BIOROUTE PRODUCTION OF BIODIESEL USING *CERBERA ODOLLAM* OIL IN PACKED BED REACTOR

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2011

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by

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Thesis submitted of the requirements for the degree
of Master of Science

DECEMBER 2011

ACKNOWLEDGEMENTS

Alhamdulillah, all praises to Allah the Al-Mighty for His strengths and blessings in completing my master research work.

First and foremost my sincere gratitude to my supervisor, Professor Dr. Azlina Harun @ Kamaruddin, who has supported me thoughout my study with her patience and knowledge whilst allowing me the room to work in my own way. I attribute the level of my Masters degree to her encouragement and effort and without her this thesis, would not have been completed or written. One simply could not wish for a better or friendlier supervisor. I also would like to convey my appreciation to my co-supervisor Associate Professor Dr. Lee Keat Teong for his advices. I am really grateful to complete my study under the supervisions of Professor Dr. Azlina Harun @ Kamaruddin and Associate Professor Dr. Lee Keat Teong.

Also I am very much indebted to Ministry of Science, Technology and Innovation (MOSTI) for providing the financial support National Science Fellowship (NSF). Sincere thanks to all technicians and staff of School of Chemical Engineering for their cooperation and warmest helping hand.

My deepest gratitude goes to my beloved family for their great support through all the times. Last but not least, thanks to all my friends in School of Chemical engineering and especially to all of my friends under the supervision of Prof. Dr. Azlina for your sincere help, concern, moral support and kindness. Thanks for the friendship and memories.

Siti Norrahmah Ab Rahaman

December 2011

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LIST OF SYMBOLS

α	Alpha (axial distance from center point which makes the design rotatable)	
β_o	Constant coefficient	
β_i	Coefficient for the linear effect	
β_{ii}	Coefficient for the quadratic effect	
β_{ij}	Coefficient for the interaction effect	
σ	Standard deviation	
χ_i , χ_j	Factors (independent variables)	
ε	Error	
ε_v	Void fraction	
τ_e	Time of exposure	
A	Substrate (triglycerides)	
A_P	Peak area of methyl palmitate	
A_O	Peak area of methyl oleate	
A_L	Peak area of methyl linoleate	
A_S	Peak area of methyl stearate	
A_M	Peak area of methyl myristate	
$A_{P.STD}$	Peak area of standard methyl palmitate	
$A_{O.STD}$	Peak area of standard methyl oleate	
$A_{L.STD}$	Peak area of standard methyl linoleate	
$A_{S.STD}$	Peak area of standard methyl stearate	
$A_{M.STD}$	Peak area of standard methyl myristate	
A_{IS}	Peak area of the internal standard	
A_x	Column cross section area	cm^2

a_m	Area of mass transfer	cm^2cm^{-3}
C_o	Substrate concentration in the bulk	mol/L
C_s	Substrate concentration at the surface of the immobilized enzyme	mol/L
$C_{P.STD}$	Concentration of standard methyl palmitate	g/ml
$C_{O.STD}$	Concentration of standard methyl oleate	g/ml
$C_{L.STD}$	Concentration of standard methyl linoleate	g/ml
$C_{S.STD}$	Concentration of standard methyl stearate	g/ml
$C_{M.STD}$	Concentration of standard methyl myristate	g/ml
C.V	Coefficient of variance	
DF	Dilution factors	
Df	Diffusivity	
D	Product (FAME)	
dS/dZ	Concentration gradient along the column length	$molml^{-1}cm^{-1}$
E	Free enzyme	
E’	Alkyl-enzyme intermediate	
EI_B	Dead-end enzyme-methanol complex	
EA	Enzyme-triglycerides complex	
E’B	Binary complex	
EA↔E’C	Transition state of reaction	
E’B↔ED	Transition state of reaction	
F1	Feed flow, C/FLAX tubing	
F2	Feed flow, C/FLAX tubing	
F3	Feed flow, Stainless Steel Piping	
F4	Transesterification product, Stainless Steel Piping	

F5	Transesterification product, Stainless Steel Piping	
F6	Transesterification product, C/FLAX tubing	
I	Inhibitor	
k_p	Reaction rate constant	s^{-1}
k_c	External mass transfer coefficient	ms^{-1}
k_{cat}	Catalytic rate constant	ms^{-1}
$K_{Mtrigly}$	Binding constant for the triglycerides	$molL^{-1}$
K_{Mmeth}	Binding constant for the methanol	$molL^{-1}$
$K_{Itrigly}$	Inhibition constant for triglycerides	$molL^{-1}$
K_{Imeth}	Inhibition constant for methanol	$molL^{-1}$
Log *P*	Partition coefficient of a given compound in the octanol and water two phase system	
M_{FAME}	Total FAME weight after reaction	g
M_{OIL}	Oil weight before reaction	g
M_P	Methyl palmitate weight	g
M_O	Methyl oleate weight	g
M_L	Methyl linoleate weight	g
M_S	Methyl stearate weight	g
M_M	Methyl myristate weight	g
n	Sample size	
N	Number of measurement	
O1	Oven	
P1	Peristalsis Pump	
r_m	Mass transfer rate	$molcm^{-3}min^{-1}$
r_r	Transesterification reaction rate	$molcm^{-3}min^{-1}$

R^2	Coefficient of determination	
S	Triglycerides concentration at bulk liquid	mol L^{-1}
S_i	Triglycerides concentration at the external surface of the immobilized enzyme	molL^{-1}
S_o	Triglycerides concentration at height of packing equal to zero	molL^{-1}
S.D	Standard deviation	
S_{trigly}	Concentration for triglycerides	molL^{-1}
S_{meth}	Concentration for methanol	molL^{-1}
T1	Temperature for inlet the PBR	
T2	Temperature for outlet the PBR	
V_i	Initial reaction rate	M/min.g.lipase
V_{max}	Maximum reaction rate	M/min.g.lipase
V	Total volume after reaction	ml
V1	Inlet close tank	
V2	Outlet open tank	
Q	Volumetric flow rate	ml min^{-1}
Z	Height of packing	cm

LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
CCD	Central composite design
CO	Carbon monoxide
DOE	Design of experiment
GC	Gas chromatography
FAME	Fatty acids methyl ester
ID	Inner diameter
IUN	Interesterification units
L	Liter
mM	miliMolar
Mtoe	Mega tonne oil energy
NO_x	Nitrogen oxide
PJ	Petajoules
PBR	Packed bed reactors
RSM	Response surface methodology
Q,Y,Z	Coded factor
rpm	Rotation per minute
SO_x	Sulfur oxide
wt%	Weight percent

PENGHASILAN BIODIESEL DARIPADA MINYAK *CERBERA ODOLLAM* DI DALAM REAKTOR LAPISAN TERPADAT

ABSTRAK

Pengurangan simpanan sumber petroleum dan peningkatan krisis makanan telah menarik perhatian para penyelidik untuk menghasilkan sumber tenaga alternatif. Biodiesel yang dihasilkan daripada minyak tumbuhan, lemak haiwan ataupun minyak daripada mikrob adalah merupakan salah satu alternatif sumber tenaga pada masa kini. Selain itu, biodiesel juga merupakan sumber mesra alam dimana kurang menghasilkan gas rumah hijau. Minyak *Cerbera odollam* telah dikenalpasti sebagai sumber minyak yang tidak boleh dimakan bagi menghasilkan biodiesel. Dalam kajian ini, *Cerbera odollam* telah diuji melalui proses transesterifikasi dengan kehadiran enzim sebagai pemangkin.

Dalam kajian ini, minyak *Cerbera odollam* telah dihasilkan dengan menggunakan unit pemerah soxhlet dan heksana sebagai pelarut. Proses transesterifikasi bersama enzim telah dijalankan dalam kajian sekelompok dengan menggunakan enzim (Lipozyme RM IM, Lipozyme TL IM dan Novozyme 435) dan pelarut (heksana dan *tert*-butanol) berlainan, bagi mengenalpasti enzim dan pelarut yang memberikan hasil lemak asid metil ester (FAME) yang maksimum. Parameter penting dalam proses transesterifikasi; suhu, kelajuan pusingan, kepekatan enzim, nisbah metanol dan minyak serta nisbah pelarut dan minyak dioptimumkan dengan metodologi permukaan sambutan (RSM) berdasarkan rekabentuk komposit berpusat (CCD). Kinetik untuk proses transesterifikasi juga dijalankan dalam kajian ini. Proses transesterifikasi berenzim dengan kajian selanjar di dalam reaktor lapisan terpadat dan kesan pemindahan jisim di dalam reaktor turut dikaji.

Sebanyak 54% minyak daripada biji *Cerbera odollam* berjaya diperolehi. Lipozyme TL IM dan *tert*-butanol dikenalpasti sebagai enzim dan pelarut paling sesuai untuk kajian ini. Sebanyak 67.05 % FAME berjaya dihasilkan dengan kombinasi Lipozyme TL IM dan pelarut *tert*-butanol. Proses transesterifikasi minyak *Cerbera odollam* dalam keadaan optimum boleh mencapai sehingga 94.75 ± 2.43 % penghasilan FAME seperti yang dicadangkan oleh RSM (R^2=0.99) ; 4.1 w/w% enzim berdasarkan berat minyak, 1:5.1 nisbah antara minyak dan metanol, 1:2 nisbah antara pelarut dan minyak, suhu sebanyak 45°C dan kelajuan 200 pusingan per minit.

Bagi kajian kinetik dalam ujikaji ini, model berdasarkan Ping Pong Bi Bi bersama perencat oleh metanol didapati sepadan dengan data kadar permulaan dan pemalar kinetik ditentukan dengan analisis regresi garis tidak lurus. Kadar tindakbalas maksimum adalah V_{max}= 17.37 mmol/ml.min, manakala pemalar minyak adalah K_{m1}=3.32 mmol/ml dan pemalar metanol adalah K_{m2}=3.00 mmol/ml. Transesterifikasi minyak *Cerbera odollam* dalam reaktor lapisan terpadat boleh mencapai sehingga 86 % penghasilan FAME dengan kelajuan kadar aliran suapan sebanyak 0.6 ml/min dan ketinggian lapisan terpadat sebanyak 8sm.

Kesan pemindahan jisim di dalam reaktor lapisan terpadat juga dikaji. Model-model untuk hasil FAME telah diuji untuk kes-kes kawalan tindak balas dan kawalan pemindahan jisim. Keputusan menunjukkan persetujuan yang baik (R^2= 0.95) diantara model kawalan pemindahan jisim dan data daripada eksperimen yang diperolehi daripada operasi enzim tersekatgerak di dalam reaktor lapisan terpadat. Didapati FAME adalah dalam kawalan pemindahan jisim. Ciri-ciri bahan bakar biodiesel yang diperolehi adalah hampir sama dengan diesel petroleum Malaysia.

BIOROUTE PRODUCTION OF BIODIESEL USING *CERBERA ODOLLAM* OIL IN PACKED BED REACTOR

ABSTRACT

Diminishing reserves of fossil fuels and arising of food crisis recently have drawn attention for researchers to produce an alternative fuels. Biodiesel which is made from vegetable oils, animal fats or microbial oils is one of the attractive alternative fuels due to this concern. Other than that, biodiesel is an environmental friendly fuel which emitted less greenhouse effect gas. *Cerbera odollam* oil was determined as a non-edible feedstock for biodiesel production. The enzymatic transesterification of *Cerbera odollam* oil as a feedstock has been studied in the present research.

Cerbera odollam oil has been extracted using n-hexane solvent in a soxhlet extractor. The enzymatic transesterification were carried out in a batch system to screen the most efficient enzyme among Lipozyme RM IM, Lipozyme TL IM and Novozyme 435 based on the highest fatty acid methyl ester (FAME) yield in the process. The best organic solvent used in this reaction was also determined in a batch process between *tert*-butanol and n-hexane to eliminate the inhibition of methanol in the reaction. Important parameters like temperature, speed agitation, enzyme concentration, methanol to oil ratio and solvent to oil ratio in enzymatic transesterification reaction were optimized by using response surface methodology (RSM) based on central composite design (CCD). The kinetic studies of enzymatic tansesterification were carried out in this research. Enzymatic transesterification of *Cerbera odollam* oil reaction was also studied in a packed bed reactor (PBR) for a continuous process. The effect of mass transfer in the PBR was also studied.

54% of *Cerbera odollam* oil was extracted from the seeds. Lipozyme TL IM and *tert*-butanol were found as the suitable enzyme and organic solvent in this study. 67.05 % FAME yield was obtained using a combination of Lipozyme TL IM and tert butanol. The transesterification reaction of *Cerbera odollam* oil can reach up to 94.75 ± 2.43 % FAME yield with the optimized condition as suggested by RSM (R^2=0.99) ; 4.1 w/w% enzyme based on oil weight, 1:5.1 oil to methanol molar ratio, 1:2 solvent to oil volume ratio, temperature of 45°C and 200 rpm agitation speed. By kinetics study of enzymatic transesterification of *Cerbera odollam* oil, a model based on Ping Pong Bi Bi with inhibition by methanol was found to fit the initial rate data and the kinetics parameters were evaluated by non-linear regression analysis. The maximum reaction rate, V_{max}= 17.37 mmol/ml.min, *Cerbera odollam* oil binding constant, K_{m1}=3.32 mmol/ml and methanol binding constant, K_{m2}=3.00 mmol/ml were obtained. The *Cerbera odollam* oil transesterification in PBR can reach up to 86% FAME yield with 0.60 ml/min feed flow rate and 8 cm PBR height. In mass transfer study, models for FAME yield showed very good agreement between mass transfer model and the experimental data obtained from PBR operation, showing that in this study the FAME yield was mass transfer controlled (R^2= 0.95). The high FAME yield in batch and continuous operation as well as the fuel characteristic meeting the standard of biodiesel proved that *Cerbera odollam* oil can be a promising feed stock for enzymatic transesterification process.

CHAPTER 1

INTRODUCTION

1.1 Renewable Energy

Fossil fuels provide the main source of energy in the world today. However, the world has been confronted with an energy crisis due to the depletion of finite resources of fossil fuel. Nowadays, the use of fossil fuels as energy is widely accepted as unsustainable due to depleting resources (Kecebas *et al.,* 2009; Demirbas *et al.,* 2010). Despite the problems with fossil fuels, mankind are using more and more energy. This is due to the fact that the world's population is growing and standards of living continue to rise. This means that, there is an increase demand for transportation, electricity, food, goods and services, all of which require energy, but the supply of fossil fuels are limited and not easily generated (Balat, 2009).

On the other hand, the use of fossil fuels also gives negative impact to the environment due to the accumulation of greenhouse gases. Apart from these emissions, fossil fuel is recognized as a major source of air contaminants including NO_x, SO_x, CO, particulate matter and volatile organic compound (VOC) (Jegannathan *et al.,* 2008; Tan *et al.,* 2010).

Hence, several studies regarding alternatives of the fuels were conducted. Biodiesel is gaining more importance as an alternative fuel due to the similarity of the characteristics compared to fossil fuels. Biodiesel can be used in diesel engine without major modification to the engine (Puhan *et al.,* 2005; No, 2010).

Biodiesel is a mixture of fatty acid methyl esters (FAMEs), has been developed as one of the most promising alternative fuel due to its limited resources of fossil fuels as well as some environmental concerns. Biodiesel is a type of fuel, which comprises of mono-alkyl ester of long chain fatty acids derived from vegetable oils or animal fats, designated by B100 as described in the ASTM 6751-02. It is also a clean engine fuel derived from renewable resources which is found superior to parafinic diesel oil in terms of the absence of sulfur-oxide, minimum aromatic content and soot particulates (Ma and Hanna, 1999; Demirbas, 2003). Biodiesel has been conventionally produced via transesterification of vegetable oils and animal fats. The products of the process are alcohol esters of vegetable oils with glycerol as a by-product.

Other than using renewable resources, biodiesel has also becoming more attractive due to its environmental benefits. There are many studies conducted on the transesterification reaction to produce biodiesel, derived from various raw materials. These raw materials include both the edible and non-edible oils. Other than becoming a promising renewable energy fuel, biodiesel can also contribute on the effort to reduce greenhouse gas effect because of less greenhouse gas generated from biodiesel (Fukuda *et al*., 2001; Lu *et al.,* 2007).

The contribution of non-edible oils such as jatropha oil and soapnut oil, which have been studied recently (Shah *et al.,* 2004; Tamalampudi *et al.,* 2008; Dizge *et al.,* 2009), will be significant as a non-edible plant oil source for biodiesel production. However, in this study, a new source for non-edible oil, *Cerbera odollam* (Sea Mango) will be investigated as a feed stock for biodiesel production. *Cerbera* *odollam* (Sea Mango) tree are widely planted by roadsides around Malaysia as an ornamental plant.

1.2 Energy Demand

Malaysia is endowed with conventional energy resources such as oil and gas as well as renewable energy resources like hydro, biomass and solar energy. However, consumption of energy is also rapidly increasing. As shown in Figure 1.1, the final energy demand is projected to grow at 3.9 percent per year, reaching 98.7 Mtoe (tonne of oil equivalent) equally to 4×10^{12} MJ in 2030, nearly three times the 2002 level. The industry sector have the highest growth rate of 4.3 percent, followed by transport at 3.9 percent, residential at 3.1 percent and commercial at 2.7 percent. Figure 1.1 shows the final energy demand (APERC, 2006).

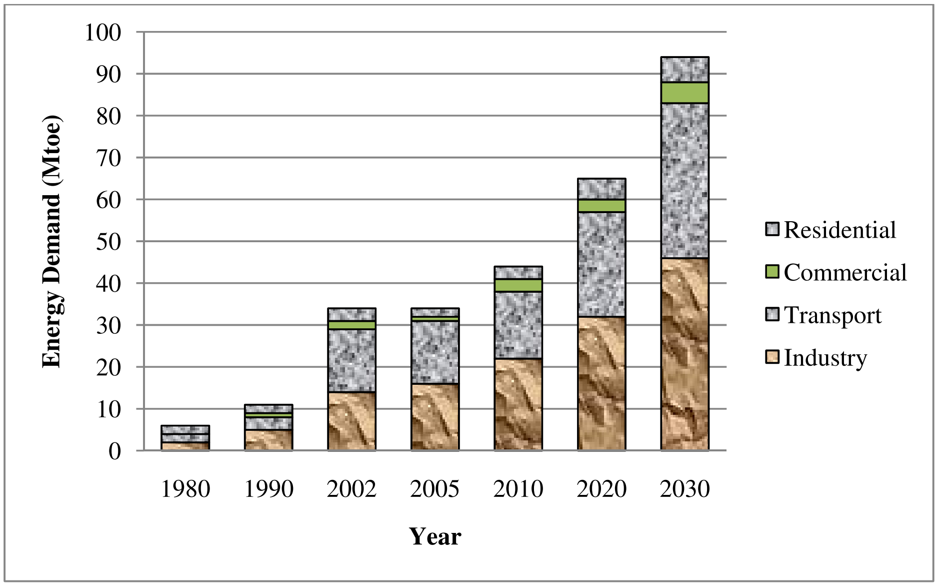


Figure 1.1: Final energy demand.
Unit toe is tonne of oil equivalent, the amount of energy released by burning one tonne of crude oil (APERC, 2006)

Final energy consumption grew at a fast rate of 5.6 percent between 2000 and 2005 to reach 38.9Mtoe in 2005. By fuel type, the trend of growth will show significant differences, with gasoline growing at 2.9 percent per year, diesel at 4.2 percent per year, and natural gas at 9.2 percent per year (APERC, 2006). Taking into account the growing energy consumption and domestic energy supply constraints, Malaysia has to figure out its renewable energy resources. The introduction of biodiesel for the transport sector in 2005 is one of the positive steps that has been undertaken to achieve sustainable energy development through diversification of fuel sources (APERC, 2006).

1.3 Enzymatic Transesterification

Several processes for biodiesel fuel production have been developed using base or acid catalyst. Although chemical transesterification using an alkali-catalytic process gives high conversion levels of triglycerides to their corresponding methyl esters in short reaction times, the reaction has several drawbacks as it is energy intensive, recovery of glycerol is difficult, the acidic or alkaline catalyst has to be removed from the product, alkaline wastewater requires treatment and free fatty acids and water interference with the reaction (Fukuda *et al.,* 2001). Moreover, the physiochemical synthesis schemes often result in poor reaction selectivity and may lead to undesirable side reactions (Noureddini *et al.,* 2005).

Enzymatic transesterification using immobilized enzyme has become more attractive for biodiesel fuel production. In particular, it should be noted that the by-product, glycerol, can be easily recovered without any complex process, in addition free fatty acids contained in waste oils and fats can be completely converted to methyl esters through transesterification reaction (Fukuda *et al.,* 2001). Figure 1.2 shows the process of transesterification of triglycerides using methanol.

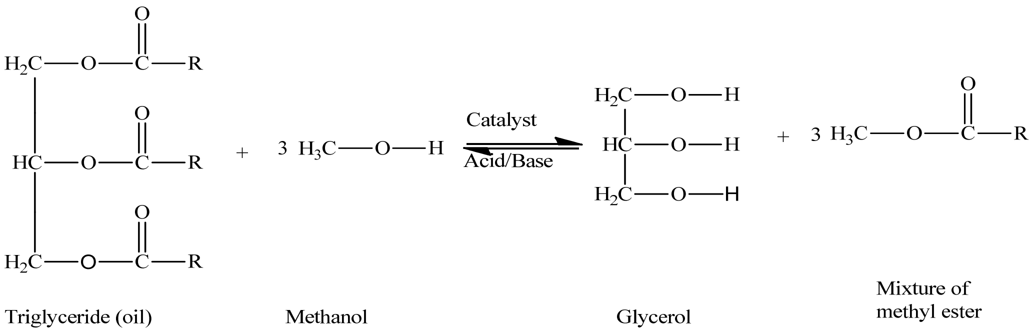


Figure 1.2: Transesterification of triglycerides using methanol

It is known that enzymes such as lipase can be used to catalyze transesterification process by immobilizing them in a suitable support. The advantage of immobilization is that the enzyme can be reused without separation. In addition, the operating temperature of the process is lower (50°C) compared to the other techniques. Disadvantages include inhibition effects which were observed when methanol was used (Nelson *et al.*, 1996; Shimada *et al.*, 2002).

The production of biodiesel using a biocatalyst eliminates the disadvantages of the alkali process by producing product of very high purity with less or no downstream operations (Fukuda *et al.*, 2001). This method of production of biodiesel using a biocatalyst was also patented by Haas (1997).

The enzyme that was found to be capable of catalyzing methanolysis is lipase which is obtained from micro-organisms like *Mucor miehei*, *Rhizopus oryzae*, *Candida antarctica*, and *Pseudomonas cepacia*. Various alcohols were introduced for the transesterification process including methanol, ethanol, iso-propanol and butanol, but methanol is considered for industrial production because of its low cost and availability.

1.4 Problem Statement

At the end of 2008, according to British Petroleum's annual Statistical Review of World Energy, the world proven oil reserves were estimated at 1.7×10^{11} and will last for only 42 years (M. Balat and H. Balat., 2010). Alternative fuels to replace fossil fuels are becoming increasingly important due to diminishing petroleum reserves. Biodiesel is generally considered to be renewable, due to the fact that its primary feedstock is from renewable resources (Gerpen, 2005). Biodiesel has been produced from a variety of vegetable sources. Vegetable oils include edible and non-edible oils. For example, soybean oil in the US, rapeseed and sunflower oils in Europe, palm oil in South-east Asia (mainly Malaysia and Indonesia) and coconut oil in the Philippines are being considered (Shah *et al.,* 2004; Barnwal and Sharma, 2005) as feedstocks. However, due to the rise in food crisis, it is important to carry out research to search for new resources from non-edible oil species. Several studies from non-edible oil have been introduced from different investigations such as jatropha oil, castor oil, pagamia pinnata oil and rubber seeds oil (Balat, 2009; Demirbas, 2009).

More than 95% of biodiesel production feedstocks come from edible oils since they are mainly produced widely and the properties of biodiesel produced from these oils are compatible to be used as diesel fuel substitute (Gui *et al.,* 2008). However, some problems such as the competition with the edible oil market, which increases both the cost of edible oils and biodiesel (Kansedo *et al.,* 2009) arise. In order to overcome the disadvantages, many researchers are interested in non-edible oils which are not suitable for human consumption because of the presence of some toxic components in the oils. Furthermore, the cost of non-edible oil crops cultivation is lower because these crops can still sustain reasonably high yield without any intensive care (Tiwari *et al.,* 2007; Gui *et al.,* 2008). *Cerbera odollam* will be used in this study, is an ornamental plant which are planted widely by roadsides in Malaysia.

In addition, petroleum-based products are the main causes of anthropogenic carbon dioxide (CO_2) emissions to the atmosphere. Today, the transportation sector worldwide is almost entirely dependent on the petroleum-derived fuels. Exhaust gases from petroleum-fuelled engines create serious environmental problem. Air pollution is one of the most serious environmental problems all over the world. The combustion of petroleum-based diesel fuel is a major source of green house gas emissions. Apart from these emissions, petroleum-based diesel fuel is also a major source of other air contaminants including carbon monoxide (CO), nitrogen oxides (NOx), sulfur oxides (SOx), particulate matter (PM), and volatile organic compounds (VOCs).Thus, the production of biodiesel as an alternative fuel has become a developing area of high concern (Chien *et al.,* 2009).

The advantages of biodiesel fuel include; safe for use in all conventional diesel engines, offers the same performance and engine durability as petroleum diesel fuel, non-flammable, reduces tailpipe emissions, visible smoke and noxious fumes and odors (Puhan *et al.,* 2005; No, 2010). Biodiesel is better than diesel fuel in terms of sulfur content (SC), flash point, aromatic content and biodegradability (Demirbas, 2003). It is also well-known that the biodiesel fuel from vegetable oil also does not produce sulfur oxide and minimize the soot particulate compared with the existing one from petroleum. Biodiesel fuel can be expected as a substitute for conventional diesel fuel for the environmental advantages (Iso *et al.,* 2001).

There are two bottlenecks in enzymatic approaches for biodiesel production. One is the high cost of lipase and its short operational life caused by the negative effects of excessive methanol and by-product glycerol. It has been demonstrated that more than 1/2 molar equivalent methanol are insoluble in vegetable oils and the immobilized lipases are easily inactivated by contacting with insoluble methanol existing as drops in the oils (Shimada *et al.,* 2002; Xu *et al.,* 2004). Previous researchers (Watanabe *et al.,* 2001) reported that two or three successive addition of methanol and more than 90% waste edible oil was successfully converted to its corresponding biodiesel, since the solubility of methanol in the alkyl esters is greater than oil, and consequently limits enzyme deactivation.

Enzymes show higher activity in relatively hydrophobic organic solvents with higher log P (>2) such as *n*-hexane and petroleum ether, and these hydrophobic organic solvents have also been tried as reaction medium for biodiesel production. However, methanol and glycerol have poor solubility in these relatively hydrophobic solvents. By-product glycerol is hydrophilic and insoluble in the oil, so it's easily adsorbed onto the surface of the immobilized lipase also leading to negative effect on lipase activity and operational stability. The negative effects on lipase activity and stability caused by methanol and glycerol cannot be eliminated and lipase still exhibit poor stability in such reaction media (Xu *et al.,* 2004). Iso *et al.* (2001) found an appreciable increase in the yield of methanolysis of triolein when using 1,4-dioxane as co-solvent, which dissolves methanol; however, a high proportion of this solvent (90%) was necessary to obtain a reasonable conversion.

In order to solve this problem, *tert*-butanol, which is a moderate polar solvent has been demonstrated in a study as an ideal solvent for lipase-catalyzed biodiesel production. With a certain amount of *tert*-butanol as the reaction medium, both methanol and by product glycerol are soluble, so the negative effect caused by methanol and glycerol on lipase catalytic activity could be totally eliminated (Xu *et al.,* 2004, Royon *et al.,* 2007; Halim and Kamaruddin, 2008). *tert*-butanol is not a substrate for the lipases because it does not act on tertiary alcohols. Moreover, *tert*-butanol is a non-toxic solvent of relatively low cost.

1.5 Research Objectives

This research focuses on enzymatic transesterification approach for biodiesel production using non-edible oil as a feed stock. *Cerbera odollam* oil is enzymatically converted to biodiesel.

The objectives of the research are:

i. To study the effects of process parameters on transesterification of *Cerbera odollam* oil with methanol using immobilized enzyme in a batch reaction and to optimize the process parameters (type of lipase, type of solvent, solvent quantity, enzyme loading, alcohol to oil molar ratio, temperature and speed agitation) using design of experiment (DOE) software.
ii. To study the enzyme kinetics and mechanism of immobilized lipase-catalyzed transesterification for biodiesel production.
iii. To study the effect of process parameters (flow rate and bed height) in packed-bed reactor and the responsibility of external mass transfer of the reaction in packed-bed reactor and to study the characteristics of biodiesel produced from *Cerbera odollam* oil.

1.6 Organization of Thesis

This thesis is divided into five chapters

Chapter 1 : Describes the renewable energy (biodiesel), energy demand in Malaysia, and potential of *Cerbera odollam* (Sea Mango) as the raw material for biodiesel production. This chapter also focuses on the problem statement and objectives of the project.

Chapter 2 : Describes literature reviewed from other researches and methods applied in the present days for the biodiesel production. Parameters optimization using Design of Experiment (DOE) is also covered in this chapter.

Chapter 3 : Describes the materials, methods, and analysis involved in the transesterification process. This chapter also explains on the chemicals and equipments required throughout the whole study.

Chapter 4 : Presents the results from experimental study. This chapter also focuses on discussion of every parameters effect on the biodiesel production.

Chapter 5 : Presents the conclusion on the study and recommendation for better improvement in the future research.

CHAPTER 2

LITERATURE REVIEW

2.1 Biodiesel

Biodiesel refers to a non-petroleum-based diesel fuel consisting of short chain alkyl (methyl or ethyl) esters, made by transesterification of vegetable oil. Alternative fuels for diesel engines are becoming increasingly important due to diminishing petroleum reserves and the environmental consequences of exhaust gases from petroleum-fuelled engines. A number of studies have shown that transesterification of triglycerides hold a potential alternative to diesel engine fuels (Fukuda *et al.*, 2001; Zhang *et al.,* 2003; Li *et al.,* 2010). Biodiesel also has similar physicochemical properties to that of the usual diesel produced from crude oil and can be used directly to run existing diesel engines without major modifications or as a mixture with petroleum diesel and produces less harmful gas emission (Atadashi *et al.,* 2011).

The world has been confronted with an energy crisis due to the depletion of finite resources of fossil fuel. Nowadays, the use of fossil fuels to generate energy is widely accepted as unsustainable due to the depleting resources as well as the accumulation of greenhouse gases in the environment (Kecebas and Alkan, 2009; Demirbas, 2010). Biodiesel has been agreed by many that it can be an alternative fuel produced from vegetable oil and animal fat. Since its primary feedstock is a vegetable oil or animal fat, biodiesel is generally considered to be renewable resource. Since the carbon in the oil or fat originated mostly from carbon dioxide in the air, biodiesel is also considered to contribute lesser to global warming than fossil fuels. Diesel engines operated on biodiesel have lower emissions of carbon monoxide, unburned hydrocarbons, particulate matter, and air toxics than when operated on petroleum-based diesel fuel (Asano *et al*., 2002).

Biodiesel can be produced by chemical methanolysis using an alkali or acid-catalysis or by enzymatic methanolysis using lipases. Currently, the main biodiesel feedstocks are classified into three categories. The three categories of feedstock are shown in Table 2.1. In this current study, the non-edible vegetable oil will be used as a feedstock.

Table 2.1: Categories of feedstocks for biodiesel production

Categories	**Types of oil**	**Reference**
Plant oil	Soybean oil	Lv *et al.,* 2008
	Jatropha oil	Shah *et al.,* 2004; Tamalampudi *et al.,* 2008
	Palm oil	Halim *et al.,* 2009; Sim *et al.,* 2009; Baiju *et al.,* 2008
	Cottonseed oil	Dizge *et al.,* 2009
	Sunflower oil	Dizge *et al.,* 2009; Wu *et al.,* 2007
Animal fat	Tallow	Da Cunha *et al.,* 2009
	Lard and grease	Lee *et al.*, 2002; Ngo *et al.,* 2008
Waste cooking oil and industrial waste oil	-	Dizge *et al.,* 2009; Lara Pizarro and Park, 2003

Enzymatic biodiesel production from raw vegetable oils has been extensively studied by many authors in recent years. Nevertheless, the raw material costs and limited availability of raw vegetable oil are recently the critical issues for the biodiesel production. Therefore, it is necessary to look for another raw material to produce biodiesel. Biodiesel can be used in different types of diesel engines with no significant drop in efficiency but significant reduction in the emissions of particulate matter, carbon monoxide and total hydrocarbons with respect to conventional diesel obtained from fossil fuel (Leung and Guo, 2006; Murillo *et al.,* 2007; Lapuerta *et al.,* 2008).

2.2 Non-Edible Oil

The properties of biodiesel also depend on the types of vegetable oils used for the transesterification process (Ramadhas *et al.,* 2006). Biodiesel is completely miscible with diesel and can also be blended in any proportion to diesel fuel.There has been several studies that used non-edible oil to produce biodiesel due to the conflict of edible oil as a competitor to food. The use of non-edible vegetable oils is of significance because of the great need for edible oil as food. In addition the selection of non-edible vegetable oil can reduce the production cost of biodiesel due to the relatively high cost of edible vegetable oils (No, 2010). Therefore, studies using non-edible oil as a raw material were conducted in this present work. Table 2.2 shows the oil and free fatty acid contents of various non-edible vegetable oils for the purpose of biodiesel production. It is apparent from the table that the oil content in non-edible oils differs from each other. The data summarized the minimum and maximum ranges of the oil content. Physical and chemical characteristics of virgin or used oils that affect the potential biodiesel production are free fatty acid (FFA) and moisture contents and other impurities as well as calorific content. Different vegetable oils with various compositions of fatty acids can be used for biodiesel production (Karmakar *et al.,* 2010).

Table 2.2: Various inedible vegetable oils with oil and free fatty acid contents

Non- Edible Vegetable Oil	**Oil Content (wt%)**	**Free fatty acid (FFA) (wt%)**	**References**
Jatropha (*Jatropha curcas*)	20–60	13.5–14.5	Moser, 2009; Singh, 2010; Azam *et al.,*2005
Karanja (*Pongamia pinnata*)	25–50	8.3–20	Srivasta, 2000; Sharma *et al.,*2008; Pinzi *et al.,* 2009
Rubber (*Hevea brasiliensis*)	40–60	17	Demirbas, 2009; Ramadhas *et al.,*2005
Castor (*Ricinus communis*)	45–50	NA	Moser, 2009; Singh, 2010
Neem (*Azadirachta indica*)	20-30	NA	Singh 2010; Azam *et al.,*2005
Cottonseed	17-25	NA	Karaosmanoglu, 1999
Mahua (*Madhuca indica*)	35-50	20	Moser 2009; Singh 2010; Azam *et al.,*2005
Cerbera odollam	54	6.4	Kansedo *et al.,* 2009

NA- Not available

For this current study, *Cerbera odollam* oil will be used in the enzymatic transesterification process to produce biodiesel. Table 2.3 shows the summary of enzymatic transesterification of edible and non-edible oils by previous researchers. Table 2.4. on the other hand shows the fuel characteristics of petrodiesel and biodiesel from various non-edible oil.

Summary of transesterification by various researchers

Table 2.3. Summary of Enzymatic Transesterification by Various Researchers

No.	**Authors**	**Oil/enzyme**	**Acyl Acceptor**	**Conversion (%)**	**Technique employed**
1	Modi *et al.,* (2007)	Jatropha oil, Novozyme 435	Ethyl acetate	91.3	Ethyl acetate having no inhibitory effects was used
2	Samukawa *et al,.* (2000)	Soybean oil, Novozyme 435	Methanol	97	Stepwise addition methanol and preincubation of enzyme in methyl oleate and soybean oil
3	Ban *et al.,* (2001)	Vegetable oil, R. oryzae	Methanol	90	Stepwise addition of methanol and application of glutaraldehyde for stability of enzyme
4	Iso *et al.,* (2001)	Triolein, P. *flourescens*	Butanol	90	Butanol was used as an acyl acceptor and no solvent was used
5	Shimada *et al,.* (2002)	Waste cooking oil, Novozyme 435	Methanol	90	Stepwise addition of methanol
6	Bako *et al.,* (2002)	Sunflower oil, Novozyme 435	Methanol	97	Stepwise addition of methanol and removal of glycerol by dialysis
7	Du *et al.,* (2004)	Soybean oil, Novozyme 435	Methyl acetate	92	A novel acyl acceptor, methyl acetate which had no inhibitory effects was used
8	Xu *et al,.* (2004)	Soybean oil, Novozyme 435	Methanol	98	Stepwise addition of methanol and removal of glycerol using the solvent, iso-propanol

S.No	Authors	Oil/enzyme	Acyl Acceptor	Conversion (%)	Technique employed
9	Li *et al.,* (2006)	Rapeseed oil, Novozyme 435 & Lipozyme TL IM	Methanol	95	Combined use of Lipozyme TL IM and Novozyme 435 along with tert-butanol as solvent
10	Royon *et al.,* (2007)	Cotton seed oil, Novozyme 435	Methanol	97	tert-Butanol was used as a solvent
11	Watanabe *et al.,* (2000)	Vegetable oil, Novozyme 435	Methanol	90–93	Stepwise addition of methanol
12	Chang *et al.,* (2005)	Canola oil, Novozyme 435	Methanol	97.9	Respone surface methodology (RSM) and five level five factor central composite rotatable design(CCRD) were employed to evaluate the effects of synthesis parameter
14	Royon *et al.*, (2005)	Cottonseed oil, Novozyme 435	Methanol	90	Yield 97% observed after 24hour. 95% yield was obtained using one step fixed bed continuous reactor with a flow rate of 9.6ml/h(g enzyme)Experiment with continuous reactor over 500 hr did not show any appreciable decrease in esters yield.

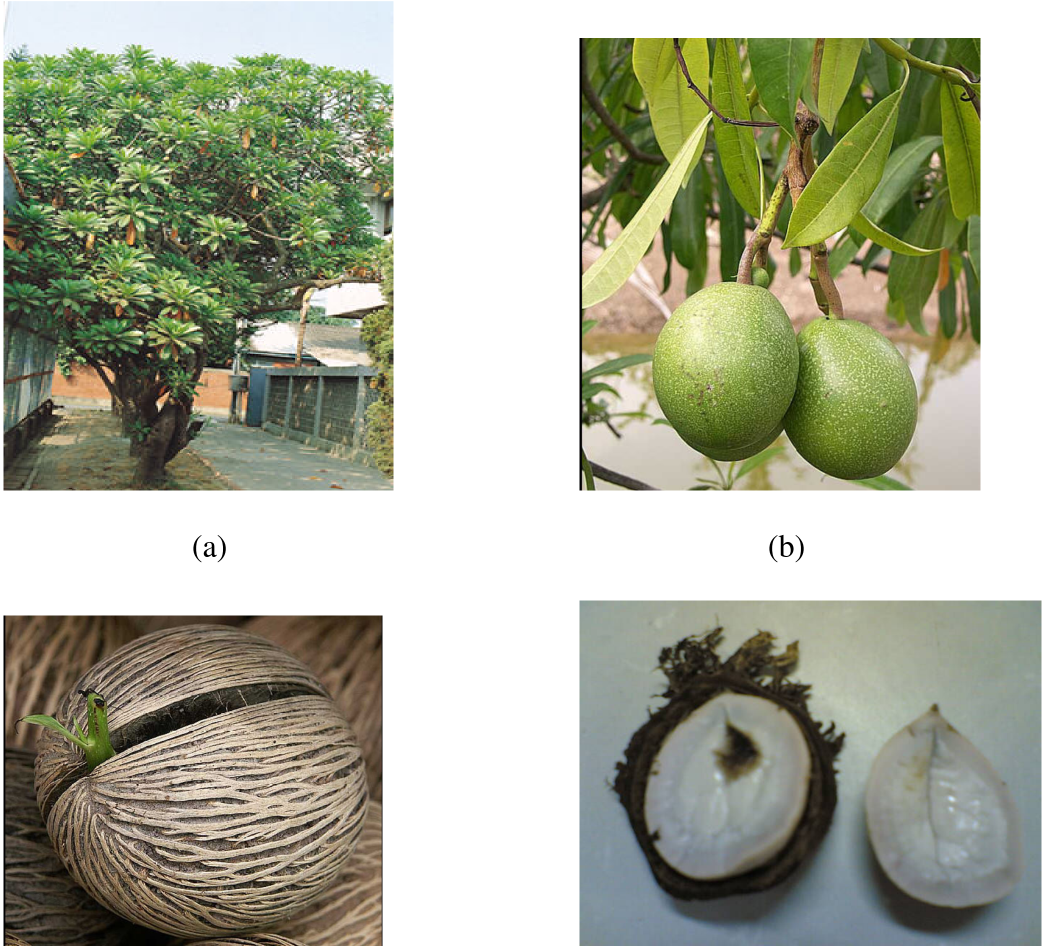
S.No	**Authors**	**Oil/enzyme**	**Acyl Acceptor**	**Conversion (%)**	**Technique employed**
15	Wang *et al.,* (2008)	Soybean deodorizer distillate, Novozyme 354 and Lipozyme TL IM	Methanol	97 84	Fine-pored silica gel and 3 °A molecular were found to be effective to control by-product water concentration and much higher biodiesel yield could be achieved with those adsorbents present in the reaction system. The highest biodiesel yield of 97% could be achieved with 3 °A molecular sieve as the adsorbent.
16	Li *et al.,* (2006)	Rapseed oil, Novozyme 435 and Lipozyme TL IM	Methanol	95	There was no obvious loss in lipase activity even after being repeatedly used for 200 cycles with *tert*-butanol as the reaction medium. Furthermore, waste oil was also explored for biodiesel production and it has been found that lipase also showed good stability in this novel system.
18	Halim and Kamaruddin (2008).	Waste cooking oil, Novozyme 435	Methanol	88	Batch and continuous packed bed reactor were carried out in this study.In the biodiesel production, enzymatic activities were influenced by excess methanol and glycerol. Tert-butanol was considered the mostsuitable solvent for this biocatalyst.

Table 2.4: Fuel characteristics of petrodiesel and biodiesel from enzymatic transesterification of various non-edible oil (Barnwal and Sharma, 2005; Reddy and Ramesh 2005; Bhatnagar *et al,*.2006)

No.	**Characteristics**	**Unit**	***Jatropha curcas***	***Pongamia glabra***	***Salvadora oleoides***	***Madhuca indica***	**Petro-diesel**
1	Density at 15 °C	kg/m^3	879.4	893.1	875.5	876.5	878.8
2	Pour point	°C	+3	+15	+3	+15	-9
3	Total sulfur	ppm	<1.0	<1.0	1200	164.8	500
4	Kinematic viscosity at 40 °C	cSt	4.34	5.13	3.25	4.98	2.85
5	Flash point	°C	135	148.0	130.0	120.0	
6	CCR	wt %	0.01	0.74	0.28	0.005	
7	Ash content	wt %	0.013	0.013	0.004	0.01	
8	Copperstrip corrosion, 2 h 100 °C		one	one	four	one	
9	Oxidation stability (UOP-413)	mg/ 100 g	0.4	3.1	14.6	2.1	
10	Molecular weight		320	358.9	258.1	294.4	266.80
11	Acid value	mg KOH/ g	0.38	0.42	0.45	0.32	
12	Cetane number		58.4	53.9	59.94	65.4	56.10 (cetane index)

2.3 *Cerberra odollam* (Sea Mango)

Cerberra odollam commonly known as the Suicide tree, Pong-pong, and Othalanga, is a species of tree native to India and other parts of Southern Asia. The tree is shown in Figure 2.1(a). It grows preferentially in coastal salt swamps and in marshy areas and can produce oil from the seeds. The fruit, when still green, looks like a small mango, with a green fibrous shell enclosing an oval kernel measuring approximately 2 cm × 1.5 cm and consisting of two cross-matching white fleshy halves. Figure 2.1(b) shows the fresh *Cerbera odollam* fruit while Figure 2.1(c) shows the dry *Cerbera odollam* fruit. *Cerbera odollam* seed is shown in Figure 2.1(d). Upon exposure to air, the white kernel turns violet, then dark grey, and ultimately brown or black. The plant as a whole yields a milky white latex (Chopra *et al.*, 1956).



(a) (b)

(c) (d)

Figure 2.1: (a) *Cerbera odollam* tree, (b) *Cerbera odollam* fruit (c) dry *Cerbera odollam* fruit and (d) *Cerbera odollam* seeds.

Cerberra Odollam Gaertner (pink-eyed *carberra,* yellow-eyed *carberra*, *odollam* tree) also sometimes called *Canberra maghas L*, belongs to the notoriously poisonous

Apocynaceae family, which also includes the yellow oleanders (*Thevetia peruviana* and *Thevetia nerifolia*) and common oleanders (*Nerium oleander* and *Nerium indicum*).

The seeds contain a non-drying oil, producing a shining flame with a pleasant nut-like odour. The Burmese use it for lighting, as cosmetics, or mixed with other oils as an insecticide or insect-repellent (Chopra *et al*., 1956). In some countries the leaves and bark are eaten for their cathartic properties. Many parts of the tree are used for the manufacture of fibre. The latex is known in India for its emetic, purgative and irritant effects (Chopra *et al*., 1958). On the Malay peninsula, the oil from the seeds of *Cerbera venenifera* is rubbed on the skin as a rubefacient and as a cure for itching, or applied to the hair as an insecticide (Minh *et al*., 1991). Figure 2.2(a) shows the dried *Cerbera Odollam* seed before the seed is blended into small particles as shown in Figure 2.2(b).


(a) (b)

Figure 2.2: (a) dry *Cerbera Odollam* seed, (b) blended *Cerbera Odollam* seed

Raw materials contribute to a major portion in the cost of biodiesel production. The choice of raw materials depends mainly on its availability and cost. The use of edible vegetable oils and animal fats for biodiesel production has recently been of great concern because of the competition with food materials. As the demand for vegetable oils for food has increased tremendously in recent years, it is impossible to justify the use of these oils for fuel purposes such as biodiesel production. Moreover, these oils could be more expensive to use as fuel. In 2008, the world faced a serious food commodity crisis and sharp increase in energy prices that induced hunger and political unrest in developing countries (Traynor, 2008).

This project addresses an alternative method for biodiesel production which uses an enzymatic approach. *Cerbera odollam* oil was enzymatically converted to biodiesel fuel via packed bed reactor (PBR) system operated in a continuous mode and under mild conditions. The main raw material chosen is *Cerbera odollam* oil, which is considered as inexpensive and non-edible feedstock. This cheap feedstock is therefore expected to make the price of biodiesel more competitive as compared to the use of raw materials from food grade vegetable oils. The production of biodiesel fuel from *Cerbera odollam* oil is considered an important step for reducing the use of edible vegetable oil.

2.4 Transesterification Process

The catalytic transesterification of vegetable oils with methanol is an important industrial method used in biodiesel synthesis. Transesterification, also called alcoholysis, is the displacement of alcohol from an ester by another alcohol in a process similar to hydrolysis, except that an alcohol is employed instead of water. Suitable alcohols include methanol, ethanol, propanol, butanol, and amyl alcohol. Methanol and ethanol are utilized most frequently, especially methanol because of its low cost and its physical and chemical advantages. This process has been widely used to reduce the viscosity of triglycerides. Table 2.5 gives the comparison of the different technologies in producing biodiesel. It shows that lipase catalytic technology gives several advantages by comparing several aspects as shown in the table over the other method used to produce biodiesel.

Table 2.5: Comparison of the different technologies to produce biodiesel (Marchetti *et al.,*2007)

Factors of consideration	**Alkali catalysis**	**Lipase catalysis**	**Supercritical alcohol**	**Acid catalysis**
Reaction temperature (^{o}C)	60-70	30-40	239-385	55-80
Free fatty acid in raw materials	Saponified products	Methyl esters	Esters	Esters
Water in raw materials	Interference with reaction	No influence	-	Interference with reaction
Yield of methyl ester	Normal	Higher	Good	Normal
Recovery of glycerol	Difficult	Easy	-	Difficult

The transesterification of vegetable oil or fat lowers the molar mass to one third that of triglyceride. The reaction can be catalyzed by either base, acid or lipase. The overall chemistry of transesterification with methanol is represented in Figure 2.3. Overall, it involves the interchange of the alkoxide group between an ester and an alcohol to give a new ester and a new alcohol. The overall reaction in Figure 2.3 consists of a number of consecutive and reversible reactions which follows the formation of diglycerides as in Figure 2.4 and the formation of monoglycerides (Figure 2.5) (Chi, 1999).

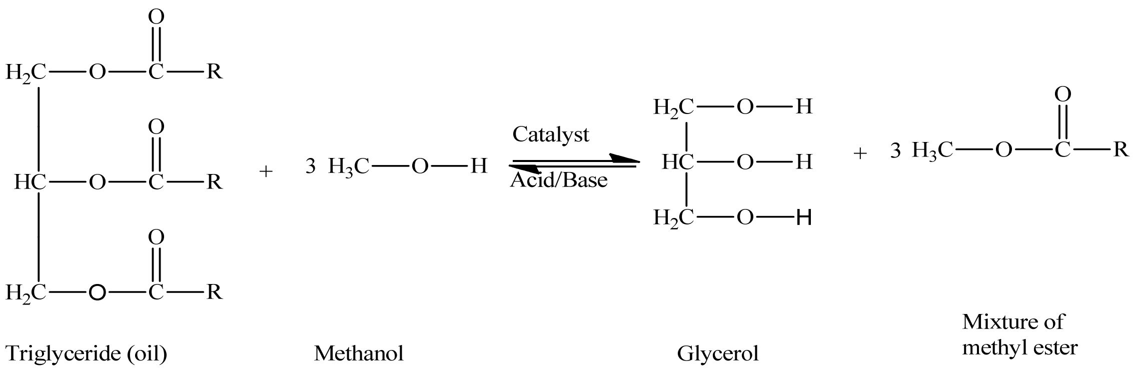


Figure 2.3. Overall transesterification reaction

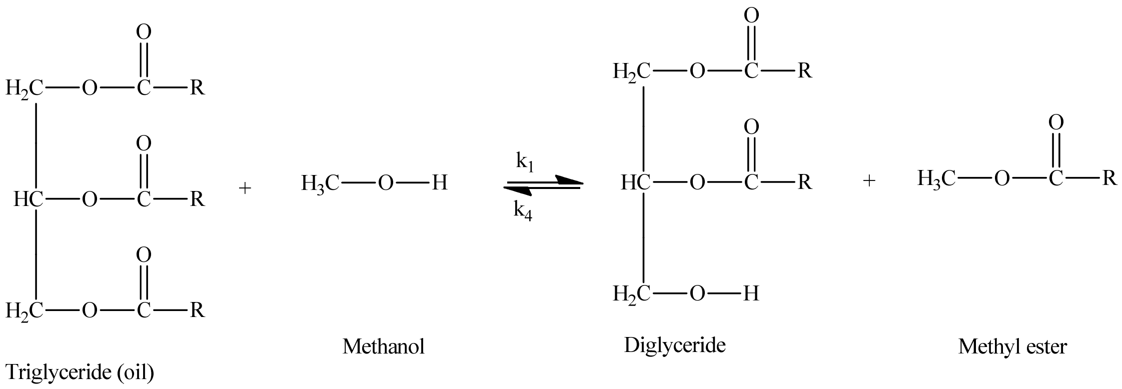


Figure 2.4. Formation of diglycerides in transesterification process

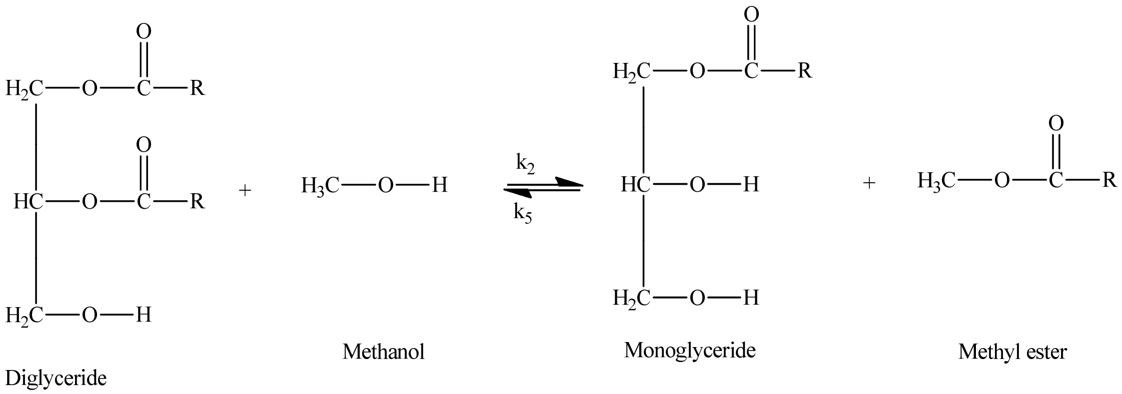


Figure 2.5. Formation of monoglycerides in transesterification process

2.4.1 Alkaline-Catalyzed Transesterification

Alkaline-catalyzed transesterification has many advantages such as; short reaction time, relatively low reaction temperature, small amount of catalyst in the reaction and with little or no darkening of colour of the oil (Singh and Padhi, 2009). High quality biodiesel can be obtained through this method. However, oil containing high amount of FFA will be converted to soap instead of oil. Soaps produced in reaction will inhibit the separation of the biodiesel, glycerin, and wash water (Canakci and Gerpen, 2003). A successful transesterification reaction produces two liquid phases; ester and crude glycerol (Demirbas, 2009).

The reaction mechanism for alkaline-catalyzed transesterification was formulated in three main steps (Ma and Hanna, 1999). The alkaline-catalyzed transesterification of vegetable oils proceeds faster than the acid-catalyzed reaction.

Most of the biodiesel is currently made from edible oils by using methanol and alkaline catalyst. However, there are large amounts of low cost oils and animal fats that could be converted into biodiesel. They often contain large amounts of FFA that cannot be converted to biodiesel using alkaline-catalyst causing a drawback for alkali catalyzed transesterification method (Demirbas, 2006; Kapilan *et al.,*2009).