BIODIESEL FROM PALM WASTE COOKING OI USING IMMOBILIZED LIPASE IN MODIFIED PVA-ALGINATE SULFATE BEADS

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A thesis submitted in fulfilment of the
requirements for the award of the degree of
Doctor of Philosophy (Bioscience)

Faculty of Science
Universiti Teknologi Malaysia

JULY 2021

ACKNOWLEDGEMENT

In the name of Allah the Most Gracious, the Most Merciful

Alhamdulillah, all praises and thanks to Allah for giving me strength to complete this thesis. First and foremost, I would like to express my sincere gratitude to Dr Nor Azimah Mohd Zain, for her guidance, support, advices, valuable comments, suggestions, provisions, patience and enthusiasism during her supervision period. Without her patience and valuable assistance, this thesis would not have been the same as presented here. It is a great honour to work under her supervision. I could not imagined having a better advisor and mentor for my Ph.D study.

To my bestfriends Balqis, Katijah,Atikah Husna, Sarina and Qistina for the sleepless nights we were working together in doing our research and always listening and giving me words of encouragement and all my fellow postgraduates' student for giving their comments and suggestion on how the project can improve.

I would like to express my eternal appreciation towards my mom Meriam binti Darus who has never gives up on me and always be there for me no matter where I am, for all her unconditional love, prayers, support and patience. I am very much thankful to my husband, Mohd Nur Hidayat Hasbollah and my son Muhammad Iman for their love, understanding, prayers and continuing support to complete this research work.

ABSTRACT

Malaysia is currently the world's second largest palm oil producer, which accounts for 39% of world palm oil production. About 0.5 million tonnes of waste cooking oil is generated annually in Malaysia. Improper disposal of waste cooking oil leads to the environmental pollution particularly in land and water. To overcome these problems, this study aimed to use palm waste cooking oil as a feedstock to produce biodiesel as an alternative to the limited and non-renewable sources of conventional petroleum. Apart from that, utilization of lipase as biocatalyst to produce biodiesel has advantages over chemical catalyst as the reaction can be performed under mild conditions and simple separation process. This study investigated the production of biodiesel from palm waste cooking oil using immobilized *Candida rugosa* lipase (CRL) in Polyvinyl Alcohol (PVA) alginate sulfate beads. The One-factor-at-a-time (OFAT) method was used in order to select a suitable range of variables before statistical analysis was performed. The Design-Expert software was used as a statistical tool to operate Central Composite Design (CCD) for optimization of significant factors. The statistical analysis was used in order to achieve maximum biodiesel production and evaluate the effect of each variable and their interaction of biodiesel yield. Four main parameters responsible for the yield of transesterification were analyzed; waste cooking oil (WCO) to methanol ratio, temperature, water content and enzyme content. The experimental results showed that the highest conversion was 85.14% under condition oil to methanol ratio 7:1, 10% of water content, 40% wt of enzyme loading and temperature 37.5°C. The regression model of the ANOVA was found to be significant with $p<0.001$ and $R^2= 0.9737$. As a conclusion, the results proved that the immobilization method of *C. rugosa* lipase in PVA-alginate-sulfate beads is reliable and can enhance conversion of palm waste cooking oil to biodiesel.

ABSTRAK

Malaysia kini merupakan pengeluar minyak kelapa sawit kedua terbesar di dunia, yang menyumbang kepada 39% daripada pengeluaran minyak sawit dunia. Kira-kira 0.5 juta tan sisa minyak masak dihasilkan setiap tahun. Masalah pelupusan sisa minyak masak yang tidak sempurna membawa kepada pencemaran alam sekitar terutamanya di darat dan air. Untuk mengatasi masalah ini, kajian ini menumpukan sisa minyak masak kelapa sawit sebagai bahan mentah bagi penghasilan biodiesel sebagai sumber alternatif kepada petroleum konvensional yang terhad dan tidak boleh diperbaharui. Selain itu, penggunaan lipase sebagai biokatalis untuk menghasilkan biodiesel mempunyai kelebihan berbanding pemangkin kimia kerana ia dilakukan di bawah keadaan reaksi yang ringan dan pemisahan produk yang mudah. Kajian ini merungkai pengeluaran biodiesel dari sisa minyak masak dari kelapa sawit dengan menggunakan *Candida rugosa* lipase (CRL) yang disekatgerak dalam manik Polivinil alkohol (PVA)-alginat-sulfat. Kaedah satu faktor pada suatu masa (OFAT) dipilih sebagai pembolehubah yang sesuai sebelum analisis statistik dilakukan. Perisisan Pakar Rujuk Rekaan digunakan sebagai alat statistik bagi mengendalikan Rekabentuk Komposit Berpusat (CCD) untuk pengoptimuman faktor-faktor penting. Analisis statistik digunakan untuk mencapai pengeluaran maksimum biodiesel dan menilai kesan setiap pembolehubah dan interaksi hasil biodiesel. Empat parameter utama yang bertanggungjawab bagi hasil transesterifikasi di analisis; nisbah sisa minyak masak (WCO) kepada metanol, suhu, kandungan air dan kandungan enzim. Keputusan eksperimen menunjukkan bahawa penukaran tertinggi adalah 85.14% dalam keadaan nisbah metanol / molar minyak 7:1, 10% kandungan air, 40% wt kandungan enzim dan suhu 37.5°C. Model regresi ANOVA didapati signifikan dengan $p<0.001$ dan $R^2=0.9737$. Sebagai kesimpulan, hasil kajian membuktikan bahawa kaedah sekatgerak *C. rugosa* lipase dalam manik PVA-alginat-sulfat boleh dipercayai dan boleh meningkatkan penukaran sisa minyak masak dari kelapa sawit kepada biodiesel.

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LIST OF ABBREVIATIONS

2LFD	-	Two-level factorial design
ANOVA	-	Analysis of variance
CCD	-	Central composite design
CRL	-	*Candida rugosa* lipase
$CuSO_4$. 5 H_2O	-	Copper (II) sulfate pentahydrate
DAG	-	Diacylglycerol
FAME	-	Fatty acid methyl ester
FESEM	-	Field Emission Scanning Electron Microscopy
FFAs	-	Free fatty acids
GC	-	Gas Chromatography
KH_2PO_4	-	Potassium dihydrogen phosphate
KH_2PO_4	-	Monopotassium phosphate
ME	-	Methyl ester
Na_2CO_3	-	Sodium carbonate
OFAT	-	One factor at a time
pH	-	Negative logarithm of hydrogen ion concentration
pNP	-	p-nitrophenol
p-NPP	-	p- nitrophenyl palmitate
PUFAs	-	Polyunsaturated fatty acids
PVA	-	Polyvinyl alcohol
RSM	-	Response surface methodology
TAG	-	Triacylglycerol
UV	-	Ultraviolet
V_{max}	-	Maximum enzyme catalysed velocity
WCO	-	Waste cooking oil

LIST OF SYMBOLS

%	-	Percent
°C	-	Degree Celsius
µm	-	Micrometre
µL	-	Microliter
g	-	Gram
H	-	Hour
M	-	Molar
min	-	Minute
mL	-	Millilitre
mL/min	-	Millimetre/minute
mm	-	Millimetre
rpm	-	Rotation per minute
v/v	-	Volume per volume
w/v	-	Weight per volume

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Many edible and nonedible oils can be used as feedstocks for biodiesel production (Vasudevan and Briggs, 2008). Using edible oil such as sunflower oil, soybean oil, rapeseed oil and palm oil has raised concern related to the use of food crops for biofuels, limited sources, competition with food consumption, and high production cost (Mittelbach, 1990). Besides, biodiesel obtained from neat vegetable oil is more costly compared to the petroleum diesel fuel (Felizardo *et al.*, 2006). According to Phan and Phan (2008) the total processing cost in producing biodiesel by using waste cooking oil (WCO) can be reduced up to two and three times less than neat vegetable oil. Thus, cheaper alternative feedstock material such as waste oils or fats or non-edible oil crops is viable option to reduce the production cost. The abundance of waste oils makes them excellent as low-cost feedstock for biodiesel production.

Currently, all waste oils are sold commercially as animal feed. But the European Union (EU) has put a ban since 2002 on feeding these mixtures to animal. It is because many harmful compounds are formed during frying process and eventually might harm human's health. This is due to the use of WCO as additives in the animal feed which is integrated into the food chain (Cvengroš and Cvengrošová, 2004). WCO is used edible vegetables oil such as palm, sunflower, soybean, rapeseed, canola and corn oils, which is no longer viable for its intended use (Refaat, 2010). WCO generated by homes and restaurants has increased rapidly due to the tremendous growth of human population (Chen *et al.*, 2009). A survey on how Malaysian community managing their WCO was conducted by Hanisah *et al.*, (2013), it was found that 17% of the respondents discarded the WCO into dustbin, 7% discarded onto soil and 60% discarded into the drainage system. Thus, the use of WCO as biodiesel feedstock could reduce problems relating to improper waste management that leads to water pollution and blockage in drainage system. This situation will disturb the aquatic ecosystem by covering the surface of water and preventing oxygen from dissolving (Yaakob *et al.*, 2013; Nur and Wan, 2016). Thus, production of biodiesel from WCO is one of the better ways to utilize them efficiently and economically.

There are several ways to produce biodiesel from WCO which is through acid, alkaline and enzyme transesterification process. Although biodiesel can be successfully produced by chemical approach, the reaction has several drawbacks such as energy intensive, difficulties in glycerol recovery, and exclusion of the catalysts from the product (Christopher *et al.*, 2014). Besides, alkaline wastewater requires treatment and high free fatty acid present in the oil will be transformed to soap and will reduce the efficiency of catalyst. WCO is usually contain high free fatty acids, water content and impurities (Gui *et al.*, 2008; Kumar and Sharma, 2008; Gebremariam and Marchetti, 2018). High FFA can leads to saponification in transesterification reaction. By using enzyme, all the FFA content in the WCO can converted to fatty acid methyl ester (FAME) (Al-Zuhair, 2008; Pourzolfaghar *et al.*, 2016). Thus, the application of enzyme transesterification of WCO for biodiesel production can overcome these problems and offer friendlier approach to the environment. The bioconversion of WCO into biodiesel involved enzyme transesterification where a reaction occurred between lipid and alcohol to produce ester and glycerol as by products (Felizardo *et al.*, 2006). However, the used of enzymes as catalyst is very expensive as the reaction takes longer time (Nelson *et al.*, 1996; Watanabe *et al.*, 2002; Jegannathan *et al.*, 2011) and the expensive soluble enzyme cannot be reused (Gebremariam and Marchetti, 2018). By immobilizing the enzyme, the catalyst can be reused a number of times without any further separation of catalyst required and reducing the additional cost (Peralta-Yahya, et *al*., 2012; Ranganathan *et al.*, 2008).

There are many studies on lipase immobilization focusing on biodiesel production. Karimi, (2016) immobilized lipase from *B. cepacia* onto mesoporous superparamagnetic iron oxide nanoparticles (SPION), and biodiesel yield is above 90% and can be reused up to 5 cycles without significant decrease of the activity. Methanolysis of palm oil, catalyzed by lipase from *Pseudomonas fluorescens* is immobilized on hybrid support polysiloxane–poly-(vinyl alcohol) showed strong transesterification yield which reached almost full conversion (98 %) in less than 24 h of reaction (Moreira *et al.*, 2007). Kuo *et al.*, (2013) immobilized *Candida rugosa* lipase on polyvinylidene fluoride membrane and a FAME yield reached to 97% after 33 h of reaction and can be reused up to 5 times without noticeable decrease of activity.

T.lanuginosus lipase immobilized onto polyglutaraldehyde activated styrene-divinylbenzene copolymer was demonstrated by (Dizge *et al.*, 2009) in methanolyiss of canola oil and obtained 97% conversion of biodiesel after 24h. *Burkolderia cepacia* immobilized in hybrid matrix of alginate and K-carrageenan was performed by Abdulla and Ravindra, (2013) in methanolysis of crude *Jathropa curcas* oil and a FAME yield of 100% was obtained after 24h reaction time.

As the cost of lipase makes up 90% of the total cost of enzymatic biodiesel production, finding a low cost support materials is crucial task in order to reduce the overall cost (Ghaly *et al.*, 2010). The choice of support material should be cost effective and offers great mechanical strength, microbial resistance, thermal stability, chemical durability, chemical functionality, hydrophobic/hydrophilic character and loading capacity (Xavier Malcata *et al.*, 1990; Jegannathan *et al.*, 2008; Zeng *et al.*, 2009). As mentioned above, synthetic polymer polyvinyl alcohol (PVA) is one of the best choice as a support material because it is non-toxic to viable cells, cheap and mechanically and chemically robust (Amanda and Wisecarver, 1992; Khoo and Ting, 2001; Long *et al.*, 2004; Bruno *et al.*, 2005; Li-sheng *et al.*, 2007).PVA is rubbery and elastic in nature. PVA is known for its biodegradable, biocompatible and eco-friendly biopolymer properties (Grande and Carvalho, 2011). PVA gel exhibits a high degree swelling in water and highly hydrophilic (Hassan. and Peppas, 2000). Thus, PVA must be crosslinked chemically or physically to make it insoluble (Gholap *et al.*, 2004).

Some researchers have produced PVA beads using PVA cross linked with boric acid (Hashimoto and Furukawa, 1987; Amanda and Wisecarver, 1992), however the beads tend to agglomerate into a mass of polymer make it difficult to break. Thus, a mixed solution of PVA and sodium alginate and a mixed solution of boric acid and calcium chloride was introduced by Long and co-workers (2004). The addition of calcium alginate in PVA solution have improve the surface properties of beads and reduce the agglomeration problem. Calcium alginate formed instantaneously when there is a contact between sodium alginate and calcium chloride solution. Previously, this immobilization matrix had successfully immobilized invertase, enhanced the beads shapes, resulting the best surface area for the enzymes, reduce cell leakage and cell agglomeration by crosslinked with sodium sulfate (Idris *et al.*, 2008; Zain *et al.*, 2011).

This study focuses on production of biodiesel from WCO using the PVA alginate sulfate beads and evaluate the feasibility of CRL immobilize in PVA-alginate sulfate beads in terms of the lipase activity within the beads. The immobilization would enhance the stability and re-usage of the enzyme while making the whole production process an eco-friendly one.

1.2 Problem Statement

The most common way to produce biodiesel is chemical or enzymatic transesterification. The common problems associated with the chemical transesterification process are difficulty in recovery of the catalyst, high energy costs and the treatment of wastewater which resulted in high production cost (Kuo *et al.*, 2015). Alkali reaction has been used widely in industrial but still it has difficulty in glycerol recovery due to formation of soap during the process. Saponification not only reduce the biodiesel production but deactivate the catalyst (Robles-Medina et al,2009; Gnanaprakasam et al , 2013) . Instead, the enzymatic approach in producing biodiesel may overcome the problems of the current chemical approach by reducing the cost for the downstream process especially in post-reaction in separation of catalyst (Akoh *et al.*, 2007; Jegannathan *et al.*, 2008; Lukovi *et al.*, 2011).

However, the biodiesel production by enzymatic means has become the major concern due to the high cost of enzymes. Therefore, the ability to repetitively reused enzyme is desirable as enzyme is generally expensive. This can be achieved via enzyme immobilization using certain suitable matrix and low cost PVA is one of the immobilization matrix that has been widely studied (Peng Ting and Gang Sun, 2000).

In this study, PVA was used as the immobilization matrix due to its high mechanical strength, good chemical stability and enhance the activity of enzymes (Idris *et al.*, 2008; Zain *et al.*, 2011). Despite the fact immobilized enzymes are cost effective as they can be reused repetitively and easily separated from the product, immobilized enzymes tend to lose their activity with respect to support and immobilization methods (Tan *et al.*, 2010). Therefore, the robustness and feasibility of immobilized *Candida rugosa* lipase in PVA-Alginate beads in different pH, temperature, stability in pH and thermal as well as reusability of immobilized lipase in producing biodiesel has become main concern. To date, the immobilization of lipase in PVA-Alginate sulfate beads in order to convert waste palm cooking oil to biodiesel has not been investigated yet. In general, a successful immobilization of the enzyme on a support increases the stability of enzymes performance in pH and temperature as well as the reusability of immobilized lipase.

1.3 Objectives

The aim of this study was to investigate the feasibility of immobilized CRL in PVA-Alginate Sulfate beads in producing biodiesel from palm WCO. Based on the research background and the problem statement, the research objectives of this study are:

a) To immobilize lipase (EC 3.1.1.3) from *Candida rugosa* (Type VII, 1746 units/mg) in PVA-alginate-sulfate and access its robustness.

b) To assess the immobilized enzyme potential for biodiesel production by using one factor at a time (OFAT) method in order to study the range of parameters involved (WCO to methanol ratio, temperature, water content and enzyme content).

c) To optimize the significant parameters influencing biodiesel production using Response Surface Methodology (RSM) by Design Expert 11.1.2.0 in order to study relationship and interaction between parameters and their effect on biodiesel yield.

1.4 Scope of Research

This study focuses on investigating the capability of immobilized *Candida rugosa* (Type VII, 1176 units/mg) in producing biodiesel from waste cooking oil (WCO). Preliminary analysis (OFAT) on working parameters that possibly affect the biodiesel optimization study were conducted namely methanol to oil ratio (1:2-1:10), temperature (30-60°C), water content (0.05-10%) and enzyme content (10-60 wt %).

Next, a statistical tool, RSM (using Design Expert 11.1.2.0) was employed in designing of experiments for the production of biodiesel. The variables that possibly affect biodiesel production were optimized through Central Composite Design (CCD). The working parameters involved in the CCD were WCO to methanol ratio (1:4 to 1:8), temperature (30-40°C), water content (2.5-12.5%) and enzyme content (10-50 wt %).

1.5 Significance of Research

This study highlights the ability of *Candida rugosa* lipase immobilized in PVA- alginates sulfate beads. So far, no attempt has been made by using this type of immobilization matrix to immobilize lipase in producing biodiesel from waste palm cooking oil. Thus, this study shows that this type of immobilization matrix can be used to immobilized lipase and improve the production of biodiesel. Parameters such as WCO to methanol ratio, temperature, water content and enzyme content were investigated and optimized using Response Surface Methodology (RSM).The findings achieved in this study will provide important information on biodiesel production by immobilized CRL in PVA-Alginate sulfate beads and help to reduce pollution in water and soil.

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