. %) is higher than soybean (22 wt %) hence the alcohol requirement for IST of rapeseed will be higher, therefore less water concentration in the solution making it more tolerant to water than soybean. Furthermore, water being a polar solvent, can reduce the solubility of the triglyceride in methanol and hence reduce the formation of esters (Musa, 2016).

4.2.3 Free Fatty Acid

The free fatty acid (FFA) content of the Soxhlet-extracted oil was determined by the procedure described in section 3.1.3 which was carried out by volumetric titration of sodium hydroxide against the oil. The content was found to be 2.1 ± 0.25%. This shows that the FFA was low enough not to cause excessive soap formation in alkaline transesterification: FFA in oil should less than 3 wt% to avoid saponification (Romano and Sorichetti, 2011). Higher FFA levels necessitate a two-step process of acid esterification to bring down the FFA, before base-

catalyzed transesterification.The cost implication of a two-step process is that it will increase the cost and time of production because each stage will require separate equipment and processing time.

4.2.4 Lipid Fractionation

Lipid fractionation of the oilseed was performed to determine the amount of lipids that can be transesterified in the sample, since not only the neutral lipids (the triglycerides) are transesterifiable, but also the phospholipids, glycolipids and the FFA. Figure 4.2 shows the lipid fraction, determined using the method described in section 3.1.4 The neutral lipids (triglyceride, diglyceride, and monoglycerides) constitute over 80% of the total lipid, while phospholipids and FFA are 17 and 2% respectively. The major constituent of the neutral lipid is the triglyceride, which accounts for over 90% of the lipid (Romano and Sorichetti, 2011). Tzen *et al.* (1993) working on another sample of rapeseed (*Brassica napus L.*), found that neutral lipids, phospholipid and FFA fraction of the rapeseed are 94.21%, 1.97%, and 0.36% respectively. However, the oil bodies for their experiment were isolated from freshly harvested seed while the experimental seed has been stored at ambient conditions in a container for several months. The lower amount of neutral lipids in our sample may be as a result of lipid degradation in the stored seed or simply because it is the sample.

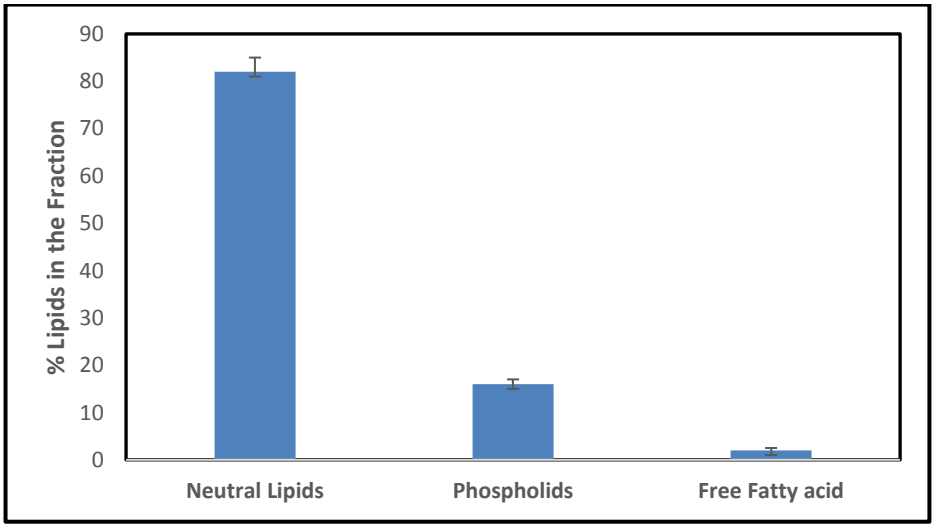


Figure 4.2 Lipid fractions of rapeseed oil

Zadernowski and Sosulski (1979) found that the proportions of polar and non-polar lipids in low erucic acid cultivars of rapeseed were 95.8% and 4.2 % respectively. The lipid fractions in oil bodies depend on the species, geographical location of the plantation, the year weather, application of fertiliser and storage duration. Phospholipids are a major component of the cell wall membranes. They consist of two fatty acids and a phosphate group attached to a glycerol backbone. The fatty acid component can be converted to FAME. However, the high

percentage of phospholipids in this study may be due to the method used in the extraction which does not allow us to separate the glycolipids from the phospholipids. In the conventional oil extraction process, gums, phospholipids, and protein in the oil are removed by water degumming. This cannot be part of an *in-situ* method.

4.3 Fatty Acid Profile

The quality of biodiesel depends on the quality of the feedstock, conversion and downstream processing. Quality parameters such as cetane number, saponification number, oxidation stability, iodine value and cold filter plugging point (CFPP) are a function of the nature of the lipids (Ramos et al., 2009). The saponification number depends on the fatty acid molecular weight and percentage composition of saturated fatty acid. Whilst the iodine value depends on the proportion of unsaturated fatty acid components, the number of double bonds and their molecular weight (Lamaisri *et al.*, 2015). Similarly, the oxidation stability and cold filter plugging point of the biodiesel is related to the degree of unsaturation of the fatty acid of the oil from which they are made. In this study, the fatty acid methyl ester profile of the rapeseed oil was determined using the gas chromatography method described in section 3.4.2. Table 4.1 shows the profile.

Table 4.1 Rapeseed methyl ester profile

Fatty Acid Type	% FAME Produced by Rapeseed	Saturation-type
Caprylic Acid (C8:0)	1.4	SFA
Capric Acid (C10:0)	2.7	SFA
Lauric Acid (C12:0)	5.7	SFA
Tridecanoic Acid (C13:0)	3.0	SFA
Myristic Acid (C14:0)	3.1	SFA
Myristoleic Acid (C14:1)	1.8	MUFA
Pentadecanoic Acid (15:0)	1.9	SFA
Palmitic Acid (C16:0)	13.2	SFA
Palmitoleic Acid (C16:1n9c)	6.5	PUFA
Heptadecanoic Acid (C17:0)	3.6	SFA
Stearic Acid (C18:0)	6.9	SFA
Oleic Acid (C18:1n9c)	23.4	PUFA
Linoleic Acid (C18:2n6c)	12.4	PUFA

Arachidic Acid (20:0)	2.1	SFA
Cis-11-Eicosenoic Acid (20:1)	2.1	MUFA
Linolenic Acid (C18:3n3)	5.6	PUFA
Behenic Acid (C22:0)	1.9	SFA
Erucic Acid (C22:1n9)	2.0	PUFA

Table 4.1 shows that oleic and palmitic fatty acids are the most abundant in the rapeseed, as would be expected. This result is in good agreement with previous studies of Ramos *et al.* (2009) which indicated that palmitic (C16:0), oleic (C18:1) and linoleic (C18:2) are the most prevalent fatty acid in rapeseed oil. Total saturated fatty acid (SFA) (Cn:0) is ~ 46%, mono-unsaturated fatty acid (MUFA) with one double bond (Cn:1) is 36%, poly-unsaturated fatty acid (PUFA) with two or three double bonds (Cn:2,3) is 18%. Therefore the total unsaturated fatty acid (MUFA + PUFA) ~ 54%. The degree of saturation of the FAME determines the cetane number of biodiesel, which is a measure of the ignition quality. Biodiesel from feedstock with high saturated fatty acid has high cetane number while biodiesel with abundant unsaturated fatty acid exhibit low cetane number (Harrington, 1986; Van Gerpen, 1996). However, biodiesels from feedstocks with highly saturated FA structures show poorer cold flow properties (CFPP) compared to that with unsaturated FA (Dwivedi and Sharma, 2016). Usually, the melting points of saturated FAs are higher. Stearic acid methyl ester has the highest cetane number 86.9 because of its full saturation and average molecular weight compared to other FAMEs. (Knothe and Steidley, 2005; Gopinath *et al.*, 2010). Fig 4.3 shows a typical rapeseed oil chromatogram. As shown in the chromatogram, the peaks of palmitic, oleic, linoleic and linoleic can be clearly separated by GC ester technique described in section 3.4.1. As expected, oleic fatty acid (C18:1) predominates in the rapeseed oil with 64%, followed by linoleic (C18:2) with 21%, linoleic (C18:3) is 8.7%, palmitic (C16:0) and stearic (18:0) are 4.1% and 1.3% respectively.

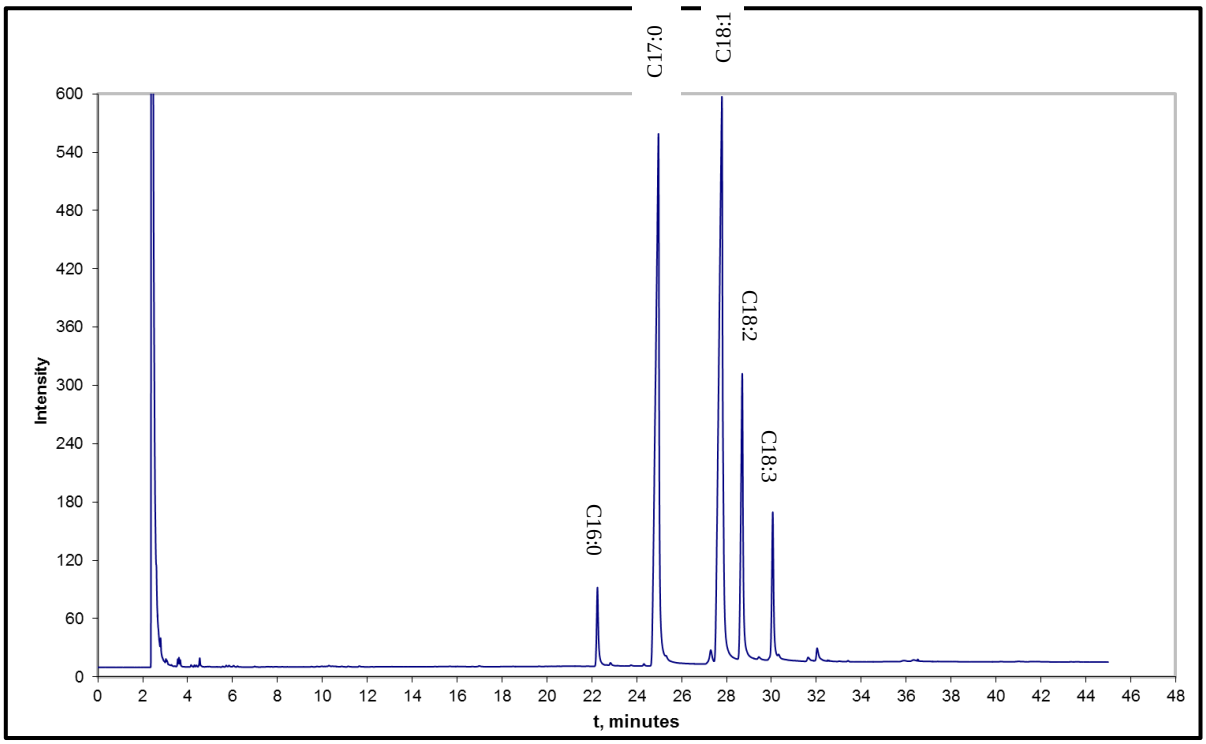


Figure 4.3 Chromatogram of Rapeseed Oil

4.4 Pre-soaking of Rapeseed for FAME Production

The aim of pre-soaking seeds prior to IST was to reduce the alcohol required to give complete or near complete transesterification. The mass transfer limitation in IST of the solid (rapeseed) and the liquid (alkaline methanol) is the reason the process requires a large excess of alcohol. Pre-soaking the seeds in methanol without the catalyst is to allow sufficient contact time between the solid and liquid to dissolve the lipid-rich cell walls before the commencement of the reaction. It has been proven by Zakaria and Harvey (2012) that no significant IST reaction occurs without the catalyst. It is reported by Salam *et al.* (2016c) that only about 6% of the alcohol is used up during IST. The rest is recovered after the separation of the FAME and glycerol. The energy cost of recovery of the excess alcohol makes IST more expensive than conventional transesterification (Haas and Wagner, 2011)

The pre-soaking study showed a 10% increase in FAME yield compared to unsoaked seeds when a lower methanol to oil molar ratio (360:1) was used at high catalyst concentration (0.1M) and high soaking time (12 h). There is no significant benefit in soaking for longer than 12 h. The reason for the large excess of methanol in IST may be that since the rate determining step is diffusion of methanol into the seed particles, the high molar ratio is needed to overcome mass transfer resistance for any significant rate of reaction (Kasim *et al.*, 2010b). It is desirable to pre-soak the seed prior to transesterification to ensure adequate mass transfer. The advantage of presoaking the seed in methanol rather than alkaline methanol is to delay the

start of the reaction, whilst facilitating transport of the methanol to the triglyceride since the soaking time are usually for 12 h and long reaction time may induce saponification. The FAME yield may be affected by adopting this approach.

Analysis of FAME yields as a function of the variables investigated in Table 4.2 using a two-level full factorial design gave a predictive equation correlating methanol to oil ratio (MR) catalyst concentration (wt/wt%) (CC) and pre-soaking time (h) (Time). The result of the predictive model for 12 h soaking time is given in equation 4.1 below:

$$Yield = 141.9 - 0.1855\text{MR} - 904CC - 4.47Time + 2.464(MR \times CC) + 0.01534(MR \times Time) + 68.2(CC \times Time) - 0.1932(MR \times CC \times Time) \quad 4.1$$

The statistical significance of each of the three variables with 2-level was estimated by the probability value (p-value) given in Table 4.2. The variables whose p-values were below 0.05 are considered to have a "significant" effect, which means 95% confidence in the data. From the Table, it can be seen that all the variables and the interaction are significant except the interaction between the methanol to oil molar ratio and time. The limitation of the model, because it is varied across only 2-levels, is that it cannot capture any curvature in the process (i.e it is mathematically impossible to obtain a unique solution when finding second order terms such as C^2).

Table 4.2 Analysis of variance for the optimisation model for reactive extraction parameters

Source	Degree of Freedom	Adjusted Sum of square	Adjusted Mean square	F-Value	P-Value
Model	7	524.75	74.96	31.56	0.000
Linear	3	466.00	155.33	65.40	0.000
MR	1	16.00	16.00	6.74	0.032
CC	1	9.00	9.00	3.79	0.087
Time	1	441.00	441.00	185.68	0.000
2-way interaction	3	42.75	14.25	6.00	0.019
MR*CC	1	20.25	20.25	8.53	0.019
MR*Time	1	2.25	2.25	0.95	0.359

CC*Time	1	20.25	20.250	8.53	0.019
3-way interaction	1	16.00	16.000	6.74	0.032
MR*CC*Time	1	16.00	16.000	6.74	0.032
Pure Error	8	19.00	2.375		
Lack-of-fit	4	58.75	14.687	6.18	0.014

The adjusted determination coefficient R^2 of the model is 96.51%, which indicates an acceptable degree of agreement between the data set and the derived predictive model equation. The model F-value of 31.56 and p-value < 0.001 indicates it is significant which means the regression model fits the data. The results show good agreement with 95.5% R^2 reported by Gupta *et al.* (2016). The lack-of-fit is not significant, as the predicted and actual yield are similar. The equation is used to plot the main effect graphs of predicted FAME yield as a function of methanol to oil molar ratio (MR), catalyst concentration (CC) and pre-soaking time (Time) in Fig. 4.4 Time zero indicates no pre-soaking which means the seed is not soaked in methanol before transesterification.

4.4.1 Effect of Methanol-to-Oil Molar Ratio (MOMR)

The FAME yield of IST increases with increasing MOMR, as shown in Fig. 4.4 This is expected, as the reaction is reversible, and the excess alcohol, apart from playing the dual role of both solvent and reagent, tends to shift the equilibrium position to the product side, thereby increasing the amount of FAME produced, by Le Chatelier's principle. However, the energy cost of separation of the reaction products will be increased. The higher the amount of unused methanol the higher the energy load required during purification at the downstream. The excess alcohol enhances the solubility of FAME in the glycerol-rich phase (GRP). Therefore the cost of production of FAME is increased by IST when operating under these conditions. The effect of MOMR in pre-soaking is statistically significant, as the p-value is 0.032. This underscores the critical role played by presoaking the seeds in methanol prior to transesterification where an increase in FAME yield at the reduction of 24% in methanol requirement is observed.

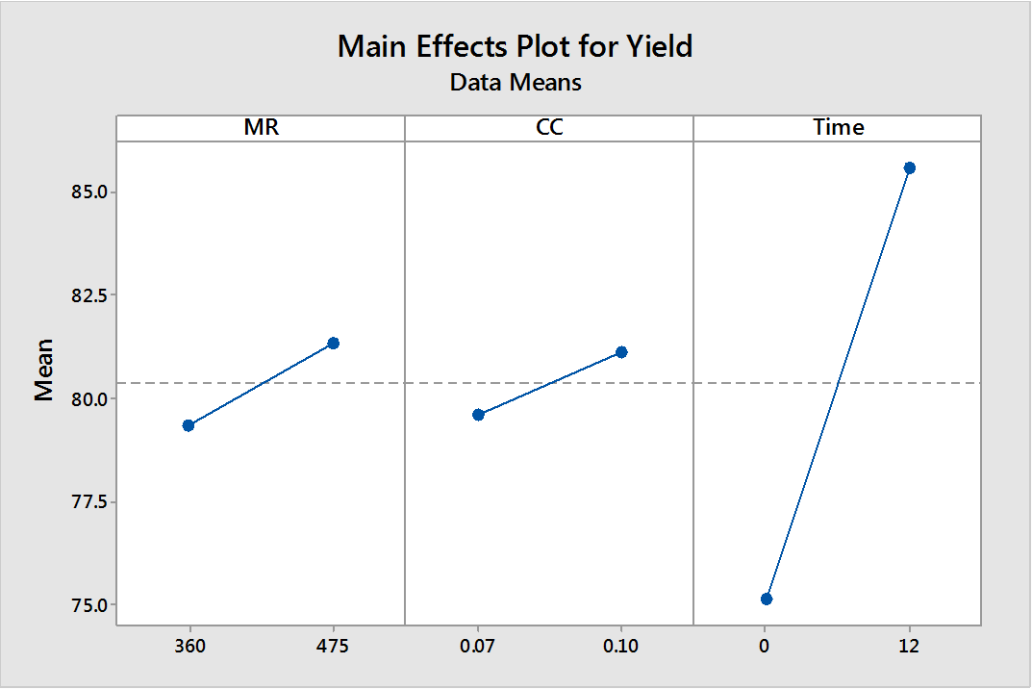


Figure 4.4 The main effect plot of MR, CC and pre-soaking time on FAME Yield (where MR is methanol to oil molar ratio, CC is catalyst concentration (M) and Time is the presoaking time (h))

4.4.2 Effect of Catalyst Concentration

The increase in NaOH catalyst concentration from 0.07 to 0.1M leads to increased FAME yield, as shown Fig. 4.4. However, there is a maximum in the FAME yield at 85%. Above this, further increases in catalyst concentration reduce the FAME yield due to the formation of soap by saponification. The effect of catalyst concentration alone in pre-soaking of rapeseed for FAME is insignificant, as can be observed in Table 4.2 where the p-value is 0.087. However, the effect becomes significant when it interacts with another variable. The explanation for this observation in the model is due to 'crossover' interaction effect where the changes in the coefficient of catalyst alone do not affect the yield, but the yield is affected when it interacts with other variables such as soaking time or methanol to oil molar ratio. The catalyst enhances the solubility of the triglyceride and methanol to form FAME. The active catalyst that attacks the triglyceride for FAME production is the methoxide ion ($^{-}OCH_3$) formed by the dissolution of sodium hydroxide in methanol. Therefore, the high concentration of the methoxide in methanol increases the FAME yield. This is further explained in the interaction effect section.

4.4.3 Effect of Pre-soaking Time on FAME Yield

The soaking time of the ground rapeseed in methanol prior to IST is statistically significant with p-value <0.001, hence it has the greatest effect on the FAME yield as shown in the main

effect plot in Fig. 4.4. The time of 12 h of soaking gave a higher maximum yield of 85% than the 75% of un-soaked seed. The soaking time enables the methanol to diffuse into the pores where the reaction takes place before diffusing out to the bulk of the liquid. This result supports the findings from pre-soaking of micro-algae (biomass) for FAME production conducted by Salam (2015). There is no significant improvement in FAME yield in soaking time that is less than 12h when compared to the FAME yield of unsoaked seed. Furthermore, comparing the effect of pre-soaking seeds prior to transesterification, FAME yield after reaction increases from 75% for the un-soaked seed to almost 80% at reduced methanol to oil ratio (360:1) and catalyst concentration of 0.07M. There is further increase in the yield to 85% at the higher molar ratio and concentration of 475:1 and 0.1M respectively. The transesterification reaction is reversible, therefore increasing one of the reactants pushes the equilibrium yield toward the product side. This result is in agreement with other researchers that worked on similar studies (Kasim and Harvey, 2011; El-Enin *et al.*, 2013). However, the reaction time is delayed by pre-soaking for 12 h, which will increase the downtime in the production. Further study is required to reduce this time to improve the efficiency of the production process.

4.4.4 Interaction Effect Plot

The interaction effect of pre-soaking shown in Fig. 4.5, below, describes the relationship between dependent variables and independent variables at constant process conditions. 4.5(a) illustrates the interaction between catalyst concentration and molar ratio on the FAME yield. It can be seen from the plot that the FAME yield increases as the methanol to oil molar ratio increases from 360/1 to 450/1 and catalyst concentration from 0.07 to 0.1M. Fig. 4.5(b) shows the interaction between pre-soaking time and MOMR on the FAME yield. The plot indicates that at high pre-soaking time of 12 h and high MOMR the FAME yield increased significantly from 75% to 85%. However, when MOMR was kept constant at 360:1 and soaking time increases to 12 h, a yield of almost 85% was still achieved compared to unsoaked FAME yield under the same conditions. This finding is significant, as it indicates that there is a way of reducing the methanol requirement of IST, which may improve the process economics of the FAME production. The interaction between MR and CC is significant as shown in Fig.4.5(c) at high CC, increase in MR increase the FAME yield, however, decreasing CC causes a slight decrease in yield as MR increases. In Fig. 4.5(d), the interaction between CC and soaking time is shown, both variables increase as the FAME yield increases. As the soaking time increases, the low CC (0.07M) favours higher FAME yields than the high CC (0.1M). This may reduce the cost of production. Also, low catalyst reduces the molecules of water that is formed from

the dissolution of sodium hydroxide in methanol. Water formation reduces the FAME yield by the formation of soap through a competing saponification reaction. Fig. 4.5(e) and (f) show that low soaking times have a less significant effect on MR and CC. In contrast to the former, the latter shows that high soaking time increase in CC shows a decrease in FAME yield. The effect of catalyst concentration is that as CC increases from 0.7 M to 0.1 M the yield of FAME increases from 80 to 85%. This is expected as more alkoxides are available to promote the transesterification reaction which translates to increase in the yield of the FAME product.

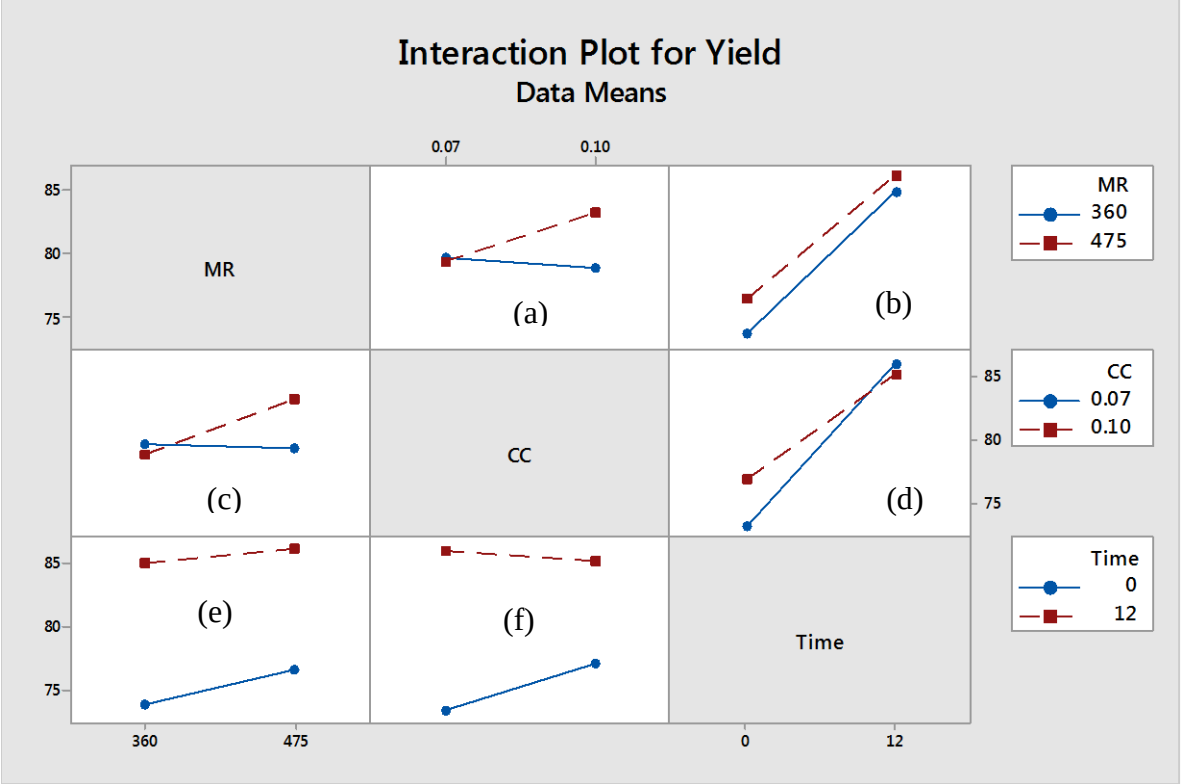


Figure 4.5 Interaction of MR, CC and Pre-soaking time effect on FAME Yield

4.5 "Fast IST" of Rapeseed for Biodiesel Production

In Fig. 4.6 below, a high molar ratio of methanol to oil of 375:1 and temperature of 60°C were used. The effect of catalyst concentration was studied. The concentrations ranged from 0.1 M NaOH catalyst concentration to benchmark against literature results (Zakaria, 2011), to a high concentration of 1.2 M NaOH that has never been used before for IST of rapeseed with low FFA. For 0.9 M NaOH concentration, a maximum FAME yield of about 90% is attained after approximately 5 min. After this, the FAME yield steadily with time. This is due to the saponification reaction, whereby the FAME produced is converted to soap and methanol as a result of high alkoxide in the reaction medium. For instance, an initial FAME yield of 90% reduces to 21% after 1 h at 0.9 M and 375/1 methanol oil to oil molar ratio. The same trend

is observed for catalyst concentrations of 0.6 M and 1.2 M with FAME yield of 75% and 70% respectively in the first 5 mins. However, the yield declined drastically to 21% and 4%, respectively, after 60 min.

The highest NaOH concentration of 1.2 M did not result in the 90%+ FAME yields that were observed with the 0.9 M concentration. This is because the saponification effect became significant earlier as a result of the high presence of methoxide in the excess methanol competing with transesterification due to relative rates of both reactions. When using 0.1 M NaOH, the FAME yield increased steadily until about 30 min, where it attained its maximum yield of 80%. This was maintained without any significant decline in yield until the end of the transesterification reaction. However, this catalyst concentration is probably too low for excessive saponification. The use of high 0.9 M concentrations of sodium hydroxide catalyst in IST can lead to acceptable 90% FAME yields within 5 min. The transesterification reaction can be more or less completed before the saponification reaction becomes significant.

The results of this study are consistent with previous literature, particularly that of Salam *et al.* (2016a), who first reported the possibility of high catalyst concentration for high FAME yield IST for non-soaked microalgae. The phenomenon was first observed for conventional transesterification of rapeseed oil by Eze *et al.* (2014).

The significance of this study is that high FAME yield can be produced rapidly using high catalyst concentration. However, the reaction must be quenched as soon as the maximum FAME yield is attained. Quenching time is important to the success of this method. Therefore the development of on-line monitoring of the reaction to knowing the right time to stop the reaction would be a significant advantage. The operating cost is increased by the cost of extra catalyst used for 'fast IST' but this cost is gained in lower reaction time. For instance, the utility (electricity, steam and process water) cost is reduced as a result of lower reaction time.

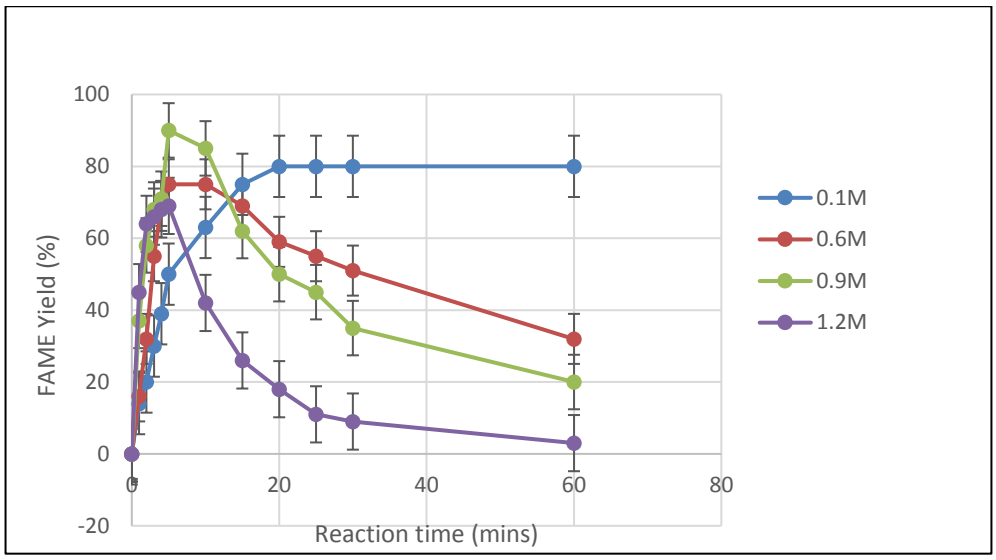


Figure 4.6 In situ transesterification of rapeseed for FAME production at different catalyst concentration and constant methanol to oil ratio (375:1), and temperature (60ºC)

4.6 On-line Monitoring of IST of Rapeseed for Biodiesel Production using FTIR

Yuan *et al.* (2014) monitored on-line FAME production by transesterification of canola oil using mid-IR spectroscopy (as mentioned in section 2.5 of the literature review). Some typical mid infra-red (IR) spectra (obtained by the FTIR method described in section 3.2.3) are shown in Fig. 4.6, below.

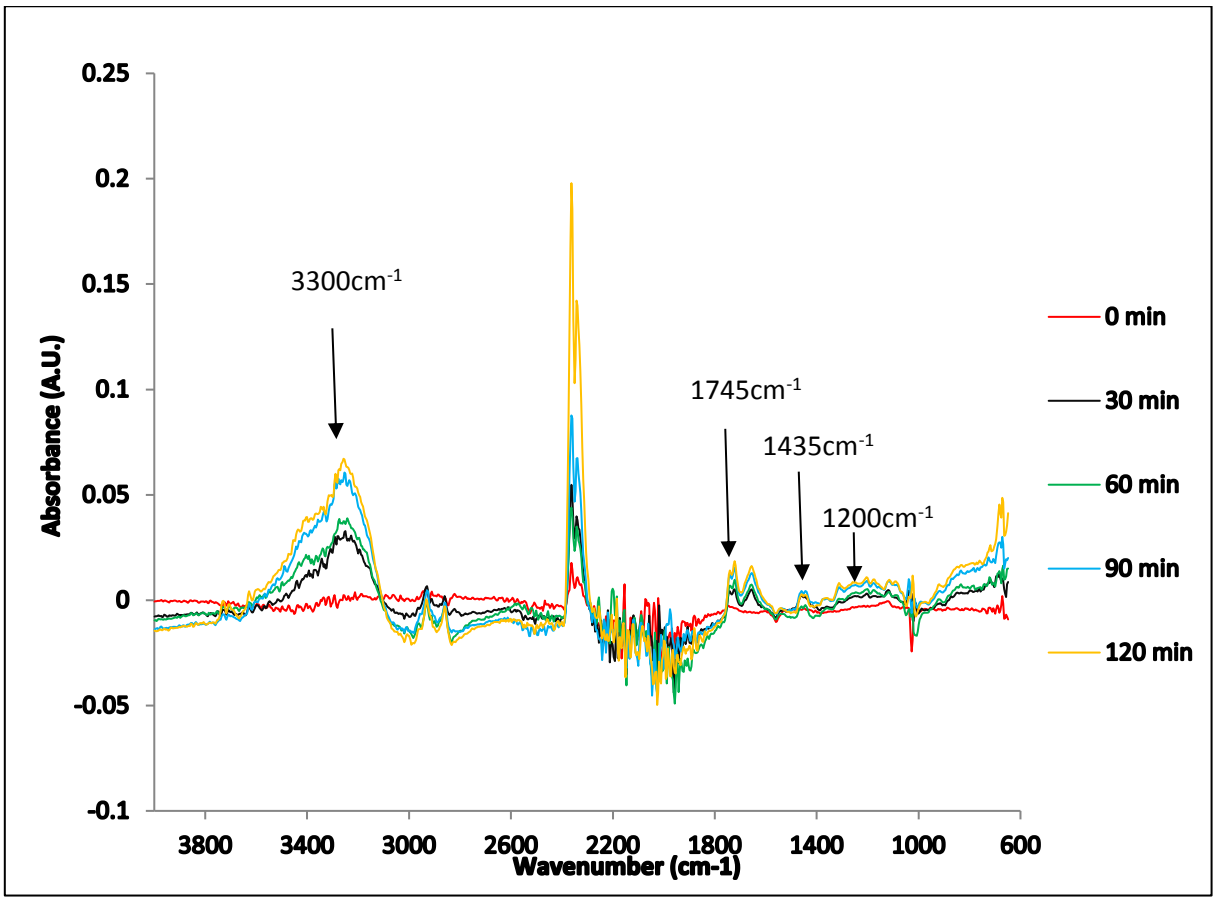


Figure 4.7 FTIR spectra for *In-situ* transesterification as a function of time for reaction condition: 375/1 MR, 0.1M CC, and 60 ºC

The clear changes in the spectra with time demonstrate that FTIR monitoring can be used here. These changes are nowhere near as significant when the reaction is conventional rather than *in situ* transesterification, as the functional groups on either side of the reaction are the same. FTIR observations of IST, however, are essentially that of an extraction process, as the FTIR monitors only the composition of the liquid phase, whereas the oil is initially inside the seed i.e. the functional groups on the reactant side are not seen by the FTIR as they are never in the liquid phase, only the products..

After 30 mins of the reaction, clear FAME characteristic peaks of carbonyl (C=O) functional groups at 1745cm^{-1} and C=C of the aromatic stretch are observed. The other peak (1435cm^{-1}) related to the carbonyl group is visible throughout the entire spectrum. This suggests the formation of the ester as it will be seen during extraction of oil. The disappearance of the triple ester group of the triglycerides in oil at 1200 cm^{-1} wavenumber and the appearance of a new signal at 1435cm^{-1} which is that of methyl ester differentiates the extraction from the transesterification reaction. The peak at 1200cm^{-1} was not distinctively clear due to the masking of the structure by fouling of the probe. The peak appeared at about 5 min of reaction

using the same condition of 70% wt/wt of NaOH. There are no further increases in all the peaks with time, with the reaction almost completed after 2 h.

The peak that distinguishes the triglyceride ester from FAME is the CH_3-O- functional group signal which occurs at 1432-1440 cm^{-1}. Other peaks apparent in the spectra are the C=O at 1715-1745 cm^{-1} and the O-H group at 3230 cm^{-1}, listed in Table 4.3 below. The latter peak is indicative of the increase in glycerol formation. The conspicuous peak at 2319 cm^{-1} is CO_2, which increases as a result of air from the reaction environment being absorbed into the reaction. Note that the region 1800-2200 cm^{-1} is the diamond region interference of the equipment.

FAMEs have a characteristic peak in the mid-IR range between 1725 – 1745 cm^{-1}. As the FAME content increases with time these peaks area increase. This is the first time that the technique has been demonstrated for IST as it has been used for conventional transesterification.

Table 4.3 Identified FTIR Peaks of IST of Rapeseed and the type of vibration (Lampman *et al.*, 2010)

Vibration Wavenumber (cm^{-1})	Type of Vibration
710	-CH_2 rocking
1070	-O-CH_2-C
1432-1440	(CO)-O-CH_3 (asymmetric bending)
1600-1660	C=C (aromatic stretch)
1725-1745	C=O Ester
2319	CO_2
3230	O-H

Apparently, the reaction can be monitored using the key functional groups stated in Table 4.3. The (CO)-O-CH_3 group at a range of 1432-1440 cm^{-1} is particularly difficult to see using the conventional liquid phase transesterification. Figure 4.8 shows the average smoothed baseline fitted plot of the FTIR spectra peak area at 1745 cm^{-1} vs. reaction time for different rapeseed particle sizes of 100, 200 and 300 µm. The spectra were pre-processed using Origin Pro software to filter out the signal noise and baseline corrected. The plot indicates that for the particle size of 300 µm the process of IST is reaction-limited rather than internal mass transfer/diffusion limited, the same is true at 200 µm. However, at 100 µm a greater proportion of triglyceride (TG) available in the solid matrix is at the surface of fewer lipid-rich cell

available for the reaction hence conversion is reduced, as shown by the peak area due to oil losses from grinding and presoaking. For the particle size of ≤ 300 µm, the IST reaction is complete after 10 min, as shown in the plot. Therefore, it will be uneconomical to reduce the particle size of the seed beyond this size. The significance of this finding is that particle size of rapeseed plays an important role in the IST of rapeseed to biodiesel, the best size being ≤ 300 µm for high FAME yields. This is the size range that was employed for all other investigation in the study.

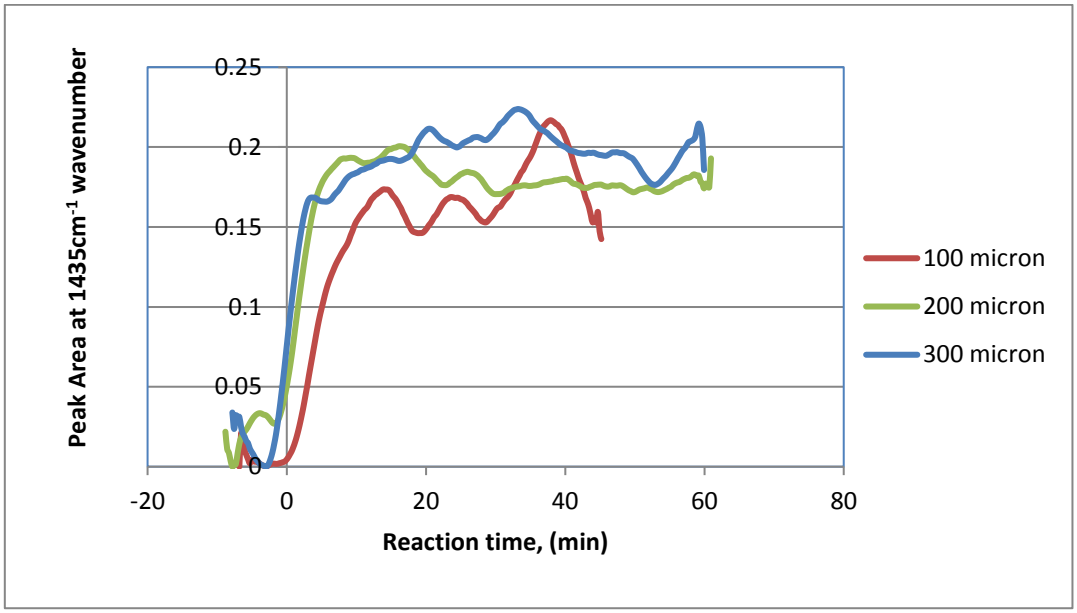


Figure 4.8 The reaction curves of in-situ transesterification of rapeseed of different particle sizes using FTIR

To quantify the FAME produced by IST using FTIR, a calibration curve was generated using known concentration of heptadecanoate FAME standard in methanol (Fig 4.9 below). The peak area was traced to the graph to give the concentration of the FAME. This was good enough to give an accurate (~90%) prediction of the concentration of FAME at the peak of 1432 cm^{-1} with percentage relative error of 0.17%. This is the only peak that gave a nearly linear response to increase in the peak area as the FAME concentration increases according to Beer-Lambert law. However, there is an exception to this rule at 80% concentration where absorbance is not linearly proportional to concentration due to the polychromatic radiation effect. The effect is a result of variation of the absorption coefficient over the specific 1432 cm^{-1} spectral bandpass. This apparent deviation as a result of failures of the spectrometer to conform to the condition under which Beer-Lambert law is derived (Lampman *et al.*, 2010).

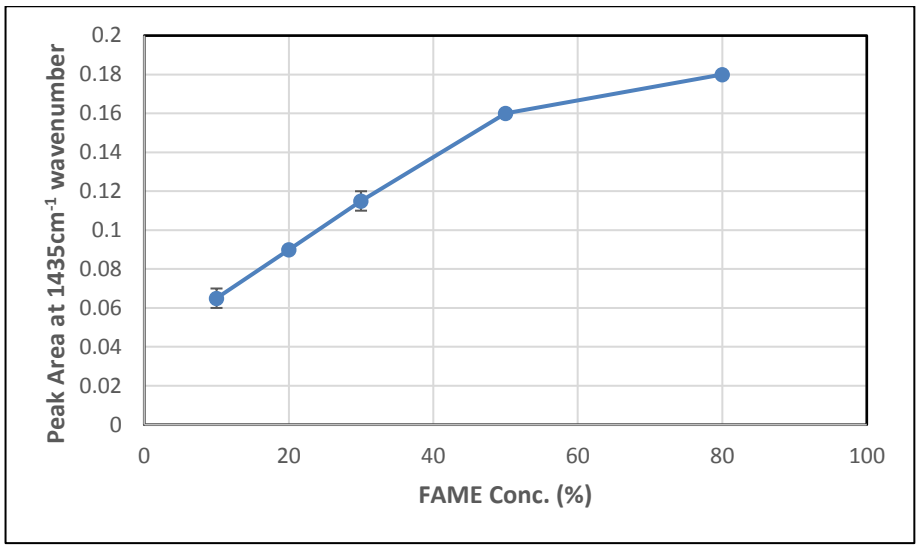


Figure 4.9 The FTIR calibration curve for heptadecanoate FAME standard.

The FAME vs time plot using the FTIR calibration curve in Fig. 4.9 for different process conditions of methanol to oil molar ratio, catalyst concentration and the temperature was given in Fig. 4.10. It can be seen from the smoothed signal curves that a lower methanol to oil molar ratio of 150:1 produces a higher FAME concentration (70%) than at a higher ratio 350:1 (20% FAME concentration), whilst keeping the other two variables of catalyst concentration and temperature constant. This result runs contrary to the literature, where higher molar ratios lead to higher FAME concentrations for IST, this is however due to the evaporation of methanol as the reaction continues. For instance, Zakaria and Harvey (2012) reported FAME yields of >85% of methanol to oil molar ratio of 450:1 after about 1 h of reaction time. This observation can be explained by the fact that minimum alcohol requirement may be needed for high yield FAME in IST but it can only be seen using online monitoring. The result may be due to the high methoxide (NaOH + MeOH) an active catalyst that attack the carbonyl group ot the triglyceride to form the intermediate complex which then react with another molecule of methanol to produce methyl ester and glycerol.The rapid increase in FAME concentration observed (based on the marked region in Fig. 4.10) over the first 5 min is seen at a high catalyst concentration of 0.5 M, followed by a steady decrease as result of saponification. This result agreed with the fast transesterification of Eze *et al.* (2014) and Salam *et al.* (2016a).

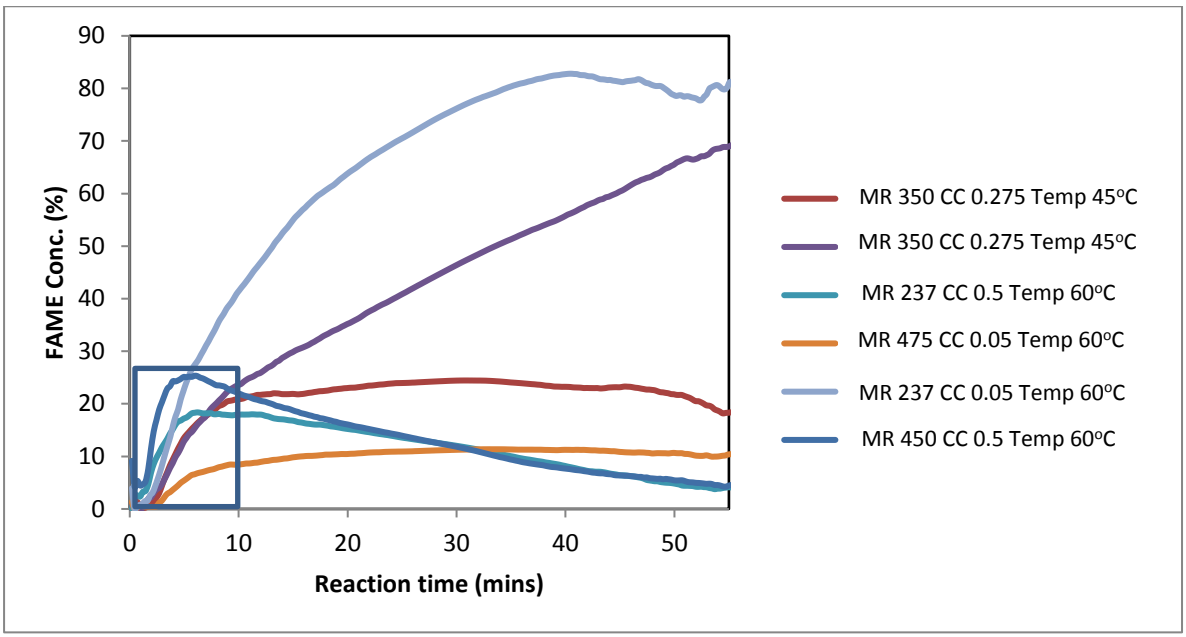


Figure 4.10 FTIR monitoring of IST of FAME production against the reaction time for different process conditions

4.6.1 Validation of on-line monitoring of IST using FTIR and GC

Fig. 4.11 shows that FTIR can be used to monitor on-line IST with a significant degree of accuracy. The study demonstrates for the first time that IR can be used to monitor IST on-line in real time, as it has been established for conventional transesterification. The benefit of this is that key compositional and quantification data can be obtained quickly and directly from the process without the destructive traditional chromatographic techniques.

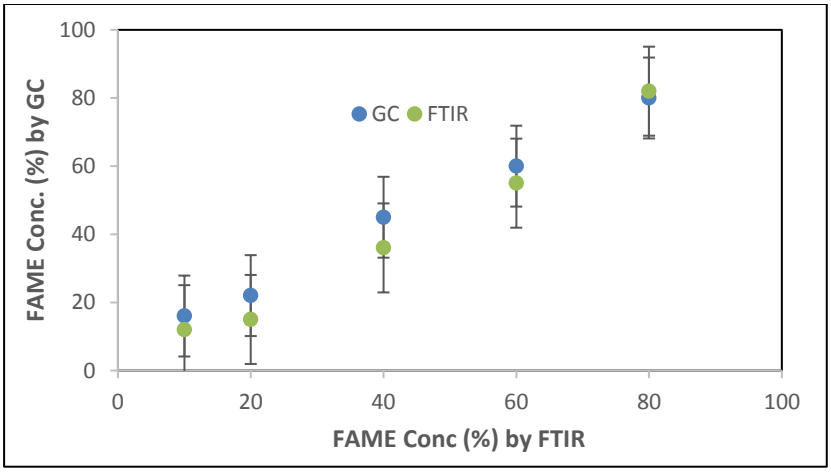


Figure 4.11 Comparison between FAME concentration as determined by GC and FTIR

This can potentially improve IST optimization particularly in the biodiesel industry where a rapid analytical technique can save production cost and time with immediate remediation action. IST is not yet developed to be used in the biodiesel industry. However there is an

evident limitation of this process as it provides only rough quantification (~ 90% accuracy) of FAME, further studies are needed to improve on the method. The usual tradeoff between accuracy and time resolution is an inherent characteristic of online/offline techniques.

4.6.2 Effect of IST reaction time on concentration

Fig. 4.12 shows that the FAME yield at 5 min increased with catalyst concentration until 70% NaOH. In contrast to this, the 1h FAME yield decreased with catalyst concentration from 8% to 96%. The reaction is initially slow, probably due to the time is taken for the diffusion of the methoxide into the seed particles to trapped triglycerides. Once there is a contact between the TG and the methoxide, the transesterification reaction proceeds rapidly until equilibrium is established. Moreover, because the IST reaction is reversible, a high concentration (usually > 8%) of methoxide species in the reaction mix can cause the formation of soap which tends to reduce the FAME yield on further reaction time. The methoxide catalyses the conversion of the FAME produced to soap and water by saponification reaction. For example, Kasim and Harvey (2011), El-Enin *et al.* (2013), and Salam *et al.* (2016a) all reported the similar effect of a decrease in FAME yield at high catalyst concentration over 1h reaction time on FAME yield. Although process conditions and feedstock differ, the main reaction is the same. It, therefore, means that economic consideration of increase catalyst cost and reduced reaction time should be considered before the selection of the suitable process condition.

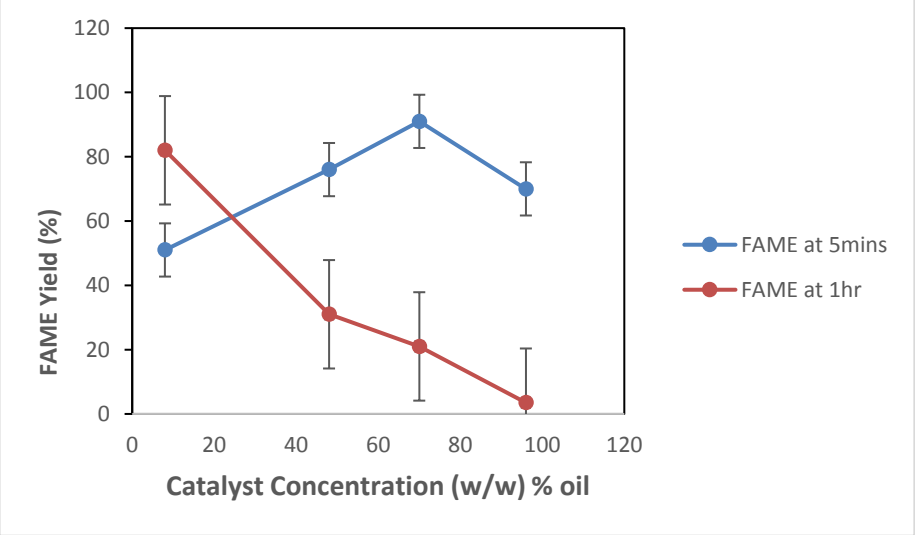


Figure 4.12 IST of rapeseed FAME yield against catalyst concentration after 5 mins and 1 h with 375:1 methanol to oil molar ratio, 60°C, and 500 rpm agitation.

4.7 Summary of Findings for Reactive Extraction

The main findings of the aspect of the research that deals with reactive extraction of rapeseed to produce biodiesel are:

- Presoaking of rapeseed in methanol prior to transesterification is shown to be a complementary method of reducing the methanol requirement in reactive extraction.
- Rapid / 'fast' reactive extraction has been identified to achieve high FAME yield (90%) at high catalyst concentration, however, this occurs within 5 mins of reaction after which the FAME yield gradually decline as a result of the saponification of the FAME.
- First-time demonstration of online monitoring of reactive extraction, via FTIR. This allowed the determination of onset of saponification and subsequent quenching of the reaction. Conventional transesterification is difficult to monitor online due to same carbonyl (C=O) functional group on triglyceride and FAME ester. However, this was possible in reactive extraction because the triglyceride is in the seed rather than being a major component of the extracted oil.

These findings represent a potential improvement to biodiesel production by reactive extraction. The second aspect of the work which investigates the combination of reactive extraction of the oilseed to produce biodiesel with reactive coupling, to convert the glycerol by-product into polyglycerol is discussed in the following sections.

4.8 Screening Study for Reactive Coupling

The glycerol acid catalysed (120-150 ^{o}C) reaction is milder in temperature requirement when compared to base catalysed (260-280 ^{o}C) as explained in section 2.4.1 of the literature review. As a result, acid catalysed transesterification is chosen because its reaction temperature is within the safety design limit of the reactive coupling rig. Fig. 4.13, below, summarises the screening study carried out to select the most compatible reaction process out of the many glycerol conversion processes with transesterification. The basis of the selection was the following criteria:

1. The probable effect of RC on the rate of transesterification
2. The product value
3. The effect of RC on the methanol requirement of IST
4. Ease of product separation
5. Compatibility of the polymerisation reaction with transesterification
6. Compatibility of the reactant with methanol

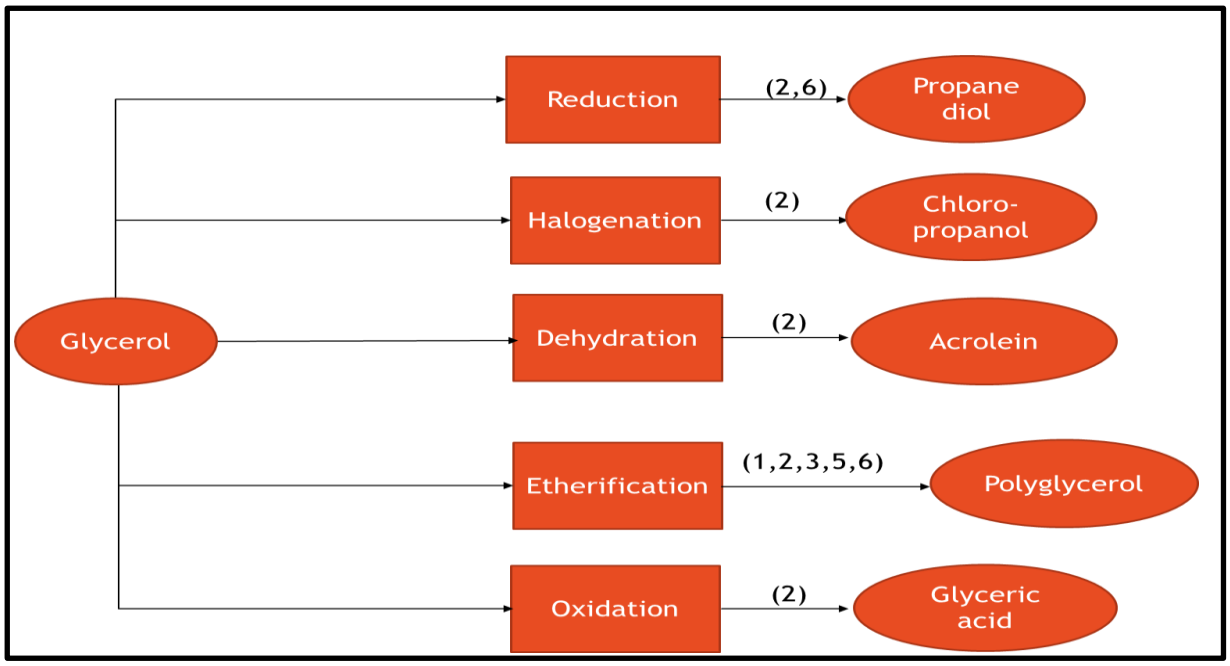


Figure 4.13 Reactive coupling screening streams. 1 – Increase in rate of IST; 2 – High product value; 3 – Decrease in methanol requirement; 4 – Ease of product separation; 5 – Reaction compatibility with IST and 6 – Reactants compatibility

The rationale for the criteria is as explained in the section that follows.

4.8.1 The rate of *In-situ* Transesterification

The rate of IST is a key factor in the efficient production of FAME. In reactive coupling, the rate of transesterification is expected to be fast because of the increased temperature (140^{o}C) at which it proceeds. Goff *et al.* (2004) show that transesterification of soybean oil at 100^{o}C with 0.5 wt% sulphuric acid catalyst loading provided over 99% conversion FAME conversion in less than 4h with 0.8 wt% FFA. The other process routes of glycerol conversion are not related to transesterification. For instance, the reduction process is the addition of hydrogen to glycerol to give either 1,2-propanediol and water or 1,3- propanediol and water or ethylene glycol and water depending on the process condition.

4.8.2 Product Value

Table 4.2 presents the estimated annual product cost of the primary product (biodiesel) and by-product (polyglycerol and DME) sale cost based on the material balance of 1-tonne biodiesel production from rapeseeds using IST approach. The evaluations were made based on the assumption of a 50,000 t/yr pilot biodiesel plant study by El-Enin *et al.* (2013). The

price values of the products were updated to price indices of 2017. The estimated annual IST operation expenditure (opex) of the plant is $43,950,950 (El-Enin *et al.*, 2013). The gross profit for reactive coupling is evaluated to be $15,636,050. There is profit after deducting the value of the product after sales from the total cost of production, which entails feedstock, utility and equipment cost. The high market value of the polyglycerol and DME from reactive coupling makes them a plausible alternative in comparison to only IST. The integration of transesterification and glycerol polymerisation/etherification reaction in a single stage makes a sound economic decision regarding overall cost of the product. Furthermore, the concept of 'pyramid of value' as described by Pagliaro *et al.* (2007b) put glycerol derivatives obtained by a chemoselective catalytic process such as propanediol, acrolein, glyceric acid, chloropropanol and polyglycerol at the top of the pyramid where usage for pharmaceutical and personal care usages resides.

Table 4.4 Estimated annual revenue of biodiesel and by-products based on El-Enin *et al.* (2013).

Material name	Market Price ($/t)	Amount/annum (t/yr)	Annual value of product ($/yr)
Biodiesel	890	50000	44,500,000
Rapeseed meal	200	64800	12,960,000
Polyglycerol	1000	1500	1,500,000
Dimethyl ether	1100	570	627,000
Total annual products value			**59,587,000**

4.8.3 Methanol Requirement

The main reason for reactive coupling apart from increasing the number products is to reduce the significant amount of alcohol (methanol) required for IST. One approach to achieving this is to remove the glycerol by-product as it is being formed during IST. This will allow the reaction to occur at a lower ratio of alcohol to oil by increasing the rate of FAME formation (Crawford *et al.*, 2006). This postulation was used to select polymerization/etherification of glycerol as a good candidate to be integrated with transesterification for FAME production.

Furthermore, the dehydration of excess methanol to form dimethyl ether another fuel gas represent a potential reduction in the amount of methanol recovery in the downstream separation of the product. This may reduce the energy cost of the entire production process. Given the preceding, the methanol requirement of coupling transesterification with glycerol polymerization for biodiesel and polyglycerol is expected to be reduced compared with normal IST reaction.

4.8.4 Ease of Product Separation

DME can be easily separated from the reaction products because it is in the gas phase. Also, simple filtration can be used to separate the rapeseed meal from other liquid products. The energy requirement for the separation of unused methanol is expected to be lower because part of the alcohol is converted to DME. The separation of other products will require fractional distillation. The conversion of crude glycerol to polyglycerol without separation in the product mixture is one of the reasons why reactive coupling is the chosen candidate. Broadly, reactions that are homogeneously catalysed are harder to separate than heterogeneously catalysed ones. For instance, PGs of different degree of oligomerisation can be formed with associated secondary dehydration products which tend to reduce product quality by colouration (Sivaiah *et al.*, 2012). The use of a solid catalyst for reactive extraction is not feasible since the feedstock in the reaction is also solid.

4.8.5 Reaction and Reactant Compatibility

The coupling of transesterification and glycerol polymerisation/etherification reactions does not inhibit the formation of FAME by transesterification and polyglycerol by polymerisation. The transesterification reaction is hindered by using another process route. For example, reduction of glycerol to produce either 1,2-propanediol, 1,3-propanediol or ethylene glycol require a condition that does not support the production of biodiesel. The addition of hydrogen to glycerol at 200^{o}C using copper chromite as a catalyst can only produce glycol as opposed to desired biodiesel (primary product) and other by-products (polyglycerol and DME). The sulphuric acid catalysed transesterification increases the yield of both FAME and glycerol. Consequently, the glycerol monomer is available for polymerisation. The reactants in the transesterification are the TG and methanol. The reactants are compatible, however, the glycerol produced is soluble in methanol, which poses the separation challenge downstream. The acid catalyst is active even in the presence of FFA (Goff *et al.*, 2004).This is a significant advantage of acid catalysed transesterification to alkaline catalyst where the base used degrades as a result of the formation of soap by reaction of the alkali with the FFA.

4.9 Reactive Coupling (RC) of Rapeseed for Biodiesel and Polyglycerol Production

RC aims to remove one of the reaction products (glycerol) from IST thereby pushing the reaction equilibrium toward the product side so that more FAME is formed using a minimum of alcohol. This goal is achieved by coupling transesterification of triglyceride in the rapeseed with polymerisation of its glycerol by-product into polyglycerol. Another driver for this study is to convert the hitherto waste crude glycerol by-product of transesterification due to the expensive refining process, into a valuable polyglycerol that can be used as a platform chemical in the production of other chemicals mainly polyglycerol esters. The refining of the crude glycerol to the purity of which it can be utilized by end users is usually expensive, particularly for small and medium scale biodiesel producers. Figure 4.14 illustrates the reaction pathways of reactive coupling.

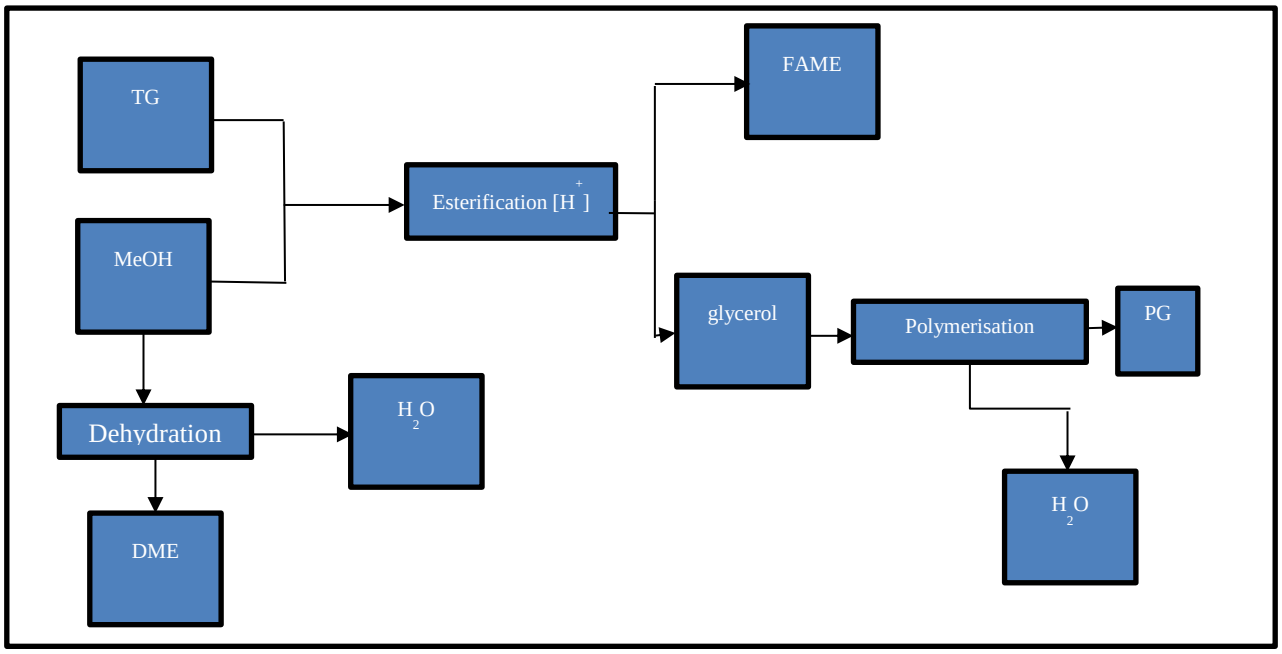


Figure 4.14 Proposed Reactive Coupling Reaction Scheme

The triglyceride in the rapeseed is reacted with methanol in the presence of a sulphuric acid catalyst to produce FAME and glycerol at 140^{o}C and 10 bar in an inert atmosphere. Sulphuric acid is used as the catalyst because alkaline catalysis for polyglycerol production requires temperatures in excess of 250^{o}C, which was above the safety design limit of the experimental rig. The glycerol is polymerised into polyglycerol and water using the active sulphuric acid in the reaction mixture as a catalyst. Furthermore, part of the unreacted excess methanol is dehydrated to produce dimethyl ether and water in another secondary reaction. The dehydration of methanol takes place in the gas phase where the calculated change in Gibbs free energy ΔG is -32.04 kJ/mol at 140^{o}C as obtained using Equation 4.1.

$$2CH_3OH \xrightarrow{H^+} CH_3OCH_3 + H_2O$$

$$\Delta G = \Delta H - T\Delta S \qquad 4.1$$

$where\ \Delta H$ is the change in enthalpy $= \sum H_{product} - \sum H_{reactant}$ (kJ/mol)

T = reaction temperature (K)

ΔS is changing in entropy $= \sum S_{product} - \sum S_{reactant}$ (kJ/K.mol)

The negative ΔG indicates that the reaction is spontaneous which means it can proceed at the given temperature condition of 140°C. The reactive coupling process not only allows the utilisation of the glycerol by-product but also produces useful secondary co-products (DME and PG) apart from the main biodiesel. This, therefore, could be the first step in achieving an integrated biodiesel-based bio-refinery. The integration of the two reaction schemes (transesterification and glycerol polymerisation), is an intensification and can potentially reduce the cost of production. However, one limiting factor to the process, particularly polyglycerol, is the formation of water in the reaction mixture which tends to retard the PG production. It was reported in the work of Lotero *et al.* (2005) that water formation inhibits the catalytic activity of sulphuric acid which catalyses the condensation polymerization reaction.

4.9.1 FTIR Analysis of the Reactive Coupling

The products of the reactive coupling experiments were analysed using FTIR. The result, as shown in Fig. 4.15, indicates the presence of the polyglycerol's characteristic functional groups in the spectrum, as identified in previous work of Salehpour and Dubé (2011) and Ardila-Suárez *et al.* (2015).

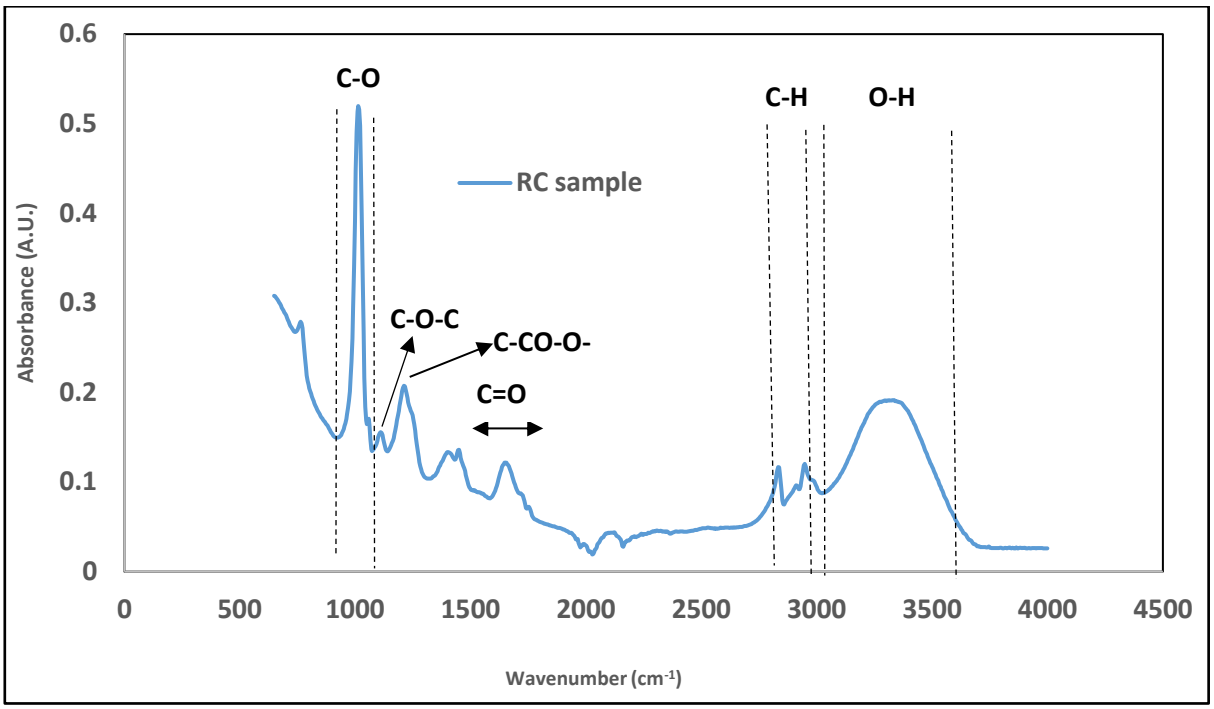


Figure 4.15 FTIR spectra of the reactive coupling sample after 4hr of reaction time methanol to oil molar ratio of 375:1, a catalyst concentration of 4.8 wt/wt% and 140ºC.

The broad OH peak at 3000-3600 cm^{-1} is associated with the hydroxyl group of polyglycerol and water. The CH at 2843-2985 cm^{-1} is due to alkyl group stretching found in polyglycerol and any other hydrocarbon, but the CO stretching at 928-1039 cm^{-1} is that of the ether linkage of polyglycerol. The other peaks in the spectrum are related to other compounds present in the product. The FAME characteristic peaks are from 1000-1800 cm^{-1}. The most obvious is the carbonyl group (C=O) of the ester, observed at 1745cm^{-1}, but there are also peaks at 1468 cm^{-1} and 1218 cm^{-1} representing (CO)-O-CH_3 and C-CO-O respectively.

4.9.2 NMR Analysis of the Reactive Coupling

The ^{1}H NMR analysis of the reactive coupling sample was obtained by the NMR spectroscopy method described in section 3.5.8. A sample spectrum is shown in Fig. 4.15 to give further evidence of the presence of polyglycerol in the product mixture, compared with the chemical shift of carbon and hydrogen that are established from standard commercial polyglycerol obtained from Solvay Chemicals, Brussels. As can be observed, both the sample and standard show the presence of polyether protons of methylene (CH_2) on a secondary carbon and methane (CH) on tertiary carbon occurs at between δ = 3.0 - 4.0 ppm with broad resonance pattern and the proton of the hydroxyl signal appeared at δ = 4.0 - 5.0 ppm. The result is consistent with Salehpour and Dubé (2011) and that of Ardila-Suárez *et al.* (2015) where the proton of methylene and methine of the polyether is shown around δ = 3.2 and 4.1 ppm and the signal of the hydroxyl ion at δ = 5 ppm.

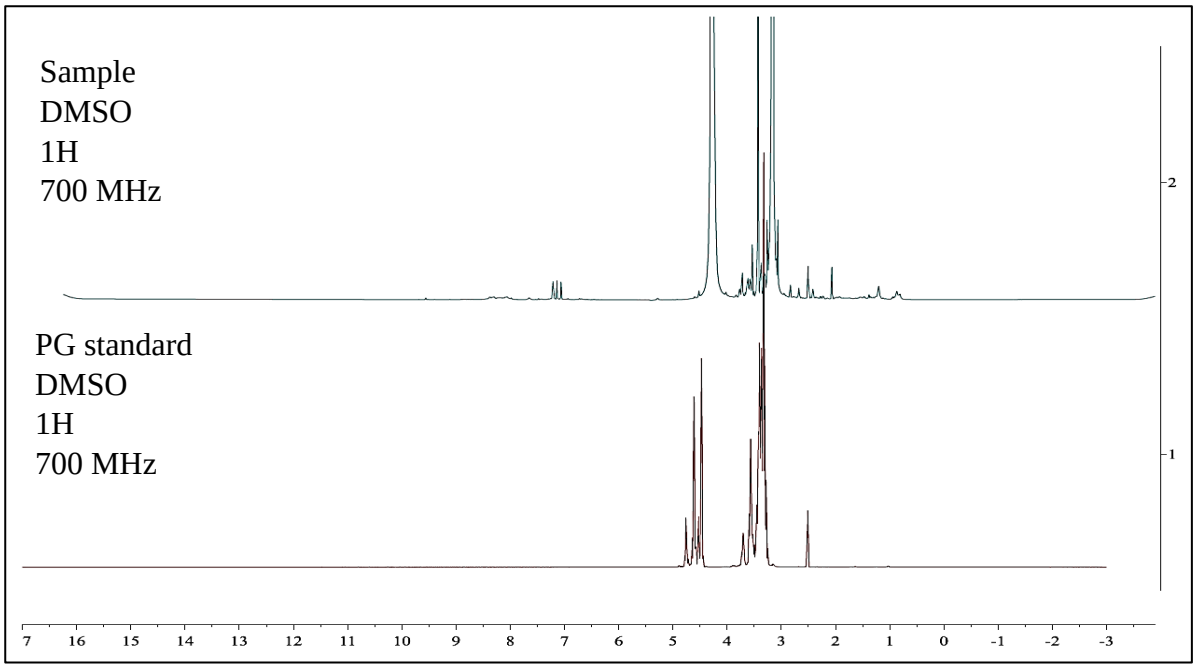


Figure 4.16 1H NMR Spectra (DMSO removed) of reactive coupling sample and commercial PG-3 from Solvay

4.9.3 Refractive index analysis of Reactive Coupling

The results below show the refractive index (RI) of product samples analysed by the method described in section 3.5.9 is as shown in Fig. 4.17. The analysis was used to rapidly monitor the polymerisation reaction.

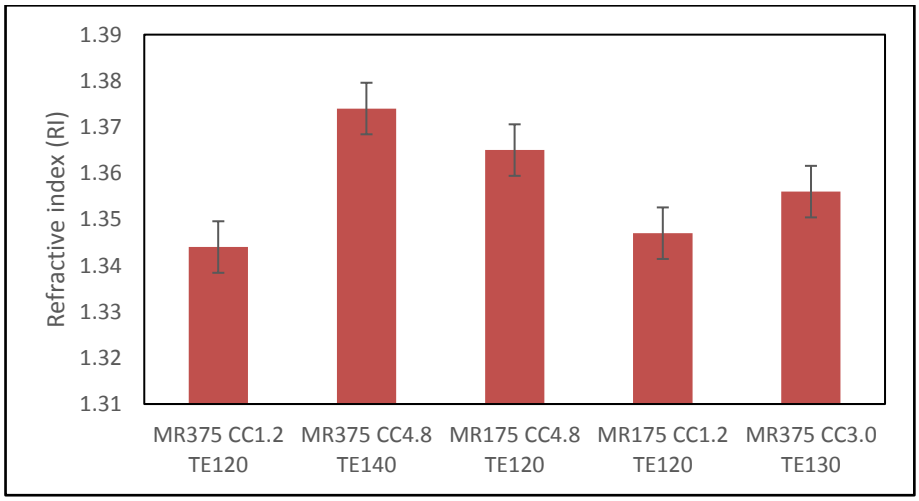


Figure 4.17 Refractive index at 23°C of reactive coupling product samples at different process condition. MR - methanol to oil molar ratio, CC – catalyst concentration, and TE- temperature.

The figure above indicates that the highest value of RI is observed for the sample with the highest methanol to oil molar ratio, catalyst concentration, and temperature while the lowest

is in the condition with the lowest methanol to oil molar ratio, catalyst concentration, and temperature. The polymerization of glycerol to polyglycerol increases with increase in the refractive index and this increases with the degree of polymerization (Hasenhuettl and Hartel, 2008). However, the condition with methanol to oil molar ratio of 375:1, catalyst concentration 1.2(v/v)% and temperature of 120^{o}C was slightly lower than that of methanol to oil molar ratio of 175:1, catalyst concentration 1.2(v/v)% and temperature 120^{o}C. This may be as a result of excess water from the polymerization of glycerol that was condensed back into the reaction mixture, therefore, dilute the mixture. Table 4.4 below gives the physical oligomers data. The dehydrated samples compared in this study contains water, methanol, sulphuric acid in addition to the reaction products formed. It can, therefore, be assumed that after purification of the crude sample of reactive coupling the refractive index may be between 1.480-1.490 to reflect the RI of a pure sample of polyglycerol. The technique may be more useful where the water is removed as it is formed in the reaction mixture, so that product samples do not contain water.

Table 4.5 Physical Properties of Oligomers (Martin and Richter, 2011)

Name	Molecular Wt. (g/mol)	Refractivity (n_D^{20})
Glycerol	$C_3H_8O_3$ (92)	1.4720
Diglycerol (DiG)	$C_6H_{14}O_5$ (166)	1.4897
Triglycerol (TrG)	$C_9H_{20}O_7$ (240)	1.4901 (40^{o}C)
Tetraglycerol (TtG)	$C_{12}H_{24}O_9$ (314)	1.4940 (40^{o}C)
Polyglycerol-3[(a)] (PG)		1.4910
Polyglycerol (this study)		1.3740[(b)]

[(a)]Commercial PG given by Solvay Chemicals
[(b)]The PG in this study is crude and hydrated.

4.9.4 LC-MS Analysis of Reactive Coupling

The mass spectrum of the reactive coupling products is shown in Fig. 4.18- 4.21, at 4 different times (1, 2, 3, and 4 h). The presence of protonated diglycerol (DiG) with m/z of 167 is seen after the first hour of the reaction. The protonated triglycerol (TrG) with m/z of 241 is also present, however, but at a lower intensity. This is due to slow rate of polymerization of glycerol to di- tri and tetra glycerol. The protonated tetra glycerol (TtG) is not yet in appreciable quantity in the reaction mix. As expected, the reactive coupling yielded many

reaction products, mainly FAME, PG and other side reaction products. Hence fragments from one component are used as a fingerprint for the identification of the component using the mass-charge ratio (m/z) ions. The m/z ion of 290 can be attributed the FAME which predominates in the reaction mixture. Cortese *et al.* (2015) used a similar method to characterise transesterification product obtained from coconut oil in the presence of polyglycerol. Their results are consistent with data obtained from TIC of the reactive coupling.

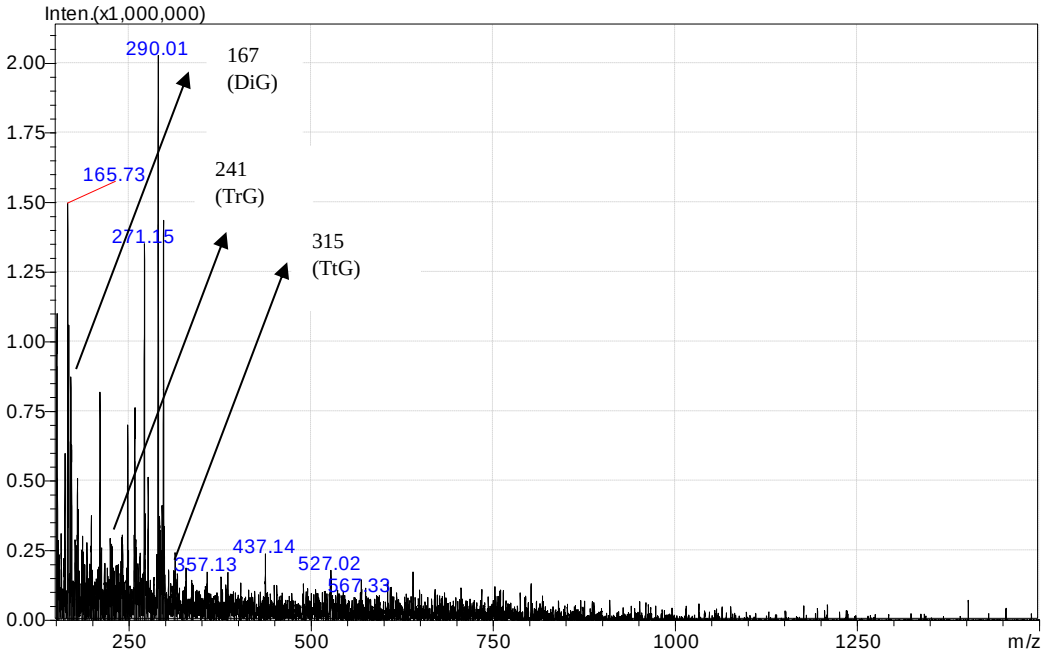


Figure 4.18 MS of reactive coupling sample obtained at 1 hr with the condition of methanol to oil ratio of 375:1, a catalyst concentration of 4.8 v/v % and temperature of 140ºC

Furthermore, Fig. 4.18 shows an increase in the intensity of the DiG at 2 h compared to 1 h of reaction. Also, a slight increase of TrG and TtG was observed at same reaction time. This may be explained by the fact that increase in reaction time enhances the rate of polymerization of the glycerol by-product from transesterification. This result seems to be consistent with the work of Medeiros *et al.* (2009) which found an increase in the oligomers of acid catalysed polymerization of glycerol as the initial reaction time increases but later decreases with time due to increase in the amount of water in the reaction medium. Another possible explanation for this observation is the break up of polyglycerol molecule forming other dehydration product such as acrolein. Also, the ester of the various fatty acids in the reaction medium is increasing thereby producing more of the glycerol which then get converted to the oligomers as explained.

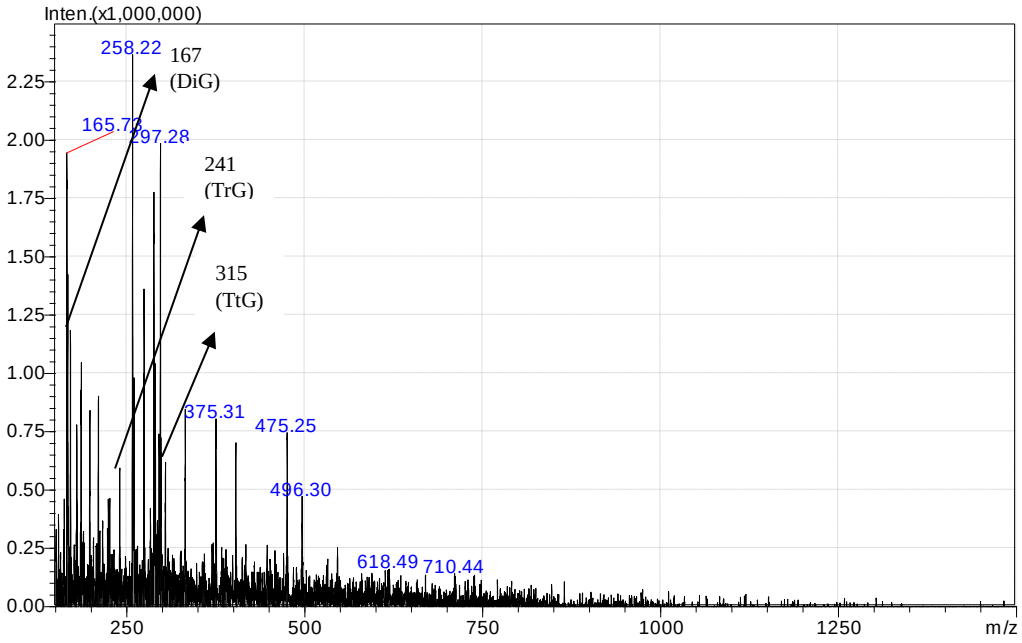


Figure 4.19 MS of reactive coupling sample obtained at 2 h with the condition of methanol to oil ratio of 375:1, a catalyst concentration of 4.8 v/v % and temperature of 140ºC.

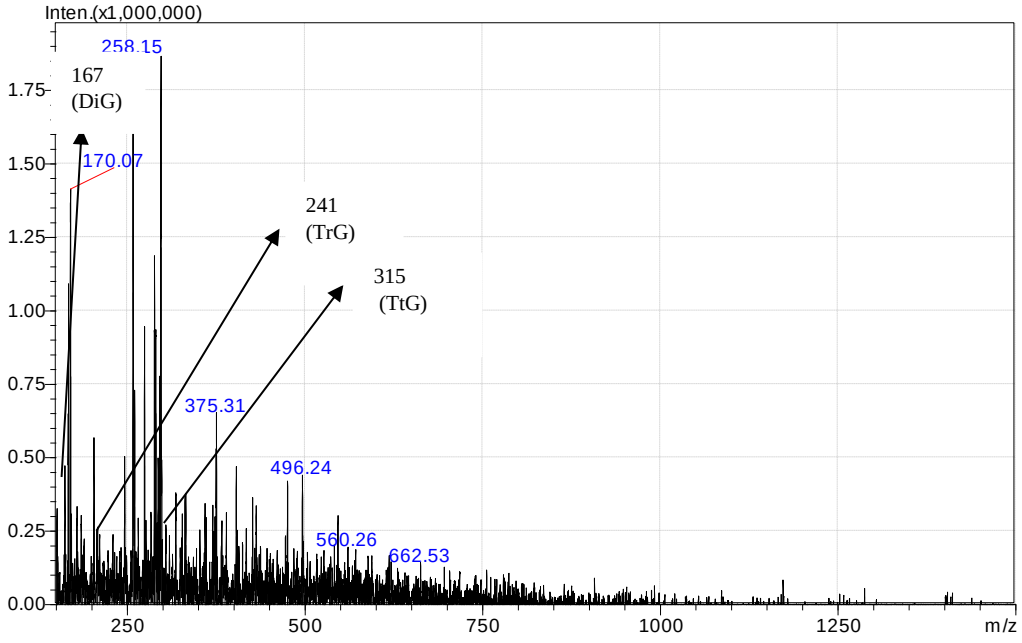


Figure 4.20 MS of reactive coupling sample obtained at 3 h reaction with the condition of methanol to oil ratio of 375:1, a catalyst concentration of 4.8 v/v % and temperature of 140ºC.

In Fig. 4.20, there is a decrease in concentration of DiG at 3 h of reaction time compared to the 2 h, a similar trend is observed for the other oligomers. This is attributed to the increase in

water in the reaction medium. The water is formed from two sources, one from the condensation polymerisation reaction and two from the side reaction dehydration of the excess methanol to form dimethyl ether.

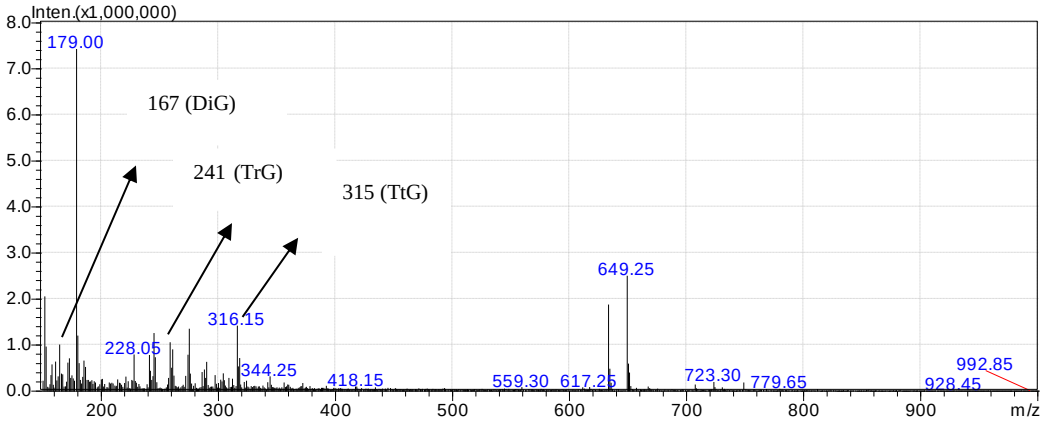


Figure 4.21 MS of reactive coupling sample obtained at 4 h with the condition of methanol to oil ratio of 175:1, a catalyst concentration of 1.2 v/v % and temperature of 140°C.

However, surprisingly, there is an increase in the concentration of the ions of the oligomers in 4 h of reaction time as seen Fig. 4.21. This inconsistency may be due to interference from the other side reaction (dehydration) in the medium because of low selectivity. The reaction is sensitive to the process condition.

4.9.5 Time Profile of Polyglycerol from Reactive Coupling

The results of analysis of the products of reactive coupling (mainly diglycerol, triglycerol and tetraglycerol) that are collectively characterised as polyglycerol oligomers using the method described in section 3.4.7 are shown in Fig. 4.22.

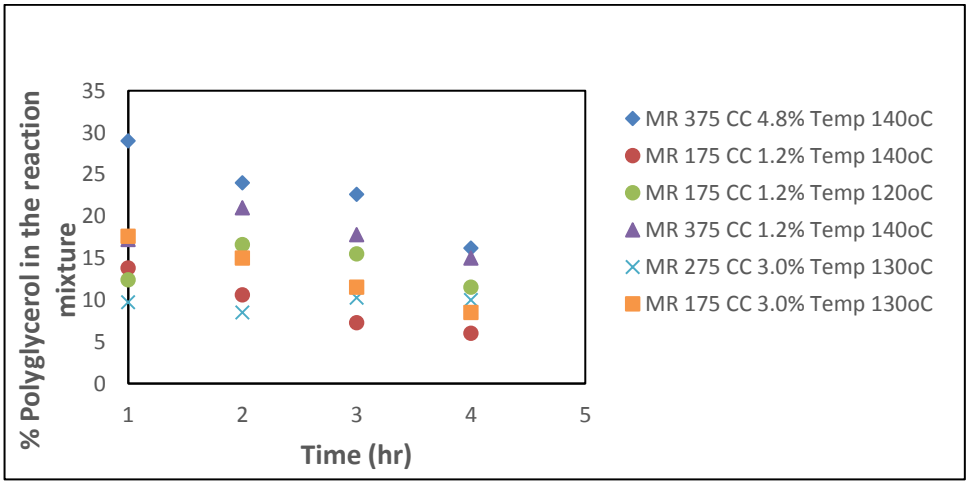


Figure 4.22 Polyglycerol concentration-time profile for various reaction conditions

The fraction of the intensity of the PG ((DiG) + (TrG) + (TtG)) to the sum of the total intensities of the set of the ions are plotted against the reaction time to monitor the changes in the concentration of the species across the reaction time for different reaction conditions.

There is a relatively high conversion of glycerol to PG in the first 1h of the reaction with the condition of MR of 375:1, CC of 4.8% and 140^{o}C. This is as a result of the increased rate of glycerol production facilitated by high catalyst concentration and temperature, which is then quickly polymerised into PG by the action of the sulphuric acid catalyst. However, the concentration of the PG decreased over time as a result of the undesired formation of water in the reaction mixture, which tends to reduce the activity of the acid catalyst for the secondary polymerization reaction. Generally, glycerol polymerisation occurs at higher temperature (> 200^{o}C) at normal atmospheric pressure using catalyst concentration of 4.8 wt %. However, the experiment is conducted at elevated pressure of 10 bar which enables us to use 140^{o}C.

There seems to be a low yield of PG at MR of 175, CC of 1.2v/v% and temperature 120^{o}C. This is because the glycerol polymerisation reaction rate is impractically slow at this condition and undesired side reaction (possible formation of acrolein) also hindered the conversion to PG. The yields in this investigation were lower than those of other studies. For instance, Salehpour and Dubé (2011) found only 30 mol% PG conversion after 10 h of reaction time with 120^{o}C and 1.2v/v% of the sulphuric acid catalyst. However, in their experiment, water was continuously taken out of the reaction mixture via a condenser. This was not possible in this study because methanol is used as both solvent and reactant. Therefore, the use of a molecular sieve that will exclude all the reactants except water may be useful in making the system water free. Although this need to be verified and it is subject of further investigation by another researcher continuing with the project.

4.9.6 Effect of Catalyst on Reactive Coupling

Prior studies indicated the importance of catalyst type and concentration on the polymerization of glycerol for the production of PG. The progress of the reaction could be followed by measuring the amount of water produced. The theoretical stoichiometry proportion of polymerisation of glycerol to produce PG and water from the equation is 2:1:1 respectively (Martin and Richter, 2011).

$$2C_3H_8O_3 \longrightarrow C_6H_{14}O_5 + H_2O$$

However, there is no time plot of theoretical polymerisation products from the stoichiometry equation in literature, probably due to formation of several secondary products.

The method described in section 3.4.5 was used to obtain the result shown in Fig. 4.23.

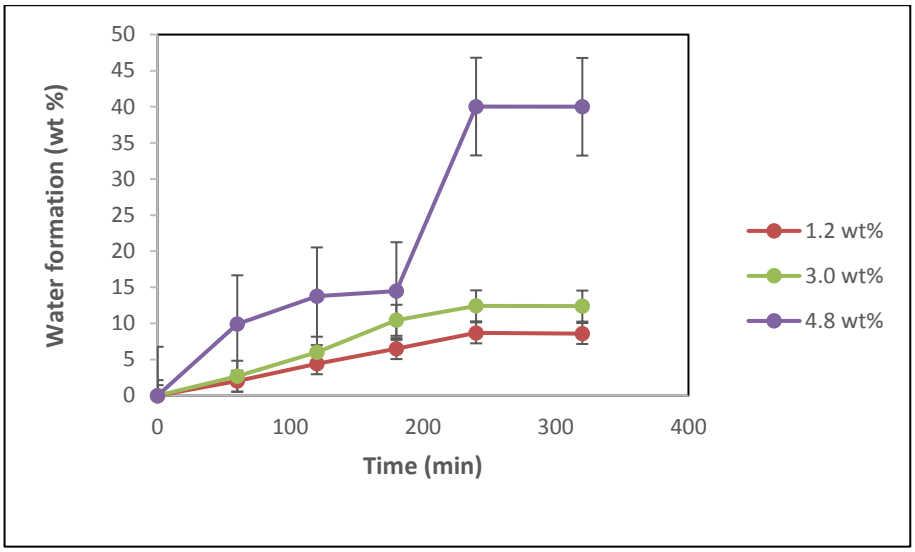


Figure 4.23 Water formation against time for reactive coupling at different catalyst concentration, constant methanol/TG molar ratio (375:1) and temperature (140°C)

The results of this study indicate that rate increases with catalyst concentration. Another significant finding in this study is that the reaction ended after 4 h, as there is no increase in conversion after that time. These results further support the work of Salehpour and Dubé (2011) that sulphuric acid catalyst at higher concentration gave the higher molecular weight polyglycerol. A note of caution is due here since water is being produced from both polymerization and dehydration reactions.

It may be difficult to conclude that the conversion is only from polymerization and this may explain why there is high water formation using the Karl Fisher apparatus. However, this is an important issue for further studies to isolate the water production from both reactions. One way to do this is to use silica gel to absorb the water, but it must be ensured that the adsorbent does not react with any of the reactants. Another way is to redesign the rig to expel the water from the reaction as it is being formed by the inclusion of condenser in the gas collection stream. However, evaporation of methanol must be avoided to prevent the reverse transesterification reaction.

4.9.7 Effect of Temperature on Reactive Coupling

A strong relationship between synthesis conditions for the PG from glycerol and the final PG properties and functionality has been reported in the literature. The current study found that there is an increase in conversion to PG as the temperature increases. This is shown in Fig.

4.23 where the water content of the sample increases from 0 to 30% as reaction temperature increases from 120^{o}C to 140^{o}C.

The catalyst concentration of 4.8v/v% sulphuric acid and 375:1 methanol to oil molar ratio were kept constant. Glycerol polymerisation occurs by liberating one water molecule for each ether linkage bond formed. These results match the earlier study of Ardila-Suárez *et al.* (2015) where they found temperature as critical synthesis parameter in determining the conversion of glycerol to PG at higher catalyst concentration.

This study is unable to demonstrate how the temperature of PG relates to the functionality and properties of the polyglycerol because this will be required to be separated from other reaction product which is beyond the scope our study. These findings may be somewhat limited by the fact that water formation is from two sources as explained in section 4.6.2.1. A further study with a focus on polymerisation reaction mechanism without interference from any side reaction is therefore suggested.

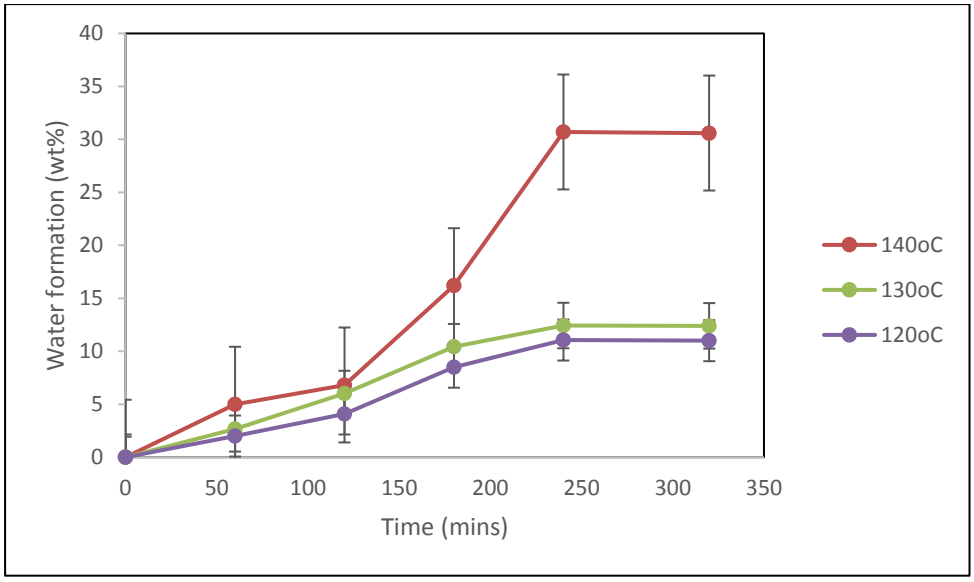


Figure 4.24 Water formation against time for reactive coupling at different reaction temperature, constant catalyst concentration (4.8 wt%) and methanol/TG molar ratio (375:1)

4.9.8 Effect of Methanol to Oil Molar Ratio on Reactive Coupling

This study was designed to determine the effect of methanol/TG on PG from the reactive coupling. In reactive extraction, FAME increases as methanol/TG increases until an optimum value is attained. However, the results of reactive extraction with reactive coupling in Fig. 4.24 seem contrary to this.

The conversion of PG for the MOMR of 375:1 and 275:1 increases with time in the first 4 h of reaction and plateau in the last hour, which tends to indicate completion of the reaction. Contrary to this trend the ratio of 175:1 resulted in water formation with time. It may be that more water is produced from the excess methanol dehydration reaction than that produced from the glycerol polymerisation reaction. High MOMR favours the production of high FAME and PG yields because the glycerol by-product of IST is the reactant for the polymerisation reaction. It may be difficult to conclude based on the trends in the graph because the lines are overlapping with less distinction among the different molar ratio. The isolation of interference of water is suggested in future studies, to be able to determine a clear effect of methanol to molar ratio on the yield of polyglycerol.

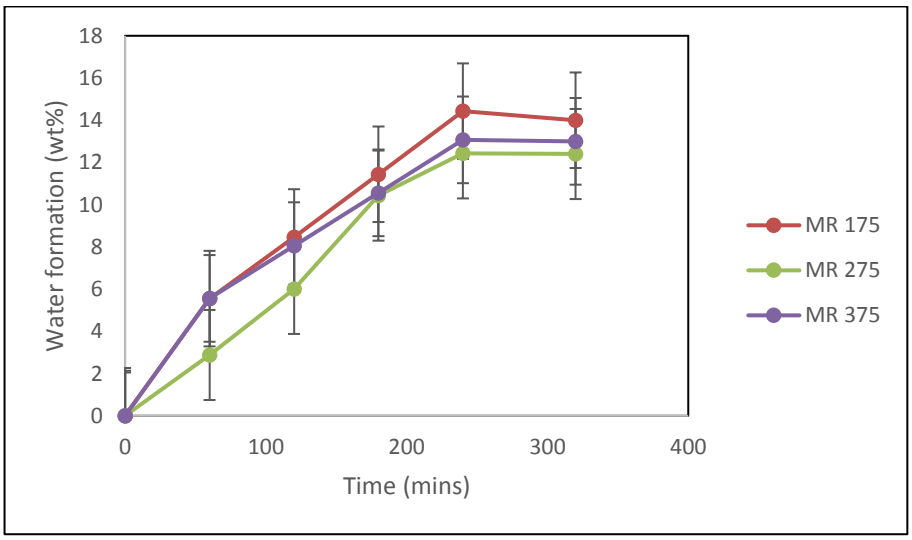


Figure 4.25 Water formation against time for reactive coupling at different methanol/TG molar at constant temperature and catalyst concentration

4.10 Gas Chromatography Analysis of Reactive Coupling Sample

The results in Fig. 4.25, below, are the GC analysis of the overhead gas collected at a constant temperature of 120^{o}C but different methanol to molar ratio and catalyst concentration after 4h. There is an increase in DME yield as the molar ratio and catalyst concentration increases, however; there is a lower yield at 275/1 MOMR than 175/1, which may be due to an error in the collection of the gas as the gas is collected by manually opening of the reactor pressure relief valve. At 375/1 MOMR there is no significant improvement in the yield when the catalyst concentration increases. This may be due to a higher volume of methanol, the amount of protons in the solution is lower than 275/1 MOMR. The reaction to form the DME proceeded in 3 stages; firstly, the protonation of the OH group of the alcohol by the acid catalyst to form R-OH_2, followed by nucleophilic attack on the carbon (SN2) to displace the

water by another molecule of the alcohol to form a C-O ether bond. The final stage is the deprotonation of the product to liberate water. Kim *et al.* (2006) demonstrated the dehydration of crude methanol (containing up to 30% water) to DME they, however, used ZSM-5 catalyst in their work.

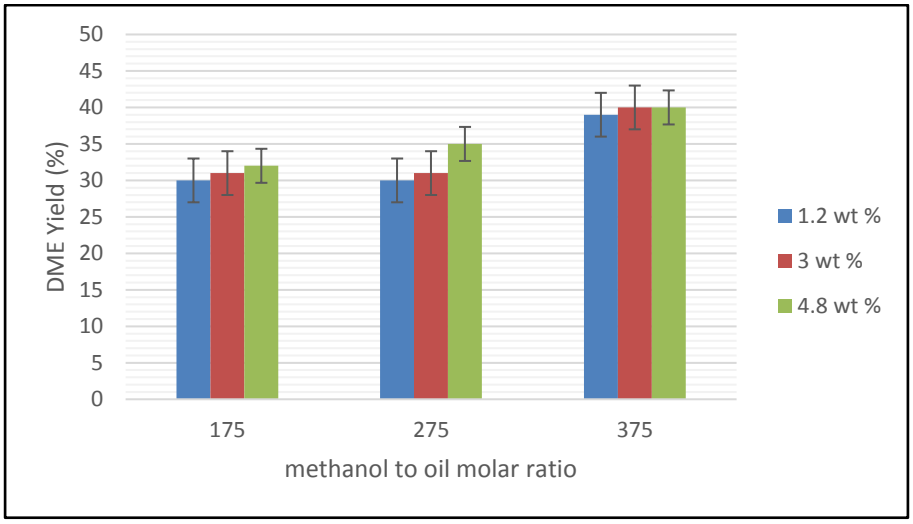


Figure 4.26 Sample DME wt/wt % of the standard 99.99% pure DME for reactive coupling samples 4 h reaction constant temperature of 120ºC, different methanol to oil molar ratios and catalyst concentration

The dehydration of methanol using concentrated sulphuric acid as a catalyst is expensive because of the cost involved in the recovery of the acid and the cost of materials for the reactor (glass-lined) which must be resistant to acid attack. However, since the product is secondary in the reactive coupling, the cost of the product is expected to offset the production and overhead cost of the primary and associated products.

Fig. 4.26 shows the DME yield at 130ºC for the MOMR 175-375 and catalyst concentration 1.2 – 4.8 v/v %. It can be seen that DME yield follows the same trend of increase in yield as molar ratio and catalyst concentration increases. However, there is a higher yield with an increase in temperature from 120 to 130ºC. Higher MOMR means more methanol is available for the dehydration reaction and higher temperature and catalyst concentration aids in the formation of DME.

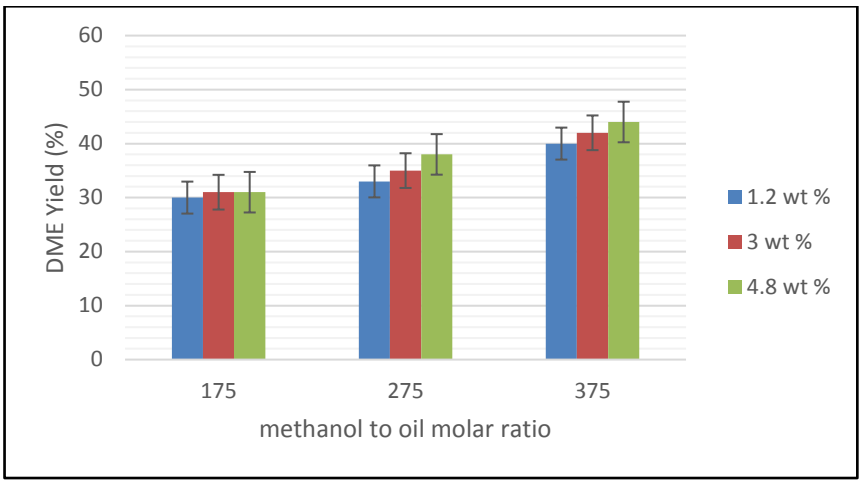


Figure 4.27 Sample DME wt/wt % of standard 99.95% pure DME for reactive coupling samples 4 h reaction constant temperature of 130ºC, different methanol to oil molar ratios and catalyst concentration

DME is usually produced from syngas using a 2-step process of producing methanol from natural gas and dehydration of the methanol using a solid acid catalyst. The direct methanol dehydration method of DME production is not as expensive as using the 2-step process. Also, it fits perfectly as a secondary product to biodiesel production in the reactive coupling. The product can be easily liquefied making it easy to transport as fuel.

Figure 4.27 shows the yield DME wt/wt % of standard DME for reactive coupling samples 4 h reaction constant temperature of 140ºC, different methanol to oil molar ratios and catalyst concentration. The high yields of DME at 140ºC, 4.8 v/v % of sulphuric acid and 375/1 attest to the effect of high molar ratio, temperature and catalyst concentration. However, a higher temperature beyond 140ºC could not be investigated because of safety limit for the design of the rig.

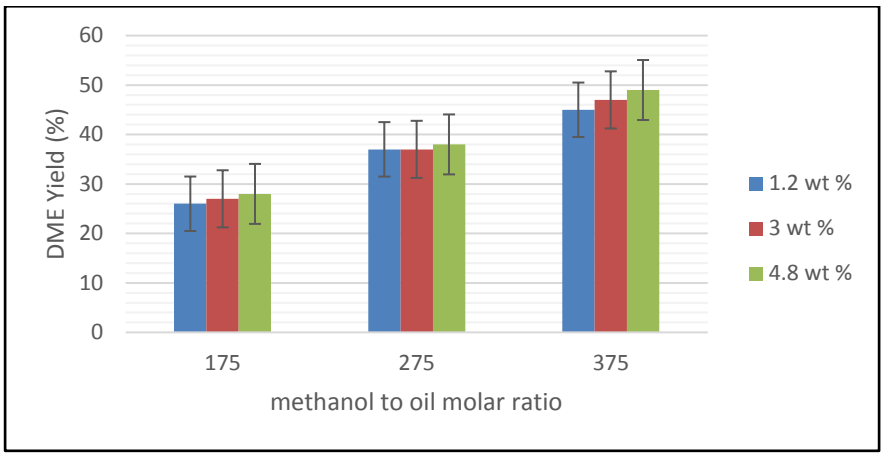


Figure 4.28 Sample DME wt/wt % of standard 99.95% pure DME for reactive coupling samples 4 h reaction constant temperature of 140ºC, different methanol to oil molar ratios and catalyst concentration

A possible explanation for this result is that acid-catalysed IST for high FAME yield occurs at high catalyst concentration with high temperature which favours the glycerol byproduct of IST and reactant for reactive coupling (Lotero *et al.*, 2005). Since only about 6% of the methanol is used for the transesterification reaction, it is, therefore, economical to ultilize part of the excess methanol to form DME in the gas phase gas phase. One of the interesting findings in this work is the production of dimethyl ether by dehydration of methanol using a sulphuric acid catalyst in addition to FAME and polyglycerol production. This is another secondary reaction that occurs due to the selectivity of the process.

No previous study combines the production of DME, FAME and polyglycerol in a single run. The findings provide evidence that not only can FAME be produced but it may be generated efficiently with DME as a secondary product. DME can be used as propane replacement in LNG and can be potentially used as fuel gas in transportation. Furthermore, DME has a higher cetane number (55 – 60) compared to methanol and LPG which are usually less than 10 (Wang *et al.*, 2011). The better ignition quality makes DME a cleaner fuel than fossil diesel fuel.

Another advantage of this process is that the energy required for the recovery of excess methanol in IST is reduced by 19% (the amount of excess methanol converted to DME). This will potentially reduce the cost of FAME production not only by this alone but with simultaneous production of other value-added products, thereby achieving the concept of a biorefinery (Pagliaro and Rossi, 2008a).

4.11 Central Composite Design of Experiment for Reactive Coupling

The optimization of the process variables (MR, CC and Temp) affecting the main reactive coupling products was performed using a central composite design of experiment and response surface methodology as described in section 3.3.1. The response that was evaluated after 4 hr of reaction was FAME yield (%), DME wt/wt (%) of the standard DME and PG wt% as analysed by GCMS

4.11.1 Effect of Reactive Coupling Process Variables on FAME Yield

The coded statistical second-order predicted model equation 4.2 below was developed using multiple regression from the central composite design of experiment. The response was percentage FAME yield, where the temperature, catalyst concentration and methanol to oil molar ratio are the process variables.

$$FAME = 79.80 + 0.80A + 9.80B + 2.60C - 2.0A^2 - 3.0B^2 + C^2 + 1.12AB - 1.37AC - 0.63BC \quad 4.2$$

Where A = methanol to oil molar ratio, B = catalyst concentration and C = temperature.

The correlation coefficient of determination R^2 was evaluated as 0.9641 using the regression model. The model F-value of 29.82 implies the model is significant. B, C and B^2 are significant terms in the model with p-values of 0.0001, 0.0026 and 0.0364 respectively. The model graph of predicted FAME vs. the actual experimental FAME values is as shown in Fig. 4.29

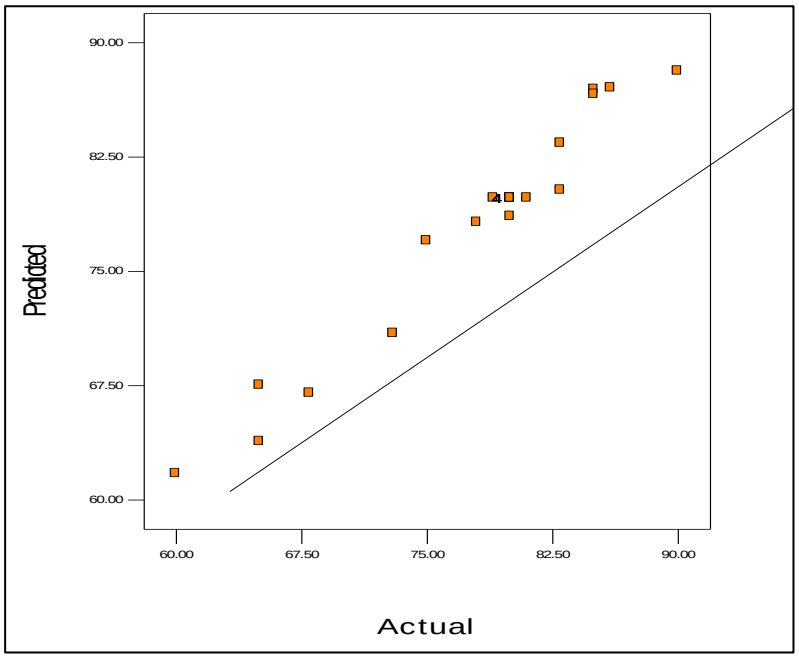


Figure 4.29 Predicted FAME yield vs. Actual Experimental FAME yield values of Reactive Coupling at different process conditions (methanol to oil molar ratio, catalyst concentration and temperature)

Fig. 4.30(a) shows the effect of catalyst concentration and methanol-to-oil molar ratio on FAME yield during reactive coupling. As expected, the FAME yield increases as catalyst concentration increases. This is because catalyst concentration is the most significant process variable in the FAME production of reactive coupling process. These results are consistent with the findings of Revellame *et al.* (2010) which showed that maximum ester conversion occurred at a high concentration of catalyst for IST of activated sludge.

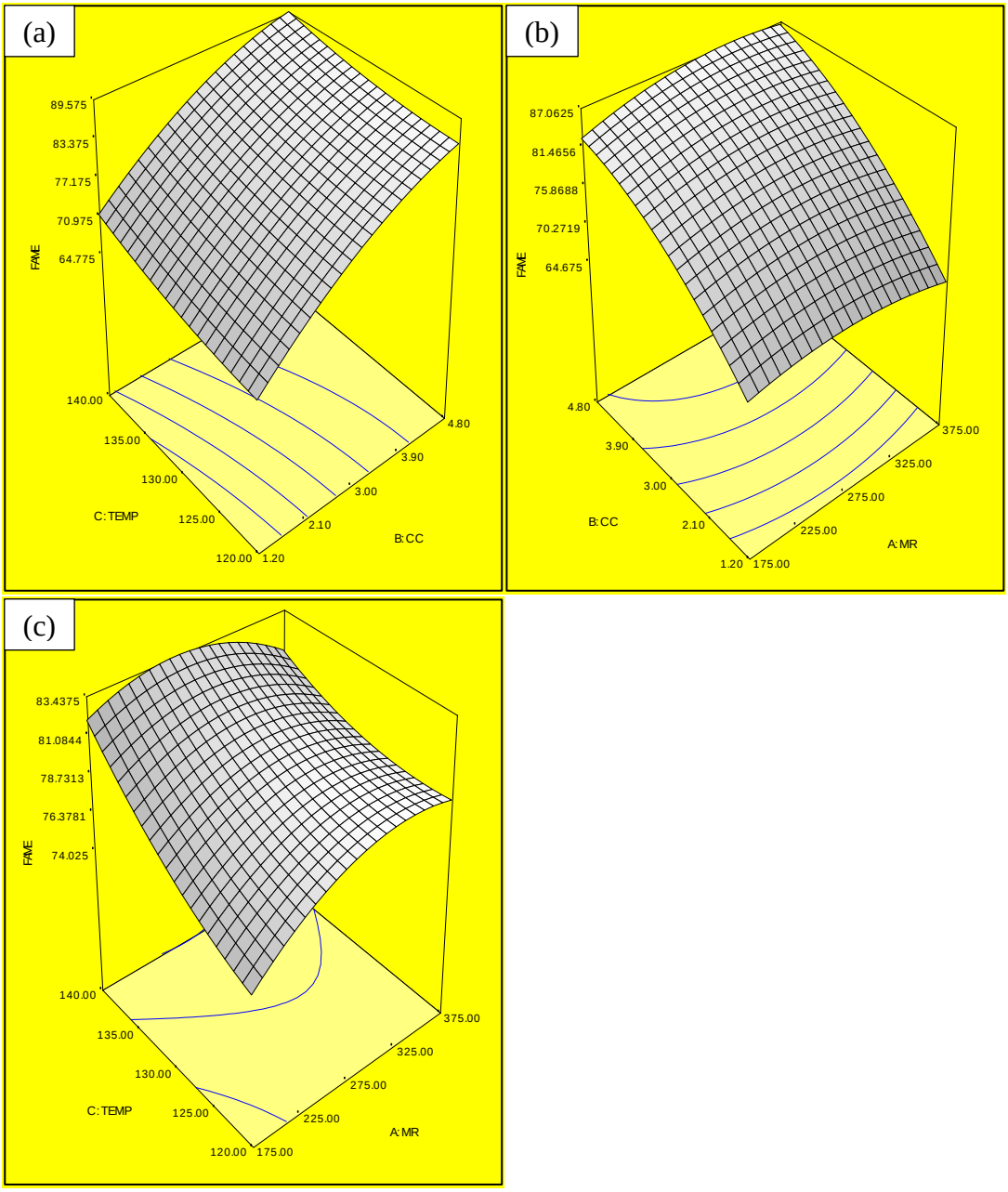


Figure 4.30 The 3D response surface plot for FAME production in reactive coupling with interaction between (a) CC and MR (b) TEMP and MR and (c) TEMP and CC

Similarly, in Fig. 4.30(b), as temperature increases the FAME yield increases, but the effect of varying the MR within this range seems insignificant. Again temperature is a significant variable with P-value of 0.0026 and MR is not a critical variable in the interaction between it and temperature. However, the maximum ester is produced at 140^{o}C and 275/1 MR. A high MR means a high cost of separation downstream of the production. Therefore a moderately low MR will reduce the cost of recovery of excess methanol.

In the interaction between temperature and catalyst concentration shown in Fig. 4.30(c), catalyst concentration is again significant in the conversion of TG to ester. The effect of an increase in temperature on FAME yield is relatively constant when compared to the catalyst

concentration. The reaction conditions for maximum FAME production according to the statistical model are at 120°C, MR 275/1 and 4.8wt/wt% catalyst concentration. The predictive maximum FAME yield of 88.13% was observed while the actual experimental value was 90%.

4.11.2 Effect of Reactive Coupling Process Variables on DME

The ANOVA for DME response surface of quadratic model is given in equation 4.3 below

$$DME = 37.79 + 5.50A - 0.20B + 3.10C + 1.27A^2 - 1.23B^2 - 0.73C^2 - 0.50AB + 1.75AC + 0.25BC \quad 4.3$$

Where A = methanol to oil molar ratio, B = Catalyst concentration (v/v) and C = Temperature (°C)

Fig 4.31 shows the plot of the predicted value of DME from the model equation 4.3 and the actual experimental values of DME. The predicted R-squared of 0.8978 is in reasonable agreement with adj R-squared of 0.9563. The model F-value of 47.14 implies that the model is significant. The implication of this is that the model can give a good representation of the actual experiment. The model shows that methanol to oil molar ratio, temperature and the combination of both variables are significant with P-values of 0.0001, 0.0001 and 0.0006 respectively.

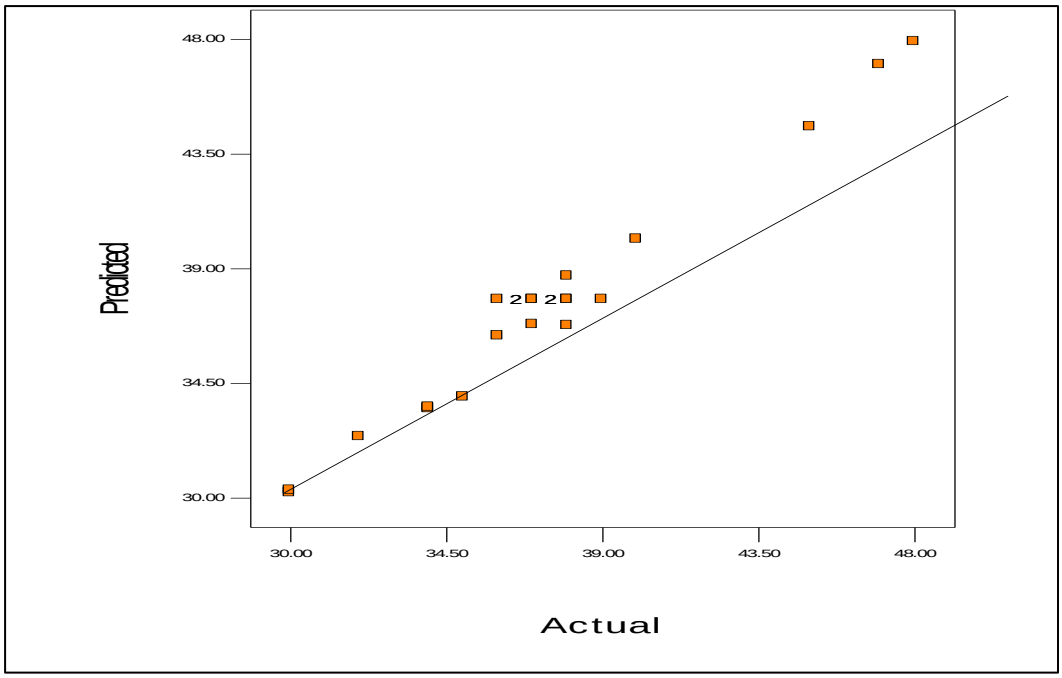


Figure 4.31 Plot of predicted DME from the model equation against the actual experimental DME values at different process conditions after 4 h of reaction time.

The Fig. 4.32 shows the response surface of DME with the interaction between catalyst concentrations, methanol to oil molar ratio and temperature.

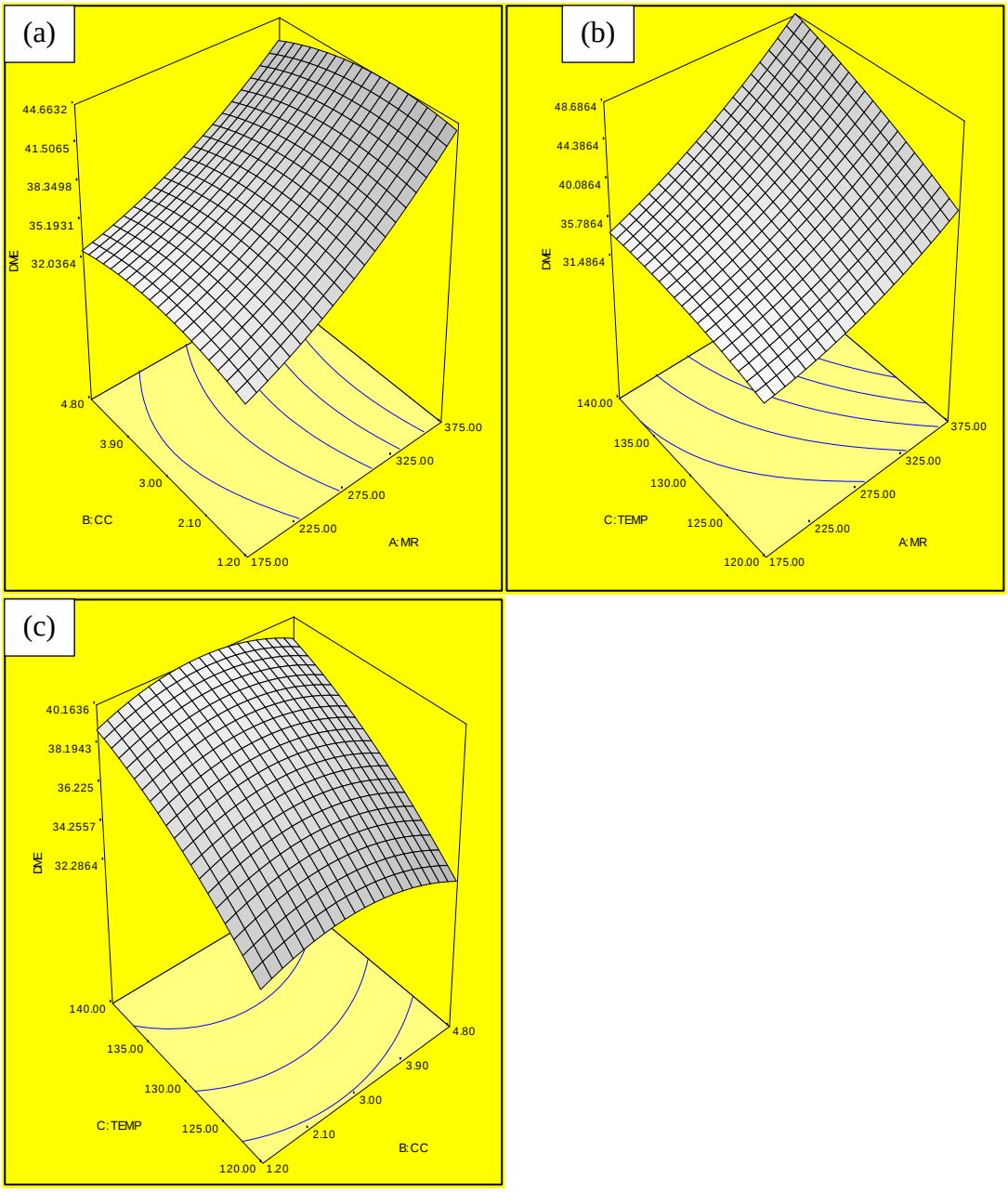


Figure 4.32 The 3D Response surface plot for DME production in reactive coupling showing the interaction between (a) CC and MR (b) TEMP and MR and (c) TEMP and CC

Fig. 4.32(a) shows a gradual increase in DME yield as the molar ratio of methanol to oil and catalyst concentration increases. Further increase beyond 3 v/v % and 275/1 in CC and MR respectively results in a decrease in DME. Process conditions for maximum DME yield based on the plot are CC of 3 v/v % and 275MR. The observed increase in DME as MR increases is attributed to the availability of more methanol for dehydration reaction which leads to the formation of more DME. These results agree with the findings of Kaewwisetkul *et al.* (2017) which showed that methanol in crude glycerol from biodiesel production could be converted to DME using macro-porous sulfonic acid ion exchange resin as a catalyst.

The present results are significant in two major respects. First, it shows that co-production of valuable secondary product apart from the main biodiesel production is possible and secondly it demonstrates the conversion of excess methanol used for reactive extraction to another product. This reduces the energy cost in recovering the methanol in the downstream separation and thereby potentially reduces the cost of production of biodiesel. Very little was found in the literature on conversion of methanol to dimethyl ether using homogeneous acid catalysts such as sulphuric acid. Most research on dehydration of methanol for DME production uses a solid catalyst. This may be the first time DME has been produced using a homogenous catalyst in *in situ* tranesterification.

The interaction between temperature and MR on DME yield is as shown in Fig. 4.32(b). Similarly to the case of the interaction between CC and MR, increasing the temperature and MR, enhances the yield of DME. The optimum DME is attained at 140^{o}C and 375/1 MR. Sabour *et al.* (2014) found that high temperature does not support complete conversion and high purity of DME. As more methanol pushes the equilibrium to the product side and the rate increases with increase in temperature hence DME yield is increased. This may be because reaction equilibria are usually a function of temperature which may explain why not all the unused methanol in reactive coupling is converted to DME.

The effect of the interaction between temperature and CC on DME yield is shown in Fig. 4.32(c). The results show that temperature is a significant factor in its interaction with CC. As can be seen from the surface plot, the DME yield increases as temperature increases while the effect of increasing CC is initially to increase the DME conversion, but at higher concentrations, it decreases. The overall conditions for optimum yield of DME based on the model are 275/1MR, 3 v/v % and 130^{o}C.

4.11.3 Effect of Reactive Coupling Process Variables on PG

The model equation for synthesis of PG from glycerol by-product of IST is given in Equation 4.4

$$PG = 14.69 + 1.89A + 0.44B + 0.86C - 0.55A^2 + 0.30B^2 - 1.40C^2 - 0.063AB + 0.16AC + 0.31BC \quad 4.4$$

Where A = methanol to oil molar ratio, B = catalyst concentration (v/v %) and C = temperature (^{o}C)

The plot of predicted PG based on the model Equation 4.4 and the actual experimental PG experimental values as obtained by MS after 4 h of reaction time is shown in Fig. 4.32. The model F-value of 4.69 implies the model is significant. There is only 1.21% chance that a "model F-value" could occur due to noise. MR and CC are statistically significant variables in the PG production during reactive coupling. The p-values are 0.005 and 0.0440 respectively. Contrary to expectation, the temperature is not a significant process variable; this may be as a result of reaching an optimum reaction temperature where the reaction cannot proceed without any further increase in the temperature. The outliers represent the condition with lowest temperature of 120^{o}C which is not sufficient for significant synthesis of PG as determined by Salehpour and Dube (2011) that 140^{o}C is the optimum temperature for H_2SO_4 catalysed glycerol polymerisation.

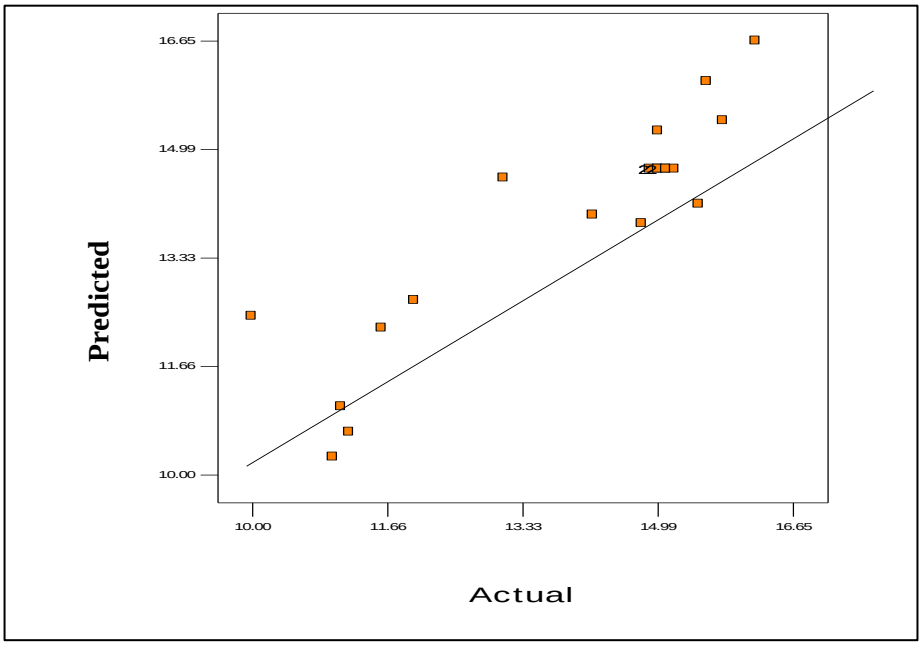


Figure 4.33 Plot of predicted PG from the model equation against the actual experimental PG values from MS at different process conditions after 4 h of constant reaction time.

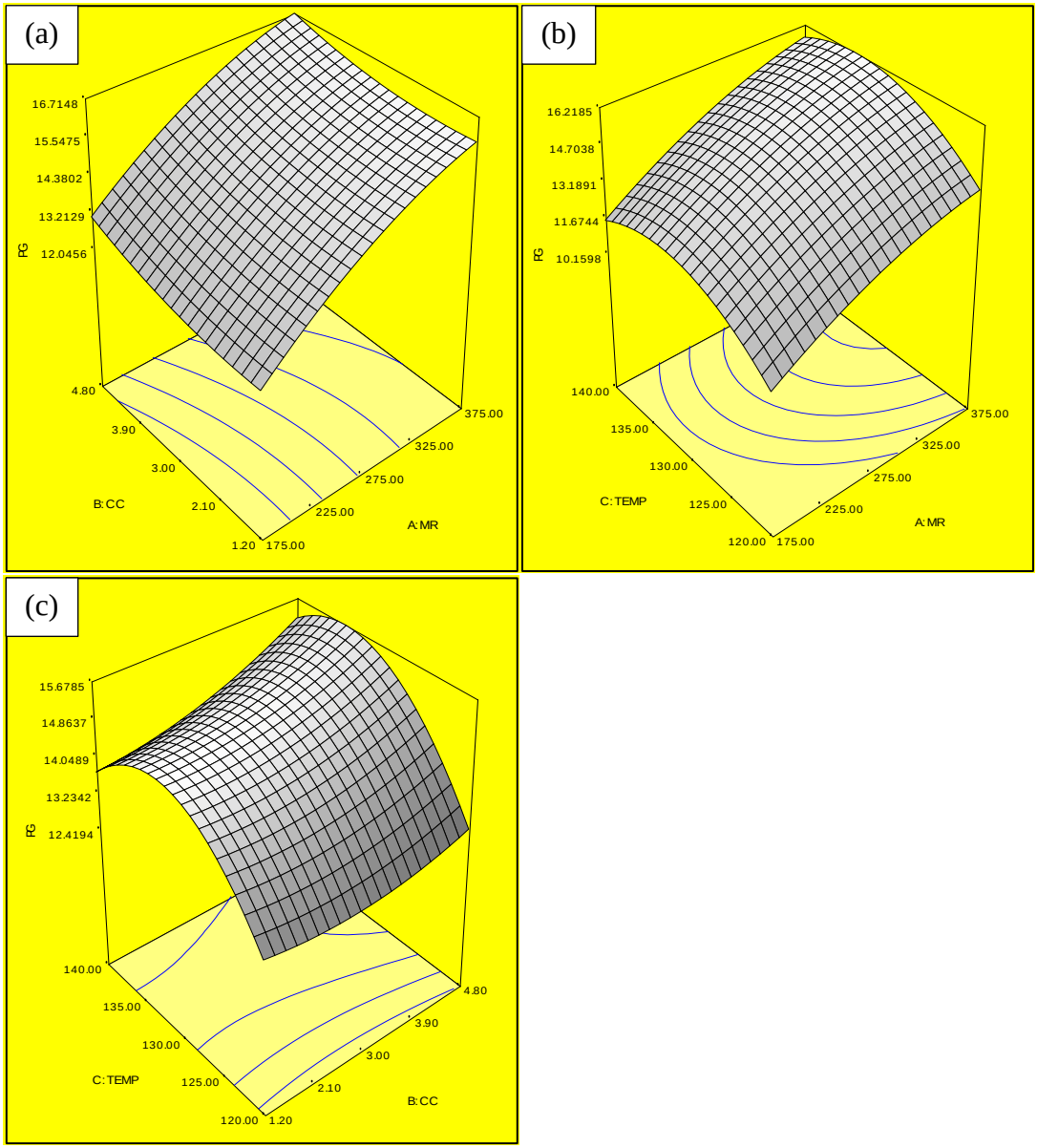


Figure 4.34 The 3D Response surface plot for PG production in reactive coupling showing the interaction between (a) CC and MR (b) TEMP and MR and (c) TEMP and CC

Fig. 4.34(a) shows that MR is significant in its interaction with CC for the PG production. The PG yield increases as the MR increases with increase in CC. The same trend is observed for FAME yield. This is due to the availability of more FAME which allows for the production of more of its glycerol by-product that is polymerised to PG. The results from previous studies show that increasing CC and MR increase FAME and glycerol yield (Kasim and Harvey, 2011; Zakaria and Harvey, 2012). This finding implies that conditions to optimise for the FAME production can also be tuned to optimise for PG production thereby intensifying the entire manufacturing process.

Fig. 4.34(b) reveals that there is a gradual increase in PG yield as the MR increases. Also, the progressive increase in temperature increases the PG. It, therefore, follows that to optimise

the PG yield the temperature and MR have to be increased. This relationship may because the acid catalysed reaction requires a high temperature for the polymerisation of the glycerol by-product to PG.

The interaction of temperature and CC on PG yield shown in Fig. 4.34(c) indicates a gradual rise in PG yield as temperature and CC increase until an optimum value is attained when a further increase in these variables shows a decrease in PG yield. This may be due to the breakup of polyglycerol bond to form other dehydrated product such as acrolein in the mixture. The response surface plot indicates that for optimum PG yield temperature and CC should be 130^{o}C and 3% (v/v) respectively.

4.12 SEM Analysis of the oilseed

To have an insight into the usefulness of the rapeseed meal, samples of ground rapeseed were examined under a light electron microscope. Fig. 4.35(a) shows fresh rapeseed with the 300-400µm particle. Fig. 4.35(b) shows the soaked rapeseed that has been used for reactive coupling but without a catalyst while Fig. 4.35(c) shows spent reactive coupling rapeseed with the acid catalyst after 4 h.

Clearly, in the SEM images Fig. 4.35(a) is the intact lipid-rich cell wall that is not ruptured. Fig. 4 .35(b) shows lipids are at the surface of the seed cell wall. Fig. 4.35(c) the cell walls are almost collapsing showing a change in the cell presumably due to oil extraction A possible explanation for this is that, before reaction, rapeseed contains mainly carbohydrate, lipid and protein (Rymer and Short, 2003). It is only the lipid that is reactively extracted from the seed. This can only occur with the aid of catalyst which when in contact with methanol protonates the carbonyl group of triglyceride lipid inside the seed cell. The adjoining carbon then becomes more electrophilic and therefore more susceptible to nucleophilic attack (S_N2).

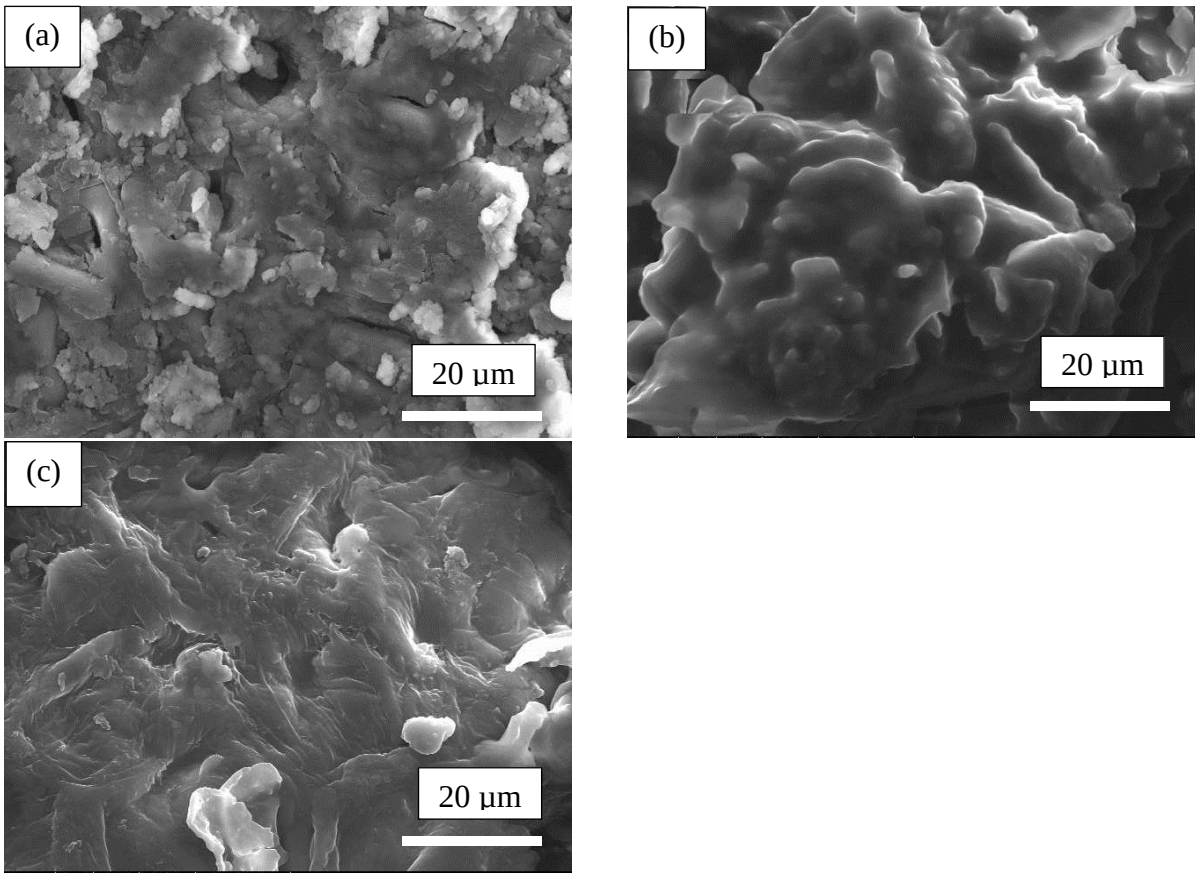


Figure 4.35 SEM images of rapeseed particle (a) fresh rapeseed (b) after soaking of seed in methanol without catalyst and (c) after reactive coupling with catalyst.

Clearly, formation of FAME is not possible without the catalyst as the lipids are still visible on the surface of the seed particle as seen in Fig. 4.35(b). However, Fig. 4.35(c) shows that the lipids presumably have been removed by reactive coupling and only the cell wall materials containing carbohydrate and protein may have been left after 4 h of reaction. Protein and carbohydrate tests are required to be carried out on the spent sample to confirm the assumption.

These findings agree with the earlier studies of Ren *et al.* (2010), which found that carbohydrate and protein are intact after reactive extraction of rapeseed. However, only SEM was used in this study. Further work should be undertaken to investigate the carbohydrate and protein content of the spent seed.

4.13 Oilseed Biorefining Concept

A biorefinery is defined as a facility that converts biomass into biofuel, power and bio-based chemicals which can be used for further production of valuable products (Pagliaro and Rossi, 2008a). This is similar to what is obtained in petroleum refineries, where varieties of fuel products and other chemicals are produced from crude oil. Reactive coupling could be the

basis of an intensified biorefining concept that allows not only biodiesel to be produced, but also a polyglycerol as bio-based chemical. The solid product, the rapeseed meal, could be used as cattle feed due to the presence of the carbohydrate and protein after the extraction of the lipids, following removal of the methanol to as low as possible. Although methanol can be detoxified in rumen, excessive amount (>1%) may be detrimental for preruminant calves and other non ruminant (Donkin *et al.*, 2009). There are multiple drivers for this concept. One is the economic factor which is to reduce process cost by the combination of many reactive steps (transesterification + polymerisation + dehydration) in one unit. Another driver is the environmental factor which is achieved by minmisation of waste (crude glycerol) by converting it into new useful bioproduct (polyglycerol). This reduces the impact of the product after use on the environment since it is degradable. Furthermore, the production of biofuel (biodiesel) helps to attain energy security since the feedstock is abundant and the use of such fuel as transport fuel decrease the effect of global warming.

4.14 Mass Balance of Reactive Coupling Components

Fig. 4.36 shows the material balance of inlet and outlet of reactive coupling based on 6g of rapeseed, 375/1 methanol to oil molar ratio and 1.5 v/v% of the sulphuric acid catalyst.

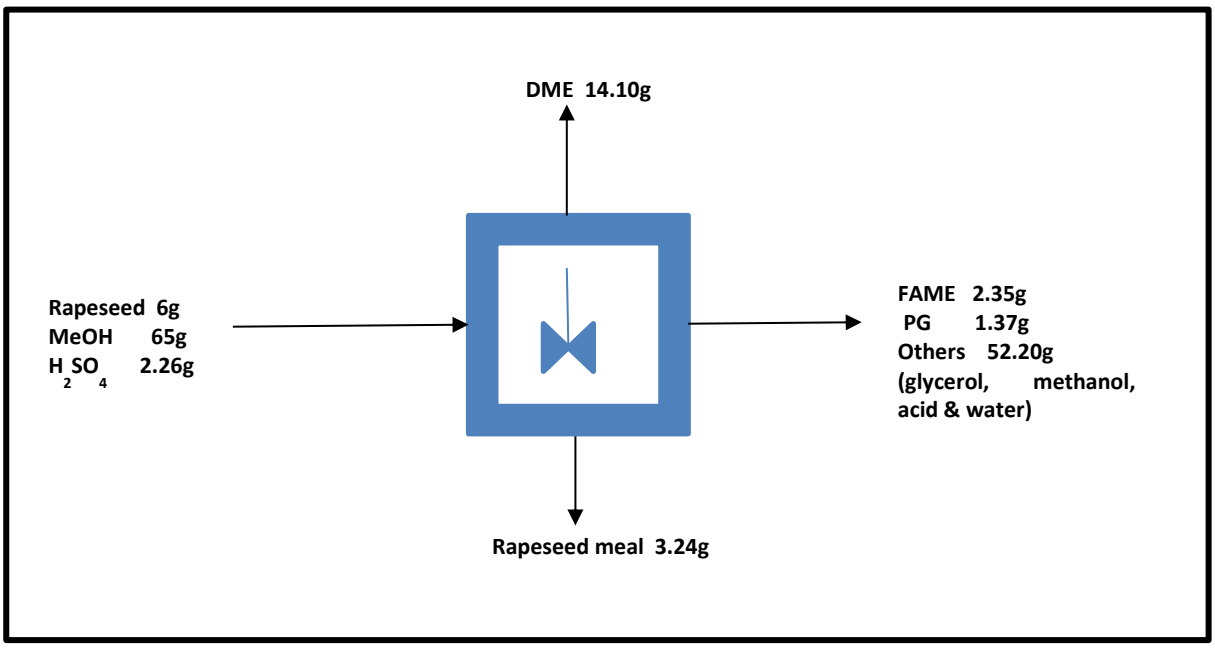


Figure 4.36 Material balance of components in reactive coupling

The analysis of the inlet and outlet component of a reactive component indicates that since the oil content in rapeseed was 46%, the theoretical maximum amount of ester possible will

be 3.76 g from 6 g of rapeseed. It follows that with the actual value of 2.35 g ester from reactive coupling, 85% yield of FAME can be attained. Only 70% of the glycerol by-product is converted to PG due to water formation during the polymerisation reaction. The actual methanol that is used for transesterification is < 4 g leaving an excess of 61 g that will be recovered at the downstream. However, because 19% of this has been converted to DME by dehydration of the excess methanol, less energy will be required to recover the unused methanol. The proof of this concept is significant in this study because the key driver for investigating reactive coupling was to reduce the amount of methanol that is required for reactive extraction.

4.15 Summary of findings for Reactive Coupling

The main findings from reactive coupling studies are as follows

1. Screening study identified the polyglycerol reaction as the best candidate for a complementary reaction, to produce an added value product from glycerol *in situ.*
2. Demonstrated that multiple useful products (e.g polyglycerol, DME and rapemeal) can be produced from reactive coupling aside from the biodiesel main product.
3. Less methanol needs to be recovered due to conversion of part of excess (19%) to DME. This represent an energy saving which potentially can reduce the overall cost of production.
4. Central composite design of experiment for reactive coupling indicated that catalyst concentration is the most significant variable for biodiesel production whilst methanol to oil molar ratio is significant for both polyglycerol and DME production.
5. SEM showed changes in the cellular structure of the spent rapemeal used for reactive coupling which presumably may indicate the extraction of the lipids and that protein and carbohydrate content are intact. However, further test needs to be carried out to confirm this assumption.

Chapter 5. Conclusions and Further Work

5.1 Conclusions

The aim of this research was to improve the process of *in situ* transesterification (IST) of rapeseed for biodiesel production. It was observed that despite the success of IST achieved by a reduction in the process steps due to direct reaction of oilseed with alcohol using a catalyst (base, acid or enzyme), a significant excess (molar ratio: 100s to 1) of methanol is required to obtain acceptable (>90%) biodiesel yields. The energy load (distillation and condensation heat) required to recover the excess or unused alcohol downstream increases the cost of biodiesel production of IST compared to conventional method. It was found that pre-soaking of the ground oilseed in methanol prior to the commencement of the transesterification reaction showed a reduction from 475:1 to 360:1 in the methanol to oil ratio with yield of about 85% biodiesel at 0.07M catalyst concentration. However, a soaking period of 12hrs was required to attain 85% yield which is higher than 75% for 'unsoaked' seeds.

On-line FTIR monitoring of IST used in this study for the first time. Reaction process was monitored by following the FTIR peak associated with CH_3-O, which occurs at a wavenumber of 1435-1440cm^{-1} which represent the methyl group stretch, the carbonyl group C=O at 1715-1745. "Fast IST" uses high catalyst (NaOH) concentrations to produce high FAME yield in relatively short times (~5 mins). Fast IST of rapeseed for biodiesel was demonstrated to be able to produce a yield of 90% in just 5mins. Fast transesterification had previously only been demonstrated for oils rather than oilseeds. This process (*in situ* fast transesterification) would have a greatly reduced number of process steps along the chain from oilseed to product, and is therefore a good example of process intensification. Other advantages include reduction in production time and elimination of the environmental impact of solvent (hexane) use in extraction. The disadvantage of this process is that the amount of (alcohol) methanol is higher than using conventional process with refined oil.

In an effort to further reduce the large excess of methanol used for IST, the reactive coupling of the glycerol byproduct of biodiesel to produce polyglycerol by coupling transesterification with polymerisation in a single stream was investigated. The driver for this study was to produce more biodiesel as the glycerol by-product is *in situ* removed by its conversion to polyglycerol thereby shifting the equilibrium, and potentially reducing the alcohol requirement of IST. The initial proof-of-concept was established which can be the basis for further investigation of the work. Biodiesel and polyglycerol were qualitatively (FTIR and NMR) and quantitatively (LCMS) determined to be present in the reaction mixture. The FTIR

analysis indicated the CO stretching at 928-1039cm^{-1} which is the footprint of the ether linkage of polyglycerol. The NMR analysis reveals the presence of polyether protons of methylene (CH_2) and methane (CH) which occurs between δ = 3.0-4.0ppm. It was also discovered that dimethyl ether (DME), which is also a potential gas fuel with higher cetane number than biodiesel, can be co-produced during reactive coupling. These findings represent a significant step toward reducing the energy requirement in recovering excess alcohol after IST because 19% of the unused methanol (by material balance calculation) was converted to DME.

The Central Composite design of experiments was used to investigate the effect and interaction of various process parameters (catalyst concentration, temperature, and methanol to oil molar ratio (MOMR) to biodiesel) in terms of polyglycerol and DME yields. It was observed that, for biodiesel production, the most significant variable over the range explored here was catalyst concentration, while for DME and polyglycerol the most important variable was molar ratio of methanol to oil. The conditions for maximum biodiesel yield of 90% were 275/1 MOMR, 4.8% catalyst concentration, and 140^{o}C. The condition for 17% polyglycerol yield were found to be 375/1 MOMR, 4.8% catalyst concentration and temperature of 130^{o}C. The conditions for DME optimised mass of 49% is 375/1 MOMR, catalyst concentration of 3% and temperature of 140^{o}C.

The study suggested that the carbohydrate and protein content of the rapeseed meal may be intact as indicated by the SEM images of the spent rapeseed after reactive extraction and reactive coupling. Thus an aspect of biorefining is achieved by using the methanol-free cake as animal feed and can improve the economy of the process as this solid stream that could be regarded as waste can be sold as animal feed.

The results of this study indicate that *in situ* transesterification can be improved upon in some ways. Firstly, pre-soaking the ground oil seed can increase biodiesel yield, whilst slightly reducing the solvent requirement. Secondly, high catalyst concentrations can be used in IST for higher yields of FAME at a higher rate provided the reaction can be monitored online to know when to quench the reaction before the onset of saponification. Thirdly, proof-of-concept of reactive coupling has been achieved. It has been shown that part of the excess alcohol can be converted to fuel gas *in situ*, whilst polymerising the glycerol byproduct of IST to value-added polyglycerol. These findings could form the basis of an oilseed-based biorefinery

The findings from this study will enrich the current research on IST. The work was the first to demonstrate online monitoring of IST using FTIR. Secondly, it further demonstrates that "fast IST" of rapeseed is possible with a high concentration of alkali catalyst after this was proved with microalgae in literature. Although this reaction can be coupled with glycerol polymerisation, it usually required higher temperature (> 250^{o}C) which above the design safety limit of reactive coupling rig. Thirdly, this study is the first to establish the concept of reactive coupling for polyglycerol formation, a process that can potentially reduce the energy load needed to recover excess alcohol after IST by utilizing part of the unused alcohol to DME fuel gas. Furthermore, the glycerol by-product of the process is converted to polyglycerol *in situ* thereby potentially improving the economics of the entire process. An aspect of biorefining can be accomplished via coproduction of biodiesel, polyglycerol and the rapeseed meal in a single stream.

5.2 Further Work

Further work is recommended in the following areas of the study

I. The limitation of the online monitoring of IST using FTIR should be further investigated to extend its use for all process conditions, particularly for high alcohol to oil molar ratio as the method is faster and more robust than the traditional GC analytical technique.

II. Re-design of the reactive coupling rig. One major limitation to product yield in reactive coupling is the production of water in both dehydration of methanol and condensation polymerisation of glycerol. The installation of e.g. Dean-Stark condenser apparatus to the rig can help remove the water as it is being produced. However, safety of the process and evaporation of methanol have to be considered in the redesigned rig.

III. To ensure high shear mixture, the mixing of the solid and liquid component of the reactants can be better improved by using overhead mechanical stirrer instead of incubation agitator or magnetic stirrer. This is recommended in future study.

IV. Fundamental understanding of glycerol polymerisation needs to be studied. Furthermore, kinetic simulation and modelling of reactive coupling will give further insight into the mechanism and dynamics of the coupled reaction. The study will enhance the prediction of the reaction rate at different process conditions.

V. Scaling up the reactive coupling from laboratory to pilot plant will enable economics analysis and life cycle assessment of the process. Data from such scale up can be compared to industrial scale.

VI. To determine the suitability or otherwise of the spent rapeseed as livestock feed, its carbohydrate, protein and glycosinolates content should be investigated.

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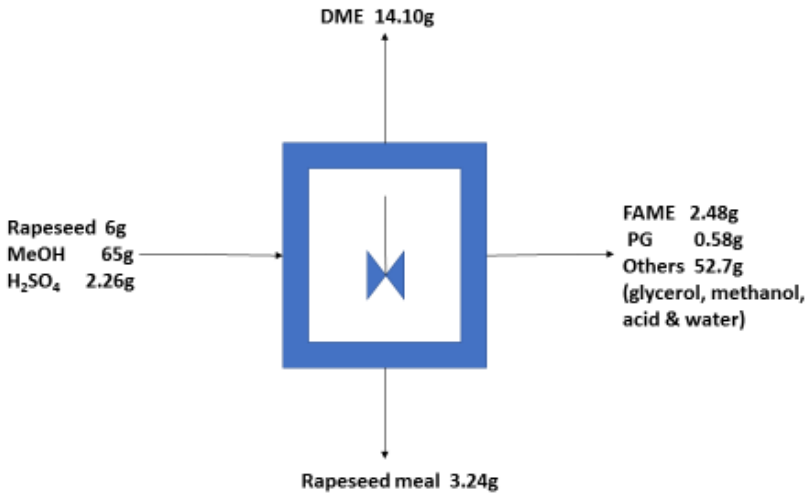
Appendix A. Calculations to Establish Parameters for IST

1. Weight of soxhlet extracted rapeseed oil = 0.092g
2. Total weight of ground rapeseed = 20g
3. $\%\, Weight\ of\ oil\ in\ seed\ = \frac{0.092}{20} \times 100\ = 46\%\ = 0.46$
4. Molecular weight of rapeseed oil (Triglyceride) = 883g/mol
5. $Moles\ of\ the\ oil\ = \frac{0.46}{883} = 5.21\ \times 10^{-4} moles$
6. Using the Zakaria's optimized condition of methanol to oil molar ratio ≡ 475:1
7. $Moles\ of\ methanol\ needed\ = 475\ \times 5.21\ \times\ 10^{-4} = 0.2475 moles$
8. Molecular weight of methanol = 32.04g/mol
9. $Mass\ of\ methanol\ = 0.2475\ \times 32.04\ = 7.928 g$
10. Density of methanol = 0.791g/ml
11. $Volume\ of\ methanol\ needed\ = \frac{7.928}{0.791} = 10 ml/g$
12. $Conc.\ of\ NaOH\ in\ methanol\ per\ gram\ of\ seed = \frac{0.1}{1000} \times 10\ \times 1 = 1 \times 10^{-3} moles$
13. Molecular weight of NaOH = 40g/mol
14. $Weight\ of NaOH\ =\ 1 \times\ 10^{-3}\ \times 40\ = 0.04 g = 40 mg$

Appendix B. Calculations for the Material Balance for Reactive Coupling



$TG + 3MeOH \rightarrow 3BD + GL$ (i) Transesterification reaction

2.76g 1.95g → 2.48g 1.95g

$2GL \rightarrow PG + H_2O$ (ii) Polymerisation reaction

(2 x 1.95 = 3.9g) GL → 0.585g (30% of 1.95)

$2CH_3OH \rightarrow CH_3OCH_3 + H_2O$ (iii) Dehydration reaction

61.1g (excess methanol) → 11.609g DME (19% of 61.1g)

2.40ton fresh Rapeseed = 1ton Biodiesel = 1.296ton Rapeseed meal (cake) @ 46% oil content in seed.

Appendix C. Conferences and Meeting Attended

1. Akeem A. Babatunde, Adam P. Harvey and Jonathan G. M. Lee (2015) Reducing the Methanol Requirement in Direct Production of Biodiesel from Rapeseed. National Biodiesel Conference (19 - 22 Jan, Forth worth Texas, USA)

2. Akeem A. Babatunde, Adam P. Harvey and Jonathan G. M. Lee (2015) *In Situ* Transesterification of Rapeseed for Biodiesel Production. 5th European Process Intensification Conference (27 Sept – 1 Oct, Nice, France).

3. Akeem A. Babatunde, Adam P. Harvey and Jonathan G. M. Lee (2017) Reactive Coupling of Rapeseed for Biodiesel and Polyglycerol Production. Process Intensification Group Meeting, Newcastle University, UK on 3rd March, 2017.