TRANSESTERIFICATION OF CRUDE PALM OIL TO FATTY ACID METHYL ESTERS (FAME) MEDIATED BY LIPASE IN BATCH AND CONTINUOUS SYSTEMS

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by

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ASTM D675	American standard for FAME
B5	A mixture composed of 5% biodiesel with 95% diesel
B20	A mixture composed of 20% biodiesel with 80% diesel
BAUN	Batch acidolysis unit NOVO
b.p.	Boiling point
CCD	Central composite design
CCRD	Central composite rotatable design
CMD	Crossed mixture design
CO	Carbon monoxide
CO_2	Carbon dioxide
CPO	Crude palm oil
CSTR	Continuous stirred tank reactor
EJ	Exjoule
EN 14214	European standard for FAME
FAME	Fatty acid methyl esters
FFA	Free fatty acids
FID	Flame ionization detector
FTIR	Fourier Transform Infrared
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
ID	Inner diameter
IRR	Initial reaction rate
IUN	Interesterification units
KF Titrator	Karl-Fischer Moisture Titrator
KLU	Kilo lipase unit
KOH	Potassium hydroxide
Lipozyme TL IM	*Thermomyces lanuginosus* immobilization on silica
Lipozyme RMIM	*Rhizomucor miehei* immobilization on anionic resin
MPOB	Malaysia Palm Oil Board
NaOH	Sodium hydroxide

Novozym 435	*Candida antarctica* immobilization on macroporous polyacrylic resin
NOx	Nitrogen oxide
PBR	Packed bed reactor
PLU	Propyl laurate unit
PORIM	Palm Oil Research Institute of Malaysia
Pred. R^2	Predicted determination coefficient
PSS	Programmed split/ splitless injector
Quad	Quadrillion Btu
RSM	Response surface methodology
RVO	Refined vegetable oil
rpm	Revolutions per minute
SO_2	Sulphur dioxide
TCD	Thermal conductivity detector
vol	Volume
WCO	Waste cooking oil
WHSV	Weight hourly space velocity
wt%	Weight percentage

LIST OF SYMBOLS

		Unit
A	Constant	min^{-1}
$[\text{A}]$	Initial concentration of substrate A	mol.L^{-1}
Adj R^2	Adjusted coefficient of determination	-
a_w	Water activity	-
B	Reactants	-
$[\text{B}]$	Initial concentration of substrate B	mol.L^{-1}
Data	Titration volume	ml
DF	Dilution factor	-
Drift	Drift value	µg.min^{-1}
E	Enzyme	-
E'	Modified form of the enzyme	-
EI	Enzyme-inhibitor complex	-
ES	Enzyme-substrate complex	-
$[\text{ESI}]$	Concentration for enzyme-substrate-inhibitor complex	-
E_0	Initial enzyme activity	mol.L^{-1}.min^{-1}
E_a	Activation energy constant	J.mol^{-1}
E_d	Denaturation energy for lipase	J.mol^{-1}
F$_1$	The factor of the reagent charged in Burette No. 1	-
$[\text{FAME}]_0$	FAME concentration at time, 0	g.L^{-1}
$[\text{FAME}]_t$	FAME concentration on the linear plot at time, t.	g.L^{-1}
$f(x_1, x_2, ...)$	Mixture variables	-
$g(z)$	Ordinary model for the process factors	-
ΔG	Standard free energy	J.mol^{-1}
h	Planck's constant = 6.63 x 10^{-34}	m^2.kg.s^{-1}
ΔH^0	Standard enthalpy (heat content) change	J.mol^{-1}
I	Inhibitor	-
K_a	Binding constant for CPO	mmol.ml^{-1}

K_b	Binding constant for methanol	$mmol.ml^{-1}$
K_I	Dissociation constant for inhibitor with enzyme	$mol.L^{-1}$
K_I^A	Dissociation constant for substrate A	$mol.L^{-1}$
K_m^A	Michaelis constant for substrate A	$mol.L^{-1}$
K_m^B	Michaelis constant for substrate B	$mol.L^{-1}$
k	Reaction rate constant	min^{-1}
k_1	Forward rate constants for formation of the ES complex	s^{-1}
k_{-1}	Reverse rate constants for formation of the ES complex	s^{-1}
k_2	Rate constant for the decomposition of ES to P	s^{-1}
k_d	Denaturation constant	s^{-1}
k_{d_0}	Constant	min^{-1}
k_B	Boltzmann constant = 1.38 x 10^{-23}	$m^2.kg.s^{-2}.K^{-1}$
Log P	Logarithm of the partition coefficient	-
M_{FAME}	Moles of FAME produced	mol
M_L	Moles of methyl linoleate	mol
M_m	Moles of methyl myristate	mol
M_o	Moles of methyl oleate	mol
M_p	Moles of methyl palmitate	mol
M_s	Moles of methyl stearate	mol
M_{CPO}	Moles of total fatty acids in crude palm oil	mol
P	Product	-
Q	Byproduct	-
R	Gas constant = 8314	$\dfrac{kg.m^2}{s^2.kg\text{-}mol.K}$
R^2	Coefficient of determination	-
S	Substrate	-
ΔS^0	Standard entropy change	$J.mol^{-1}.K^{-1}$
T	Operating temperature	K

t	Time	s
t_o	Initial Time	s
Δt	Reaction time taken to produce $[FAME]_t$	min
V_{max}	Maximum reaction rate for CPO and methanol	$mmol.ml^{-1}.min^{-1}$
v	Initial rate of reaction	$mol.ml^{-1}.min^{-1}$
v_0	Constant	$mol.ml^{-1}.min^{-1}$
Wt_1	Weight of the sample before the sample is injected for titration	g
Wt_2	Weight of the remaining sample after the sample is injected for titration	g
x	Independent variables	-
y	Predicted response	-

Greek letters

β_o	Plane interception coefficient	-
β_i	First-order model coefficient	-
β_{ii}	Quadratic coefficient for the factor i	-
β_{ij}	Linear model coefficient for the interaction between factors i and j	-
β_x	Partial regression coefficient	-
ε	Random error term	-
τ	Half life time	min
ψ	Lipase activity coefficient	-

TRANSESTERIFIKASI MINYAK KELAPA SAWIT MENTAH KE LEMAK ASID METIL ESTER (FAME) MENGGUNAKAN PERANTARAAN LIPASE DALAM SISTEM KELOMPOK DAN SELANJAR

ABSTRAK

Harga minyak mentah yang meningkat akibat daripada penyusutan simpanan bahan api fosil dan kadar penggunaan tenaga yang semakin meningkat di serata dunia telah menarik perhatian penyelidik-penyelidik untuk menghasilkan biodiesel sebagai sumber tenaga alternatif. Transformasi minyak kelapa sawit kepada produk yang berharga seperti biodiesel membantu meningkatkan nilai dagangan minyak kelapa sawit. Penghasilan biodiesel dengan 'teknologi hijau' seperti transesterifikasi berenzim dengan minyak kelapa sawit mentah (CPO) sebagai bahan mentah telah dikaji dalam penyelidikan ini. Lipozyme TL IM terbukti sebagai pemangkin yang sesuai bagi transesterifikasi CPO dengan hasil lemak asid metil ester (FAME) yang stabil sebanyak 54.84 - 63.14% pada suhu 30 - 60°C. Pemulihan Lipozyme TL IM mudah untuk penggunaan berulang dan kos lipase yang murah (USD 125.91/kg) berbanding dengan Novozym 435 (USD 1671.19/kg). Perencatan akibat kesan metanol dapat diatasi dengan mengaplikasikan rekabentuk campuran silang (CMD) di mana 96.15% hasil FAME dicapai pada 0.37: 0.10: 0.53 kadaran CPO: metanol: tert-butanol. Proses beroperasi dengan had pemindahan jisim yang minimum dapat dicapai pada 150 rpm, 6.65 jisim% bebanan enzim dan 4 jam masa tindakbalas untuk menghasilkan 96.15% hasil FAME. Model matematik dengan mempertimbangkan kesan pengaktifan dan perencatan lipase dapat meramalkan hasil FAME dengan perubahan suhu dan masa tindakbalas ($R^2 = 0.99$). Sehubungan itu, suhu 40°C merupakan suhu kendalian optimum kerana kadar perencatan lipase sebanyak 1.10 x 10^{-2} jam^{-1} pada suhu 40°C dapat diatasi dengan 2.66% hasil FAME/min.

Pemangkinan lipase pada transesterifikasi CPO dengan perantaraan tert-butanol dapat dijelaskan dengan mekanisme ping-pong bi-bi dengan 1.45 mmol/ml.min kadar tindakbalas maksimum, V_{max}, 1.59 mmol/ml nilai pemalar CPO, K_a dan 1 mmol/ml nilai pemalar metanol, K_b. Transesterifikasi CPO di dalam sistem selanjar reaktor lapisan terpadat menunjukkan kecekapan pemangkinan optimum pada hasil FAME 90% dengan kadar aliran penyuapan 0.70 ml/min, ketinggian lapisan terpadat 3.50 sm dan saiz mangkin 355 µm. Pencapaian hasil FAME yang tinggi dalam kajian ini telah menunjukkan kemungkinan untuk menghasilkan biodiesel daripada CPO melalui transesterifikasi berenzim.

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ABSTRACT

The rapid increase in the crude oil prices due to diminishing reserves of fossil fuels and the increased rate of world energy consumption have drawn attention of the researchers towards biodiesel as an alternative energy source. The transformation of palm oil into value-added product biodiesel can help to increase its commercial value of palm oil. The green technology of enzymatic transesterification with crude palm oil (CPO) as feedstock for biodiesel production has been studied in the present research. Lipozyme TL IM was found as a suitable biocatalyst for CPO transesterification with stable FAME productivity of 54.85 - 63.14% within the temperature range of 30 - 60°C. The recovery of Lipozyme TL IM for repeated usage was simple and relatively cheap (USD 125.91/kg) as compared to the competitive Novozym 435 (USD 1671.19/kg). Methanol inhibition was alleviated successfully using CMD where 96.15% FAME yield was achieved at 0.37: 0.10: 0.53 proportions of CPO: methanol: tert-butanol. Process operation with minimum mass transfer limitations was defined at 150 rpm, 6.65 wt% enzyme loading and 4 hr reaction time in order to attain 96.15% FAME yield. A kinetic model that accounted for both lipase activation and deactivation effects was proposed and FAME yield corresponding to temperature and reaction time variation was predicted from the model (R^2 = 0.99). The most suitable operative temperature was 40°C because the lipase denaturation rate of 1.10×10^{-2} hr^{-1} at 40°C was overwhelmed with the gain of 2.66% FAME yield/min. The lipase-catalyzed transesterification of CPO mediated with tert-butanol could be elucidated by ping-pong bi-bi mechanism with maximum

reaction rate, $V_{max} = 1.45$ mmol/ml.min, CPO binding constant, $K_a = 1.59$ mmol/ml and methanol binding constant, $K_b = 1$ mmol/ml. The CPO transesterification in continuous PBR system showed high catalytic efficiency with 90% FAME yield and produced at 0.70 ml/min feed flowrate, 3.50 cm PBR height and 355 μm biocatalyst particle size. The high FAME yield achieved in this study shows that it is feasible to produce biodiesel from CPO via enzymatic transesterification.

CHAPTER 1

INTRODUCTION

1.1   Biodiesel fuel: a clean and promising renewable energy source

The U.S. Energy Information Administration declared that total world energy consumption in 2005 was 488 EJ (exjoule, 10^{18}J) or 463 Quad (quadrillion Btu, 10^{15} Btu). World consumption is expected to surpass 60% increase in energy consumption or equivalent to 650 EJ by 2025 and this value reflects the degree of industrialization, efficiency of primary energy source used and energy conservation (Energy Information Agency, 2007). In view of the worldwide energy supply, crude oil accounted for 35% of the total energy consumption in 2002. Natural gas, coal and nuclear accounted for 21%, 23% and 7% respectively whereas renewable energy contributed to 14% (U.S. Department of Energy, 2004; Energy Information Agency, 2007). Annual energy consumption values for selected countries are shown in Figure 1.1. On top of the annual energy consumption between 1985 and 2005, China and India achieved an overwhelming of 250 % increased in energy demand compared to 18 % in Europe (Energy Information Agency, 2007). Currently, available fossil fuel sources are estimated to become depleted in the next century, with petroleum reserves depleted within 40 years (BP, 2005; Energy Information Agency, 2007) . As a consequence, crude oil prices have risen from less than $20/ barrel in the 1990s to nearly $100/ barrel in 2007 (Kinney and Clemente, 2005).

In addition to the energy security crisis, severe climate changes were the consequences of greenhouse gases due to fossil fuels consumption (Drapcho *et al.*, 2008; Arent *et al.*, 2010; Meunier, 2007; Kriegler, 2010; Ellington *et al*., 1993). As

an alternative, biodiesel fuel produced on a sustainable basis offers the benefits of environmentally friendly (Zhang, 2003), energy independency and security, economic and social cohesion (Howell and Jobe, 2005). These factors have created the driving force and motivation for this alternative fuel-biodiesel production.
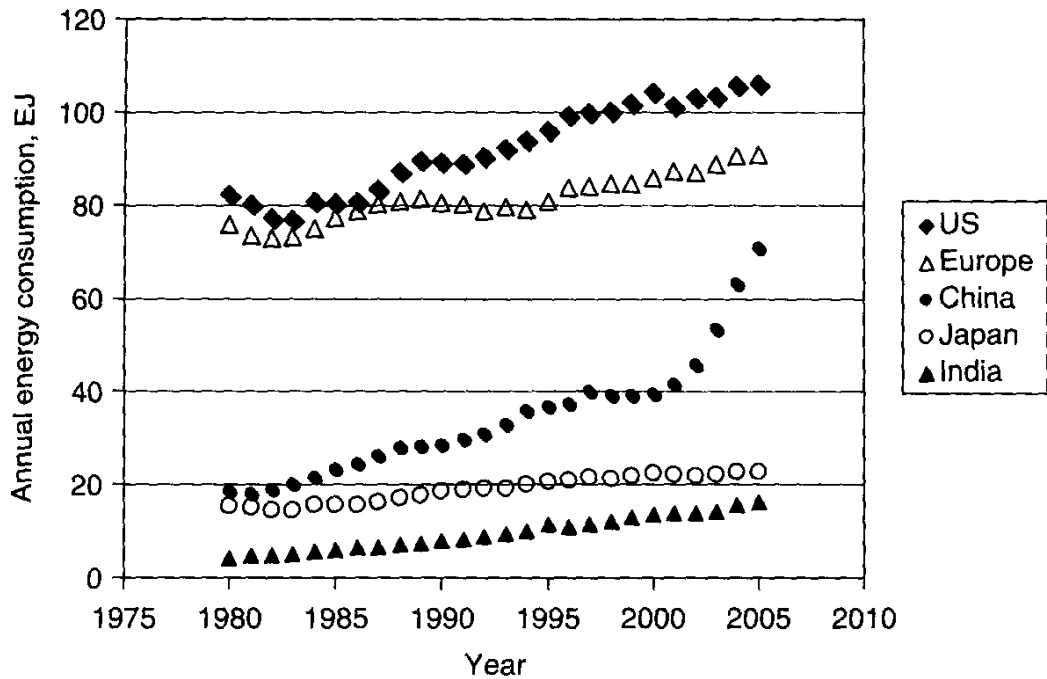
Figure 1.1: Annual energy consumption values for selected countries. (Adapted from Energy Information Agency, 2007)

Biodiesel, chemically defined as monoalkyl esters of long chain fatty acids, are derived from renewable feedstocks from vegetable oils or animal fats (Ganesan *et al.*, 2009). Biodiesel is an environmentally friendly and a renewable fuel that gives a promising alternative fuel for compression-ignition engines. Generally, biodiesel resulted from vegetable oil has viscosity close to diesel fuel, possesses high cetane number and flash point (Meher *et al.*, 2006; Demirbas, 2009). The utilization of biodiesel possesses several distinct technical advantages and disadvantages compared to petro diesel (Table 1.1).

Table 1.1: List of advantages and disadvantages of biodiesel as engine combustion fuel compared to petrodiesel. (Meher *et al.*, 2006; Demirbas, 2009)

Advantages	Disadvantages
• Derivation from renewable resource, thus in the aim of sustainability and dynamic energy preservation.	• Its inherent higher price (offset by legislative and regulatory incentives/ subsidies)
• Biodegradability	• Slightly increased NO_x exhaust emissions
• Reduction of most exhaust emissions	• Instability when exposed to air (oxidative stability)
• Higher flash point for safer handling and transport purpose	• Cold flow properties especially relevant in North America.
• High cetane number resulted in high combustion efficiency	
• Excellent lubricity compared to greatly reduced lubricity of petrodiesel fuels.	

Global economic growth factor is forcing higher burning fossil fuels and thus greenhouse gases are released at a life-threatening rate and causing global warming. In terms of impacts on environmental hazard, biodiesel production can be more environmentally benign than fossil fuel technologies for several reasons. Biodiesel production from biomass is carbon neutral where the CO_2 produced as fuel is combusted, is offset by the carbon absorbed as the biomass is grown (Tillman *et al.*, 2006). Besides, bioconversion processes produce less hazardous compounds and less environmental pollutions are involved. In contrast, extraction of fossil fuels without the execution of sustainable practices places a heavy toll on the environment (Drapcho *et al.*, 2008). Therefore, by considering the process as well as the compound itself, biodiesel is considered as a clean fuel that can combat global warming and stabilize the climate through the reduction of CO_2 emissions (Jacquet, 2007; Drapcho *et al.*, 2008; Arent *et al.*, 2010; Meunier, 2007; Kriegler, 2010; Ellington *et al.*, 1993).

The utilization of biodiesel would largely enhance the commercial value for fats and oils, the need to build biodiesel plants and the employment of human capital to operate these plants. Thus, using biodiesel would gain economic benefits for farmers, local communities and the nation as a whole. Increased utilization of biodiesel results in significant economic benefits for both the urban and rural sectors, as well as the balance of trade (Howell and Jobe, 2005).

Coupled with political considerations over the security of energy supplies and price volatility, environmental concerns related to global warming and sustainability are the momentums to move the worlds' energy consumption away from fossil fuels. The initiatives and efforts taken across the European and Asian countries in developing and promoting biodiesel production so as to reduce dependency on energy supply from fossil fuels are reviewed in the following section.

1.2   Biodiesel developments to meet energy challenges

The factors of fuel prices volatility, the desire for energy independence and security, encourage several countries to dramatically step up for targets and mandates for biofuels (Bockey, 2005). The global biodiesel industry has grown significantly over the pass decade and estimated to reach 37 billion gallons by 2016 at an annual growth of 42% (Sims, 2007). The European Union was the global leader in biodiesel production with 3.2 million tons in 2005 and 4.9 million tons in 2006 (European Biodiesel Board, 2006). Germany alone accounted for half of the global biodiesel production in 2005. Several countries in the EU dramatically stepped up targets and mandates for biofuels policies in 2005-06. French established a 5.75% of biodiesel blending by 2008 whereas Germany mandated 4.4% biodiesel blending. EU

countries have also been enacting biofuel tax exemptions (Bockey, 2005). The United States has increased biodiesel production from 2 million gal in 2000 to 250 million gal in 2006 (Carriquiry, 2007). The blending mandates have also initiated in several biodiesel producer countries such as Brazil, Canada, Colombia, Malaysia and Thailand. Malaysia started using B5 or 5% biodiesel blending in 2005 (Körbitz, 2005).

Recently, a rapid expansion in production capacity is being observed in developing countries. In China, a vegetable fat chemistry plant was started in June 2001 and has established a capacity 10,000 tons/year. The Kyoto city initiated biodiesel production from recycled frying oil and has established the B20 mixture for 81 city buses since 2000 (Körbitz, 2005). As reported at the International Biofuel Conference in 1998, the biodiesel produced from Palm Oil Research Institute of Malaysia's (PORIM) pilot plant has promising results in diesel engines. The suitability of palm oil as biodiesel feedstock with encouraging results for trial run on diesel engine and the limitations of biodiesel feedstock in Europe had significantly increased the palm oil exports which gave an additional venue for Malaysia's palm oil industry (Malaysia Palm Oil Council, 2007). Alternatively, Malaysian government launched National Biofuel Policy in promoting the biodiesel production from palm oil in research stages by PORIM until the establishment of a few biodiesel plants in Malaysia (National Biofuel Policy, 2006). Prospects and impacts of biofuel from palm oil especially to Malaysia are elaborated and discussed in detail in the following section.

1.3   Prospects of biodiesel from palm oil

Malaysia is endowed with abundant supplies of non-renewable energy resources, especially oil and gas. However, the non-renewable fossil fuels (current oil and gas reserves) are expected to be depleted within 17 and 37 years respectively. From 1998 to 2001, electricity accounted for 18% of the fuel energy consumption, averaging 28,500 in the Malaysia energy sector, therefore Malaysia is expected to become an importer of crude oil when its domestic petroleum consumption exceeds production (Earth Trends Country Profiles, 2003). With the knowledge that these non-renewable fossil fuel resources will exceed their threshold capacity, Malaysian government has to optimize the use of its huge quantities of palm oil as renewable energy resource (Malaysia Palm Oil Board, 2009).

In view of the usage of biodiesel would reduce dependency on imported crude oil, there must be a balance to be sought between agricultural, economic development and the environment (Basiron, 2007). Malaysia Palm Oil Board (MPOB) has dramatically taken leading steps and efforts in research and development from as early as 1987 to explore biodiesel production from palm oil. Malaysia is well-known as world's largest producer and exporter of palm oil with crude palm oil production dramatically increased from 8.3 million tonnes in 1998 to 15.8 million tonnes in 2009 (Malaysia Palm Oil Board, 2009). In 2008, palm oil contributed to about 13% of the total world production of oils and fats and it is competitive to soybean oil as the most important vegetable oil (Santosa, 2008; Sumathi *et al.*, 2008).

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Biodiesel produced from palm oil meets full EN 14214 and ASTM D675 specifications. Research studies by MPOB claimed that palm oil methyl esters used as diesel substitute resulted in promising and satisfying engine performance through a long term assessment of the effect of biodiesel on vehicle maintenance, operating costs, fuel consumption and exhaust emission (May *et al.*, 2005; Kalam and Masjuki, 2005; Crabbe *et al.*, 2001). These studies concluded from test run on biodiesel fuel engines mounted on passenger buses with milestones of 300,000 km. This also included very detailed tests on bus fleets, which were started by Daimler-Benz as early as 1987 (Körbitz, 2005). Other benefits with the used of palm oil biodiesel includes the great reduction in CO_2, CO, unburnt hydrocarbon, SO_2, particulates and air toxics and biodegradable (Kalam and Masjuki, 2005). The palm oil fuel has experienced intensive development from research to the first commercial small scale plant by Carotino Sdn. Bhd. and the plant started production of 3,000 tons per annum by August 2002. The plant expanded to 120,000 tons per annum in 2006. In addition, the world first integrated commercial normal and winter-grade biodiesel plant was successfully launched by Carotino Sdn. Bhd. in August 2006 (Choo *et al.*, 2009).

The successful studies and developments in biodiesel production from palm oil has attracted keen interest by the Malaysian government to dramatically step up and release the National Biofuel Policy in March 2006 (Choo *et al.*, 2009). The National Biofuel policy with envisions to reduce the dependency on fossil fuels, with the use of environmental friendly, sustainable and viable sources of energy. The policy also aims to enhance prosperity and well-being of the stakeholders in the agriculture and commodity based industries through stable and remunerative prices (National Biofuel Policy, 2006). Five Strategic Thrusts were embedded in the

National Policy to sustain and establish the biodiesel industry. Government have mandated the used of 5% biodiesel from palm oil blending with 95% petroleum diesel or B5 in land and sea transport as well as in the industrial sector (Malaysia Palm Oil Board, 2009). Government and private sectors would provide enough funding adequate for the research, development and commercialization of biofuel technologies to enable an increase use of biofuel. Malaysia's interest to become an active biofuel exporter and thus, the establishment of plants for biodiesel production is encouraged and facilitated. The utilization of biofuel will reduce the use of fossil fuels and consequently enhance the quality of the environment (Abdullah *et al.*, 2009a).

Various conversion methods on renewable feedstocks have been established for biofuel production with chemical reaction being the most popular route. However, biodiesel synthesis method of enzymatic transesterification is environmental benign, requires mild operating conditions of low energy input and the ease of glycerol separation from biofuel. There is no need of deodorization nor neutralization of the product necessary compared to chemical process (Du *et al.*, 2004; Fukuda *et al.*, 2001; Wardle, 2003). In the present study, the research is focused on using enzymatic transesterification for biodiesel production throughout the investigation. Therefore, literatures on the effects of crucial parameters such as lipids, lipase sources, methanol to oil molar ratio, operating temperature and water content on transesterification are emphasized. In addition, current techniques which are adopted for process enhancements and process optimization, the available system configurations for continuous transesterification reaction are discussed and reviewed in Chapter 2.

1.4   Problem statement

Enzymatic transeserification reaction for FAME production is not commercially adopted, mainly due to the expensive cost of immobilized lipase and relatively slow reaction rate compared to alkaline-catalysed transesterification. Therefore, this study was carried out to identify the problems occurred during the enzymatic transesterification process and appropriate solutions to the problems will be suggested.

In previous researches, effort was carried out mostly on transesterification of refined vegetable oil or waste cooking oil by expensive immobilized lipase *Candida antarctica* (Novozym 435). Waste cooking oil (WCO) although cheap but the feedstock is not adequate in supply and its chemical composition is inconsistent. Besides, high free fatty acids (FFA) in WCO may cause the hydrolysis reaction in competition with the transesterification reaction and thus, lead to low FAME yield. Therefore, crude palm oil (CPO) which is high in triglyceride content (>96%) and extremely low in FFA is chosen as a potential feedstock for transesterification. The cost for CPO resulting from palm oil extraction is relatively low compared to the refined vegetable oil (RVO) that undergoes processes such as degumming, bleaching, deoderization and hydrogenation. Although CPO is cheap, it is not a favourable feedstock for research due to the phospholipids and degummed oil contents inhibit the lipase used for the enzymatic transesterification. The semi-solid form of CPO at room temperature is quite viscous and difficult to handle since the diffusion of reactants from bulk liquid to biocatalyst could lead to significant mass transfer limitations. Therefore, CPO is recommended for a pretreatment process prior to the transesterification reaction.

A widely adopted lipase isolated from *Candida antarctica* is an excellent enzyme for transesterification with more than 90% of fatty acid methyl esters (FAME) yield, but the production cost of the lipase is expensive (USD 1671.19/ kg) (Haas *et al.*, 2006) and the ease of lipase inactivation with mild methanol to oil molar ratio of 1.5:1. These adverse effects have hindered the repeated use of lipase *C. antarctica* for a prolong period of operation and thus, enzymatic transesterification for biodiesel production is not economically feasible. Alternative lipase sources need to be screened with characteristics of cheap in production cost, high resistant to feedstock and product inhibitions, maintaining high catalyzing efficiency even for extended period of operation and achieved high FAME yield. Relatively cheap lipase of *Thermomyces lanuginosus* (USD 125.91/ kg) (Haas *et al*., 2006) compared to lipase *C. antarctica* is a better choice for transesterification.

Due to the ease of lipase inactivation with mild methanol concentration, step-wise methanol addition method and the use of organic solvent as reacting medium are the initiatives adopted to increase the viability of enzyme transesterification in biodiesel production. Batch wise methanol addition technique for high methyl ester yield is a compromise between methanol dosage and addition time so as to solubilise all the added methanol into oil. The method is time consuming and requires intensive monitoring to avoid excessive insoluble methanol droplets in reaction mixture. Organic solvent, for instance, tert-butanol is able to dilute both hydrophilic methanol and hydrophobic CPO into the reaction mixture, but the excessive use of tert-butanol may result in dilution effect and could lead to low conversion. Besides, the subsequent immersion of insoluble byproduct, glycerol tends to bind to the lipase structure and causes very slow or no catalytic activity. Therefore, all the components

in the reaction mixture present in appropriate proportions are of paramount importance to ensure complete solubility between reactants (CPO, methanol) with products (FAME, glycerol) into organic solvent. Crossed mixture design (CMD) is adopted to optimize the mixture proportions and operating parameters simultaneously.

Most of the transesterification studies were focused on optimizing the operating temperature merely to accelerate and maximize catalyzing activity of lipases without considering thermal stability of lipases due to high temperature operation. Lipase is sensitive and is reported to be merely inactivated at moderate temperature operation of 50°C. The enzymatic transesterifiation operates under the most desirable operating temperature able to attain high FAME productivity as a whole besides preserving the lipase catalyzing activity.

Continuous stirred tank reactor (CSTR) for FAME production usually results in low FAME yield compared to batch system which is mainly due to the relatively short retention time of substrate in the reactor. In addition, the procedures for lipase recovery in CSTR system are difficult and expensive while the process scale-up could be unreliable. Continuous system integrated with the bed of immobilized lipase particles being packed inside the reactor helps to minimise the methanol inhibition to lipase due to low differences between substrate and product concentration in the whole reactor. The *in-situ* immobilized lipase separation can be carried out with packed bed reactor (PBR) while the corporation of membrane in CSTR to recover lipase for reusability have caused an expensive capital cost and mass transfer limitations. The effective enzyme loading required for high methyl esters yield was

largely reduced in PBR compared to CSTR. Continuous system with all the lipase particles packed inside the PBR offers the ease of reactants mass transfer to lipase active site for catalyzing activity. Whereas, in CSTR, the lipase shearing resulted from collisions between biocatalyst particles and impeller besides the adsorption and tendency for the lipase particles to accumulate at the membrane surface have largely reduced the theoretical catalytic activity. Key factors for continuous system are simultaneously identified and optimized to maximize the FAME production rate.

1.5   Objectives

The project was aimed to initiate renewable fuel production from crude palm oil (CPO) via enzymatic transesterification. Process enhancement and optimization in batch and continuous systems were carried out using statistical approach. In order to achieve the main objective, a number of specific objectives are required and they are listed as follows:

1.) To compare the catalyzing efficiency on FAME production from CPO and methanol for both free and immobilized lipases from three different species (*Candida antarctica, Rhizomucor miehei, Thermomyces lanuginosus*).

2.) To optimize composition of the reaction mixture (tert-butanol, CPO and methanol) and process parameters simultaneously for high catalyzing activity using crossed mixture design (CMD).

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3.) To identify external mass transfer control and lipase loading conditions in batch system for high FAME yield using factorial experimental design approach.

4.) To study the thermal effects on lipase and to determine the optimum operating temperature that simultaneously achieve high FAME yield and preserves high catalyzing activity of lipase.

5.) To deduce the kinetics and mechanism for lipase-catalyzed transesterification of CPO under reaction-controlled condition, which then leads to identification of types of inhibition exerted from both substrates and product on the lipase catalyzing rate.

6.) To study and optimize the enzymatic transesterification of CPO process in continuous packed bed reactor using factorial experimental design.

1.6   Scope of the study

Overall, the FAME production from CPO and methanol were initiated in batch reactor and the reaction was then transferred to continuous packed bed reactor (PBR) system with the aim of obtaining high production rate and high FAME yield.

Scope 1: Identifying the most potential lipase for CPO transesterification. Few commonly utilized lipases (from: *C. antarctica, T. lanuginosus* and *Rhizomucor miehei*) in free and immobilized forms were studied for their catalyzing specificity in transesterification reaction. The most desirable immobilized lipase that can withstand

the highest methanol to oil molar ratio while achieving the highest FAME yield at the lowest reaction temperature was identified. Since the transesterification reaction is reversible, the excess molar methanol concentration is necessary to drive the FAME production into completion. The desired lipase was further examined for the degree of methanol inhibition when susceptible to the extremely high methanol to oil molar ratio of 35:1.

Scope 2: Process optimization, thermal and kinetic studies in batch system. Optimization on transesterification reaction in batch system was carried out with the study on independent variables of feed compositions (methanol, tert-butanol, CPO), operating temperature (30 – 60 °C) and reaction time (0.50 - 12 hr). The crossed mixture design (CMD) was employed to optimize feed compositions of reaction mixture and operating parameters simultaneously. Transesterification reaction at the minimum mass transfer limitations was developed by optimizing process parameters of agitation speed, enzyme loading and reaction time using factorial experimental design. The optimum mixture compositions which were defined by CMD were adopted in the process optimization study. In the thermal effects study, the most desirable operating temperature for transesterification reaction was identified which produces high FAME yield while preserving the catalyzing activity of lipase. The thermal stability and activity of immobilized lipase at different operating temperature and period of heat treatment was modelled for an enzymatic reactor. The kinetic parameters and suitable kinetic mechanism based on varians were deduced for the lipase-catalyzed transesterification of CPO with methanol in tert-butanol solvent system. Consequently, types of inhibitions exerted by substrates of CPO and

methanol as well as byproduct of glycerol were evaluated using reciprocal plots of Lineweaver-Burk plot and Hans-Woolf plot.

Scope 3: To study CPO transesterification in a continuous PBR system and process optimization. A packed bed reactor suitable for continuous production of FAME from CPO was fabricated and reaction was conducted. The product recovery after the reaction was also carried out. An empirical model that revealed the process performance with key parameters was developed for PBR system with the aid of statistical design. The obtained model equation was simulated for desirable product yield and the simulated results were compared with the experimental data.

1.7 Organization of the thesis

The thesis comprises of five chapters. Chapter 1 (Introduction) explains the importance of biodiesel as sustainable fuel for future besides incentives and energy policies taken nation wide to secure energy independency. Research developments until the establishment of biodiesel plant using palm oil feedstock in Malaysia are described to clarify the prospects of biodiesel production from palm oil. Difficulties occurred with enzymatic transesterification technology are identified in problem statement. Objectives are listed down and strategies are proposed with scope of the study, to solve the problems for the current research.

Chapter 2 (Literature Review) is mainly on the literature survey, information on theories and principles involved in kinetics for lipase-catalyzed reaction, thermodynamic effects on reaction, key parameters that affecting process efficiency and significant findings from the previous researches on enzymatic transesterification

for biodiesel production. Description about the modeling tools of factorial design, crossed mixture design (CMD) and response surface methodology (RSM) are included.

Chapter 3 (Material, Experimental Methods and Analysis) is a compilation of all the materials, equipments and chemicals used in the experiment. All the procedures from feedstock pretreatment, conducting transesterification reaction for optimization, kinetic studies and thermodynamic studies in batch system are mentioned in this chapter. Lastly, the experimental setup and operation for continuous PBR as well as the product analyses are presented.

Chapter 4 (Results and Discussion) is a discussion section for the experimental work done based on the data analysis. This chapter includes the lipase screening for high FAME yield, defining optimum mixture compositions to minimise methanol and glycerol inhibitions, process optimization at minimum external mass transfer and intra-particles mass transfer limitations, studies on the thermal effects and the lipase kinetics to understanding process behaviour. Performance of transesterification reaction in continuous PBR is optimized with statistical analysis, effect of external mass transfer in PBR system is evaluated and concluded with long hours' operation.

Chapter 5 (Conclusions and Recommendations) highlight and emphasize the dominant observations for the experimental studies with specific remarks based on the previous research works. Recommendations are included for future work of biodiesel production via enzymatic transesterification.

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CHAPTER 2

LITERATURE REVIEW

2.1 Biodiesel production routes

The substitution of vegetable oil for diesel fuel in diesel engines has shown to encounter problems of high viscosities, low volatilities and polyunsaturated character (Srivastava and Prasad, 2000). Oils with high in polyunsaturates have higher nitrogen oxide emissions once burned. However, these oil characteristics can be modified for improved diesel engine performances through pyrolysis, microemulsification and transesterification (Fukuda *et al.*, 2001).

2.1.1 Pyrolysis

Pyrolysis is defined as the conversion of one substrate into another by the application of thermal energy in the absence of oxygen. Generally, pyrolysis is carried out under flow of nitrogen (Srivastava and Prasad, 2000; Schwab *et al.*, 1988). Soybean oil, cotton seed oil, rapeseed oil and other oils are thermally cracked with similar chemical compositions to fossil fuels (Ma and Hanna, 1999). However, pyrolyzed vegetable oils are contaminated with trace amounts of sulphur, water, sediment and these could lead to corrosion problems in the engine. This process usually requires high maintenance cost especially the distillation unit for various fractions separation (Ma and Hanna, 1999). The removal of oxygen during the thermal processing of pyrolysis eliminates the benefits of using oxygenated fuel.

2.1.2 Microemulsion

A microemulsion is defined as a colloidal equilibrium dispersion of optically isotropic fluid microstructures with dimensions generally 1 - 150nm range formed spontaneously from two normally immiscible liquids and one or more ionic or non-ionic amphiphiles (Schwab *et al.*, 1987). Ionic and non-ionic microemulsions of aqueous ethanol in soybean oil have good performances as No. 2 diesel where No. 2 diesel is known as diesel fuel oil. Emulsion of 53% (vol) alkali-refined and winterized sunflower oil, 13.3% (vol) 190-proof ethanol and 33.4% (vol) 1-butanol when undergoing 200 hrs laboratory screening endurance test, irregular injector needle sticking, heavy carbon deposits, incomplete combustion and an increase of lubricating oil viscosity were reported (Ziejewski *et al.*, 1984).

2.1.3 Transesterification

Transesterification is the most widely used process producing biodiesel from vegetable oils. Transesterification is the alcoholysis of triglyceride esters and resulting in a mixture of mono-alkyl esters and glycerol (Fukuda *et al.*, 2001). This process has been used to reduce the viscosity of triglycerides and enhancing the physical properties of renewable fuels with the aim to improve the performance in engine (Clark *et al.*, 1984). Short-chain alcohol especially methanol and ethanol are utilized due to its low cost, easy availability and its physical and chemical advantages (Fukuda *et al.*, 2001). Transesterification can be carried out through variety of ways in the presence of alkali catalyst, acid catalyst, biocatalyst, heterogeneous catalyst or using alcohols under supercritical conditions without catalyst.

Microemulsions of vegetable oils would reduce the biofuel viscosity but engine performance problems of injector coking and carbon deposits still persist (Bala, 2005). Pyrolysis of triglycerides produces compounds with incompatible biodiesel quality in terms of ash, carbon residues and pour point (Schwab *et al.*, 1987). Among all the conversion methods, transesterification of vegetable oils is the most promising route to produce biofuel with similar properties and performances as hydrocarbon based diesel fuels (Fukuda *et al.*, 2001).

2.2   Transesterification in biodiesel production

Transesterification is not a newly discovered process but the reaction has been conducted as early as 1853 by scientists E. Duffy and J. Patrick. The process of converting vegetable oil into biodiesel fuel is termed as transesterification. There are numerous transesterification citations in the scientific and patent literature (Ma *et al.*, 1998; Freedman *et al.*, 1986; Schwab *et al.*, 1987). The transesterification reaction with alcohol as acyl acceptor consists of a number of consecutive, reversible reactions. The mechanism is initiated with the released of one mole methyl esters from the conversion of triglycerides to diglycerides, followed by second and third methyl ester yield from the conversion of diglycerides to monoglycerides and from monoglycerides to glycerol (Freedman *et al.*, 1986; Noureddini and Zhu, 1997).

2.2.1   Chemical-catalyzed transesterification

The effects of the different acyl acceptor, molar ratio, catalyst loading and reaction temperature on to the reaction rate were the concerned parameters to be examined by researchers (Freedman *et al.*, 1986). Catalyst transesterification is the most often used method to produce fatty acid methyl ester (FAME) and the alkalis

used include sodium hydroxide (NaOH), potassium hydroxide (KOH), carbonates and alkoxides such as sodium methoxide, sodium ethoxide and sodium butoxide (Fukuda *et al.*, 2001). For an effective alkali-catalyzed transesterification, the reaction mixture consisted of triglycerides and alcohol must be anhydrous and low in free fatty acids (FFA) (Ma *et al.*, 1998; Wright *et al.*, 1944) to eliminate the occurrence of saponification reaction that involved soap production. The catalyst is consumed during the soap formation and thus reduces the catalytic efficiency, causing increase in fluid viscosity, formation of gels and renders the separation of esters and glycerol (Ma and Hanna, 1999; Fukuda *et al.*, 2001).

Acid-catalyzed transesterification is used when triglycerides have relatively high water and FFA (Aksoy *et al.*, 1988; Freedman *et al.*, 1984). Acids used for transesterification include sulphuric, phosphoric, hydrochloric and organic sulfonic acids (Fukuda *et al.*, 2001). Researchers reported that acid-catalyzed transesterification was particularly important when conducting the reaction with low grade material such as sulphur olive oil (Aksoy *et al.*, 1988). The acid-catalyzed process is not favourable because the process is corrosive and the reaction rate was reported to be 4000 times slower than alkali-catalyzed transesterification with the same amount of catalyst (Formo, 1954).

2.2.2 Supercritical transesterification

Supercritical state of transesterification without using any catalyst was developed by Saka and Kusdiana (2001) under reaction temperature of 350°C and molar ratio of methanol to rapeseed oil of 42:1 (Saka and Kusdiana, 2001). Non-polar triglycerides can be well solvated with supercritical methanol to form single

phase oil to methanol mixture, thus the conversion rate of oil to methyl esters increased dramatically (Kusdiana and Saka, 2001). In addition, the purification of products after the process is simple to accomplish and the reaction is environmentally friendly. However, the requirements of high temperature of 350°C and operating pressure of 45 MPa with the supply of excessive amount of methanol are prerequisite to drive the supercritical state of transesterification reaction (Fukuda *et al.*, 2001).

2.2.3   Enzymatic transesterification

Several researchers have attempted to produce biodiesel using extracellular lipase enzyme for effective methanolysis reaction. The successful implementation of enzymatic transesterification was able to offset several drawbacks occurred during the chemical transesterification in biodiesel production. In contrast to chemical transesterification, lipase-catalyzed processes are promising due to high selectivity of lipase in reaction under mild operating conditions (Salis *et al.*, 2003; Jaeger and Eggert, 2002; Schimd *et al.*, 2002; Salis *et al.*, 2004). Besides, recovery of FAME is simple to accomplish (Fukuda *et al.*, 2001). When compared to base catalysis, FFA concentration in the oil is not critical to enzymatic transesterification because fats containing triglycerides and FFA can be enzymatically converted to biodiesel in a one-step-process. Lipases are able to catalyze both transesterification and esterification reactions (Szczesna Antczak *et al.*, 2009). Production of cheaper and robust lipase preparations together with system development that favor for long-term, iterative use of biocatalyst can give rise to the replacement of chemical processes with enzymatic route (Gerpen, 2005; Meher *et al.*, 2006; Ma and Hanna, 1999; Ranganathan *et al.*, 2007; De Greyt, 2004; Marchetti *et al.*, 2007; Akoh *et al.*, 2007).

The comparison in catalyzing behaviours and operating conditions between chemical catalysis, supercritical alcoholysis and lipase catalysis processes for FAME production is summarized in Table 2.1.

Table 2.1: Comparison between chemical catalysis, supercritical alcoholysis and lipase-catalysis methods for biodiesel fuel production. (Fukuda *et al.*, 2001; Ganesan *et al.*, 2009)

Criterion	Acid-catalysis	Alkali-catalysis	Supercritical alcoholysis	Lipase-catalysis
1. Reaction temperature	• 55-80 °C	• 60-70 °C	• 239-385 °C	• 30-40 °C
2. FFA in raw materials	• Esters	• Saponified products	• Esters	• Methyl esters
3. Water on raw materials	• Interfere with the reaction	• Interfere with the reaction	• Not stated	• No influence
4. Yield of methyl esters	• Normal	• Normal	• Good	• Higher
5. Recovery of glycerol	• Difficult	• Difficult	• Not stated	• Easy
6. Purification of methyl esters	• Repeated washing	• Repeated washing	• Not stated	• None
7. Production cost of catalyst	• Cheap	• Cheap	• Medium	• Relatively expensive

2.3   Biogreen technology: enzymatic transesterification

The enzyme catalyzing triglycerides with methanol appears to be in accordance with a successive reaction mechanism (Kaieda *et al.*, 1999). Triglycerides and partial glycerides are first hydrolyzed by lipase to partial glycerides and FFA respectively. The intermediate reaction includes the alcoholysis of FFA with methanol and methyl esters are synthesized (Kaieda *et al.*, 1999). In brief, triglycerides contained in vegetable oil react with an alcohol (usually