The control of hydrolysis in eliminating FFA from acidic oils using CAL-B lipase supported on a 2D/3D nanocatalyst and in a membrane reactor.

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Abstract

Biodiesel is the most successful drop-in biofuel used in transportation. It can reduce GHG emissions in transportation by 50 to 90% depending on the type of feedstock used. Waste cooking oils and fats containing free fatty acids (FFA) are less expensive feedstocks for biodiesel production than refined vegetable oils. The major issue that limits the use of these oils as feedstock is the interference of FFAs with widely used base catalyzed reaction processes. The FFAs consume base catalyst, produce water of neutralization and form soaps that create emulsions downstream in the process reducing process yields.

There is an important need to develop technologies that reduce the FFA content in these oils to below 0.5 wt%; the accepted limit for a feedstock to be processed by the base catalysed reaction. Enzymes are an efficient and environmentally friendly catalyst for FFA esterification. However, they are prone to deactivation with methanol and also catalyze the hydrolysis of esters and triglycerides to FFA. Using them to pre-treat oils and fats remains a challenge: in the presence of water, enzymes can readily produce FFAs from lipids. The objective of this work was to investigate two enzymatic processes to pre-treat acidic oil below the FFA requirement of 0.5 wt%. In this study, two different continuous systems, a packed bed reactor (PBR) and membrane reactor (MR) were used in FFA enzymatic esterification to meet the 0.5 wt% requirement, improve the reusability of enzymes and reduce catalyst cost.

The esterification in the PBR was carried out using CALB immobilized on a new 2D/3D nanoplatelet support (TAN). The enzyme was covalently bonded to the TAN using a hydrophobic epoxy ligand. Acidic oil containing canola oil and 2.5 wt% FFA was used as the feedstock for the esterification. It was found that the FFA concentration met the quality specification of <0.5 wt%

using CALB-TAN, while it did not using the commercial Novozym 435. The surface fluid velocity was found to have an effect on the removal of water from the PBR reactor. When the velocity was too low, water was retained in the reactor and the FFA conversion was low, when it was too high the reaction time for esterification was not sufficient. It was found that feed velocity of 3 to 6 x 10^{-5} m/s met the 0.5 wt% requirement. In the PBR, the use of CALB-TAN successfully eliminated the hydrolysis of TG and achieved the continuous esterification of FFA for 42 days.

In the MR, acidic oil containing canola oil and 10 wt% FFA was used as the feedstock for the esterification. The enzyme adsorbed on the surface of the polar phase containing glycerol and water and was successfully retained in the reactor by a 0.2-micron ceramic membrane. The addition of glycerol increased the polarity of the dispersed phase in the reactor, bounded water, and retained the liquid enzyme in the reactor. However, the added glycerol in the reactor increased the operating pressure of the reactor. The operating pressure was reduced by adding biodiesel to the feedstock prior to treatment. The lowest level of FFA from the 10 wt% FFA feedstock was 0.68 wt%. This would require a second polishing step to reach the required 0.5 wt%.

The PBR and MR using CALB are technologies that limit the hydrolysis at low FFA concentrations and are promising for the pre-treatment of acidic feedstocks in base catalysed biodiesel processes.

Resume

Le biodiesel est un biocarburant qui peut réduire les émissions de GES de 50 à 90% selon le type de matière première utilisé dans sa production. Les huiles de cuisson et les graisses contenant des acides gras libres (AGL) sont des matières premières moins coûteuses et plus abondantes pour la production de biodiesel que les huiles végétales raffinées. Le problème principal qui limite l'utilisation de ces huiles en tant que matière première est l'interférence des acides gras libres dans les procédés catalysés par une base. Les AGL consomment le catalyseur, produisent de l'eau de neutralisation et forment des savons qui créent des émulsions, réduisant ainsi les rendements.

Il existe un besoin important de développer des technologies qui réduisent la teneur en acides gras libres dans ces huiles à moins de 0,5% en poids; la limite acceptée pour la matière première à traiter par les réactions catalysées par base. Les enzymes sont des catalyseurs efficaces et écologiques pour l'estérification des AGL. Cependant, ils sont sujets à la désactivation avec le méthanol et catalysent également l'hydrolyse des esters et des triglycérides en AGL. Les utiliser pour prétraiter les huiles et les graisses reste un défi; en présence d'eau et à faible teneur en AGL, les lipides produiront des AGL. L'objectif de ce travail était de trouver deux voies enzymatiques pour le prétraitement au-dessous de l'exigence de 0,5% AGL. Dans cette étude, deux systèmes continus différents, un réacteur à lit garni (PBR) et un réacteur à membrane (MR) ont été utilisés dans l'estérification enzymatique des AGL pour améliorer la réutilisation des enzymes et réduire les coûts du catalyseur.

L'estérification dans le PBR a été réalisée à l'aide de CALB immobilisé sur un nouveau support de nanoplaquettes 2D/3D (TAN). L'enzyme a été liée par covalence au TAN en utilisant un ligand époxy hydrophobe. Dans le réacteur à membrane, l'estérification a été réalisée en utilisant CALB

dans une formulation liquide. De l'huile acide contenant de l'huile de canola et 2,5% en poids de AGL a été utilisée en tant que matière première pour l'estérification. L'estérification en utilisant CALB-TAN et Novozym 435 commercial dans un PBR ont également été comparées dans l'étude. Il a été constaté que la concentration en FFA répondait aux spécifications <0,5% en poids en utilisant CALB-TAN, alors que celle produite utilisant Novozym 435 ne rencontrait pas la spécification. Le système avec CALB-TAN présentait une efficacité d'élimination de l'eau élevée de 75 %. La vitesse d'alimentation s'est avérée avoir un effet sur l'élimination de l'eau du réacteur PBR. Lorsque la vitesse était trop basse, l'eau était retenue dans le réacteur et la conversion en FFA était faible, quand elle était trop élevée, le temps de réaction pour l'estérification n'était pas suffisant. Une vitesse d'alimentation de 3 à 6 x 10^{-5} m/s s'est avérée optimale pour rencontrer la cible de 0.5 %. Dans le PBR, l'utilisation de CALB-TAN a réussi à éliminer l'hydrolyse des TG et à obtenir une estérification continue de AGL pendant 43 jours.

Dans le MR, de l'huile acide contenant de l'huile de canola et 10% en poids de FFA a été utilisée comme matière première pour l'estérification. L'enzyme adsorbée à la surface du glycérol polaire et des gouttelettes d'eau a été retenue avec succès dans le réacteur par une membrane céramique de 0,2 micron. L'ajout de glycérol forme des gouttelettes polaires qui ont retenu avec succès l'enzyme liquide dans le réacteur. L'introduction de glycérol dans le réacteur a augmenté la pression de fonctionnement du réacteur. La pression a été réduite en ajoutant du biodiesel dans l'alimentation avant le traitement. Le niveau le plus bas de AGL provenant de la charge de 10% en poids de FFA était de 0,68% en poids. Cela nécessiterait une seconde étape de polissage pour atteindre les 0,5% en poids requis. Le PBR et le MR utilisant CALB sont des technologies qui limitent l'hydrolyse à de faibles concentrations en FFA et sont prometteuses pour le prétraitement des matières premières acides dans les procédés de biodiesel catalysés par une base.

Statement of Contributions of Collaborators

I hereby declare that I am the sole author of this thesis. I performed all the experiments and the data analysis. I have written the chapters contained in this thesis.

Dr. Andre Y. Tremblay supervised this thesis project and provided continual guidance and support. He also made many editorial comments and corrections to the written work presented. His day-to-day guidance, discussion and never ending support have resulted in tremendous improvements of the thesis.

Acknowledgements

I wish to express my deepest gratitude to my research supervisor, Dr. Andre Tremblay, a respectable, responsible and resourceful scholar, who has provided me with valuable guidance and support in the completion of this project. I am profoundly indebted to him for the assistance he rendered during my tough times over the two years of my research. Without his enlightening instruction, impressive kindness, endless patience and ongoing encouragement, I could not have completed my thesis. It was a great pleasure to work with a professor like him.

I would also like to thank the support staff within the department of Chemical and Biological Engineering, Franco Ziroldo and James Macdermid, for their outstanding support when faced with technical difficulties.

Last but not least, I would like to thank my parents and friends for their constant support and encouragement.

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Nomenclature

This section lists some of the most frequently used abbreviations in this thesis. Other less-common abbreviations used in the thesis are specified at their first appearance.

List of abbreviations

ASTM – American Society for Testing and Materials

C_{acid} – the concentration of the hydrochloric acid (mol/L)

CALB-TAN – an immobilized enzyme of *Candida antarctica* lipase B immobilized on silylated twinned alumina nanosheets

CBBG – Coomassie Brilliant Blue G-250

C_{FFA} – the concentration of FFA (wt %)

MR – Membrane reactor

ETES – 2-(3,4-Epoxycyclohexyl)ethyltriethoxysilane

FAAE – Fatty acid alkyl ester

FAEE – Fatty acid ethyl ester

FAME – Fatty acid methyl ester

FER – Fossil energy ratio

FFA – Free fatty acid

GHG – Greenhouse gas

HCl – Hydrochloric acid

ID – Inside diameter

KOH – Potassium hydroxide

m_{product} – the weight of the sample (g)

Δm_{water} – the total amount of water increased in the mixture after going through the column (g)

$\Delta m_{\text{water FFA reacted}}$ – the total amount of FFA reacted in the mixture after going through the column in grams, that is, the amount of water produced during the reaction in grams

$m_{\text{water in product}}$ – the amount of water in the feed (g)

$m_{\text{water in non-polar phase}}$ – the amount of water in the non-polar phase (g)

$m_{\text{water in polar phase}}$ – the amount of water in the polar phase (g)

$m_{\text{water in product}}$ – the total amount of water removed from the column (g)

MW_{FFA} – the molecular weight of FFA, 282.5 g/mol

$C_{\text{FFA in feed}}$ – the FFA concentration in the feed (wt%)

$C_{\text{FFA in product}}$ – the FFA concentration after esterification (wt%)

Δn_{water} – the total amount of water increased in the mixture after going through the column in moles

$\Delta n_{\text{water FFA reacted}}$ – the total amount of FFA reacted in the mixture after going through the column in moles, that is, the amount of water produced during the reaction in moles

PBR – Packed bed reactor

PBS – Phosphate buffer solution

PFAD – Palm fatty acid distillate

PM – Particulate matter

ppm – Parts per million

TG – Triglyceride

V_{acid} – the volume of the FFA (L)

WCO – Waste cooking oil

WZ – Tungstated zirconia

η – the water-removal ratio (%)

Chapter 1 Introduction

1.1 Introduction

There is a pressing need to develop carbon-neutral renewable fuels due to climate change and regional geopolitical instabilities in the world. Biodiesel is a renewable, clean-burning, and biodegradable energy source. It can be blended with fossil fuels and used directly in diesel engines to reduce greenhouse gas emissions. [1] Biodiesel can be used as an alternative energy source to reduce the world's dependence on fossil fuels.

Biodiesel is composed of fatty acid alkyl esters (FAAE) obtained from the transesterification of vegetable oils and animal fats with short-chain alcohols, such as methanol and ethanol. Commercially, biodiesel is mostly produced by the alkaline catalysis of vegetable oils that contain low concentrations of free fatty acids (FFA) [2]. The main concern in using edible vegetables as feedstocks is associated with their potential impact on the food supply [2]. Moreover, the high cost of vegetable oils also hinders the widespread use of biodiesel. Therefore, waste cooking oils (WCO) are of increasing interest as a feedstock for its production. It has been reported that WCO is 40-60 % less expensive than vegetable oil and WCO biodiesel has a higher greenhouse gas (GHG) reduction than edible oils. The use of biofuels reduced from 35 to 90 % of the GHG emission compared to fossil fuels [3], [4]. Suppliers of WCO release it at a low cost to avoid the disposal fees [5]. However, the existence of a considerable amount of FFA in WCO affects the viability of alkaline catalysis as a method of transformation [6]. The neutralization of FFA consumes base catalysts and produces soap, leading to phase separation issues that reduce biodiesel yields. Thus, before introducing FFA containing feedstocks into a base catalyzed transesterification process, a pre-treatment procedure to convert the FFA in waste cooking oils to fatty acid methyl esters (FAME) is indispensable.

1.2 Objectives

The objective of this thesis is to develop a treatment process to reduce FFA in synthetic acidic oils for their use in base catalysed biodiesel processes.

The first objective is to investigate the performance of FFA esterification using a novel immobilized lipase – CALB-TAN covalently bonded to 2D/3D nanosheet structures in a packed bed column. The results will be compared to the reaction using a commercial immobilized enzyme – Novozym 435, as well as the use of CALB-TAN in a batch reactor.

The second objective is to investigate FFA esterification in a continuous enzyme membrane reactor using liquid CALB. A microfiltration ceramic membrane of with a pore size of 0.2 µm will be used in the experiments. The enzymatic esterification in the membrane reactor will be compared to that in a batch reactor.

1.3Thesis structure

This thesis is divided into five chapters.

Chapter 2 is a literature review, of the properties and the benefits of biodiesel, the feedstocks used in biodiesel production, the issues caused by low-quality feedstocks and pre-treatment technologies for feedstocks containing FFAs.

Chapter 3 covers the FFA reduction in acidic oil using CALB lipase immobilized on a 2D/3D nanocatalyst placed in a packed bed reactor. The effects of variables such as the molar ratio of MeOH to FFA fed to the reactor, along with the residence time and velocity are discussed.

Chapter 4 covers the FFA esterification of acidic oil using a CALB liquid enzymes formulation in a membrane reactor. The effects of the flow rate, the molar ratio of MeOH to FFA, glycerol and biodiesel are discussed.

Chapter 5 contains conclusions, and recommendations for future work.

Chapter 2 Overview

2.1 Definition and properties of biodiesel

Biodiesel is a renewable fuel comprised of fatty acid alkyl esters (FAEE). According to the California Air Resources Board, biodiesel is the most successful renewable fuel for transportation, reducing CO_2 emissions to the atmosphere by 50 to 90% compared to petrodiesel [7]. It is produced by the transesterification of vegetable oils and animal fats reacting with short-chain alcohols. Biodiesel can be blended with fossil fuels and used directly in existing diesel engines [8]. With minor engine modifications, pure biodiesel (B100) can be used as a direct substitute for petrodiesel.

Biodiesel is unique as a fuel as it is not a distillate. In order to be called biodiesel, an FAEE mixture must meet American Society for Testing and Materials (ASTM) specification D6751 (Table 2.1) or the European Specification EN14214 (Table 2.2). These norms pose strict standards on impurities in pure biodiesel such as mono-, di-, and tri- glycerides, glycerol and free fatty acids content [9]. Biodiesel is usually produced using methanol or ethanol as alcohols in the transesterification process. Fatty acid methyl esters (FAME) are produced when methanol is used in the reaction and Fatty Acid Ethyl Esters are produced (FAEE) when ethanol is used.

Table 2.1 American biodiesel standard ASTM D6751 [10]

Property	Test Method	Limits		Units
		min	max	
Calcium & Magnesium, combined	EN 14538	–	5	ppm (µg/g)
Flash Point (closed cup)	D 93	93	–	°C
Alcohol Control (one to be met):				
1. Methanol Content	EN 14110	–	0.2	% (m/m)
2. Flash Point	D93	130	–	°C
Water & Sediment	D 2709	–	0.05	% (v/v)
Kinematic Viscosity, at 40 °C	D 445	1.9	6.0	mm2/sec.
Sulfated Ash	D 874	–	0.02	% (m/m)
Sulfur:				
S 15 Grade	D 5453	–	0.0015	% (m/m)
S 500 Grade	D 5453	–	0.05	% (m/m)
Copper Strip Corrosion	D 130	–	3	No.
Cetane	D 613	47	–	–
Cloud Point	D 2500	Report		°C
Carbon Residue, 100% sample	D 4530	–	0.05	% (m/m)
Acid Number	D 664	–	0.05	mg KOH/g
Free Glycerin	D 6584	–	0.020	% (m/m)
Total Glycerin	D 6584	–	0.240	% (m/m)
Phosphorus Content	D 4951	–	0.001	% (m/m)
Distillation-Atmospheric equivalent temperature 90% recovery	D 1160	–	360	°C
Sodium/Potassium, combined	EN 14538	–	5	ppm (µg/g)
Oxidation Stability	EN 15751	–	3	hours
Cold Soak Filtration	D7501		360	seconds
For use in temperatures below -12 °C	D7501	–	200	seconds

Table 2.2 European biodiesel standard EN 14214 [10]

Property	Test method	Limits		Unit
		min	max	
Ester content	EN 14103	96.5	–	% (m/m)
Density at 15°C	EN ISO 3675, EN ISO 12185	860	900	kg/m3
Viscosity at 40°C	EN ISO 3104, ISO 3105	3.5	5.0	mm2/s
Flash point	EN ISO 3679	120	–	°C
Sulfur content	EN ISO 20846, EN ISO 20884	–	10.0	mg/kg
Carbon residue (in 10% dist. residue)	EN ISO 10370	–	0.30	% (m/m)
Cetane number	EN ISO 5165	5 1	–	–
Sulfated ash	ISO 3987	–	0.02	% (m/m)
Water content	EN ISO 12937	–	500	mg/kg
Total contamination	EN 12662	–	24	mg/kg
Copper strip corrosion (3 hours, 50°C)	EN ISO 2160	–	1	class
Oxidative stability, 110°C	EN 14112	6.0	–	hours
Acid value	EN 14104	–	0.50	mg KOH/g
Iodine value	EN 14111	–	120	g I/100 g
Linolenic acid content	EN 14103	–	12	% (m/m)
Content of FAME with ≥4 double bonds		–	1	% (m/m)
Methanol content	EN 14110	–	0.20	% (m/m)
Monoglyceride content	EN 14105	–	0.80	% (m/m)
Diglyceride content	EN 14105	–	0.20	% (m/m)
Triglyceride content	EN 14105	–	0.20	% (m/m)
Free glycerine	EN 14105; EN 14106	–	0.02	% (m/m)
Total glycerine	EN 14105	–	0.25	% (m/m)
Alkali metals (Na + K)	EN 14108; EN 14109	–	5.0	mg/kg
Earth alkali metals (Ca + Mg)	EN 14538	–	5.0	mg/kg
Phosphorus content	EN 14107	–	10.0	mg/kg

2.2 History of biodiesel

The transesterification of triglycerides was first carried out in 1853 by Duffy and Patrick [11]. Rudolph Diesel invented the diesel engine in the 1890s. In its original version, the diesel engine burnt pure vegetable oil. Vegetable oils were first used in diesel engines to power heavy duty vehicles. However, considering the high cost of these oils, petrodiesel became the dominant fuel in diesel engines during the early to the middle twentieth century [11]. Transesterified vegetable oils were first used to power heavy duty vehicles in South Africa in the 1930s [11].

In the late twentieth century, the energy and environmental crises due to the excessive over-consumption and intensive exploitation of fossil fuels challenged many nations to act to develop a renewable fuel industry in order to replace our use of fossil fuels. Since then, governments issued a series of regulations and legislations, such as tax preferential treatments for renewable fuels, resulting in the growth of the biofuels industry, especially biodiesel, an alternative energy source to conventional diesel for transportation [12].

2.3 Benefits of biodiesel

2.3.1 Air quality

Particulate matter (PM) that is less than 2.5 μm in diameter (PM2.5) can enter the deeper parts of the human lung, leading to negative health effects. Studies indicate that PM2.5 in exhaust using B20 blend (20 % soy-based biodiesel and 80 % petrodiesel) can be reduced by 65-80 %, compared to petrodiesel exhaust [13].

2.3.2 Waste minimization

Considerable quantities of waste cooking oils are produced in restaurants and catering operations. The degradation of these oils is difficult in conventional wastewater treatment plants. Many countries have banned their disposal due to the potential blockage of sewers by solidified fats and oils and the technical difficulties they pose in water treatment plants [14]. The use of waste cooking oil in biodiesel production not only helps to avoid high disposal fees for waste producers; it also reduces the cost of feedstock and recovers useful energy at the same time.

2.3.3 Environmental risk

The combustion of conventional petrodiesel produces a great amount of carbon dioxide, a GHG that is linked to climate change. Moreover, if the sulfur content of the fuel is high, the SO_x in the

exhaust will cause acid rain. These environmental issues are gradually threatening the survival and development of mankind. Several studies have investigated GHG and sulfur reduction of biodiesel. It was found that compared to petrodiesel, biodiesel had a GHG reduction up to 94 % and a sulfur reduction up to 100 %. Additionally, the GHG and sulfur emissions reductions were associated with the proportion of biodiesel in the petrodiesel blend. Increasing the percentage of neat or pure biodiesel in petrodiesel decreased the emissions of GHG and SO_X. [15]

Biodiesel is also safer to ship compared to petrodiesel because it is biodegradable and relatively non-toxic. Thus, spills of biodiesel do not require intense emergency response clean-up activities compared to spills involving crude oil[16].

2.3.4 Energy efficiency

Biodiesel can be produced domestically. The fossil energy ratio (FER) has been used to measure the energy balance of a fuel [17]. It is defined by the following equation:

$$\text{The fossil energy ratio (FER)} = \frac{\text{the energy output of the final fuel product}}{\text{the fossil energy required to produce the fuel}} \quad (2.1)$$

Pradham et al. 2009, reported that biodiesel produced by soybean oil had an FER of 3.2, that is, for each unit of fossil fuel energy consumed to produce biodiesel we gain 3.2 times the energy. Petrodiesel's FER is 0.83; that is, more energy is required to produce the fuel than it delivers [17]. Additionally, Gahlaut et al. [18] found that the FER of biodiesel from waste cooking oil was 4.3. The fossil energy ratios of biodiesel and petrodiesel have shown that biodiesel, especially biodiesel from waste cooking oil, has a very high energy efficiency.

2.3.5 Feedstocks for biodiesel production

Various animal fats and plants rich in plant oil can be regarded as potential feedstocks to produce biodiesel [2], [15]. The selection of raw materials lies in their availability and cost in the local area [19]. The main biodiesel feedstocks used in different countries are listed in Table 2.3.

Table 2.3 Current potential feedstocks for biodiesel worldwide [20]

Country	Feedstock
Argentina	Soybeans
Brazil	Soybeans/palm oil/castor/cotton oil
Canada	Rapeseed/animal fat/soybeans/yellow grease and tallow/mustard/flax
China	Jatropha/waste cooking oil/rapeseed
France	Rapeseed/sunflower
Germany	Rapeseed
Greece	Cottonseed
India	Jatropha /Pongamia pinnata (karanja)/soybean/rapeseed/sunflower/peanut
Indonesia	Palm oil/jatropha/coconut
Ireland	Frying oil/animal fats
Italy	Rapeseed/sunflower
Japan	Waste cooking oil
Malaysia	Palm oil
Mexico	Animal fat/waste oil
New Zealand	Waste cooking oil/tallow
Philippines	Coconut/jatropha
Singapore	Palm oil
Spain	Linseed oil/sunflower
Sweden	Rapeseed
Thailand	Palm/jatropha/coconut
UK	Rapeseed/waste cooking oil
USA	Soybeans/waste oil/peanut

According to the characteristics of the raw materials listed in Table 2.1, there are four main categories of fats and oils used in producing this fuel.

1. Edible vegetable oils: coconut oils, canola oils, soybean oils and so on.
2. Non-edible vegetable oils: waste cooking oils, cotton oils and so on.
3. Animal fats: tallow, fish oils, chicken fats and so on.
4. Oils from other sources: algae, fungi, bacteria and so on.

As seen in Figure 2.1, the cost of feedstock represents 75 % of the total cost of the final biodiesel [15]. One of the effective methods to enhance the economic viability of biodiesel lies in using waste cooking oil as biodiesel feedstock. WCO is 2 to 3 times less expensive than vegetable oil [4]. Additionally, WCO, as an "urban crop", has high availability, as it can be widely collected from the restaurants, hotels and cafeterias in metropolitan areas. It was shown in the literature that 123,000 tons of waste cooking oil was produced per year in the United States, while approximately 135,000 tons of WCO was generated per year in Canada and 700,000-1,000,000 tons/yr in the EU countries [14]. Further, the utilization of WCO to produce biodiesel recovers the energy from the waste and avoids the blockage of the municipal sewage system, water pollution and landfills.

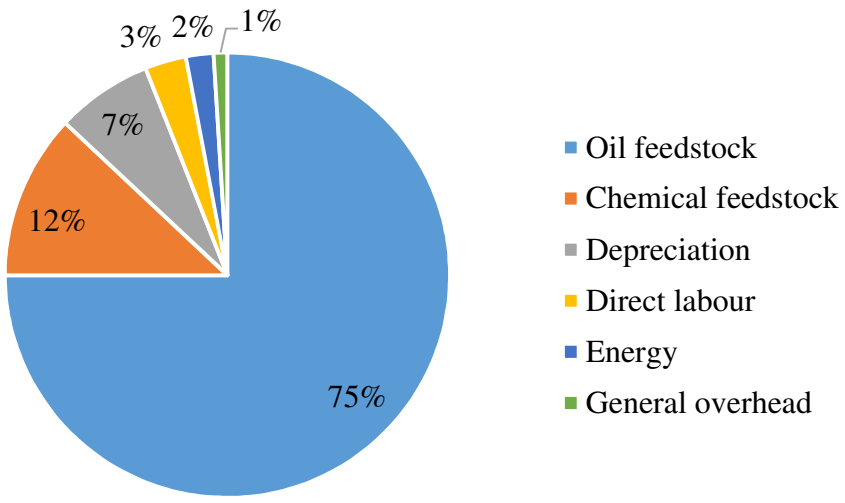


Figure 2.1 General cost breakdown for production of biodiesel [15]

2.4 Saponification and hydrolysis

The majority of biodiesel is produced commercially through alkaline transesterification. Under alkaline conditions, free fatty acids are neutralized and form water and soaps.

In producing biodiesel using base catalysis, the presence of 3% or greater free fatty acids in a feed mixture results in an inevitable loss of catalysts, along with a viscosity increase of the reactive mixture, the formation of gels and soap separation issues [21]. These separation issues lead to the considerable loss of triglycerides contained in the feedstock. Furthermore, water produced during FFA neutralization leads to the hydrolysis of triglycerides and other intermediates involved in producing the esters. This hydrolysis forms more FFA, as seen in figure 2.2 below [21], [22].

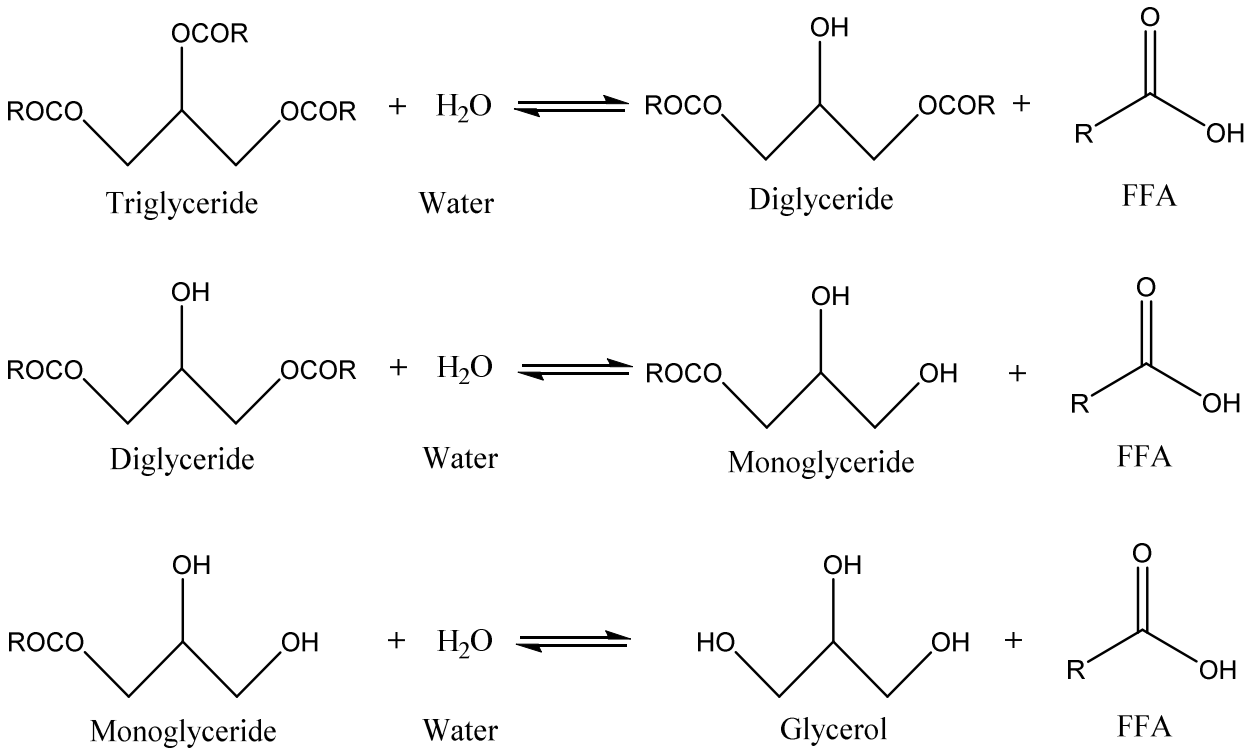


Figure 2.2 Hydrolysis triglycerides and intermediates involved in the formation of biodiesel.

2.5 Pre-treatment technologies for acidic biodiesel feedstocks

A pre-treatment step is most often performed in order to utilize FFA containing feedstocks in base-catalyzed biodiesel processes. This step involves either the removal of the FFA or its transformation into the product ester. While some references suggest the reduction of FFA to

below 3% [20] it is suggested by other sources that process economics are favourable when FFAs are reduced to less than 0.5% [23]. The removal of FFA can be done by distillation [24] and/or solid absorbents [25]. The transformation of FFAs into useful triglycerides and esters can be done by glycerolysis [26], homogeneous and heterogeneous acid catalysis [27], reaction in supercritical methanol [28], and by using ion exchange resins [29] and enzymes [30] as catalysts. Methods that convert FFAs to esters are favoured as they convert contaminants into products. These methods are described in the following sections.

2.5.1 Distillation

Yuan et al. [24] demonstrated that distillation could be used to reduce FFA content in waste rapeseed oil, as shown in Figure 2.3. Gums were removed by degumming with 0.7% w/v NaCl at 70-75 °C, 400 rpm, followed by centrifugation and drying under vacuum for 20-30 min. The distillation of the vacuum-dried oil was carried out under a pressure of 5 mmHg at 220 °C for 1.5 h. The FFA concentration after distillation was reduced from 29 wt% to 1.15 wt%. This oil would need further processing to be used as a feedstock in base catalyzed biodiesel processes.

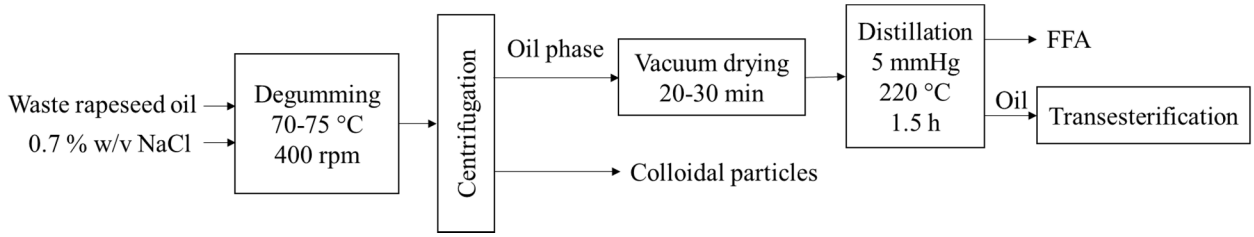


Figure 2.3 The distillation pre-treatment of waste rapeseed oil.

Due to the complicated process and the harsh conditions of high temperature and vacuum, distillation is usually not favored to reduce FFA in acidic feedstocks.

2.5.2 Glycerolysis

Glycerolysis is a process that converts FFA to mono-, di-, and triglycerides by esterification with glycerol in the presence of a catalyst, as shown in Figure 2.4. Felizardo et al. [26] investigated the glycerolysis reaction of acidulated soap-stock having a 20-50 % FFA concentration. The effects of reaction temperature, glycerol concentration, types and concentration of catalysts, stirring intensity and reaction time were studied in their work. A decrease in FFA concentration was shown as the temperature increased from 180 °C to 220 °C. However, when the temperature was higher than 220 °C, the final FFA concentration was no longer affected by the temperature. Additionally, the use of zinc or zinc acetate catalysts dramatically decreased the FFA concentration in the soap-stock, but no significant effects were observed when different types or amount of catalysts were used in the experiments. The process reduced the FFA concentration from 50 % to 1.1 % by using 0.1 % zinc or zinc acetate catalysts, at 220 °C, 500 rpm mixing. However, at these temperatures, oxidation of the feedstock and by-product formation is a concern.

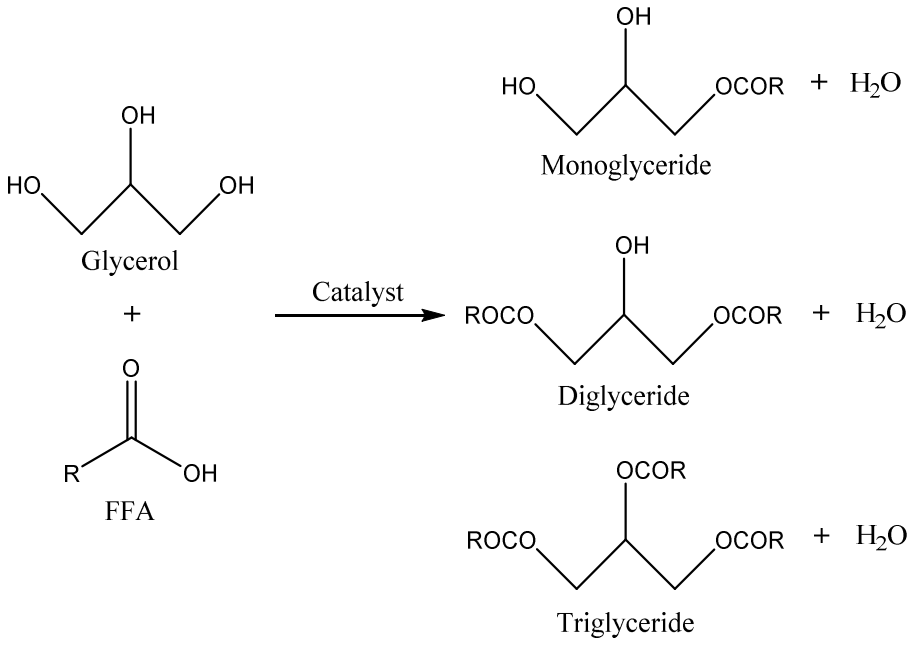


Figure 2.4 Glycerolysis reaction

2.5.3 Solid adsorbents

A base-treated adsorbent comprised of inorganic porous supports (amorphous, silicas or aluminas, clays, diatomaceous earth, etc.) was developed to remove the trace FFA along with soaps, phosphorous, metal ions and color bodies from glyceride oils [31]. The sorbent was easily dispersed in the oil to react with FFA, and the soap formed by the reaction was then adsorbed by the adsorbent, leading to an effective retention of the FFA.

Furthermore, in a study by Soh et al. 2011 [25] four adsorbents with different surface areas and pore sizes were used to reduce the concentration of FFA in a WCO containing 1.3% FFA (Table 2.4). It was found that the absorbents with large surface areas and small pore sizes such as activated carbon and silica gel performed better for FFA reduction, whereas the ones with a small surface area and large pore size had low adsorption capacity resulting in poor FFA reduction. According to the experimental results, silica gel was shown to be the most effective adsorbent. Increasing the dosage of adsorbent favored the adsorption of FFA. However, considering the economic aspect and slurry formation in the oil at high adsorbent concentration, the most effective dosage of silica gel was 20 wt%. This concentration of silica gel would create a considerable amount of waste in a commercial process even if it were reused several times.

Table 2.4 Physical properties

Adsorbent	surface area (m^2/g)	Average pore diameter (nm)	Type of porous material
Activated carbon	888.4	3.018	Mesopores
Silica gel	412.0	2.912	Mesopores
Activated bleaching earth	199.2	7.205	Macropores
Aluminium oxide	0.1	7.571	Macropores

2.5.4 Homogeneous acid catalysis

Liquid acid catalysts, such as sulfuric acid and hydrochloric acid, have been used commercially as catalysts in biodiesel production since base-catalyzed transesterification poses many problems for the reaction of feedstocks containing high FFA concentrations [6]. The acid catalysts can catalyze the esterification of FFA and transesterification of oils simultaneously [6], [32]. In the study of Marchetti and Errazu [33], the reaction of the acidic oil containing 10.68 wt% FFA was successfully carried out using sulfuric acid as a homogeneous acid, achieving 96% conversion to biodiesel.

Di Serio et al. [34] investigated Cd, Mn, Pb and Zn carboxylic salts catalyzed reaction and found that catalytic activities were affected by the Lewis acidity of the metals and the molecular structure of the anion. The experimental results revealed that the cation metals having a complex stability constant with dibenzolimetane between 8.6 and 10.23 showed the best catalytic activity, and the stearates was a prior choice compared to acetates due to its high solubility in oil. The best performance was obtained using $Pb(Ac)_2$, leading to 96 % FAME yields and less than 1 % FFA concentration at 210-220 °C in 200 min with 0.0004:1 catalyst to oil weight ratio.

However, the homogeneous acid catalyzed process is sensitive to the presence of water [35]. Canakci and VanGerpen [36] found that the reaction was affected when the water content was higher than 0.5 wt%, while completely inhibited at a water content of over 5%. In addition, the removal of the liquid acid catalyst is also a great challenge. The previous study showed that a 10,000 tons/yr capacity biodiesel plant required about 1000 t/yr CaO to neutralize the acid catalyst, and the by-product was also a serious issue to deal with [34]. Additionally, the very acidic conditions require specialty materials that resist corrosion [37]. An additional processing issue are

the hazards of dehydrating methanol with sulfuric acid. This can lead to the formation of dimethyl ether which has a boiling point of – 24 °C and can pose an explosion hazard.

2.5.5 Heterogeneous acid catalysis

In comparison with homogeneous acid catalysts, heterogeneous acid catalysts are easy to separate and recover from the reaction system. They can also be used in a continuous reaction system, which is more cost-effective.

Suwannakarn et al. [38] carried out FFA reduction with methanol using a commercial tungstated zirconia (WZ) solid catalyst in a well-stirred semi-batch reactor at 130 °C and atmospheric pressure. Methanol was continuously added using a syringe pump, and the unreacted methanol and produced water were removed by vaporization. Both esterification and transesterification were catalyzed by the WZ catalyst. The conversion of esterification was found to be 85 %, which was 4 times greater than that of transesterification using the WZ catalyst at 130 °C and 1 atm after 2 h. The catalyst retained over 60 % activation after three 2-h reaction cycles, and the activity could be completely recovered by calcination.

Park et al. [39] reported the utilization of heterogeneous catalysts in a continuous flow process, as shown in Figure 2.5. The catalytic activity of several inorganic heterogeneous acid catalysts were tested in an autoclave batch reactor. These were: SO_4^{2-}/Al_2O_3, SO_4^{2-}/SiO_2, H-zeolite, SO_4^{2-}/ZrO_2, WO_3/SiO_2 and Cs-heteropoly acid. The results showed that the WO_3/ZrO_2 catalyst had the highest activity with an FFA conversion of over 90 %. In the packed-bed continuous reactor, a pellet-type support instead of a powder-type WO_3/SiO_2 was used, due to the possible loss of powder-type enzyme and the potential blockage caused by the powder. According to the experimental results, the FFA conversion was only 80 % for the first 24 h. It then decreased to 65% after 140 h in the

continuous reaction system. It was found that the pellet-type WO_3/SiO_2 had a smaller surface area and lower pore size distribution, leading to a lower activity compared to the powder-type and a lower FFA conversion in the packed-bed system. Another important factor that affects the FFA conversion was the mass transfer limitations in the packed-bed reactor. In spite of the relatively low FFA conversion compared to the batch reaction, the packed-bed continuous reactor was a promising reaction system for biodiesel production, due to its low cost and large-scale production. And also, in this case, the catalyst can be easily recovered and regenerated by re-calcination.

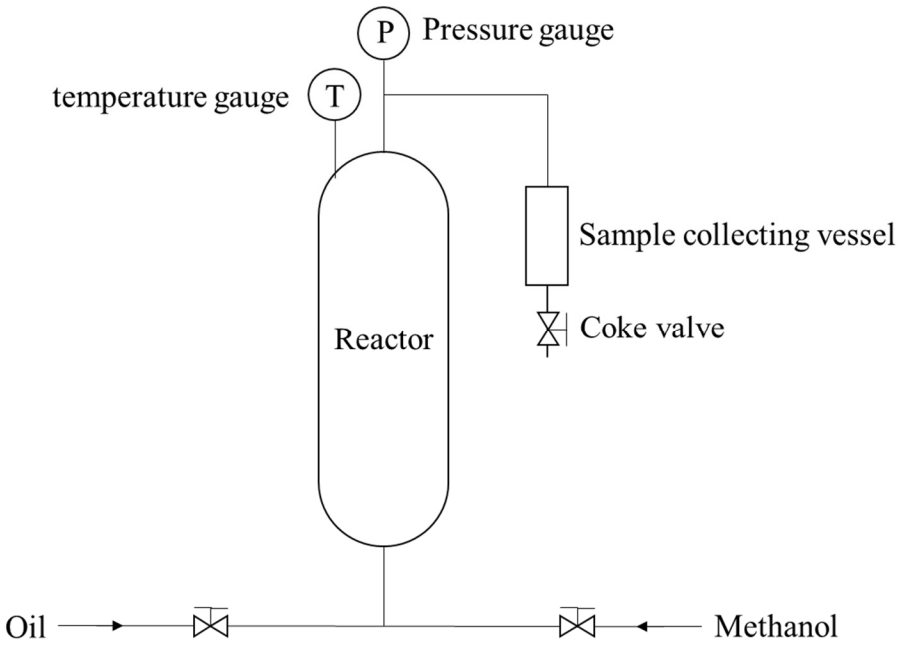


Figure 2.5 The apparatus of the PBR for the continuous production of biodiesel [39]

2.5.6 Supercritical methanol

Using supercritical methanol, the oil or FFA react with methanol spontaneously, without the need for a catalyst. Unlike the catalytic methods, the supercritical methanol has a high tolerance to FFA and water, and the esterification and transesterification can take place simultaneously [11]. Kusdiana and Saka [40] studied the effect of water on a catalytic process and a non-catalytic

supercritical process. Results showed that the presence of water did not affect the FAME conversion in the supercritical process, and the FAME conversion was 95.8 % using the feedstock with 20 % FFA and 61 % water, whereas the reaction did not take place with the same feedstock using alkaline or acid catalysts.

Kusdiana and Saka [41] have developed a two-step process (seen in Figure 2.6), that is, hydrolysis followed by esterification, to achieve high conversion at milder conditions. They found that the reaction rate and FAME yield were higher in esterification than that in transesterification. Therefore, in this case, they first converted oil/fat to FFA by hydrolysis. The supercritical methyl esterification was carried out at 270 °C, 17 MPa and a relatively low methanol to oil molar ratio (10:1) for 20 min, and the FAME yields were over 95 %.

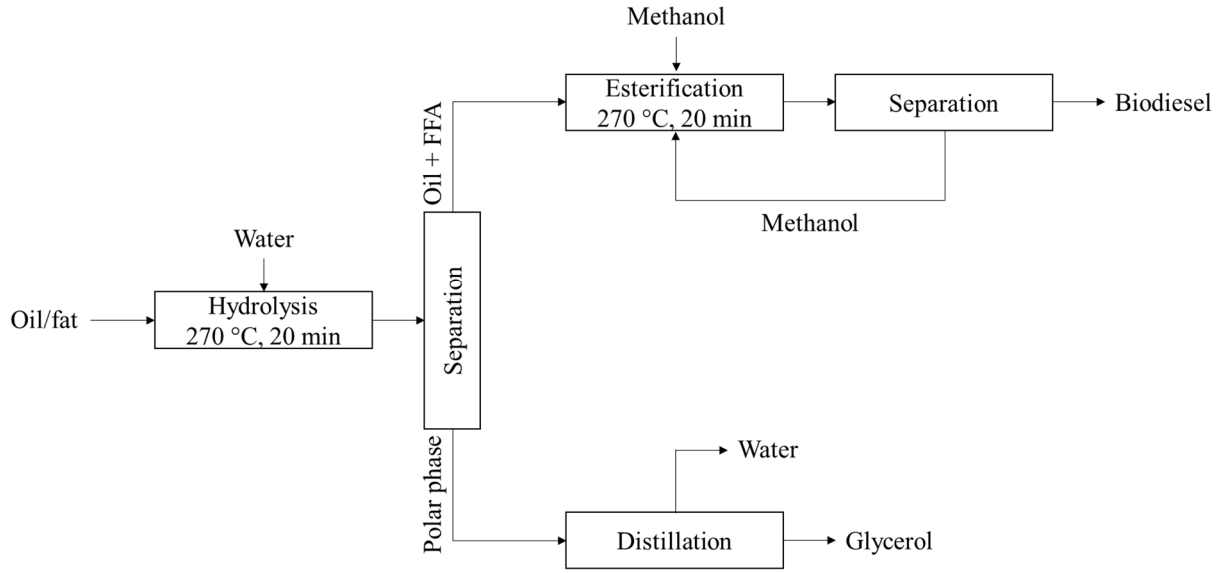


Figure 2.6 Schematic process of biodiesel production by two-step preparation [41]

A high conversion (up to 99 %) can be obtained in a short residence time using supercritical methanol. Nonetheless, the reaction requires high temperature (177-327 °C), high pressure (8-35 MPa) and a high molar ratio of methanol to oil (20-40:1) [11], [42].

2.5.7 Ion exchange resin

Ion exchange resins are a potential choice for FFA reduction due to their high tolerance to FFA, relatively low cost and good conversion. Talukder et al. [43] investigated the esterification of palm fatty acid distillate (PFAD) with 97 % FFA concentration catalyzed by Amberlyst 15 (acidic styrene-divinylbenzene sulfonated ion-exchange resin). The maximum biodiesel yield was 97 % at 60 °C for 6-8 h using 30 % Amberlyst 15. The catalyst was reused for over 15 cycles and no activity loss was observed. A study [29] reported that the activity of ion exchange resins was associated with the average pore size and magnitude of the BET surface area. The authors examined the activity of different resins in FFA esterification, including Amberlyst-15 (A-15), Amberlyst-16 (A-16), Amberlyst-35 (A-35) and Dowex HCR-W2. From the results, the resins with large average pore diameters and high BET surface area had high activities for esterification, since the large pore diameter allowed for the diffusion of FFA molecules into the pores and improved the contact between catalyst and reactant. The activity order was found to be A-15 > A-35 > A-16 > Dowex HCR-W2, and the highest FFA conversion was 45.7 % at 60 °C with 2 wt% A-15.

2.5.8 Enzymes

Enzymatic esterification is considered to be a promising method to reduce the FFA in acidic oil since enzymes are environmentally friendly and the reaction can be carried out under mild conditions [44]. According to the literature [45], enzymatic reactions are usually carried out at a moderate temperature ranging from 20 °C to 70 °C.

Esterification and transesterification are reversible reactions. Increasing the concentration of alcohol facilitates the forward reaction. However, Shimada and co-workers [46] reported enzyme denaturation caused by excess methanol. More specifically, undissolved methanol droplets can

form a dispersed phase in the mixture and inactivate the enzyme. This occurred when the mixture contained over 1.5 molar equivalents of methanol to oil. The optimized molar ratio of MeOH to oil varied from 3:1 to 7:1, depending on the tolerance of different enzymes to alcohols [45]. Deng et al. [47] investigated the alcoholysis performance of different lipases using different alcohols. *Thermomyces lanuginosa* lipase (Lipozyme TL IM; Novozyme A/S, Bagsvaerd, Denmark), *Rhizomucor miehei* lipase (Lipozyme RM IM; Novozyme A/S), *Candida antarctica* lipase (Novozym 435; Novozyme A/S), *Pseudomonas cepacia* lipase (Lipase PS-C; Amano, Nagoya, Japan) and *Pseudomonas fluorescens* lipase (Lipase AK-C; Amano) were tested in a batch reactor with 7 alcohols, including straight and branched-chain primary and secondary alcohols. Results showed that the reaction with methanol, absolute ethanol and 1-propanol using Novozym 435 had the highest FFA conversion of over 90 %. Besides, the reaction with primary alcohols had a higher conversion than that with secondary or branched alcohols since the primary alcohols had lower sterically hindrance. Primary alcohols are more nucleophilicity and this led to a lower degree of hydrolysis. Several studies have tried to overcome the inhibition caused by excess methanol. Watanabe and co-workers [48] demonstrated that the lipase-catalyzed reaction was successfully carried out by three-step methanolysis, and achieved 93.8 % conversion for degummed oil and 95.9 % conversion for refined oil in a batch reactor. The stepwise addition of methanol was also applied to the reaction with waste edible oil in a fixed-bed bioreactor, and it was found a higher conversion was obtained by 90.4 % [49]. Further, some researchers used methyl or ethyl acetate as acyl acceptors instead of directly adding methanol into the reaction system and reached over 90 % of the yields [50], [51]. Hsu et al. [52] found that the methanol inhibition on free *Pseudomonas cepacia* lipase was eliminated by immobilization. In addition, adding a solvent that

increases the solubility of methanol in the reaction mixture is also a feasible method to solve the methanol inhibition problem [45].

Lipase needs to be activated by water, whereas the accumulation of water during the reaction is the reason of the triglyceride hydrolysis [47], [53]. Nielsen et al. [54] investigated the connections between water and FFA concentration using Eversa Transform lipase. The esterification of 2 wt% FFA residue in crude FAME was carried out in a stirred batch reactor. The results showed that 650 ppm water was produced by esterifying 1 wt% of FFA. The water produced during the reaction was continuously removed by air stripping. It was found that the FFA concentration were positively correlated with the water content was less than 0.25 wt%. When the water content controlled by air stripping was less than 500 ppm, the FFA concentration was less than 0.25 wt%. In another study, a higher conversion was observed by adding molecular sieves directly to the reaction mixture in order to remove water [52].

An enzyme can be immobilized on specific matrix to improve its activity and stability. Hsu and co-workers [52] investigated the immobilization of *Pseudomonas cepacia* lipase (PS-30) on a phyllosilicate sol-gel matrix and its application in catalysing the reaction of tallow and yellow grease feedstocks. The results indicated that a high ester yield of 92-94 % was achieved with methanol using immobilized lipase PS-30 while only 45-47 % conversion was obtained using free enzyme. Enzyme immobilization also improved the reusability of the enzyme. No reduction in yields was observed after 5 cycles using immobilized lipase PS-30, whereas, the conversion dramatically decreased with free lipase after the first cycle.

The main restriction in using enzyme catalysis on an industrial scale is the high cost of enzymes. In order to improve the enzyme reusability and the feasibility of using enzymes in this application , Nordblad et al. [55] performed the esterification of acidic oil containing 11 wt% FFA in a

continuous packed-bed reactor using Novozym 435. The results revealed that the FFA conversion was 92.6 % in the first day and 84.8 % after 4.4 days. The catalytic efficiency was found to be 1.2 m^3 oil per kg catalyst, and the total amount of oil processed during 4.4 days was 4.85 L. However, the FFA concentration after the treatment was about 1-2 wt%, which did not meet the specification of less than 0.5 wt%.

Chapter 3 FFA reduction of acidic oils using immobilized enzyme in a continuous packed bed reactor (PBR).

Manuscript for submission, J. Zhou, K. Roebuck, A.Y. Tremblay.

Abstract

The elimination of free fatty acids (FFA) from acidic oils remains an issue in their pre-treatment to produce biodiesel. The formation of water produced as a result of the esterification of these acids leads to the hydrolysis of the oil which further generates FFAs. Enzymatic catalysts that participate in hydrolysis can increase the residual concentration of FFA in the reacted mixture. New catalysts that resist hydrolysis are required to minimize the residual FFA in these oils. A catalyst was specifically designed to perform this task. *Candida antarctica* lipase B immobilized on a porous 3D nanosheet structure of (CALB-TAN) using a highly hydrophobic epoxy ligand was synthesized. The CALB-TAN catalysts were housed in a packed-bed column. The optimum reaction conditions were obtained by evaluating the residence time and the molar ratio of methanol to FFA. The lowest FFA concentration was 0.22 wt% from a feed containing 2.5 wt% FFA. The packed columns were used for up to 42 days without a substantial decrease in the catalyst activity. The experimental data showed that 75 % of the water produced during the reaction process was removed from the PBR. The FFA esterification catalyzed by CALB-TAN in the packed-bed column was compared with CALB-TAN in a batch reactor and Novozym 435 in a PBR system. The FFA concentration met the standard specification of <0.5 % at a residence time of 12-30 min corresponding to 2.3 to 6.8×10^{-5} m/s using CALB-TAN in the PBR and over 100 min in the batch, while the Novozym 435 in the PBR could not meet the FFA specification using.

3.1 Introduction

Biodiesel is a clean-burning, biodegradable and non-toxic source of energy. It is a mixture of mono-alkyl esters whose purity is defined by American (ASTM) and European (EN) standards [1]. It is primarily produced by the transesterification of animal fats or vegetable oils with short-chain oils as seen in Figure 3.1 [56]. Most commercial biodiesel is produced using base catalysts such as sodium methoxide, NaOH or KOH. The price of feedstocks is the greatest contributor to the cost of biodiesel. The proportion of the feedstock cost compared to the overall cost has increased from 60 % to 85 % in the past 20 years [57]. The increasing price of vegetable oils and the food vs. fuel debate have driven manufacturers to find alternative feedstocks such a greases and waste cooking oils (WCO). However, the use of base catalysis in transforming these feedstocks is problematic. Soap is formed when FFA is neutralized by a base catalyst, see Figure 3.2. The presence of soap causes separation issues downstream and greatly affects biodiesel yields [58]. Thus, an FFA-removal process is indispensable for the alkaline transesterification of these acidic oils. Lam et al. [6] report that feedstocks containing 0.5 wt% FFA or less can be processed economically in base-catalysed processes.

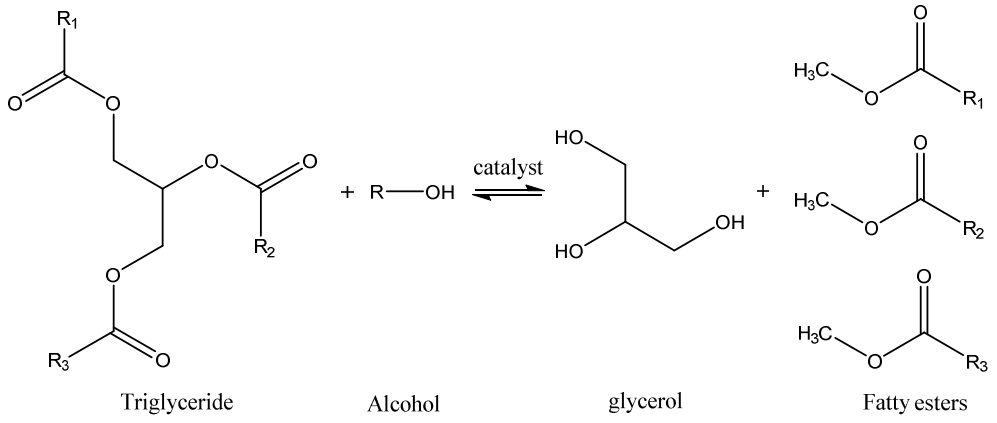


Figure 3.1 Transesterification reaction

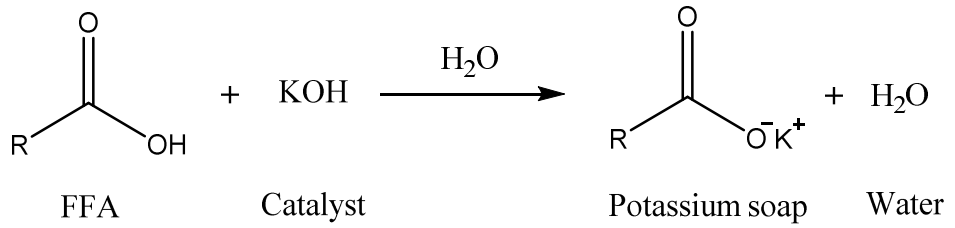


Figure 3.2 Saponification reaction

FFAs can be removed from acidic feedstocks by physical adsorption or by reaction to esters. The adsorption of FFAs produces wastes that are difficult to dispose of and results in a loss of feedstock. Esterification of FFAs produces fatty acid alkyl esters, the desirable end products of the production process. The principles of green engineering favour the transformation of acids into final products. The esterification reaction, shown in Figure 3.3, has been used to treat FFA-containing feedstocks. Strong acids [59] and enzymes [56] have been used as catalysts in the reaction. Although the reaction proceeds without the need for catalysts in supercritical methanol [60], the pressures required to maintain supercritical conditions affect the cost and safety of this approach. Enzyme catalysis offers many advantages compared to other esterification technologies. Enzymes are highly selective, can perform transformations at mild conditions and can be easily disposed of after use. Nielsen et al. [54] have used a liquid lipase formulation containing *Candida sp.* and obtained good FFA reduction in a batch reactor. However, enzymes tend to be expensive and must be reused to treat many batches of feedstock. Their separation from reaction mixtures greatly affect the economics of their use in industrial applications. The immobilization of enzymes onto substrates facilitates their recovery from the reaction system. Lipase can be immobilized onto different supports (silica beads, ion-exchange resin, alumina, etc.) to improve reaction efficiency by providing a rigid external backbone for the enzyme [6].

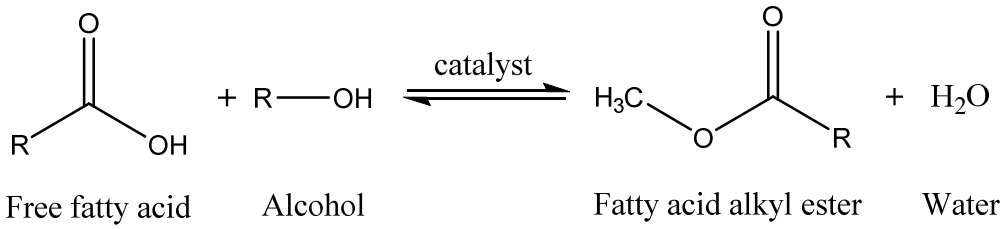


Figure 3.3 Esterification reaction

Many commercial enzyme supports are hydrophilic. Water can be preferentially adsorbed onto the support. If it is not readily removed from the vicinity of the enzyme, hydrolysis of esters and triglycerides will be favored over esterification. This causes difficulties in reaching very low levels of FFA in acidic oils. In experiments aimed at reducing FFAs in acidified oils, Nordblad et al. [55] found that in the methanolysis of an acidic oil with Novozym 435, the concentration of FFA first decreased due to esterification, then increased due to hydrolysis. Novozyme 435 is CALB immobilized onto a hydrophilic macroporous acrylic polymer resin (Lewatit VP OC 1600, Bayer) by Novozymes [61]. The increase in FFA was due to the uptake of water on the support. An acceptable lower limit of FFA was reached after 30 min but could not be maintained as the FFA concentration increased due to hydrolysis.

In this work, *Candida antarctica* lipase B (CALB) was immobilized onto nanoplatelets arranged in a 2D/3D hierarchical structure. FFA oil esterification was carried out in a continuous packed bed reaction system using a silylated-twinned alumina nanosheets immobilized enzyme. The objective of this work is to demonstrate the performance of the FFA reduction in a continuous packed bed reaction system. The impact of the reactant flow rate in the PBR and the methanol-to-FFA ratio were investigated to achieve the optimal conditions. The reusability of CALB-TAN catalyst was also determined by the experiments. Furthermore, the calculation of water balance

was carried out since the accumulation of water in the packed bed column would affect the reaction. The FFA reduction using CALB-TAN in a PBR system was compared with CALB-TAN in a batch reactor and Novozym 435 in a PBR system.

3.2 Materials and methods

3.2.1 Materials

Cetyl trimethylammonium bromide (CTAB), sodium hydroxide pellets, potassium hydroxide pellets, anhydrous ethanol, oleic acid (technical grade, 90%), bromophenol blue, phenolphthalein, Novozymes CALB L (aqueous lipase solution from C. sp.), and Novozym 435 (C. antarctica immobilized on Lewatit VP OC 1600, polymethylmethacrylate (PMMA)) were purchased from Sigma-Aldrich (Canada). Aluminum chloride hexahydrate (99%, nitrogen flushed) was purchased from Acros Organics. Phosphate buffer solution (PBS) (potassium phosphate monobasic, sodium hydroxide, 0.05 M, pH 7.00 at 25 °C), Methanol (99.9 %, 0.2 micron filtered), 1-propanol, 2-propanol, hydrochloric acid (2N standard solution), and cellulose filter paper (Whatman #1) were purchased from Fisher Scientific (Canada). 2-(3,4-Epoxycyclohexyl)ethyltriethoxysilane (ETES) was purchased from Gelest, Inc. (Pennsylvania). HYDRANAL^{TM} Coulumat Oil anolyte and Coulumat CG catholyte for water test were purchased from Honeywell (Canada). Refined canola oil (Mazola) containing no additives or preservatives was purchased from Loblaw Co. Inc. All materials were used as purchased. The refined canola oil was spiked with 2.5 wt% oleic acid to simulate a low-grade acidic oil.

3.2.2 Batch experiments

The batch experiments containing 40 g of acidic oil with methanol (MeOH to FFA molar ratio = 6:1) were carried out in a 250 ml round-bottom flask. The batch reactor was immersed in a 45 °C hot water bath at 700 rpm. 0.3 wt% CALB-TAN catalyst based on the weight of acidic oil, were

prepared for the batch reaction as shown in our previous work. During 0-24 h, samples of 0.4-0.6 g were collected at intervals for acid and water analysis. After 24 h, the reaction was stopped and the enzyme was recovered by centrifugation for the subsequent batch runs.

3.2.3 PBR experiments

The PBR esterification was carried out in a 45 °C hot water bath. The CALB-TAN was packed in a polytetrafluoroethylene tube of 1/16 or 1/8 inch inside diameter. Two 1/16 inch ID columns were packed each using forty milligrams of CALB-TAN as packing. The length of these columns were both 6 cm. One column was labeled T1 and the other T2. The length of the 1/8 inch ID column was 3 cm and 80 mg of CALB-TAN was used in its fabrication. This column was labeled T3. The acidic oil pre-mixed with MeOH was pumped into the column at a given flow rate via KDS-410 syringe pump (KD Scientific, Inc., USA). The column was operated in a down-flow configuration. The products were collected sequentially from the outlet using 50 ml centrifuge tubes. A schematic diagram of the experimental apparatus is reported in Figure 3.4. The effect of molar ratios of MeOH to FFA in the range of 3:1-7.5:1 was investigated. Different residence times were obtained by changing the infusing flow rate of the pump.

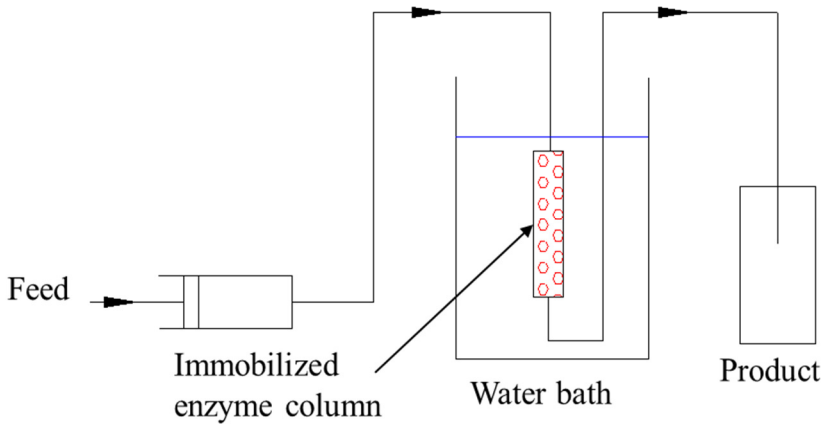


Figure 3.4 Scheme of the PBR system apparatus.

3.2.4 Determination of acid concentration

The titration of FFA in the samples was performed according to the modified method Cc 17-79 by Van Gerpen et al. [62]. A certain amount of sample weighed by an analytical balance was diluted with 10 ml isopropanol in a 15 ml centrifuge tube. The tube was shaken until the sample was dissolved. The mixture was transferred into a 125 ml Erlenmeyer flask for titration.

Stock solutions of 0.01 N potassium hydroxide (KOH) in isopropanol and 0.01 N hydrochloric acid (HCl) in isopropanol were prepared. Phenolphthalein and bromophenol blue were diluted in isopropanol as pH indicators.

The phenolphthalein solution was added into the Erlenmeyer flask and the sample was titrated with 0.01 N KOH in isopropanol to the endpoint. Then the bromophenol blue was added and the 0.01 N HCl in isopropanol was used to titrate the mixture to the endpoint. The volumes of acid and base were recorded for the calculation of FFA concentration.

The FFA concentration was calculated by the following equation:

$$C_{\mathrm{FFA}} = \frac{C_{\mathrm{acid}} V_{\mathrm{acid}} MW_{\mathrm{FFA}}}{m_{\mathrm{sample}}} \times 100\ \% \tag{3.1}$$

where C_{FFA} and C_{acid} are the concentration of FFA (wt %) and acid (mol/L), V_{acid} is the volume of the FFA (L), MW_{FFA} is the molecular weight of FFA (282.5 g/mol), and m_{sample} is the weight of the sample obtained by the analytical balance (g).

3.2.5 Determination of water concentration

The water concentration was determined by Titroline Karl-Fisher Trace Titrator (Schott Instruments, Germany) using fresh HYDRANAL™ Coulumat Oil anolyte and Coulumat CG catholyte from Honeywell Canada.

Before the water test, the phases were separated by centrifugation. The polar and non-polar phases were aspirated with a syringe and injected into the titrator. The water concentration was read from the screen in ppm.

3.3 Results and discussions

3.3.1 Characteristics and reusability of CALB-TAN catalysts

In order to prevent the accumulation of water onto the substrate, CALB was covalently bonded to a hydrophobic support. In addition to this, in order to further promote water release form the catalyst surface, the substrate was selected to be a flat 2D nanosheet. These nanosheets were synthesized in 2D/3D superstructures with high flow-through characteristics, which permitted the release of the dephased water droplets from the core of the support. It has been reported that twinned alumina nanosheets are isotropic and have a very high porosity (79-88 %) [63].

As mentioned in our previous work, the CALB-TAN catalyst has a hydrophobic layer formed by ETES-silylation, which inhibits the approach of free water and thus avoids the hydrolysis of FAME and TG seen in Figure 3.5.

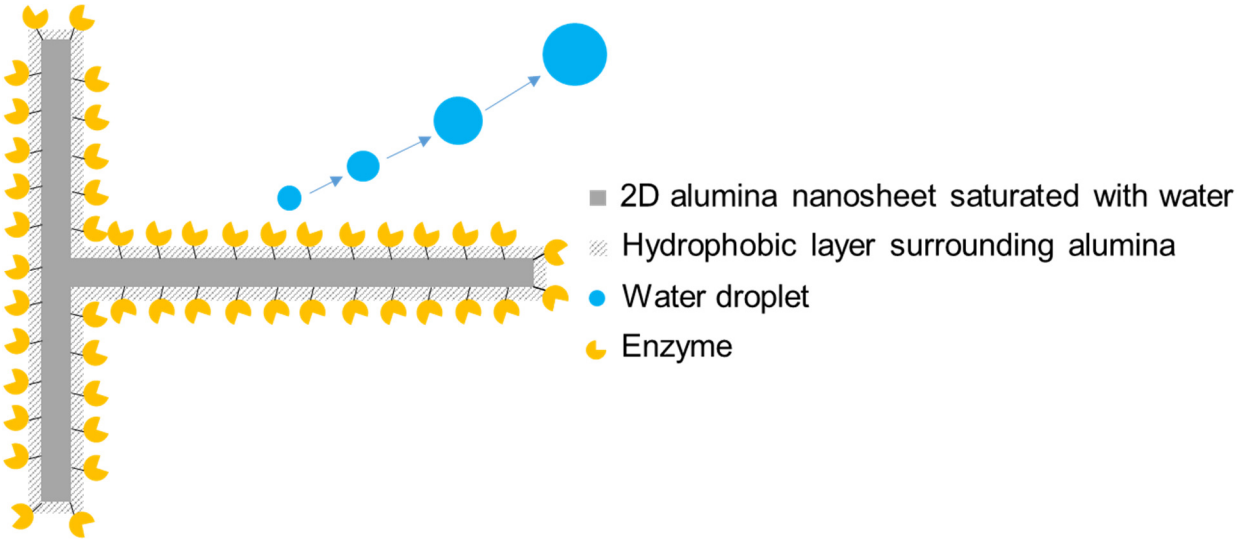


Figure 3.5 Schematic diagram of CALB-TAN. The water produced during the reaction was rejected by the hydrophobic layer formed by the ETES spacer arms.

3.3.2 PBR process

1. The effect of methanol to FFA molar ratio

Methanol, as a low-cost short-chain alcohol, is widely used in the biodiesel industry [64]. From the reaction equation, increasing the concentration of alcohol will drive the reaction forward. However, the irreversible enzyme loss of activity has been reported due to the excess of methanol [54], [65]. Deng et al. [47] found that different enzymes had different tolerability of methanol.

In order to investigate the tolerability of methanol, the experiment was carried out with various molar ratios of MeOH to FFA (3:1, 4.5:1, 6:1, 7.5:1) at 7 µL/min, 45 °C. 40 mg CALB-TAN was packed into a column of 1/16 in inside diameter labeled T1.

Figure 3.6 summarizes the FFA concentrations during a continuous run at different molar ratios of MeOH to FFA. The FFA concentration in the feedstock was 2.5 wt%. In the first 468 h, at a 6:1 molar ratio of MeOH to FFA, the FFA concentration was around the threshold level, which was 0.5 wt% (5000 ppm). When the molar ratio decreased to 3:1, the FFA concentration increased to

1.1 wt% (11000 ppm). Where after, the molar ratio increased to 4.5:1, the FFA concentration decreased to around 0.86 wt% (8600 ppm). But when the molar ratio was up to 7.5:1, the FFA concentration kept increasing over time, indicating the irreversible loss of enzyme activity.

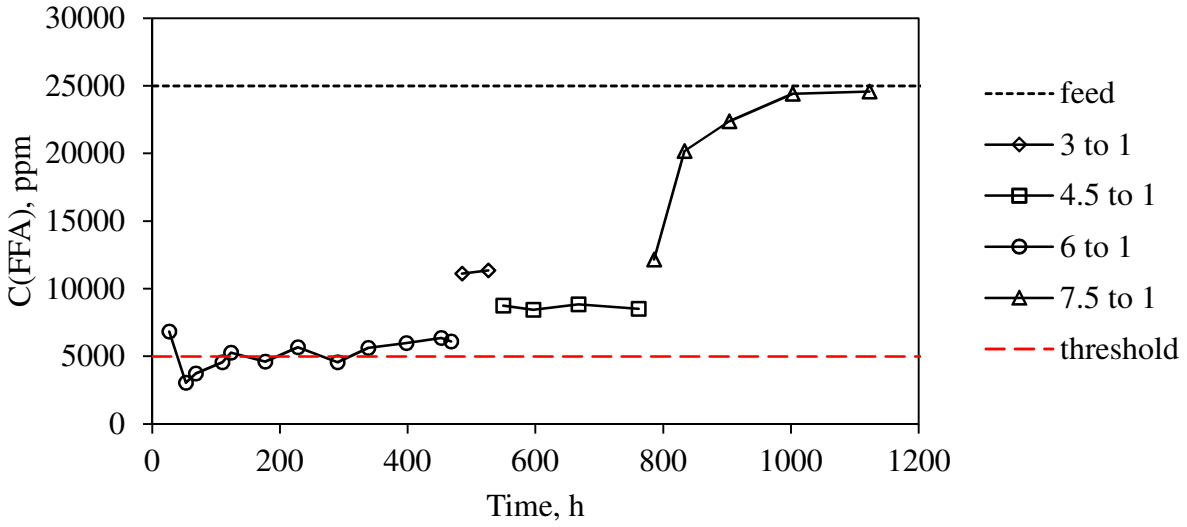


Figure 3.6 FFA concentration at the exit of the T1 PBR over time.

The methanol to FFA ratio changed during the run. Figure 3.7 shows the FFA concentrations at different MeOH to FFA molar ratios. At a molar ratio of less than 6:1, the FFA concentration decreased as the molar ratio increased, while after that, the FFA concentration increased due to the enzyme inactivation caused by excess methanol, as shown in Figure 3.6. Thus, the optimal molar ratio of MeOH to FFA was found to be 6:1.

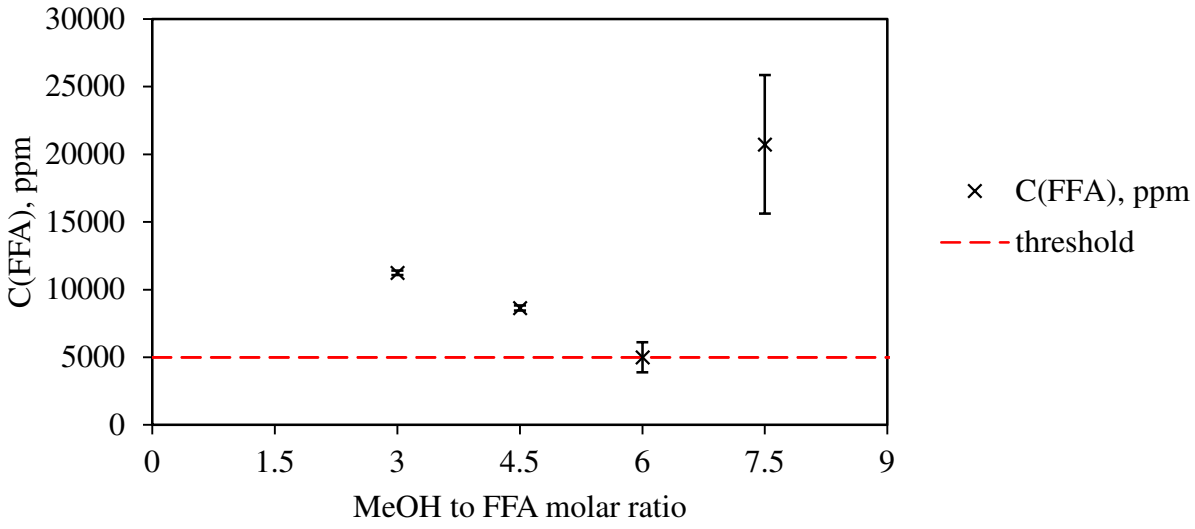


Figure 3.7 Average FFA concentrations at the exit of the T1 PBR at different MeOH to FFA molar ratios.

2. The effect of residence time

Residence time is an important parameter in the PBR system, which affects the reaction efficiency [66]. In this work, different residence times were obtained by changing the feed flow rates. Reducing the flow rate increases the residence time and facilitates the reaction. This caused water accumulation in the column, which in turn decreased the contact between FFA and enzyme, leading to the increase of FFA concentration. The lowest FFA concentration of 0.22 wt% was achieved at a residence time of 25 min, as seen in Figure 3.8.

A batch reaction using 40 mg CALB –TAN was conducted to compare with the continuous reaction. In the batch reaction, the threshold level of 0.5 wt% FFA was reached after 100 min, which was four times longer than that in the PBR system. Further, the unavoidable enzyme loss during the recovery process restricted the reusability of enzyme, which increases the cost of the batch process relative to the continuous PBR process.

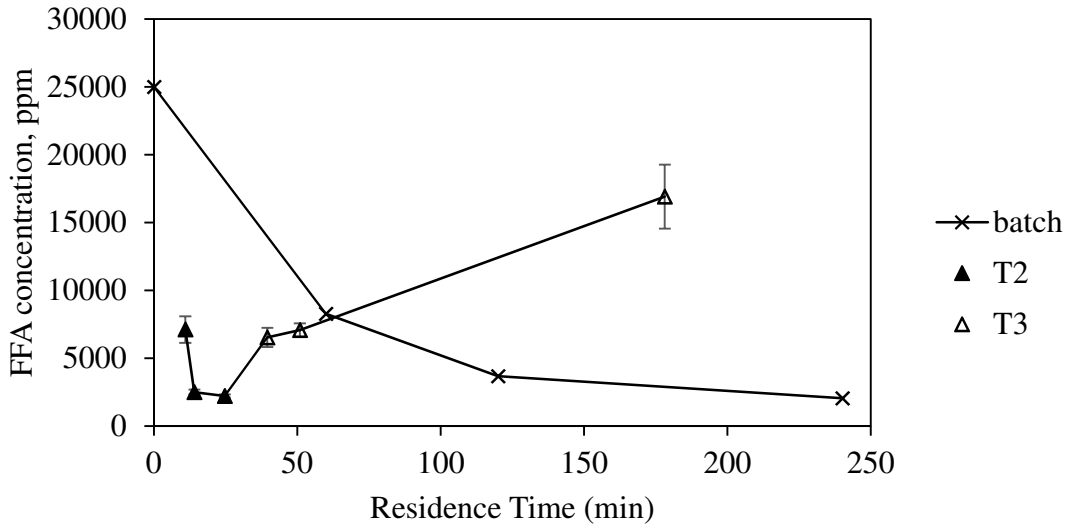


Figure 3.8 FFA concentration with 6:1 MeOH/FFA molar ratio at different residence times. The batch reaction was carried out in a 250 ml round-bottom flask at 45 °C, 600 rpm.

3. The effect of the diameter of the column

The reactions were carried out at 7 µL/min in the columns with different inside diameter (ID) – 1/8 in and 1/16 in. From the results showed in Figure 3.9, the FFA concentration of the reaction in the 1/16 in column maintained around 0.25 wt%, which met the standard specification of <0.5 wt%. The FFA concentration of the reaction in the 1/8 in column was shown to be higher and had more fluctuations, likely due to the low mass transfer coefficient in the column with a large inside diameter.

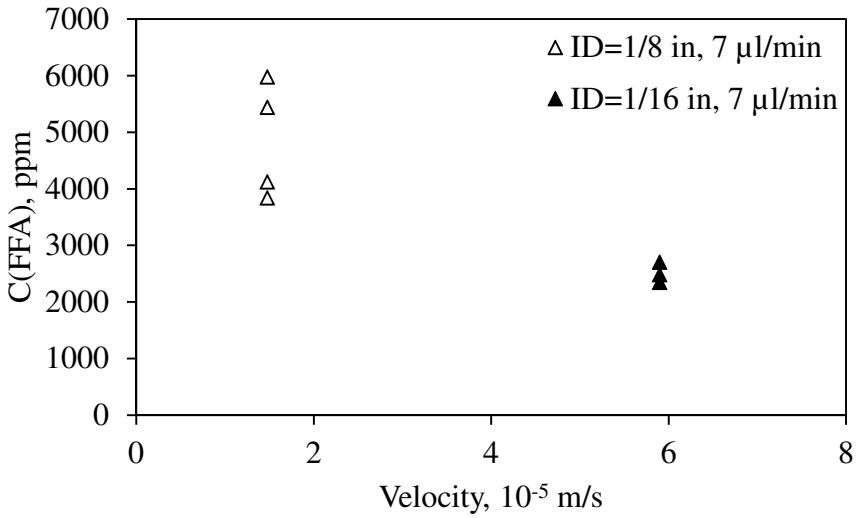


Figure 3.9 FFA concentration of the reactions in columns T2 and T3.

4. The effect of the velocity

Figure 3.10 showed the FFA concentration varied with velocity. When the velocity was less than 3.4×10^{-5} m/s, the FFA concentration decreased as the velocity increased. The lowest FFA concentration was obtained as 0.23 wt% at 3.4×10^{-5} m/s. After that, the FFA concentration went up as the velocity increased. The threshold was reached at 2.3 to 6.8×10^{-5} m/s.

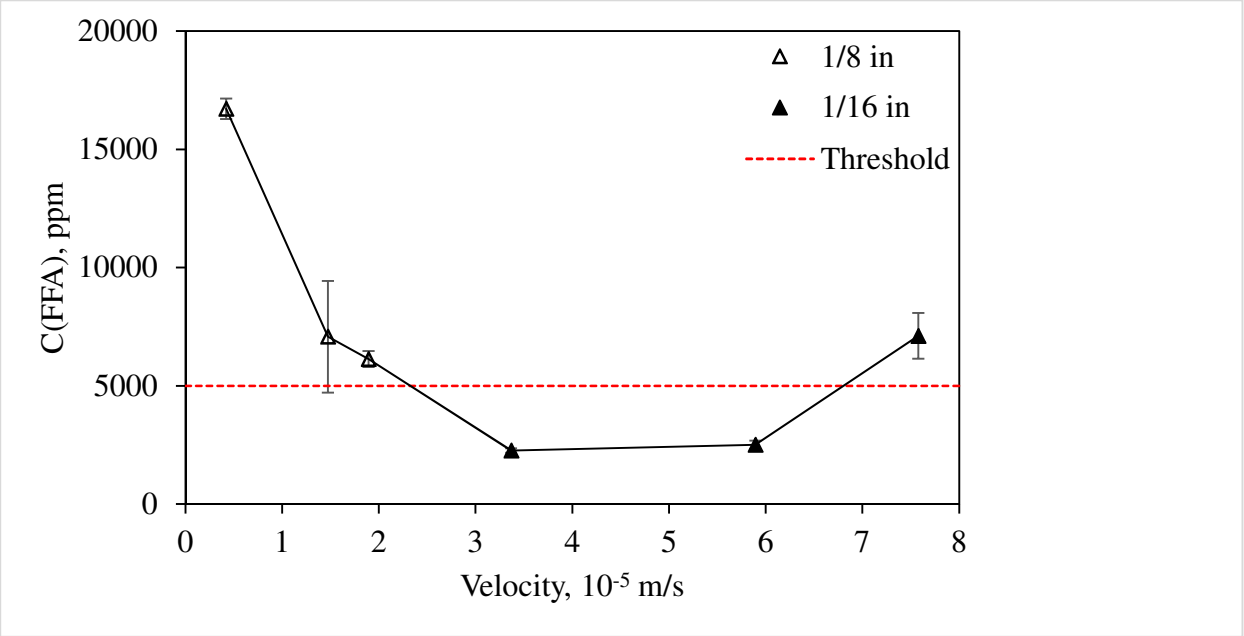


Figure 3.10 The FFA concentrations at different velocities in columns T2 and T3.

5. Water reduction in the column

Figure 3.11 and Figure 3.12 show the reaction rate of the esterification in the PBR over time. In general, the FFA reaction rate increased as the feed flow rate increased, and the water production rate had the same tendency as the FFA reaction rate. The produced water firstly accumulated to form a water drop and then removed from the column resulting in the swing of the water production rate at the same flow rate.

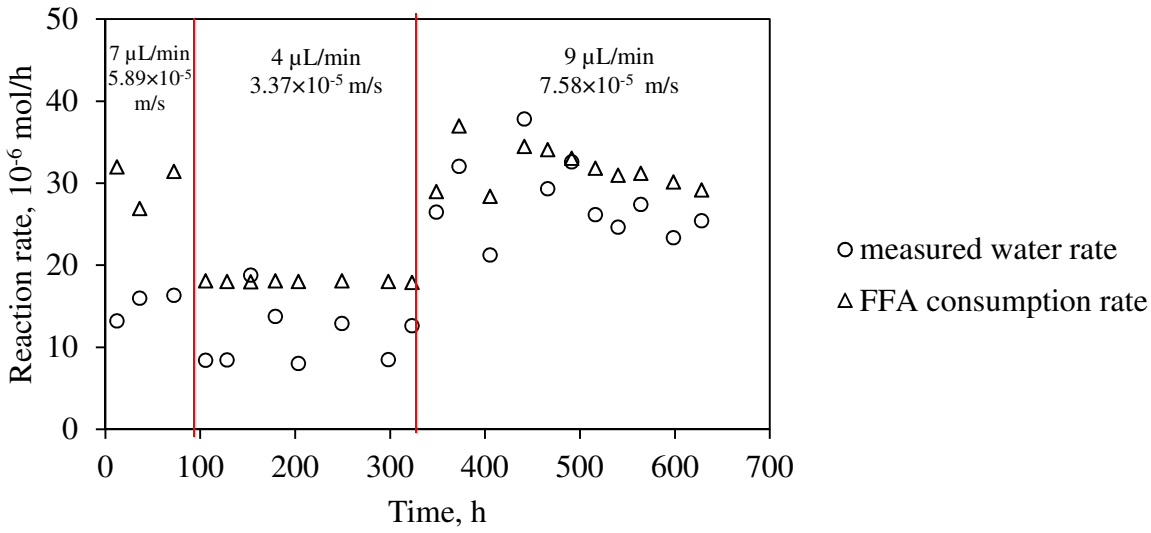


Figure 3.11 Reaction rate in T2. The flow rate of each period was recorded in µL/min and m/s.

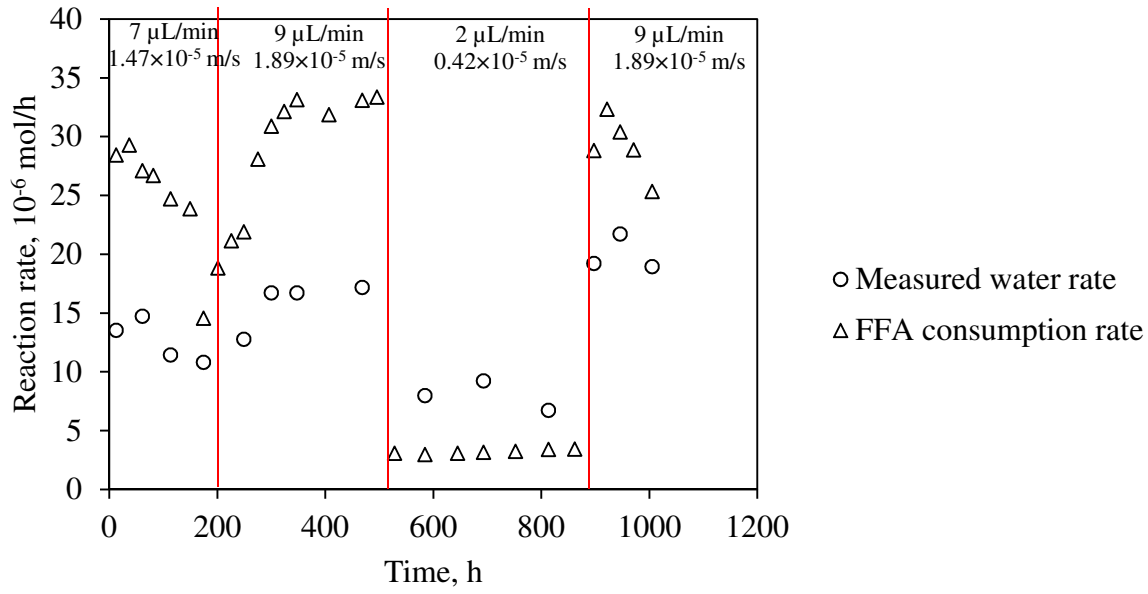


Figure 3.12 Reaction rate in T3. The flow rate of each period was recorded in µL/min and m/s.

In order to investigate the FFA reduction efficiency in the column, the water balance was derived as follows.

The total amount of water removed from the column was calculated by the following equation:

$$m_{\text{water in product}} = m_{\text{water in polar phase}} + m_{\text{water in non-polar phase}} \quad (3.2)$$

where $m_{\text{water in product}}$ is the total amount of water removed from the column (g), $m_{\text{water in polar phase}}$ and $m_{\text{water in non-polar phase}}$ are the amount of water in the polar and non-polar phase (g).

The average water concentration in the feedstock was 250 ppm. The total amount of water increased in the sample was calculated by equation (3.3) and (3.4):

$$\Delta m_{\text{water}} = m_{\text{water in product}} - m_{\text{water in feed}} \quad (3.3)$$

$$\Delta n_{\text{water}} = \frac{\Delta m_{\text{water}}}{MW_{\text{water}}} \quad (3.4)$$

where Δm_{water} and Δn_{water} are the total amount of water increased in the mixture after going through the column in grams and moles, $m_{\text{water in feed}}$ is the amount of water in the feed (g), and MW_{water} is the molecular weight of water.

The water produced by the esterification was calculated based on the FFA concentration. According to the reaction formula, one mole of FFA reacted produces one mole of water. Thus, the amount of water produced was calculated by equation (3.5):

$$\Delta n_{\text{water FFA reacted}} = \frac{(C_{\text{FFA in feed}} - C_{\text{FFA in product}}) m_{\text{product}}}{MW_{\text{FFA}}} \quad (3.5)$$

where $\Delta n_{\text{water FFA reacted}}$ is the amount of water produced during the reaction (mol), $C_{\text{FFA in feed}}$ is the FFA concentration in the feed (2.5 wt%), $C_{\text{FFA in product}}$ is the FFA concentration after esterification (wt%), m_{product} is the weight of the sample obtained by the analytical balance (g). MW_{FFA} is the molecular weight of FFA (282.5 g/mol).

The water-removal ratio was calculated using the following equation:

$$Water\ removal\ ratio = \frac{\Delta n_{\text{water}}}{\Delta n_{\text{water FFA reacted}}} \tag{3.6}$$

Figure 3.13 and Figure 3.14 show the water removal ratio of each sample. The total water balance indicated that 75 % of the water produced by esterification was removed from the 1/16-in column and 70 % was removed from the 1/8-in column. Both columns had relatively the same water-removal ratio. Since the water came out intermittently as droplets it is difficult to estimate the amount of water on the catalyst. However, as seen in figure 3.12 the presence of water in the column did not affect the reaction rate for FFA as it was the same at 9 µL/min before and after the excursion to 2 µL/min and this was after 42 days of operation (1000 h).

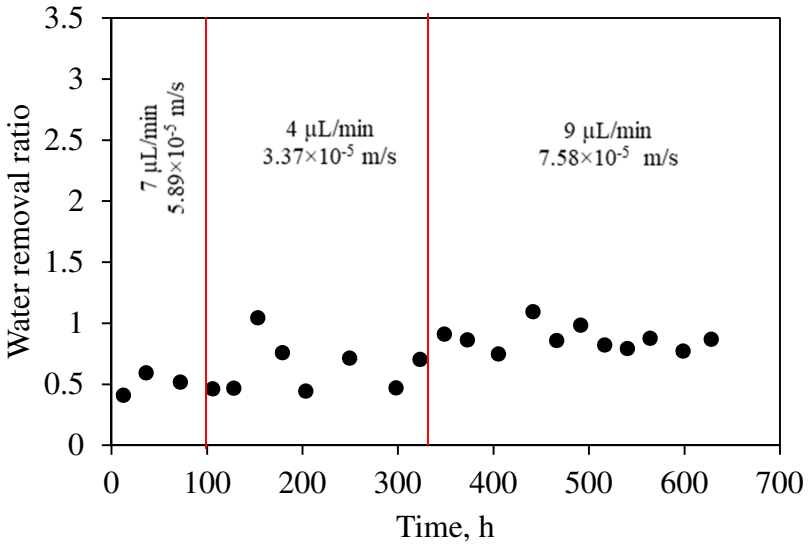


Figure 3.13 Water removal ratio in column T2. The flow rate of each period was recorded in µL/min and m/s.

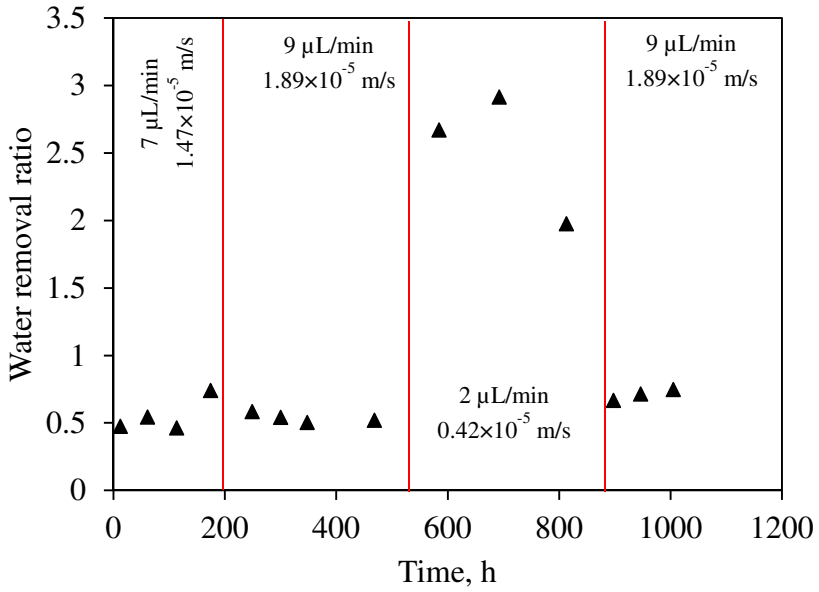


Figure 3.14 Water removal ratio in column T3. The flow rate of each period was recorded in µL/min and m/s.

6. The comparison of CALB-TAN and Novozym 435

The CALB-TAN catalyzed reaction performance was compared with the Novozym 435 catalyzed run. The reactions were carried at 7 µL/min in 1/16 in inside diameter columns and the results were summarized in Figure 3.15. The FFA concentration maintained around 0.5 wt% in the CALB-TAN catalyzed run, while in the Novozym 435 catalyzed run, the lowest FFA concentration was 1.37 wt%, which was much higher than that in the CALB-TAN run, and the FFA concentration was not maintained at a 1.37 wt% but fluctuated with time.

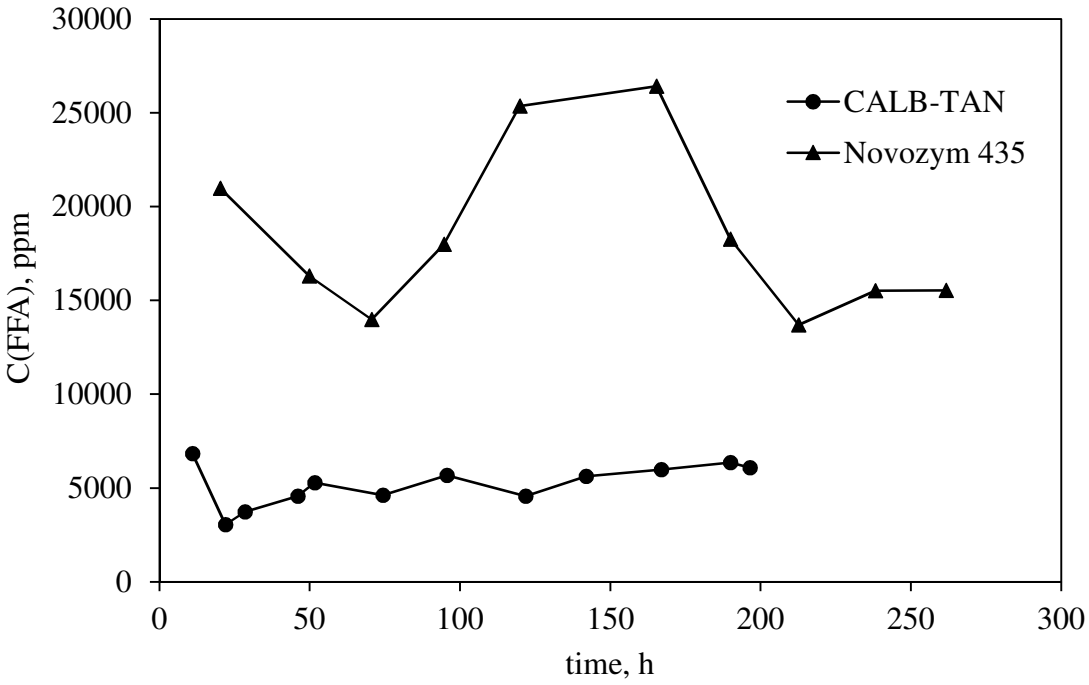


Figure 3.15 FFA reduction of CALB-TAN (T1) and Novozym catalyzed reaction systems. The same amount of catalysts were used in the both runs. The molar ratio of methanol:FFA was 6:1.

3.4 Conclusions

The PBR column filled with silylated TAN-supported enzyme was successfully applied to the FFA esterification of acidic oil. The FFA concentration decreased below the threshold (0.5 wt%) at the residence time ranging from 12-30 min in the PBR, while this took over 100 min in the batch system. The lowest FFA concentration of 0.23 wt% in the PBR system was obtained at a residence time of 14 min in a 45℃ water bath using the MeOH to FFA molar ratio of 6:1 in a 1/16-inch column. According to the calculation of water balance, the 1/16-in PBR system was able to remove 75 % of the water from the column, and the 1/8-in system had a water removal ratio of 70 %. Compared to the PBR using the commercial Novozym 435, the esterification using CALB-TAN

was superior in FFA esterification and a lower FFA concentration was obtained in the CALB-TAN run. Besides, it was demonstrated that the CALB-TAN catalyst retained its activity for 42 days.

Chapter 4 Comparison of acidic oil esterification using a liquid lipase formulation in a batch and a membrane reactor (MR)

To be submitted, J. Zhou, A.Y. Tremblay.

Abstract

Candida antarctica lipase B (CALB) is capable of converting FFA to esters through esterification. Liquid enzymes are more cost effective than supported enzymes but more difficult to separate from reacted oil mixtures compared to immobilized enzymes. In this work, a continuous stirred tank reactor equipped with a ceramic membrane was used to implement the continuous pre-treatment of FFA for eventual use in base catalysed biodiesel processes. Enzymatic membrane reactors have the advantage of shifting the reaction equilibrium by removing products and by keeping the enzyme catalyst in the reactor. However, the pore size of the membrane used must be slightly smaller than the size of the enzyme in order to assure its retention. Ultrafiltration membranes having a molecular weight cut-off of 10 to 15 kDa are typically used to retain lipases. Such a membrane has a tremendous pressure drop when used in filtering vegetable oils as these are 50 times more viscous than water at 45 °C [67]. The aim of this work is to develop a process that uses a 0.2 micron microfiltration membrane in an enzyme reactor to esterify FFAs in acid feedstocks. The performance of the liquid enzyme was tested by the reaction of the enzyme with the acidic oils containing 2.5, 5, 10, 20, and 30 wt% FFA and different alcohols (methanol/ethanol) in a stirred batch reactor. The results showed that the enzyme had a tolerance to acid an acid concentration of 10 wt%, and the conversion was higher using methanol than ethanol as an alcohol. The experimental results showed that the addition of glycerol to the MR increased the FFA conversion, while the viscosity of the reaction mixture also increased, resulting in an increase in the operating pressure in the reactor. The issue of excessive pressure in the reactor during the

esterification was solved by adding biodiesel to the acidic oil. The highest conversion of FFA into FAME in the continuous membrane system was 92.05 % at a residence time of 9.3 hours, glycerol/enzyme mass ratio of 2.6:1, methanol/FFA molar ratio of 3:1. At these conditions, the FFA conversion in the MR was maintained over 80 % for 5.2 cycles and 6 cycles after adding biodiesel to the feedstock This was compared to a batch reactor where conversion was higher but only maintained for 4 cycles. The enzymatic esterification in the MR has the advantages of high efficiency and easy recovery of liquid enzyme compared to the complex recovery of catalyst in the batch reactor.

4.1 Introduction

Recently, biodiesel has drawn increased attention in solving the climate change problems posed by fossil fuels [20]. It is a sulfur-free, biodegradable and renewable alternative to petrodiesel [3], [68]. However, the main hurdle to its commercialization is the high cost of the feedstock [69], [70]. It is reported that the production cost has been reduced to less than half by substituting refined vegetable oils with waste cooking oil (WCO) [32]. Furthermore, deriving biodiesel from the WCO recuperates the energy in the waste and provides a way out of the disposal issues of WCO which are not easily biodegraded in landfills and cannot be discharged [2], [9].

Most biodiesel is produced commercially using methanol alkaline catalysis. However, the free fatty acids (FFA) in the WCO will cause saponification in the presence of base catalysts, having an adverse impact on the yields. Therefore, several approaches have been adopted for WCO pre-treatment, such as adsorption, acid catalysis, supercritical methanol, ion exchange resin, reactive extraction and enzymatic esterification [32], [44]. The advantages and disadvantages of these catalysts were discussed in Chapter 2 of this thesis. Enzymes were selected as a preferred catalyst since they are environmentally-friendly and highly-efficient in esterifying FFA to FAME under mild conditions [71]. However, their use in reducing FFA to levels that are permissible for base catalysis (0.5 wt%) is limited due to their ability to hydrolyze esters and triglycerides into FFAs in the presence of water. To avoid this issue enzymatic processes treating high FFA oils completely hydrolyze the acidic oil to FFA, dry the hydrolysis product and then transform the FFA to esters (biodiesel).

The high cost of enzymes implies they must be recycled. Immobilization has been widely used to improve the reusability of enzymes, since the immobilized enzyme preparation can be separated from the reaction mixture more easily than free enzymes. However, the support material is

relatively fragile, particularly when the immobilized enzyme is introduced in a stirred batch reactor or continuous stirred tank reactor (CSTR). The support material tends to break down due to the high shear rate generated by the mixer. The enzyme stability can be enhanced by a low stirring rate at the expense of mass transfer efficiency, which has an adverse effect on the reaction rate [54]. Fatimah et al. [72] reported that the shear stress in continuous systems can be reduced by a packed-bed reactor. However, there would be a risk of high pressure drop caused by the loss of structural integrity of the support due to water formation and the accumulation of glycerin and insoluble components in the column [54]. Free liquid lipase offers advantages over the immobilized enzyme, due to its lower cost and stability under high shear rates. It was found that the *Candida antarctica* lipase B was able to catalyze both the esterification and transesterification of oils [73]. The FFA methyl esterification required less enzymes and it was 10 times faster than the TG transesterification.

Nielsen et al. [54] used 1 wt% liquid lipase from *Candida antarctica* to carry out the FFA esterification in a well-mixed stirred tank reactor at 500 rpm, and the reaction achieved a high FFA conversion of 95 %. Ninety five percent of the enzyme activity was in an emulsion phase between the glycerol-water phase and FAME phase after the gravity separation. However, the emulsion phase has a low volume and is difficult to recover, also the enzyme is amphiphilic and bridges the polar and non-polar phase in the mixture. Therefore, the emulsion phase contains a mixture of glycerol-water and itself and is fed to the next run, resulting in an eventual decrease in FFA conversion. This drop can be ascribed to the unavoidable loss of enzyme and the accumulation of water and glycerol from the reaction. Since the recovery step is relatively complicated when using liquid enzymes in a batch process, the FFA esterification in a continuous reaction system is of great interest.

In this work, the FFA esterification in a continuous stirred tank reactor using liquid lipases is presented. A ceramic membrane was used to retain the lipase in the reactor. It was reported that the ceramic membrane had good thermal and chemical stability, good tolerance to high pressure, and high resistance to fouling [74]. Rios et al. [75] indicated that the selectivity of the membrane was related to the relative size of membrane pores and molecules in the solution. Since plenty of enzymes have a molecular weight between 10 to 80 kD, an ultrafiltration membrane with a molecular cut-off between 1 to 100 kD is most commonly used in a membrane reactor. The viscosity of the oil is too high to use membranes having small pore sizes. Some researchers [76] achieved the separation of reacted biodiesel and glycerol using ceramic membranes in the microfiltration ranges. A 0.2 µm membrane could achieve a 99.6 % separation of glycerol in a 5 % ethanol in biodiesel feed.

The objective of this work is to investigate the FFA esterification using liquid lipase in a continuous membrane reactor using a microfiltration membrane. Glycerol, as a water binder, was added to the reaction system. Additionally, the effects of flow rate, organic solvent and the enzyme reusability were also discussed. Correspondingly, the FFA conversion in the continuous membrane reactor was compared to the reaction in a batch reactor.

4.2 Materials and methods

4.2.1 Materials

Refined canola oil without additives or preservatives (Mazola) was purchased from Loblaw Co. Inc. Potassium hydroxide pellets, oleic acid (technical grade, 90%), bromophenol blue, phenolphthalein, and Novozymes CALB L (aqueous lipase solution from *Candida sp.*) were purchased from Sigma-Aldrich (Canada). The composition of the lipase solution is shown in Table 4.1. Methanol (99.9 %, 0.2 micron filtered), 2-propanol, hydrochloric acid (2N standard solution)

and Coomassie Brilliant Blue G-250 assay reagent were purchased from Fisher Scientific (Canada). HYDRANAL™ Coulumat Oil anolyte and Coulumat CG catholyte for water test were purchased from Honeywell (Canada). Ceramic membrane filters (0.2 micron, 47 mm) were purchased from Sterlitech Corporation (USA). Canola-based biodiesel was purchased from Milligan Biofuels (Canada). All materials were used as purchased. The refined canola oil was spiked with oleic acid to simulate a low-grade acidic oil.

Table 4.1 The composition of aqueous lipase solution from Candida sp.

Ingredients	Concentration, %
Lipase, triacylglycerol	6
Water	44
D-Glucitol	25
Glycerol	25
Sodium benzoate	0.2
Potassium (E,E)-hexa-2,4-dienoate	0.1

4.2.2 Effect of FFA concentration and type of alcohol

In order to investigate the effect of FFA concentration and type of alcohol on conversion to FAME, several test were performed at increasing FFA concentration with methanol and ethanol as an alcohol. These tests were carried out in a round-bottom flask using 40 g of acidic oil with various concentrations of FFA (2.5, 5, 10, 20, 30 wt%), with a molar ratio of alcohol (MeOH or EtOH) to FFA of 3:1, and catalyzed with 1 wt% Novozym CALB L liquid lipase (w/w of acidic oil) for 24 h. The mixtures were stirred at 700 rpm and 45 °C. Samples containing 0.4-0.6 g were collected at 0, 0.5, 1, 2, 4, 8 and 24 h for the acid and water analysis.

4.2.3 Continuous membrane reactor

The esterification reaction mixture comprised acidic oil (containing 10 wt% FFA), 3 molar equivalents of methanol (based on FFA) and 0 or 5 wt% biodiesel (based on the acidic oil). The MR esterification was carried out in a 70 ml cylindrical stirred cell. A 0.2-micron ceramic membrane was placed at the bottom of the reactor. The reactor was immersed in a 45 °C hot water bath and stirred at 700-rpm using a magnetic stirrer. The cell was filled with the esterification reaction mixture along with 1 wt% Novozym CALB liquid catalyst (based on the acidic oil in the cell) and a given amount of glycerol or biodiesel. During the experiment, the reaction mixture was pumped into the cell at a given flow rate using a KDS-410 syringe pump (KD Scientific, Inc., USA). The reactor was operated in a down-flow configuration. The permeate was collected sequentially from the outlet using centrifuge tubes for the FFA analysis. A schematic diagram of the experimental apparatus is shown in Figure 4.1. The effect of methanol, glycerol, organic solvent and the flow rate were investigated. The reaction conditions are given in detail in Table 4.2.

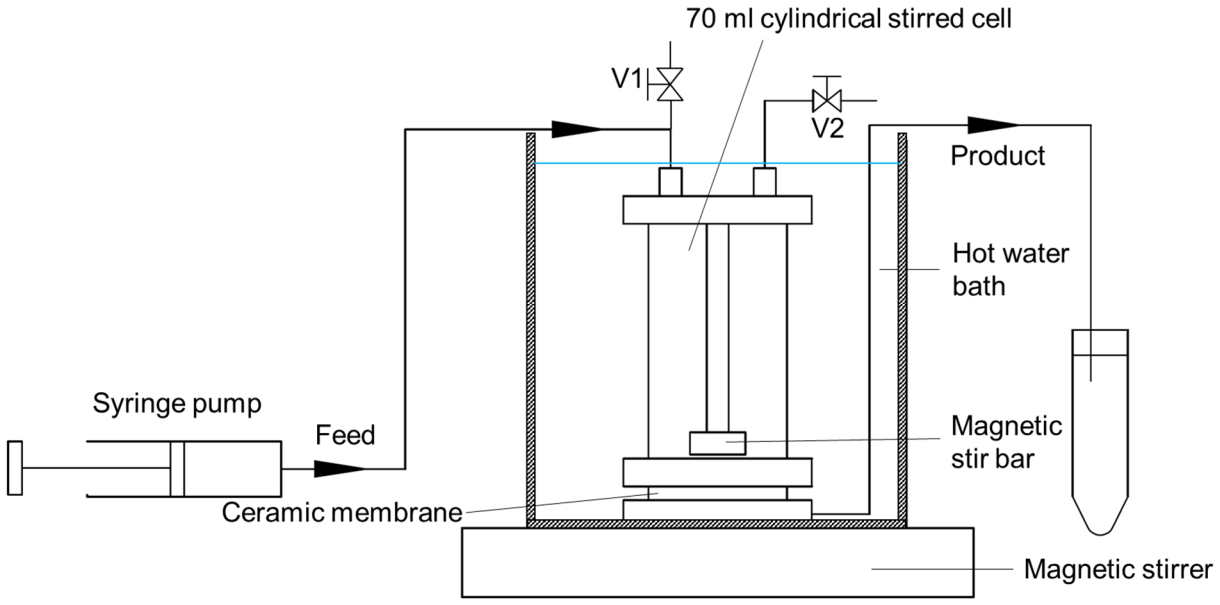


Figure 4.1 Process for the MR system apparatus (V1, V2: valves).

Table 4.2 MR conditions

#Run	Label	Flow rate, μL/min	Mass ratio of glycerol to enzyme	Molar ratio of MeOH to FFA	Mass ratio of biodiesel to acidic oil
1	200-0G-3M-0B	200	0	3:1	0
2	125-0G-3M-0B	125	0	3:1	0
3	125-1G-3M-0B	125	1:1	3:1	0
4	125-2G-3M-0B	125	2:1	3:1	0
5	125-2.6G-3M-0B	125	2.6:1	3:1	0
6	125-2.6G-6M-0B	125	2.6:1	6:1	0
7	125-2.6G-3M-0.05B	125	2.6:1	3:1	1:20
8	125-0G-3M-0.05B	125	0	3:1	1:20

4.2.4 Cleaning of the experimental module and the membrane

After each run in the MR, the experimental unit was immediately cleaned to preserve the equipment and restore the permeability of the used membrane. Firstly, the MR and membrane were washed with water and detergent until most biodiesel was eliminated. Then, the MR was rinsed with deionized water and the ceramic membrane was soaked in isopropanol for 12 h for further cleaning. The membrane was placed on a dry paper and isopropanol was removed by capillarity. The membrane was then used directly in the MR. The module and membrane were cleaned with isopropanol.

4.2.5 Batch reaction

The esterification reaction mixture comprised acidic oil (containing 10 wt% of FFA) and 3 molar equivalents of methanol (based on FFA). The batch reaction was conducted in the same cell as shown in Figure 4.2. There were no inputs or exits from the cell during the batch reaction. The batch reactor was immersed in a 45 °C hot water bath. The water bath was placed on a magnetic stirring mechanism to cause an agitation of 700 rpm for the reaction mixture. The reaction mixture, 1 wt% Novozym CALB liquid catalyst (based on the acidic oil) and 0 or 2.6 equivalents of glycerol (based on the catalyst) were fed to the reactor for 10 h. During 0-10 h, samples of 0.4-0.6 g were collected at regular intervals for FFA analysis. After 10 h, the enzyme was separated from the reacted mixture by gravity and recovered for the subsequent batch runs. The reaction conditions are shown in Table 4.3.

Table 4.3 Batch conditions

Operating parameter	value
Acidic oil (10 wt% FFA)	70 ml
Enzyme solution	1 wt %
The molar ratio of MeOH to FFA	3:1
The mass ratio of glycerol to enzyme solution	0 and 2.6:1

4.2.6 Determination of acid concentration

The titration of FFA in the samples was according to method Cc 17-79 by Van Gerpen et al. [62]. A sample was weighed by an analytical balance and diluted with 10 ml isopropanol in a 15 ml centrifuge tube. The tube was shaken until the sample was dissolved. The mixture was transferred into a 125 ml Erlenmeyer flask for titration.

Solutions of 0.01 N potassium hydroxide (KOH) and 0.01 N hydrochloric acid (HCl) in isopropanol were used in the titration. Phenolphthalein and bromophenol blue were diluted in isopropanol and used as pH indicators.

The phenolphthalein solution was added into the Erlenmeyer flask and the sample was titrated with 0.01 N KOH in isopropanol to the endpoint. Bromophenol blue was added and the 0.01 N HCl in isopropanol was used to titrate the mixture to the endpoint. The volume of acid and base was recorded to calculate the FFA concentration of the sample.

The FFA concentration was calculated by the following equation:

$$C_{\mathrm{FFA}} = \frac{C_{\mathrm{acid}} V_{\mathrm{acid}} MW_{\mathrm{FFA}}}{m_{\mathrm{sample}}} \times 100\ \% \qquad (4.1)$$

where C_{FFA} is the concentration of FFA (wt %) and C_{acid} the acid (HCl) (mol/L), V_{acid} is the volume of the FFA (L), MW_{FFA} is the molecular weight of FFA (282.5 g/mol), and m_{sample} is the weight of the sample obtained by the analytical balance (g).

4.2.7 Determination of water concentration

The water concentration was determined by Karl Fisher analysis using a Titroline Karl-Fisher Trace Titrator (Schott Instruments, Germany).

The reacted mixture collected in the centrifuge tube was separated into the oil phase and non-polar phase by centrifugal separation. A 0.1-0.5 g sample was taken from each of the two phases using a needle and injected in the Titroline Karl-Fisher Trace Titrator. The exact mass of the sample used for the water titration was obtained by measuring the mass difference of the syringe before and after the injection and entered into the titrator. After 5-15 min, the water concentration was read directly from the instrument in ppm.

4.2.8 Protein test in methanol and ethanol

The protein in the permeate and retentate was detected by Coomassie Brilliant Blue G-250 (CBBG) assay reagent. For a single test during the MR reaction, 5 ml of CBBG was injected into the reactor. The color of CBBG changed from reddish violet to blue in presence of protein.

4.3 Results and discussions

4.3.1 Lipase performance

Figures 4.2 to 4.5 show the results of methyl esterification and ethyl esterification of the acidic oil containing 2.5, 5, 10, 20 and 30 wt% FFA in the batch reactor. From the results of 2.5, 5 and 10 wt% FFA methyl esterification runs in Figure 4.2, the FFA concentration in the process rapidly decreased in the first 0.5 h and reached a minimal level of 0.16 to 0.22 wt% FFA in each run. In

addition, the FFA concentration was maintained at 0.16 to 0.27 wt% after 24 h, which was below the threshold for base catalysis of 0.5 wt%. However, when the FFA concentration of the feedstock increased to 20 wt%, the content of the remaining FFA in the mixture after 24 h was 3.36 wt%, which was higher than the threshold. In the methyl esterification in which the FFA concentration of the acidic oil was 30 wt%, the residual FFA concentration after 24 h was 26.85 wt%.

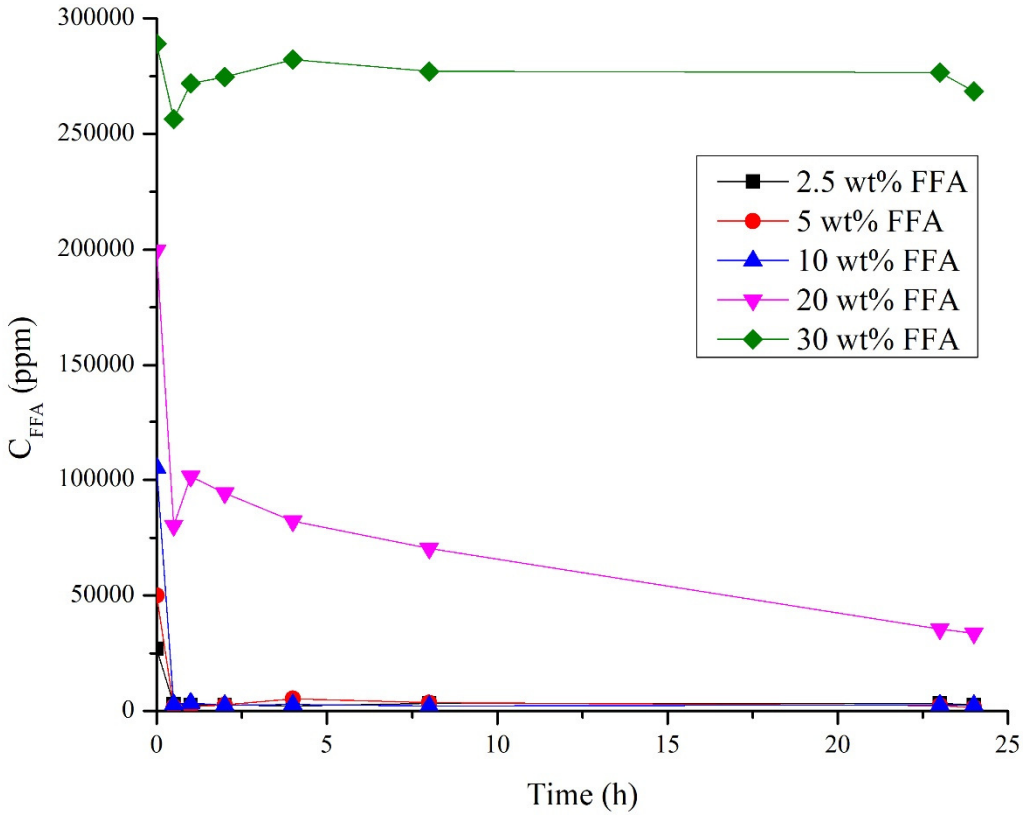


Figure 4.2 FFA concentration versus time for methyl esterification.

From the results of 2.5 wt% and 5 wt% FFA ethyl esterification runs in Figure 4.3, the FFA concentration in the process rapidly decreased to 0.42-0.88 wt% in the first 0.5 h and it maintained at 0.27 to 0.28 wt% after 24 h, which was below the threshold (0.5 wt%) but slightly higher than

that in methyl esterification (Figure 4.2). When the FFA concentration in the feedstock was more than 5 wt%, the conversion of ethyl esterification of the same feedstock was significantly lower than that of methyl esterification (Figure 4.2). Further, when the FFA concentration in the feedstock was more than 20 wt%, the conversion after reacting for 24 h was almost zero. The results indicated that under the same conditions, ethanol was not as effective as methanol in performing the esterification. Lipases work best when there is a two phase system. Ethanol is a better compatibilizing agent than methanol so the reaction with ethanol is not as effective as with methanol.

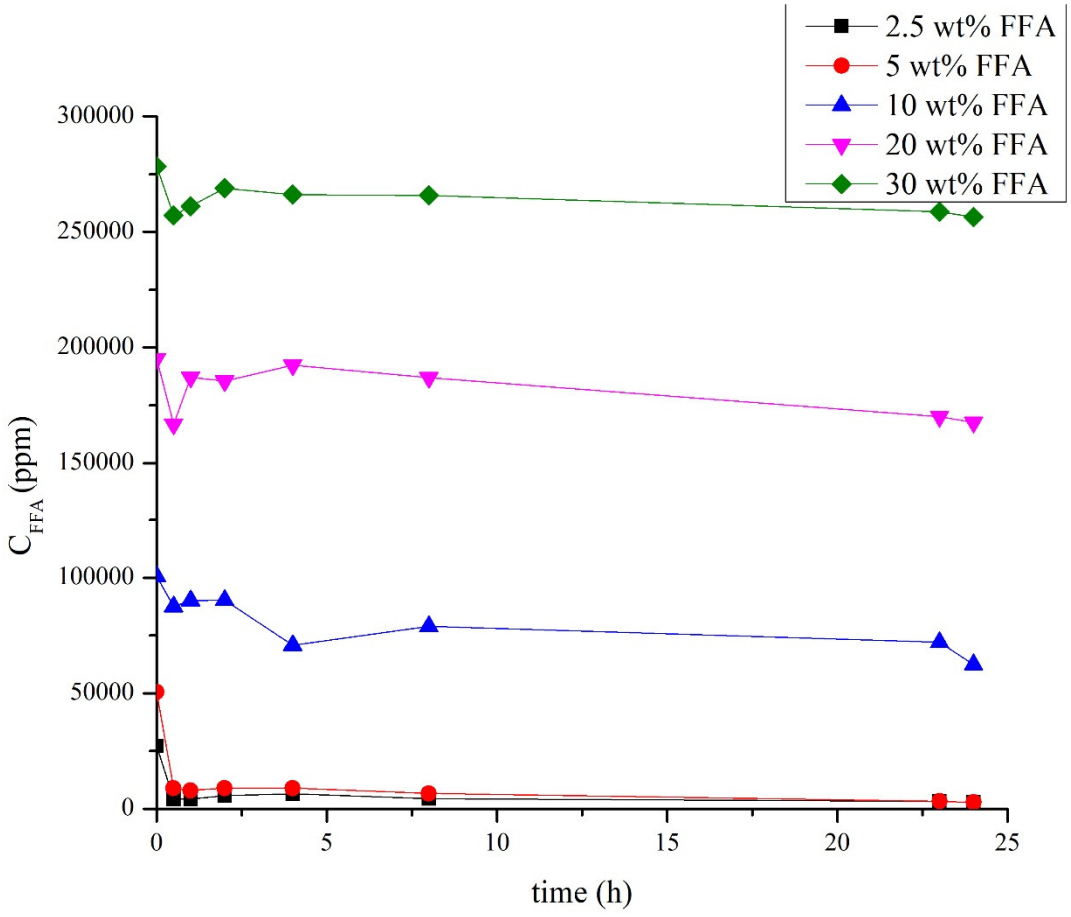


Figure 4.3 FFA concentration versus time for ethyl esterification.

It was reported that the water was a critical factor in maintaining the lipase activity during the esterification, besides, the amount of water required in the media depended on the characteristics of the lipases [47]. Figure 4.4 and Figure 4.5 summarize the FFA and water concentrations of the samples that were taken during the methyl and ethyl esterification. The initial water content in the feedstock was 482 ± 129 ppm. Compared to the esterification of the acidic oil containing low FFA concentration (2.5, 5 and 10 wt% FFA in Figure 4.4; 2.5 and 5 wt% in Figure 4.5), the esterification of the acidic oil containing high FFA concentration (20 and 30 wt% FFA in Figure 4.4; 10, 20 and 30 wt% in Figure 4.5) produced relatively more water, which favored the hydrolysis of triglyceride (TG) but inhibited the activity of lipase.

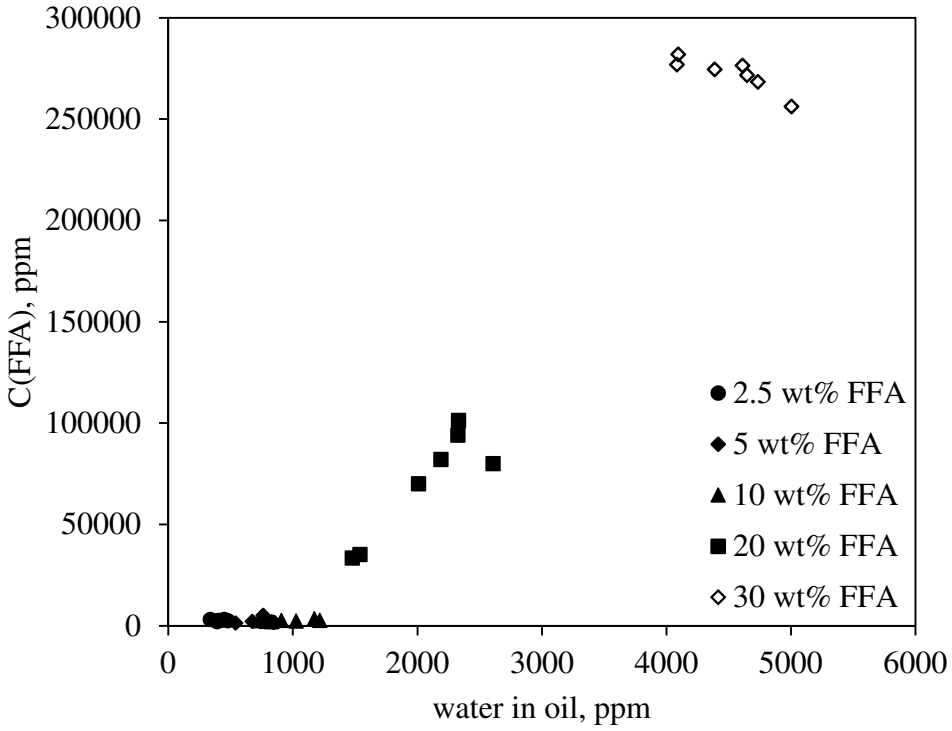


Figure 4.4 FFA concentration versus water concentration in FFA methyl esterification.

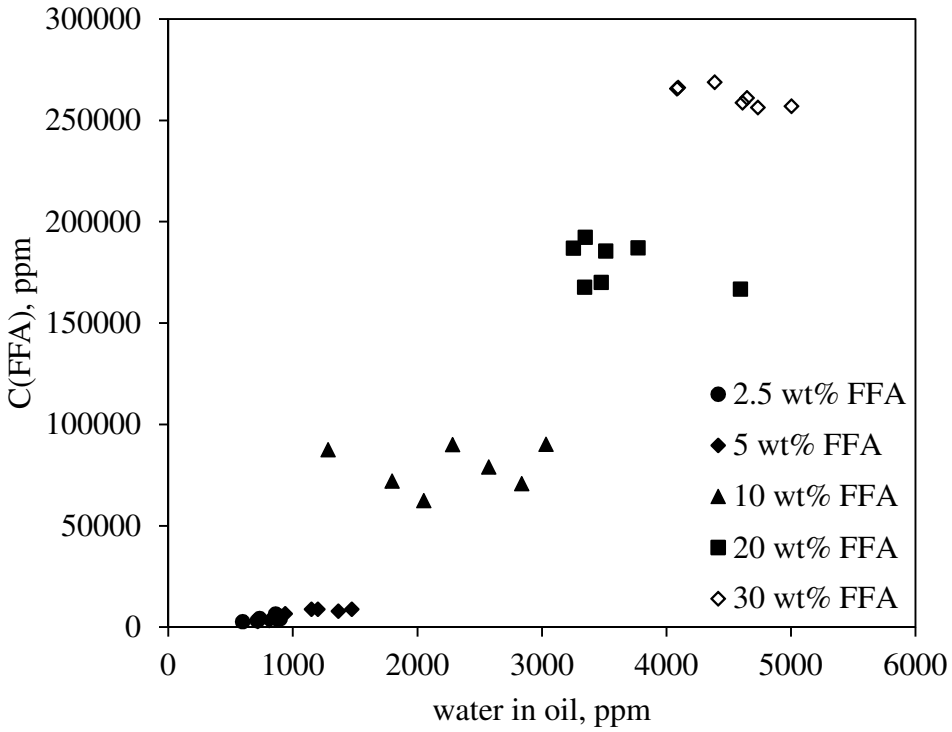


Figure 4.5 FFA concentration versus water concentration in FFA ethyl esterification.

In summary, the water tolerance of Novozym CALB L liquid lipase was around 1500 ppm, according to Figure 4.4 and Figure 4.5 and the enzymatic esterification of FFA with methanol was superior to ethanol. As the FFA concentration in the feedstock increased, the FFA conversion decreased, which was likely due to the increase in the amount of alcohol and the water produced in the mixture. The amount of methanol increased as the initial amount of FFA increased since the molar ratio of MeOH to FFA was 3:1. Since the hydrolysis could be triggered by accumulated water that increased as the initial FFA concentration in the feedstock increased and considering the tolerance of the enzyme to water, the acidic oil containing 10 wt% FFA was chosen as the feedstock for the continuous enzymatic methyl esterification.

4.3.2 Membrane reactor

Figure 4.6 shows the FFA conversion at flow rates of 200 and 125 µl/min, which corresponded to residence times of 350 and 560 min calculated by equation (4.2). Obviously, the FFA conversion of the reaction with a flow rate of 125 µl/min was higher than that with a flow rate of 200 µl/min. Therefore, the flow rate was set to 125 µl/min in the subsequent runs.

$$\text{residence time} = \frac{\text{volume of the cell}}{\text{flow rate}} \tag{4.2}$$

The number of cycles is given by:

$$\text{cycles} = \frac{\text{time on stream}}{\text{residence time}} \tag{4.3}$$

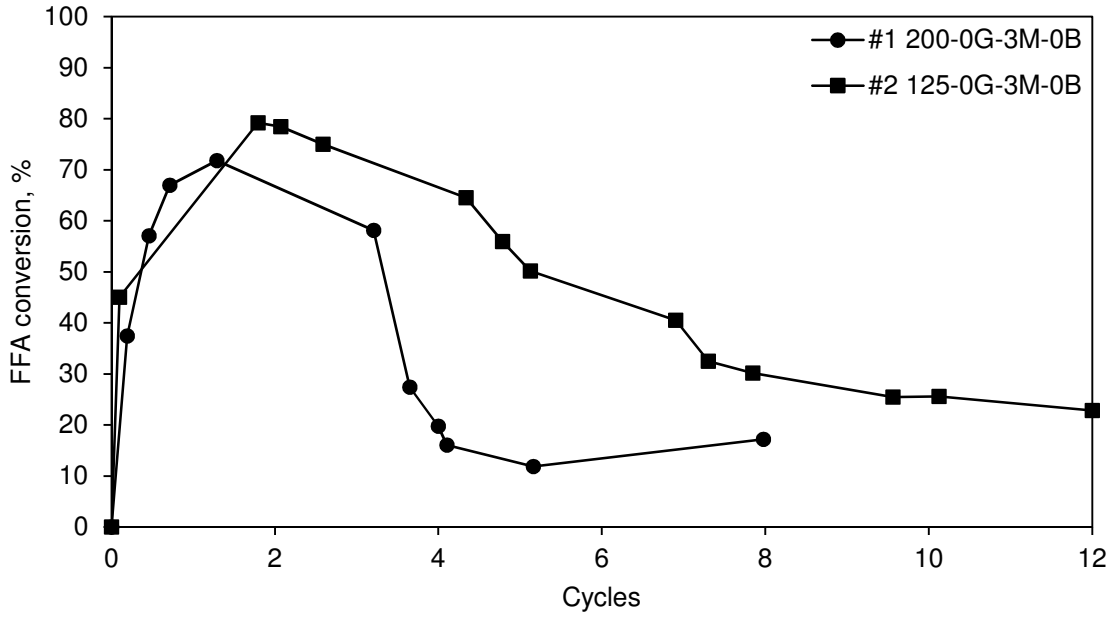


Figure 4.6 FFA conversion at different flow rates. #1 was operated at 200 µL/min and #2 was operated at 125 µL/min. The molar ratio of methanol to FFA was 3:1 and no biodiesel or glycerol were added.

Figure 4.7 shows the effect of glycerol on the FFA conversion. The addition of glycerol to the membrane reactor facilitated the esterification, and the FFA conversion increased as the glycerol

to catalyst mass ratio increased from 0 to 2.6:1. The maximum FFA conversion of 93.16 % was obtained at the mass ratio of 2.6:1 (#5). Besides, the FFA conversion was maintained above 80 % for 5.2 cycles whereas it was only 4 cycles at the mass ratio of 1:1 and 2:1. It was reported that the glycerol had a higher affinity to water and FFA than mono-glyceride and di-glyceride, and that it could act as a substrate for glycerolysis [77]. According to another study [54], most of the enzyme activity was located in an emulsion phase between the oil and water phase due to the enzyme's amphiphilicity. The glycerol was able to compatibilize the FFA and enzyme in a polar phase. The addition of glycerol in the MR decreased the FFA concentration from 2.2 wt% to 0.68 wt%.

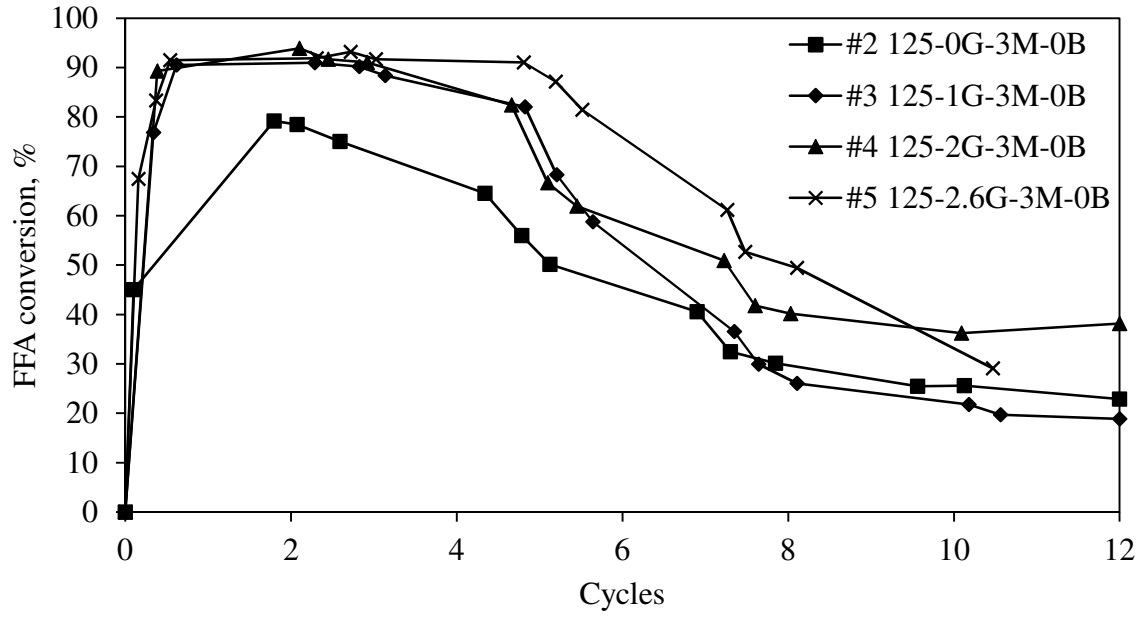


Figure 4.7 The effect of glycerol on the FFA conversion. The mass ratios of glycerol to enzyme of #2, #3, #4 and #5 were 0, 1:1, 2:1 and 2.6:1 respectively. The molar ratio of methanol to FFA was 3:1 and no biodiesel was added.

However, according to Figure 4.8, the pressure in the reactor increased as the mass ratio of glycerol to enzyme increased, owing to the high-viscosity glycerol that formed a gel on the surface of the membrane during the process.

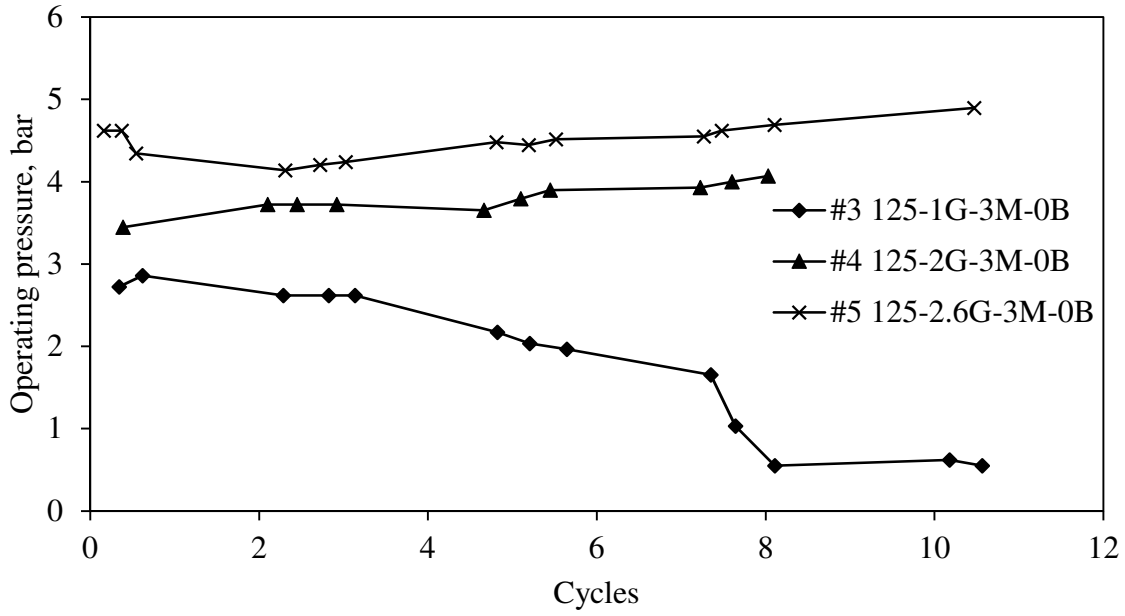


Figure 4.8 The pressure of the FFA methyl esterification using different amount of glycerol. The mass ratios of glycerol to enzyme of #3, #4 and #5 were 1:1, 2:1 and 2.6:1. The molar ratio of methanol to FFA was 3:1 and no biodiesel was added.

In order to lower the pressure across the membrane, biodiesel was added into the feedstock to reduce the viscosity of the mixture. Biodiesel is an ideal solvent as it would be readily available being a product of the process, and there would be no need to remove later in the process. As seen in Figure 4.9, the pressure of #5 was around 4.83 bar, while dropped to less than 2.76 bar after the addition of biodiesel. According to the FFA conversion in Figure 4.10, the introduced biodiesel slightly inhibited the FFA esterification, resulting in a lower FFA conversion of #8 compared to #2, which was also observed in #5 and #7. The FFA conversions in both #5 and #7 were over 80 %, however in #7, the high FFA conversion (>80%) lasted for 5.5 cycles, which was a slightly longer than the 5.2 cycles in #5.

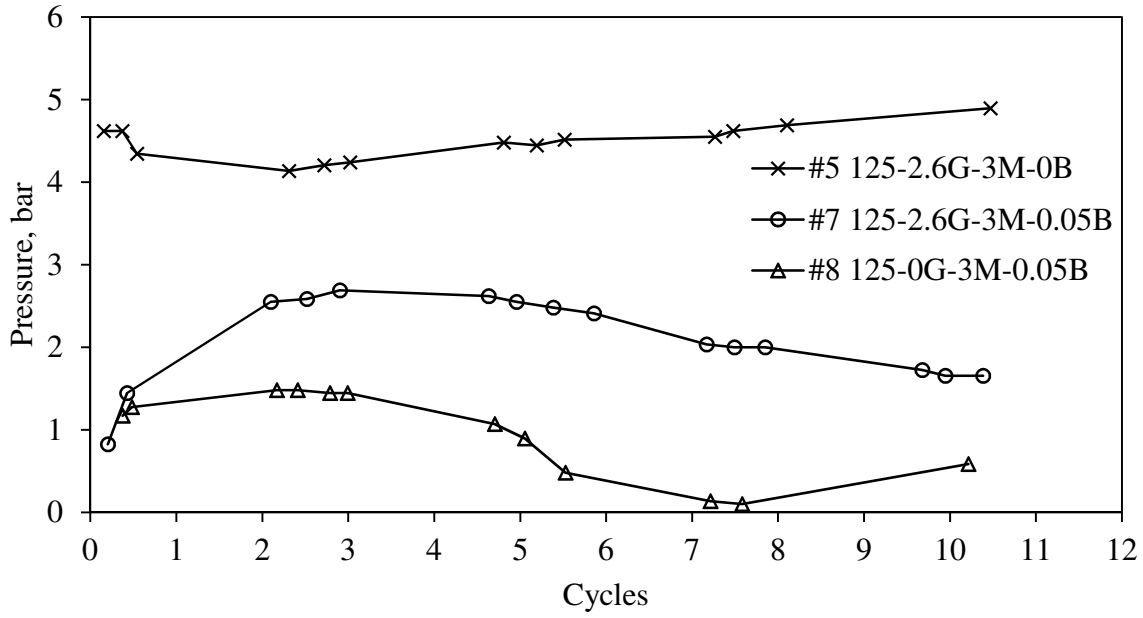


Figure 4.9 The effect of biodiesel on the pressure. The molar ratio of methanol to FFA was 3:1. #5: 2.6:1 mass ratio of glycerol to catalyst, no biodiesel; #7: 2.6:1 mass ratio of glycerol to catalyst, 1:20 mass ratio of biodiesel to acidic oil; #8: no glycerol, 1:20 mass ratio of biodiesel to acidic oil.

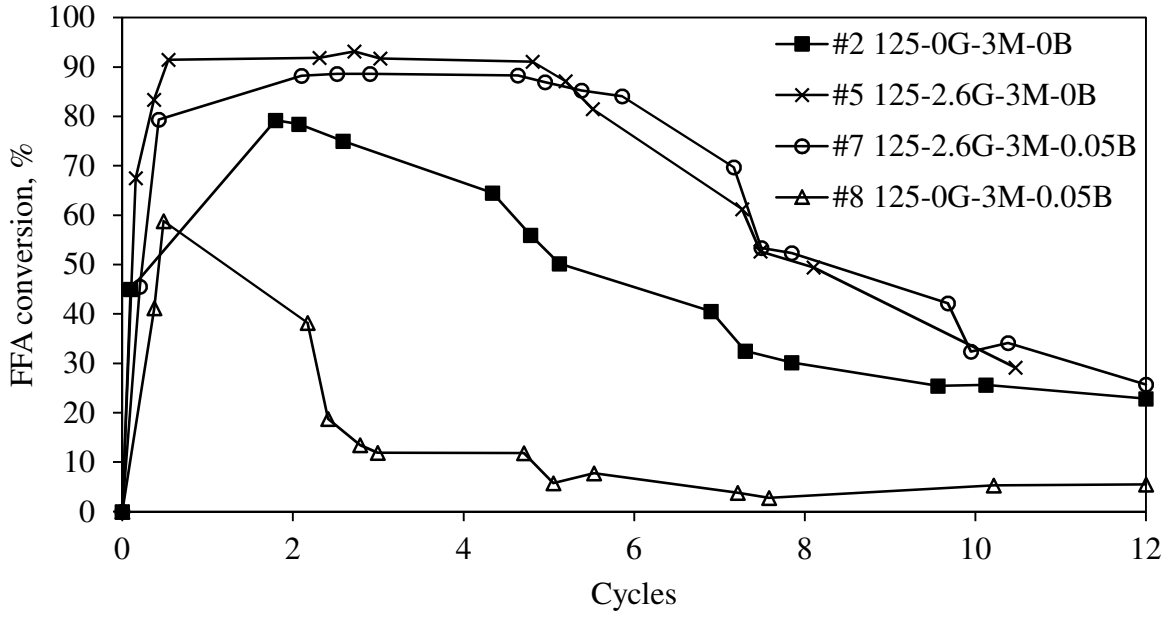


Figure 4.10 The effect of biodiesel on FFA conversion. The molar ratio of methanol to FFA was 3:1. #2: no glycerol, no biodiesel; #5: 2.6:1 mass ratio of glycerol to catalyst, no biodiesel; #7: 2.6:1 mass ratio of glycerol to catalyst, 1:20 mass ratio of biodiesel to acidic oil; #8: no glycerol, 1:20 mass ratio of biodiesel to acidic oil.

Figure 4.11 shows the effect of MeOH contents on FFA conversion during the esterification. It was obvious that the FFA conversion was higher in #5 at a molar ratio of MeOH to FFA of 3:1 than that in #6, which was carried out at a molar ratio of 6:1. The FFA conversion was less than 70 % in #6, mainly due to the loss of enzyme activity caused by excess methanol [65].

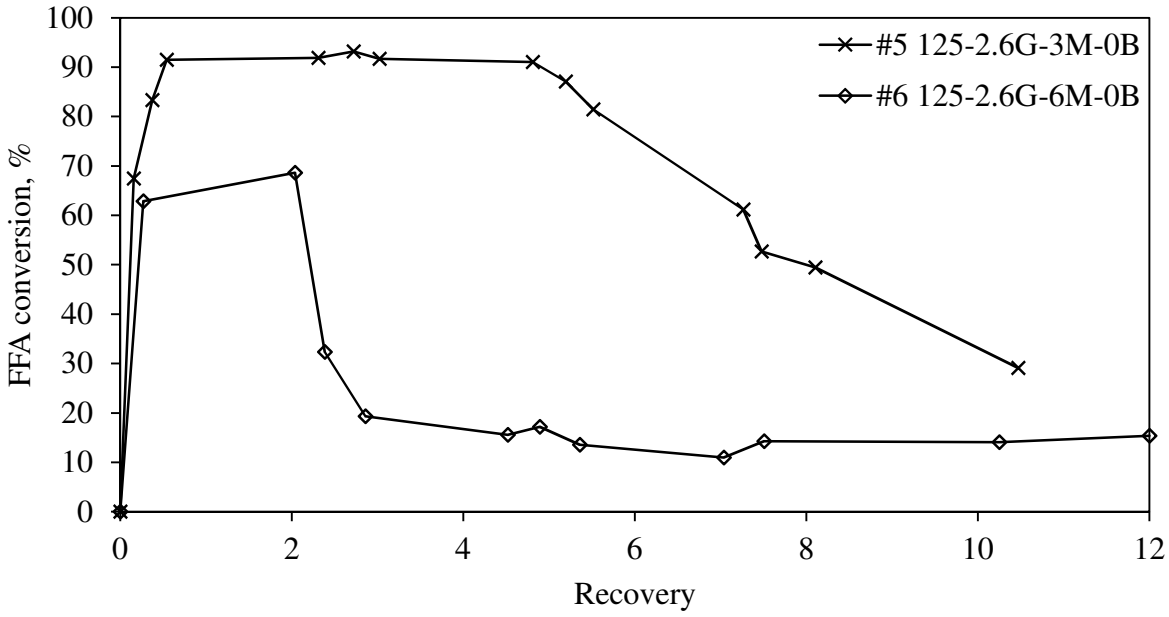


Figure 4.11 The effect of methanol contents on FFA conversion. The molar ratios of MeOH to FFA of #5 and #6 were 3:1 and 6:1. The mass ratio of glycerol to catalyst was 2.6:1 and no biodiesel was added.

Figure 4.12 summarizes the average FFA conversion and the average pressure in each run. The highest average conversion was 92.05 wt% using 2.6:1 mass ratio of glycerol to catalyst and 3:1 malar ratio of MeOH to FFA at 125 µl/min (#6). By adding 5 wt% biodiesel into the acidic oil, the average pressure decreased from 4.21 to 2.62 bar (#7). The average FFA conversion 88.41 wt%, which was not affected much by biodiesel.

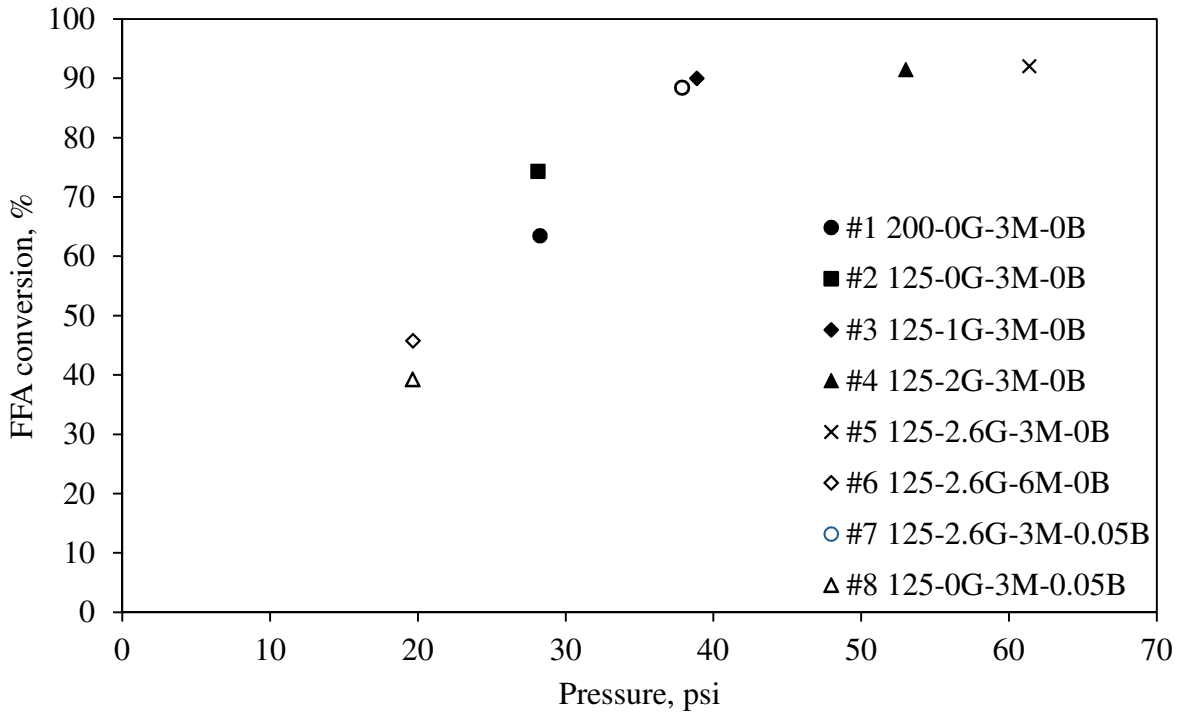
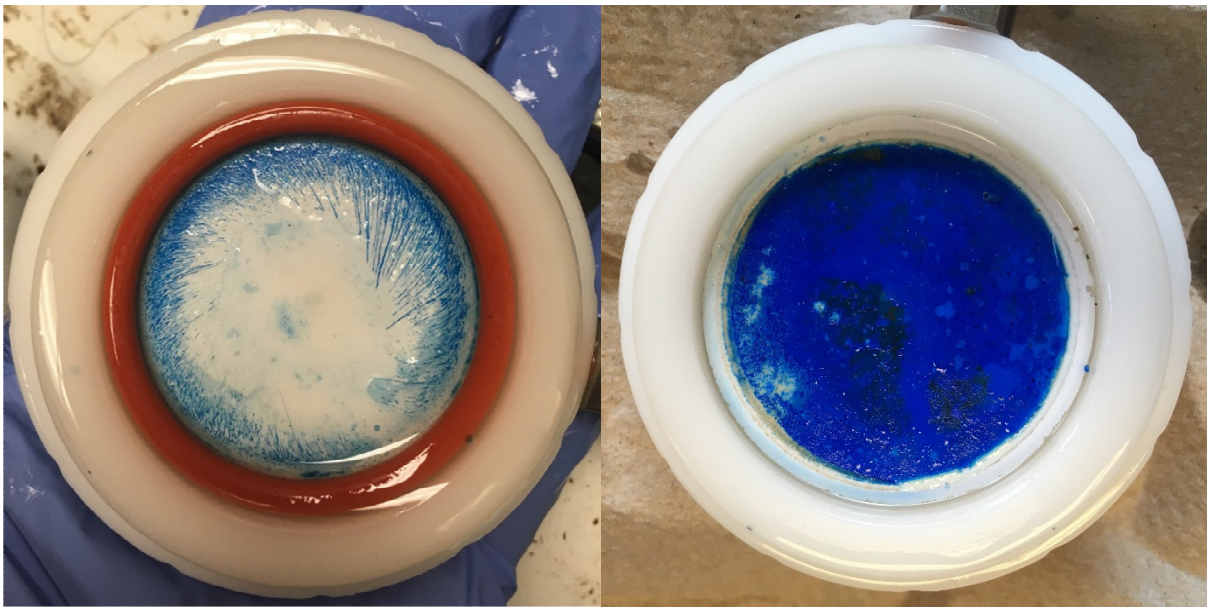


Figure 4.12 The average pressure vs. the average FFA concentration in each run. The average FFA concentration of each run was the average of the four highest FFA conversions in the samples. And the average pressure was the average of the pressures corresponding to the four highest conversions.

4.3.3 Enzyme distribution

The results of the protein test showed that the CBBG in the retentate turned blue, while it didn't turn blue in the permeate, indicating that the enzyme was retained at the membrane surface.

Figure 4.13 shows the protein distribution on the surface of the membrane. With agitation, seen in Figure 4.13 (a), the proteins were dispersed in the cell, thus only a few left on the surface of the membrane. But according to Figure 4.13 (b), when there was no mixing, the glycerol and enzyme accumulated on the suface of the membrane and formed a gel, which led to the membrane fouling and the pressure rising to over 100 psi during the reaction.



(a) With 700 rpm stirring (b) Without stirring

Figure 4.13 Protein distribution after the reaction. The reaction was carried out using 2.6:1 mass ratio of glecerol to catalyst and 3:1 molar ratio of MeOH to FFA at 125 µl/min.

It was reported that the enzyme was amphiphilic located between the oil phase and the polar phase [54], [78]. The added glycerol was dispersed uniformly in the reactor by stirring. It was miscible with the water in the mixture to form more polar glcerol-water droplets, which were able to adsorb the amphiphilic enzymes. Therefore, the enzyme could be retained in the reactor even if the size of the 0.2 µm ceramic membrane used in the experiment was much larger than the size of the enzyme, as shown in Figure 4.14. After the reaction, the lipophilic FAME molecule returned to the oil phase and passed through the membrane with the unreacted TG, while the produced water remained in the droplet and the enzyme continued to catalyze the esterification of other FFA molecules in the mixture.

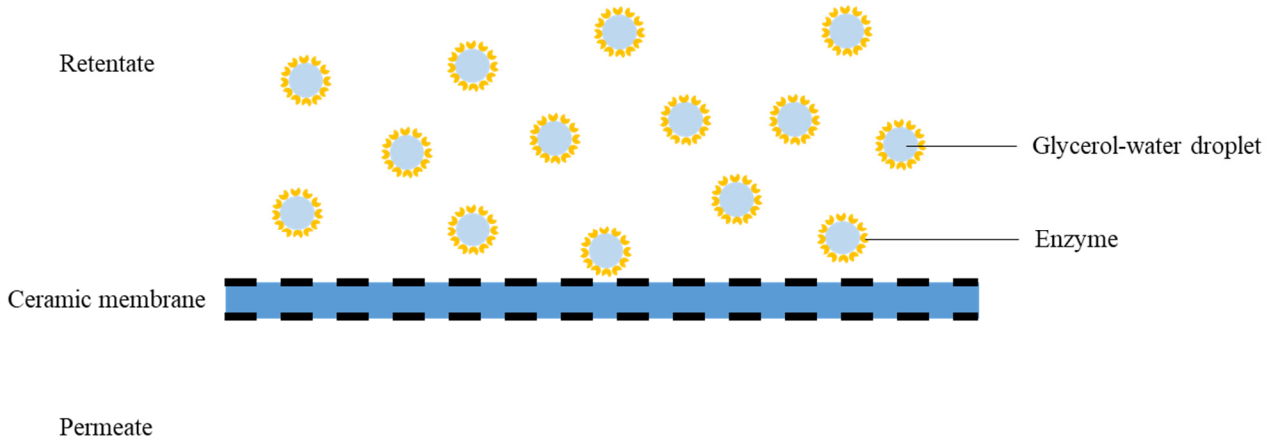


Figure 4.14 Conceptual diagram of membrane reactor.

The enzyme emulsion was retained in the cell by the ceramic microfiltration membrane with a pore size of 0.2 microns which is signoficantly larger than the size of the enzyme. The agitation provided a good mass transfer for the reaction.

4.3.4 The comparison of the reactions in the batch and continuous membrane reactor

The batch reactions were carried out in the membrane reactor under the same conditions as that in run #5. The enzyme after one reaction was separated by centrifugation and used for the subsequent runs. Each batch run was reacted for 560 min, which was the same as the residence time in the MR.

In the first 4 cycles, the FFA conversion in the batch was higher than that in the continuous membrane reactor, as shown in Figure 4.15. However, after 4 cycles, the FFA conversion in the batch significantly decreased. The decrease in FFA conversion could be ascribed to the accumulation of water during each reaction and enzyme loss during the transferring step. In addition, the enzyme transfer from batch to batch was not easy to operate in the industrial pre-treatment process. Unlike the batch, the membrane reactor could retain the enzyme in the reactor

using a ceramic membrane and achieve continuous pre-treatment of the acidic oil, which was convenient and could be used in industry.

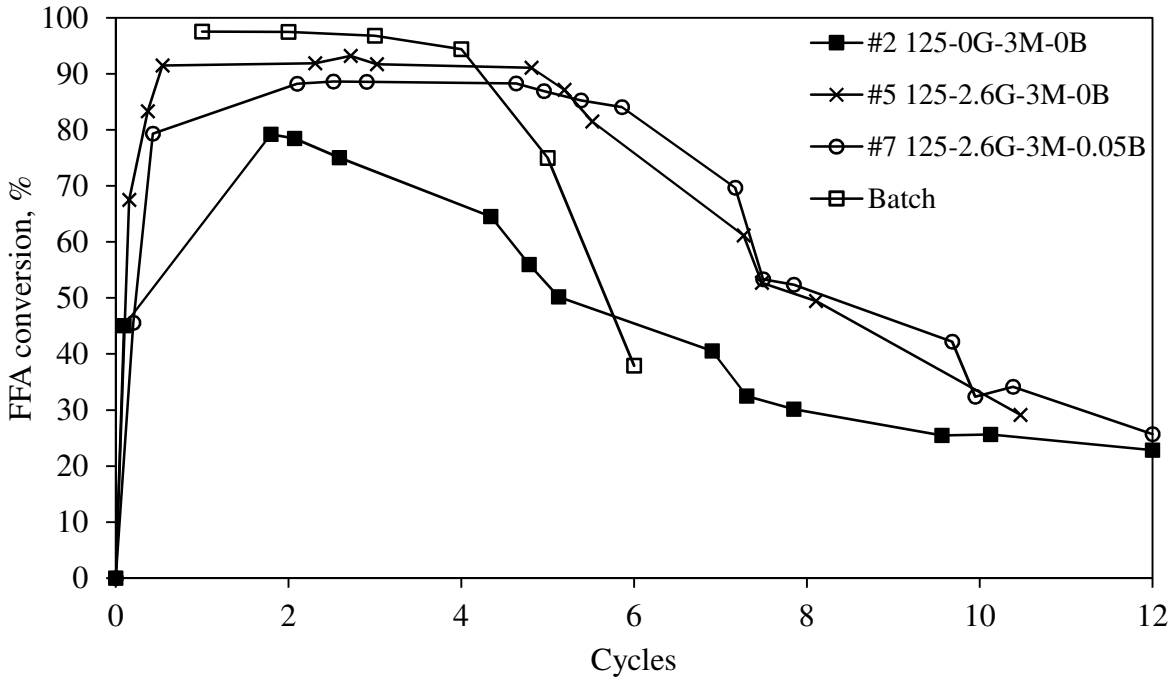


Figure 4.15 FFA conversion in the batch and MR. #2 was carried out using a MeOH to FFA molar ratio of 3:1. #5 and batch runs were carried out using 2.6:1 mass ratio of glecerol to FFA and 3:1 molar ratio of MeOH to FFA. #7 was carried out using 1:20 mass ratio of biodiesel to acidic oil, 2.6:1 mass ratio of glecerol to catalyst and 3:1 molar ratio of MeOH to FFA. #2, #5 and #7 were carried out at 125 µl/min.

4.4 Discussion

The liquid enzymes used in our work were commercially available. It was reported that the enzyme required an aqueous environment to maintain its activity and the glycerol could act as a stabilizer to prevent enzymatic denaturation [79], [80]. The free liquid enzymes have many advantages over immobilized enzymes. Nielsen et al. [80] demonstrated that the addition of an extra solid support can decrease the reaction rate. Besides, the support materical itself and the immobilization process

increased the cost of the preatment techonology. Although the non-immobilized enzymes are less expensive than the immobilized enzymes, they are still more expensive than the chemical catalyst such as acid and base. Therefore, in order to improve the competitiveness of non-immobilized enzymes, the enzyme recovery is required.

In this work, the ceramic membrane with a pore size of 0.2 μm successfully retained the enzymes in the MR by adding glycerol. The reactor was based on the general principle that polar particles formed immiscible droplets in the hydrophobic environment [81]. In the oil-rich reaction system, when the small amount of glycerol added were mixed with the hydrophilic unreacted methanol and produced water, the polar droplets with lager sizes were formed and dispersed in the oil phase by agitation. The amphiphilic enzymes were distributed between the two phases.The droplets were not only the carrier of the enzymes, but also prevented the enzymes with a size of 10 to 80 kD from passing through the 0.2 μm ceramic membrane. Additionally, the droplets could absorb the water generated during the esterification to avoid the hydrolysis of TG. Theoretically, three hydroxyl groups in one molecule of glycerol combined with three molecules of water. As the esterification proceeded, when the hydroxyl groups were saturated with water, the excess water accumulated in the oil phase, leading to the hydrolysis of TG and a decrease in FFA conversion after a few cycles.

4.5 Conclusions

It was obvious that the addition of glycerol could maintain the high FFA conversion for a longer time, since it combined with water to form a uniformly dispersed polar phase. The enzymes were able to be adsorbed on the surface of the polar droplets and retain in the reactor in spite of using 0.2 μm ceramic membrane. The biodiesel was successful in reducing the viscosity of the oil permeating through the membrane which in turn reduced the pressure in the reactor. The highest

FFA conversion was achieved using 3:1 molar ratio of methanol to FFA and 2.6:1 mass ratio of glycerol to catalyst at a flow rate of 125 µL/min. The FFA conversion was maintained over 80 % for 5.2 cycles. With the addition of biodiesel into feedstock, the conversion was slightly lower but maintained over 80 % for nearly 6 cycles. Compared to the MR runs, the batch runs had higher FFA conversion, however, it only maintained for 4 cycles.

Chapter 5 Conclusions and recommendations

5.1 Conclusions

The esterification of FFA in acidic oils was carried out using a new immobilized CALB-TAN catalyst in a PBR system. The FFA concentration after reaction was reduced below the threshold level for base transesterification (0.5 wt%).

It was found from the experimental results that the lowest FFA concentration of 0.23 wt% was obtained at the following conditions: 14-min residence time in a 1/16-inch column, 45℃ reaction temperature and 6:1 MeOH to FFA molar ratio. The FFA concentration was under the threshold after a 14-30 min residence time. The water balance showed that 75 % of water produced during the reaction was removed from the column. The high water-removal ratio enhanced the contact between the catalysts and the FFA and favored esterification. The catalyst was able to retain its activity for 42 days. In addition, the FFA esterification using the CALB-TAN in the PBR showed a better performance than the commercial Novozyme 435 in the PBR.

The use of free liquid lipase-catalyzed esterification in a membrane reactor was also investigated. The reaction using acidic oils containing different amounts of FFA was performed and the results indicated that the production of water increased as the FFA concentration increased. Methanol showed a better performance during the FFA esterification than ethanol, especially when the initial FFA concentration was higher than 5 wt%. Considering the enzyme activity and the effect of alcohols, an acidic oil containing 10 wt% FFA was used as the feedstock in continuous reaction studies. It was found that the added glycerol could combine with water to form a polar phase dispersed in the oil phase. The dispersed polar droplets became enzyme binders that prevented the enzymes from passing through the membrane, while they also increased the viscosity of the mixture, leading to a pressure increase. Biodiesel was added to the feedstock to reduce the viscosity

of the mixture in the membrane reactor. The result was a decrease in the operating pressure of the reactor. For the membrane reactor: The FFA conversion was over 80 % for 5.2 cycles at 45℃, 125 µL/min, 3:1 molar ratio of MeOH to FFA, 2.6:1 mass ratio of glycerol to enzyme. The batch reactor could only maintain 80% conversion over 4 cycles. The addition of 5 wt% biodiesel to the feed mixture reduced the pressure in the membrane reactor and maintained the FFA conversion over 80 % for 6 cycles.

5.2 Recommendations

Little information on FFA esterification in acidic oils in a continuous PBR, especially in a continuous membrane reactor, is available in the literature. During the MR runs using 10 wt% acidic oil, none of the results met the FFA requirement of <0.5 wt%. The lowest FFA concentration was 0.68 wt% in the continuous run. Thus, a two-step esterification should be further studied.

A mixture of canola oil and FFA was used to simulate WCO. The reaction with actual WCO from restaurants would be more complicated due to the oxidation at high temperature and the potential enzyme inhibition caused by contaminants in the oils. A future study should examine the reaction of actual WCO in a two-step esterification process to reduce the FFA to an acceptable level for base transesterification.

Chapter 6 References

[1] J. Saleh, A. Y. Tremblay, and M. A. Dubé, "Glycerol removal from biodiesel using membrane separation technology," *Fuel*, vol. 89, no. 9, pp. 2260–2266, 2010.

[2] M. M. M. Gui, K. T. T. Lee, and S. Bhatia, "Feasibility of edible oil vs. non-edible oil vs. waste edible oil as biodiesel feedstock," *Energy*, vol. 33, no. 11, pp. 1646–1653, Nov. 2008.

[3] A. Demirbas, "Political, economic and environmental impacts of biofuels: A review," *Appl. Energy*, vol. 86, no. SUPPL. 1, pp. S108–S117, Nov. 2009.

[4] A. N. Phan and T. M. Phan, "Biodiesel production from waste cooking oils," *Fuel*, vol. 87, no. 17–18, pp. 3490–3496, 2008.

[5] G. Toop, S. Alberici, M. Spoettle, S. Huygen van, and W. Ulf, "Trends in the UCO market," 2013.

[6] M. K. Lam, K. T. Lee, and A. R. Mohamed, "Homogeneous, heterogeneous and enzymatic catalysis for transesterification of high free fatty acid oil (waste cooking oil) to biodiesel: A review," *Biotechnol. Adv.*, vol. 28, no. 4, pp. 500–518, Jul. 2010.

[7] "LCFS Pathway Certified Carbon Intensities." [Online]. Available: https://www.arb.ca.gov/fuels/lcfs/fuelpathways/pathwaytable.htm. [Accessed: 15-Aug-2018].

[8] I. M. Atadashi, M. K. Aroua, and A. A. Aziz, "High quality biodiesel and its diesel engine application: A review," *Renew. Sustain. Energy Rev.*, vol. 14, no. 7, pp. 1999–2008, Sep. 2010.

[9] A. Tafesh and S. Basheer, "Pretreatment Methods in Biodiesel Production Processes," Springer, Berlin, Heidelberg, 2013, pp. 417–434.

[10] P. I. Lacey and S. A. Howell, "Fuel Lubricity Reviewed," 1998.

[11] A. Demirbaş, "Biodiesel from vegetable oils via transesterification in supercritical methanol," *Energy Convers. Manag.*, vol. 43, no. 17, pp. 2349–2356, Nov. 2002.

[12] W. Kdrbltz, "BIODIESEL PRODUCTION IN EUROPE AND NORTH AMERICA, AN ENCOURAGING PROSPECT," *PERGAMON Renew. Energy*, vol. 16, pp. 1078–1083, 1999.

[13] N. Traviss, B. A. Thelen, J. K. Ingalls, and M. D. Treadwell, "Biodiesel versus diesel: A pilot study comparing exhaust exposures for employees at a rural municipal facility," *J. Air Waste Manag. Assoc.*, vol. 60, no. 9, pp. 1026–1033, Sep. 2010.

[14] A. Chhetri, K. Watts, and M. Islam, "Waste Cooking Oil as an Alternate Feedstock for Biodiesel Production," *Energies*, vol. 1, no. 1, pp. 3–18, Apr. 2008.

[15] M. Mofijur, A. E. Atabani, H. H. Masjuki, M. A. Kalam, and B. M. Masum, "A study on the effects of promising edible and non-edible biodiesel feedstocks on engine performance and emissions production: A comparative evaluation," *Renew. Sustain. Energy Rev.*, vol. 23, pp. 391–404, Jul. 2013.

[16] D. Bailey and G. Solomon, “Pollution prevention at ports: clearing the air,” *Environ. Impact Assess. Rev.*, vol. 24, no. 7–8, pp. 749–774, Oct. 2004.

[17] A. Pradhan *et al.*, “Energy Life-Cycle Assessment of Soybean Biodiesel,” 2009.

[18] A. Gahlaut, V. Kumar, D. Gupta, and N. Kumar, “Life Cycle Analysis and Feasibility of the Use of Waste Cooking Oil as Feedstock for Biodiesel,” *Int. J. Adv. smart Converg.*, vol. 4, no. 1, pp. 162–178, May 2015.

[19] P. Cao, M. A. Dubé, and A. Y. Tremblay, “High-purity fatty acid methyl ester production from canola, soybean, palm, and yellow grease lipids by means of a membrane reactor,” *Biomass and Bioenergy*, vol. 32, no. 11, pp. 1028–1036, Nov. 2008.

[20] A. E. Atabani, A. S. Silitonga, I. A. Badruddin, T. M. I. Mahlia, H. H. Masjuki, and S. Mekhilef, “A comprehensive review on biodiesel as an alternative energy resource and its characteristics,” *Renew. Sustain. Energy Rev.*, vol. 16, no. 4, pp. 2070–2093, May 2012.

[21] L. C. Meher, D. Vidya Sagar, and S. N. Naik, “Technical aspects of biodiesel production by transesterification—a review,” *Renew. Sustain. Energy Rev.*, vol. 10, no. 3, pp. 248–268, Jun. 2006.

[22] D. Y. C. Leung, X. Wu, and M. K. H. Leung, “A review on biodiesel production using catalyzed transesterification,” *Appl. Energy*, vol. 87, no. 4, pp. 1083–1095, Apr. 2010.

[23] A. Abbaszaadeh, B. Ghobadian, M. R. Omidkhah, and G. Najafi, “Current biodiesel production technologies: A comparative review,” *Energy Convers. Manag.*, vol. 63, pp. 138–148, Nov. 2012.

[24] X. Yuan, J. Liu, G. Zeng, J. Shi, J. Tong, and G. Huang, “Optimization of conversion of waste rapeseed oil with high FFA to biodiesel using response surface methodology,” *Renew. Energy*, vol. 33, no. 7, pp. 1678–1684, Jul. 2008.

[25] L. Soh Kheang, F. Subari, and S. A. Aishah Syed Kadir, “PRE - TREATMENT OF PALM OLEIN - DERIVED USED FRYING OIL AS A FEEDSTOCK FOR NON - FOOD APPLICATIONS,” 2011.

[26] P. Felizardo *et al.*, “Study on the glycerolysis reaction of high free fatty acid oils for use as biodiesel feedstock,” *Fuel Process. Technol.*, vol. 92, pp. 1225–1229, 2011.

[27] Y. Liu, E. Lotero, and J. G. Goodwin, “A comparison of the esterification of acetic acid with methanol using heterogeneous versus homogeneous acid catalysis,” *J. Catal.*, vol. 242, pp. 278–286, 2006.

[28] A. Demirbas, “Biodiesel from waste cooking oil via base-catalytic and supercritical methanol transesterification,” 2009.

[29] N. Özbay, N. Oktar, and N. A. Tapan, “Esterification of free fatty acids in waste cooking oils (WCO): Role of ion-exchange resins,” *Fuel*, vol. 87, no. 10–11, pp. 1789–1798, Aug. 2008.

[30] J. Price, B. Hofmann, V. T. L. Silva, M. Nordblad, J. M. Woodley, and J. K. Huusom, “Mechanistic modeling of biodiesel production using a liquid lipase formulation,”

Biotechnol. Prog., vol. 30, no. 6, pp. 1277–1290, Nov. 2014.

[31] D. A. Denton, "Use of base-treated inorganic porous adsorbents for removal of contaminants," 03-Apr-1991.

[32] A. Talebian-Kiakalaieh, N. Aishah, S. Amin, and H. Mazaheri, "A review on novel processes of biodiesel production from waste cooking oil," 2013.

[33] J. M. Marchetti and A. F. Errazu, "Esterification of free fatty acids using sulfuric acid as catalyst in the presence of triglycerides," *Biomass and Bioenergy*, vol. 32, no. 9, pp. 892–895, Sep. 2008.

[34] M. Di Serio, R. Tesser, M. Dimiccoli, F. Cammarota, M. Nastasi, and E. Santacesaria, "Synthesis of biodiesel via homogeneous Lewis acid catalyst," *J. Mol. Catal. A Chem.*, vol. 239, no. 1–2, pp. 111–115, Sep. 2005.

[35] E. F. Aransiola, T. V Ojumu, O. O. Oyekola, T. F. Madzimbamuto, and D. I. O. Ikhu-Omoregbe, "A review of current technology for biodiesel production: State of the art," *Biomass and Bioenergy*, vol. 61, pp. 276–297, 2014.

[36] M. M. Canakci and J. Van J. Van Gerpen, "BIODIESEL PRODUCTION VIAACID CATALYSIS," *Trans. ASAE*, vol. 42, no. 5, pp. 1203–1210, 1999.

[37] M. G. Kulkarni, R. Gopinath, L. C. Meher, and A. K. Dalai, "Solid acid catalyzed biodiesel production by simultaneous esterification and transesterification," *Green Chem.*, vol. 8, no. 12, p. 1056, Dec. 2006.

[38] K. Suwannakarn, E. Lotero, K. Ngaosuwan, and J. G. Goodwin, "Simultaneous free fatty acid esterification and triglyceride transesterification using a solid acid catalyst with in situ removal of water and unreacted methanol," *Ind. Eng. Chem. Res.*, vol. 48, no. 6, pp. 2810–2818, 2009.

[39] Y.-M. Park, D.-W. Lee, D.-K. Kim, J.-S. Lee, and K.-Y. Lee, "The heterogeneous catalyst system for the continuous conversion of free fatty acids in used vegetable oils for the production of biodiesel," 2008.

[40] D. Kusdiana and S. Saka, "Effects of water on biodiesel fuel production by supercritical methanol treatment," *Bioresour. Technol.*, vol. 91, no. 3, pp. 289–295, 2004.

[41] D. Kusdiana and S. Saka, "Two-Step Preparation for Catalyst-Free Biodiesel Fuel Production," *Appl. Biochem. Biotechnol.*, vol. 115, no. 1–3, pp. 0781–0792, 2004.

[42] K. De Boer and P. A. Bahri, "Supercritical methanol for fatty acid methyl ester production: A review," 2011.

[43] M. M. R. Talukder, J. C. Wu, S. K. Lau, L. C. Cui, G. Shimin, and A. Lim, "Comparison of novozym 435 and amberlyst 15 as heterogeneous catalyst for production of biodiesel from palm fatty acid distillate," *Energy and Fuels*, vol. 23, no. 1, pp. 1–4, 2009.

[44] S. Chongkhong, U. Kanjaikaew, and C. Tongurai, "A Review of FFA Esterification for Biodiesel Production," 2012.

[45] A. Guldhe, B. Singh, T. Mutanda, K. Permaul, and F. Bux, "Advances in synthesis of

biodiesel via enzyme catalysis: Novel and sustainable approaches," 2014.

[46] Y. Shimada *et al.*, "Conversion of vegetable oil to biodiesel using immobilized *Candida antarctica* lipase," *J. Am. Oil Chem. Soc.*, vol. 76, no. 7, pp. 789–793, Jul. 1999.

[47] L. Deng, X. Xu, G. G. Haraldsson, T. Tan, and F. Wang, "Enzymatic production of alkyl esters through alcoholysis: A critical evaluation of lipases and alcohols," *JAOCS, J. Am. Oil Chem. Soc.*, vol. 82, no. 5, pp. 341–347, May 2005.

[48] Y. Watanabe, Y. Shimada, A. Sugihara, and Y. Tominaga, "Conversion of degummed soybean oil to biodiesel fuel with immobilized Candida antarctica lipase," *J. Mol. Catal. B-Enzymatic*, vol. 17, no. 3–5, pp. 151–155, 2002.

[49] Y. Watanabe, Y. Shimada, A. Sugihara, and Y. Tominaga, "Enzymatic conversion of waste edible oil to biodiesel fuel in a fixed-bed bioreactor," *J. Am. Oil Chem. Soc.*, vol. 78, no. 7, pp. 703–707, Jul. 2001.

[50] W. Du, Y. Xu, D. Liu, and J. Zeng, "Comparative study on lipase-catalyzed transformation of soybean oil for biodiesel production with different acyl acceptors," *J. Mol. Catal. B Enzym.*, vol. 30, no. 3–4, pp. 125–129, Aug. 2004.

[51] M. K. Modi, J. R. C. Reddy, B. V. S. K. Rao, and R. B. N. Prasad, "Lipase-mediated conversion of vegetable oils into biodiesel using ethyl acetate as acyl acceptor," *Bioresour. Technol.*, vol. 98, no. 6, pp. 1260–1264, Apr. 2007.

[52] A. F. Hsu, K. Jones, W. N. Marmer, and T. A. Foglia, "Production of alkyl esters from tallow and grease using lipase immobilized in a phyllosilicate sol-gel," *J.Am.Oil.Che.Soc.*, vol. 78, no. 6, pp. 585–588, 2001.

[53] F. Hasan, A. A. Shah, and A. Hameed, "Industrial applications of microbial lipases," *Enzyme Microb. Technol.*, vol. 39, no. 2, pp. 235–251, Jun. 2006.

[54] P. M. Nielsen, A. Rancke-Madsen, H. C. Holm, and R. Burton, "Production of Biodiesel Using Liquid Lipase Formulations," *J. Am. Oil Chem. Soc.*, vol. 93, no. 7, pp. 3–8, 2016.

[55] M. Nordblad, A. K. Pedersen, A. Rancke-Madsen, and J. M. Woodley, "Enzymatic pretreatment of low-grade oils for biodiesel production," *Biotechnol. Bioeng.*, vol. 113, no. 4, pp. 754–760, 2016.

[56] K. F. Haigh, G. T. Vladisavljević, J. C. Reynolds, Z. Nagy, and B. Saha, "Kinetics of the pre-treatment of used cooking oil using Novozyme 435 for biodiesel production," *Chem. Eng. Res. Des.*, vol. 92, no. 4, pp. 713–719, 2014.

[57] M. Canakci and H. Sanli, "Biodiesel production from various feedstocks and their effects on the fuel properties," *J. Ind. Microbiol. Biotechnol.*, vol. 35, no. 5, pp. 431–441, May 2008.

[58] A. Karmakar, S. Karmakar, and S. Mukherjee, "Properties of various plants and animals feedstocks for biodiesel production," *Bioresour. Technol.*, vol. 101, no. 19, pp. 7201–7210, Oct. 2010.

[59] S. Chongkhong, C. Tongurai, P. Chetpattananondh, and C. Bunyakan, "Biodiesel

production by esterification of palm fatty acid distillate," *Biomass and Bioenergy*, vol. 31, no. 8, pp. 563–568, Aug. 2007.

[60] V. F. Marulanda, G. Anitescu, and L. L. Tavlarides, "Investigations on supercritical transesterification of chicken fat for biodiesel production from low-cost lipid feedstocks," *J. Supercrit. Fluids*, vol. 54, no. 1, pp. 53–60, Jul. 2010.

[61] B. Chen, J. Hu, E. M. Miller, W. Xie, M. Cai, and R. A. Gross, "*Candida antarctica* Lipase B Chemically Immobilized on Epoxy-Activated Micro- and Nanobeads: Catalysts for Polyester Synthesis," *Biomacromolecules*, vol. 9, no. 2, pp. 463–471, Feb. 2008.

[62] J. Van Gerpen and J. Van Gerpen, "Biodiesel processing and production," *Fuel Process. Technol.*, vol. 86, no. 10, pp. 1097–1107, Jun. 2005.

[63] K. Roebuck and A. Y. Tremblay, "Highly permeable twinned alumina nanoparticles for the precoat filtration of fine colloids," *Sep. Purif. Technol.*, vol. 182, pp. 197–206, Jul. 2017.

[64] Y. Wang, S. Ou, P. Liu, F. Xue, and S. Tang, "Comparison of two different processes to synthesize biodiesel by waste cooking oil," *J. Mol. Catal. A Chem.*, vol. 252, no. 1–2, pp. 107–112, Jun. 2006.

[65] B. Castillo López *et al.*, "Production of biodiesel from vegetable oil and microalgae by fatty acid extraction and enzymatic esterification," *J. Biosci. Bioeng.*, vol. 119, no. 6, pp. 706–711, 2015.

[66] H. Chen, H. Ju, T. Wu, … Y. L.-B. R., and U. 2010, "Continuous production of lipase-catalyzed biodiesel in a packed-bed reactor: optimization and enzyme reuse study," *downloads.hindawi.com*, 2010.

[67] S. N. Sahasrabudhe, V. Rodriguez-Martinez, M. O'Meara, and B. E. Farkas, "Density, viscosity, and surface tension of five vegetable oils at elevated temperatures: Measurement and modeling," *Int. J. Food Prop.*, pp. 1–17, Dec. 2017.

[68] M. Canakci, "Combustion characteristics of a turbocharged DI compression ignition engine fueled with petroleum diesel fuels and biodiesel," *Bioresour. Technol.*, vol. 98, no. 6, pp. 1167–1175, Apr. 2007.

[69] K. Jacobson, R. Gopinath, L. C. Meher, and A. K. Dalai, "Solid acid catalyzed biodiesel production from waste cooking oil," *Appl. Catal. B Environ.*, vol. 85, no. 1–2, pp. 86–91, Dec. 2008.

[70] D. Y. C. Leung and Y. Guo, "Transesterification of neat and used frying oil: Optimization for biodiesel production," *Fuel Process. Technol.*, vol. 87, no. 10, pp. 883–890, Oct. 2006.

[71] H. Taher and S. Al-Zuhair, "The use of alternative solvents in enzymatic biodiesel production: a review," *Biofuels, Bioprod. Biorefining*, vol. 11, no. 1, pp. 168–194, Jan. 2017.

[72] S. Fatimah, A. Halim, A. H. Kamaruddin, and W. J. N. Fernando, "Continuous biosynthesis of biodiesel from waste cooking palm oil in a packed bed reactor: Optimization using response surface methodology (RSM) and mass transfer studies," 2008.

[73] Y. Watanabe, T. Nagao, Y. Nishida, Y. Takagi, and Y. Shimada, "Enzymatic production of

fatty acid methyl esters by hydrolysis of acid oil followed by esterification," *JAOCS, J. Am. Oil Chem. Soc.*, vol. 84, no. 11, pp. 1015–1021, 2007.

[74] M. Zielińska and M. Galik, "Use of Ceramic Membranes in a Membrane Filtration Supported by Coagulation for the Treatment of Dairy Wastewater.," *Water. Air. Soil Pollut.*, vol. 228, no. 5, p. 173, 2017.

[75] G. M. Rios, M. P. Belleville, D. Paolucci, and J. Sanchez, "Progress in enzymatic membrane reactors – a review," *J. Memb. Sci.*, vol. 242, no. 1–2, pp. 189–196, Oct. 2004.

[76] M. C. S. Gomes, N. C. Pereira, and S. T. D. de Barros, "Separation of biodiesel and glycerol using ceramic membranes," *J. Memb. Sci.*, vol. 352, no. 1–2, pp. 271–276, Apr. 2010.

[77] F. Gö??üs, S. Fadılo??lu, and O. N. Çiftçi, "Change in glyceride composition of olive pomace oil during enzymatic esterification," *Nahrung/Food*, vol. 48, no. 3, pp. 205–208, Jun. 2004.

[78] T. M. Deboni, E. A. C. Batista, and A. J. A. Meirelles, "Equilibrium , Kinetics , and Thermodynamics of Soybean Oil Deacidi fi cation Using a Strong Anion Exchange Resin," *Ind. Eng. Chem. Res.*, 2015.

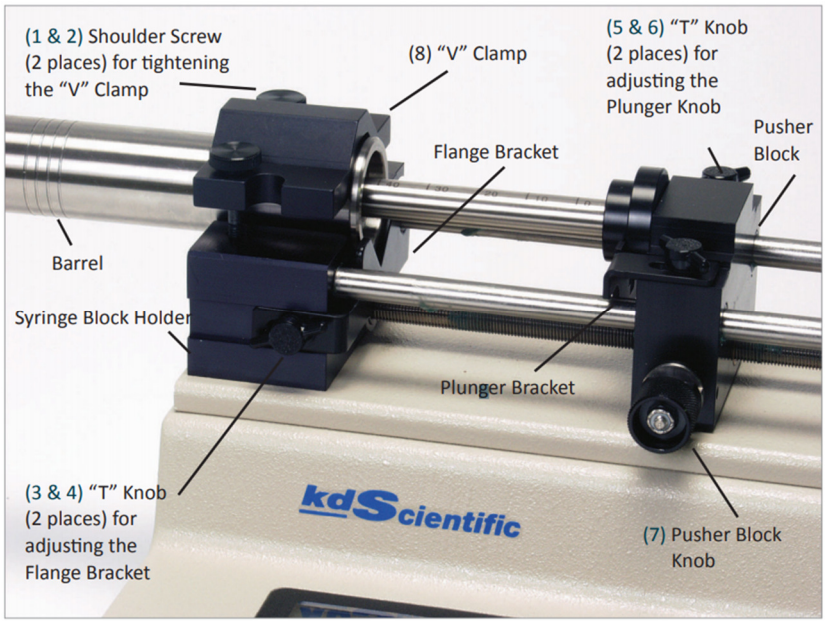
[79] D. von der Haar, A. St??bler, R. Wichmann, and U. Schweiggert-Weisz, "Enzymatic esterification of free fatty acids in vegetable oils utilizing different immobilized lipases," *Biotechnol. Lett.*, vol. 37, no. 1, pp. 169–174, 2015.

[80] P. M. Nielsen, J. Brask, and L. Fjerbaek, "Enzymatic biodiesel production: Technical and economical considerations," *Eur. J. Lipid Sci. Technol.*, vol. 110, no. 8, pp. 692–700, Aug. 2008.

[81] I. M. Atadashi, M. K. Aroua, and A. A. Aziz, "Biodiesel separation and purification: A review," *Renew. Energy*, vol. 36, no. 2, pp. 437–443, Feb. 2011.

Appe□dix

Steps for the operation of KD Scientific 410 pump



Note: these steps will be explained with an example in parentheses { }, and the figure above shows the clamping for the stainless steel syringe.

PBR system

1. Choose a syringe that is suitable for the pump (diameter / length) and install it on the pump.
2. Install the valves and hoses.
3. Open the air valve and turn on the pump.
4. Loosen the Pusher Block Knob (7) by rotating the knob 90° clockwise and infuse 40 ml feedstock by pulling out the Pusher Block.

5. Engage the Pusher Block by pulling the Plunger Knob (7) out and rotate the knob 90° clockwise.
6. Click on *select* to program the pump.
7. Move the cursor with the arrows to *Dia* and write a diameter for the syringe {e.g., 28.25 mm varies depending on the syringe}.
8. Move the cursor with the arrows to *Mode* and click on *select*.
9. Choose *Vol* mode and enter the volume and flowrate for the program {e.g., 40 ml and 7µL/min}.
10. To start the pump, click on *run/stop*.
11. The pump will run automatically until program complete. To stop the pump during the run, press *run/stop*.

MR system

Note: the first few steps for operating the MR system are the same as step 1 to 7 in the PBR system.

1. Enter the volume of the syringe {e.g., 40 ml varies according to the syringe} and press *select*.
2. Move the cursor to *Mode*, press *select* and find the *Prgm* option. Click again on *select*.
3. Once in *Prgm*, press *select*. Then there should be *Step* on the screen. Press *select* again to start the programming sequence.
4. The next option is *Num. of steps*. Enter the desired number {e.g., 2 steps}

5. The machine will then ask for *Edit Step 1*, click on *select*.
6. Now, set the desired duration with the numbers on the pump and press enter this time {e.g., 8 min}
7. For all the next steps, use *enter* to enter what you choose.
8. *Edit Step 1* should appear on the screen. Press *Enter*.
9. The pump will introduce the Infuse or Withdraw option. Choose the required process {e.g., Withdraw}.
10. Then enter the flow rate for the beginning and end of the process {e.g., 5000 µL/min for both}.
11. The next option will ask Pinout; just make sure it's *"1Pinout: 1 = H, 6 = H"*, then weigh in.
12. The pump then designates the choice of an active or inactive pause (e.g., Pause: inactive}.
13. Then it will ask if a loop is needed. Choose yes or no and save the first step {e.g.: No}.
14. Next move on to *Next Step*.
15. Press enter to continue *Edit Step 2*.
16. Now set up step 2. Enter the time {e.g.: 320 min}.
17. Select *Infuse* this time since at step 9 it was *Withdraw*.
18. Write a flow rate of 125 µL / min for start and end.

19. Press *enter* for pinout and inactive for pause option.
20. Click on *Yes* for Loop.
21. The pump will ask for Loop to step; put *Step 1* since this will program a continuous Loop.
22. Then a loop count will be asked; write the desired number {e.g.: 20} (Note: loop count is the number of loops to be repeated. If n Loop is written, the pump will make n + 1 cycle where n is the number entered in the pump).
23. Save the step and click on *Done* to complete the programming.
24. To start the pump, click on *run/stop*.
25. The pump will run automatically until program complete. To stop the pump during the run, press *run/stop*.

Note: The flows entered in the machine must be very accurate, otherwise an error could be seen especially during a long process, so the rounding of significant figures is very important. This is to say, rounding must be applied which offers a minimum margin of error, since the pump only takes 4 significant digits.