



**UNIVERSITI PUTRA MALAYSIA**

***TRANSESTERIFICATION OF PALM OIL USING FREE AND IMMOBILIZED  
LIPASE FROM PSEUDOMONAS SPECIES FOR BIODIESEL PRODUCTION***

**NIDA JAFRI**

**IB 2013 6**



**TRANSESTERIFICATION OF PALM OIL USING FREE AND  
IMMOBILIZED LIPASE FROM PSEUDOMONAS SPECIES FOR  
BIODIESEL PRODUCTION**

**By**

**NIDA JAFRI**

**Thesis Submitted to the School of Graduate Studies, Universiti  
Putra Malaysia, in Fulfilment of the Requirements for the Degree of  
Master of Science**

**February 2013**

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Master of Science

**TRANSESTERIFICATION OF PALM OIL USING FREE AND  
IMMOBILIZED LIPASE FROM PSEUDOMONAS SPECIES FOR  
BIODIESEL PRODUCTION**

By

**NIDA JAFRI**

**February 2013**

**Chairman: Norhafizah Abdullah, PhD**

**Faculty: Institute of Bioscience**

Refined palm oil (RPO) was used as the feedstock for the synthesis of fatty acid methyl ester (FAME) commonly called as biodiesel by transesterification process. The proximate fatty acid composition of RPO used showed it contained high amount of oleic acid and palmitic acid at 54% and 33%, respectively indicating a good potential of raw feedstock for biodiesel production. The transesterification reaction was done using a mixture containing RPO and methanol in *n*-hexane as a medium with lipase as catalyst in a batch STR. The scouting of lipase was conducted in the same set-up with lipase from *Pseudomonas* species (PS) and *Penicillium camembertii* (PC). The scouting result showed efficient conversion of FAME by lipase PS as compared to lipase PC. Therefore all the subsequent work

used lipase PS. Effects of molar ratio of RPO to alcohol, molar concentration of RPO, amount of lipase dosage and reaction time on the composition of biodiesel were investigated. The optimum conditions obtained were as follows: methanol to RPO molar ratio- 3:1, RPO concentration- 0.9 M, enzyme dosage- 50 units/mg at reaction temperature- 40°C after 72 hours. The composition of FAME was determined by gas chromatography-mass spectrometry (GC-MS) and results showed a total FAME yield of 92%. An eco-friendly immobilized lipase was also designed by entrapment technique in calcium alginate beads in an attempt to further improve the yield of biodiesel and reusability of lipase. Transesterification was performed using the same optimized reaction conditions as in the free lipase experiment. The experimental result showed a maximum biodiesel yield of 97% in STR which was higher than the yield given by free lipase PS in STR at 92%. The reusability and stability of immobilized lipase was tested for consecutive transesterification experiment. A yield of 77% was obtained after the fourth batch cycle. There was no physical deformation in the structure of the beads which indicated a high mechanical stability of the beads even after four batch cycles under continuous mixing at 150 rpm. A preparatory scale-up of transesterification reaction was also done in an oscillatory flow reactor (OFR) using free and immobilized lipase PS. The main purpose of using OFR was to improve mixing condition with higher reaction volume and shortening overall reaction time. The reaction was conducted with 2.5 kg of RPO at molar ratio of 3:1 in *n*-hexane medium. Maximum conversion of 98% and 97% was obtained after a reaction time of 2 hrs from free-suspended lipase and

immobilized lipase, respectively. This study showed lipase PS exhibited excellent catalyst quality for FAME production and a further improvement in productivity when immobilized lipase was used. There was a 37 fold of improvement in FAME productivity when using OFR as compared to STR. The OFR also reduced the reaction time and gave a good biodiesel yield. Results from this study showed a positive indication for the potential use of OFR in industry for biodiesel production.



Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia  
sebagai memenuhi keperluan untuk ijazah Master Sains

**TRANSESTERIFIKASI MINYAK KELAPA SAWIT DENGAN  
MENGUNAKAN LIPASE BEBAS DAN TERPERANGKAP DARIPADA  
SPESIS PSEUDOMONAS UNTUK PENGHASILAN BIODIESEL**

Oleh

**Nida Jafri**

**Februari 2013**

**Pengerusi: Norhafizah Abdullah, PhD**

**Fakulti: Institut Biosains**

Minyak kelapa sawit bertapis (RPO) telah digunakan sebagai bahan mentah untuk sintesis asid lemak metil ester (FAME) yang biasanya dikenali sebagai biodiesel daripada proses transesterifikasi. Komposisi asid lemak RPO daripada analisis hampiran menunjukkan ia mengandungi jumlah asid oleik dan asid palmitik yang tinggi, iaitu pada 54% dan 33%, masing-masing. Ini menunjukkan potensi yang baik sebagai bahan mentah untuk penghasilan biodiesel. Tindak balas transesterifikasi telah dilakukan dengan menggunakan campuran yang mengandungi RPO dan metanol dalam n-heksana sebagai medium dengan lipase sebagai pemangkin dalam reaktor tangki teraduk (STR) kelompok. Pencarian lipase telah dijalankan dengan

menggunakan eksperimen yang sama daripada spesies *Pseudomonas* (PS) dan *Penicillium camembertii* (PC). Keputusan kajian menunjukkan kecekapan penukaran FAME yang efisien oleh lipase PS berbanding dengan lipase PC. Oleh itu kesemua eksperimen yang berikut akan menggunakan lipase PS. Kesan nisbah molar PO kepada kepekatan alkohol, kepekatan molar RPO, jumlah dos lipase dan masa tindak balas terhadap komposisi biodiesel telah diselidik. Keadaan optimum yang diperolehi adalah seperti berikut: nisbah molar metanol kepada RPO-3:1, kepekatan molar RPO- 0.9 M, dos lipase -50 unit/mg pada tindak balas selepas 72 jam dan suhu pada 40°C. Komposisi FAME telah ditentu mengguna alatan oleh kromatografi gas-spektrometri jisim (GC-MS) dan keputusan menunjukkan hasil FAME keseluruhan sebanyak 92%. Lipase terperangkap yang mesra alam turut direka oleh teknik perangkap dalam manik kalsium alginat dalam usaha untuk meningkatkan lagi hasil biodiesel dan kebolegunaan semula enzim lipase. Transesterikasi dilakukan menggunakan keadaan tindakbalas optimum yang sama seperti dalam eksperimen lipase bebas di atas. Hasil kajian menunjukkan penghasilan biodiesel maksimum yang lebih tinggi pada 97% yang lebih tinggi oleh lipase PS terperangkap berbanding dengan lipase bebas pada 92%. Kebolegunaan semula dan kestabilan lipase terperangkap telah diuji dalam empat eksperimen transesterifikasi secara berterusan. Hasil sebanyak 77% telah diperolehi selepas empat kitaran kelompok. Tiada perubahan dalam bentuk fizikal dan struktur manik alginat yang mengandungi lipase dan ini menunjukkan kestabilan mekanikal yang bagus walaupun selepas empat kitaran proses transesterifikasi di bawah keadaan

adukan berterusan pada 150 rpm. Eksperimen penskalaan tindak balas transesterifikasi juga dilakukan dengan menggunakan reaktor aliran ayunan (OFR) bagi kedua-dua jenis lipase PS bebas dan lipase PS terperangkap. Tujuan utama OFR digunakan adalah untuk meningkatkan lagi keefisienan pencampuran dalam isipadu tindak balas yang lebih tinggi dan memendekkan masa tindak balas secara keseluruhan. Tindak balas telah dijalankan dengan menggunakan 2.5 kg RPO, nisbah molar metanol kepada minyak sebanyak 3:1 dan *n*-heksana sebagai medium. Penukaran maksimum sebanyak 98% dan 97% telah diperolehi selepas masa tindak balas 2 jam dihasilkan oleh lipase PS bebas dan lipase PS terperangkap, masing-masing. Kajian ini menunjukkan lipase PS mempunyai kualiti pemangkin yang sangat baik untuk penghasilan biodiesel dan penambahbaikan dalam produktiviti apabila lipase terperangkap digunakan. Peningkatan penghasilan biodiesel merekodkan gandaan produktiviti sebanyak 37 kali apabila OFR digunakan, berbanding dengan STR. OFR juga dapat mengurangkan masa tindak balas dan memberikan hasil biodiesel yang baik. Hasil kajian ini boleh digunakan sebagai satu indikasi yang positif dalam potensi penggunaan OFR sebagai reaktor diperingkat industri bagi pengeluaran biodiesel.



## ACKNOWLEDGEMENTS

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

All praise and glory goes to Almighty Allah (Subhanahu Wa Ta'ala) who gave me the courage and patience to carry out this work. Peace and blessings of Allah be upon his last Prophet Muhammad (PBUH) and all his Sahaba (companions) who devoted their lives towards the prosperity and spread of Islam.

First and foremost gratitude is to the esteemed university, Universiti Putra Malaysia for my admittance, and to its learned faculty members for imparting quality learning and knowledge with their valuable support and able guidance that has led my way through this point of undertaking my research work. My deep appreciation and heartfelt gratitude goes to my thesis supervisor Dr. Norhafizah Abdullah and for her constant endeavour, guidance and the numerous moments of attention she devoted throughout the course of this research work. Her valuable suggestions made this work interesting and knowledgeable for me. Working with Dr. Norhafizah in a friendly and motivating environment was really a learning experience.

I extend my deepest gratitude to my thesis co-supervisor Dr. Tinia Idaty Md. Ghazi for her constructive and positive appreciation, extraordinary attention and thought-provoking contribution in my research. It was surely an honour and an exceptional learning to work with her.

Family support plays a vital role in the success of an individual. I owe thanks to my relatives and my friends for their help, motivation and pivotal support. I would like to thank my parents, siblings, family members and last but not the least my loving friends from the core of my heart. Their prayers and encouragement always helped me take the right steps in life.

May Allah help us in following Islam according to Quran and Sunnah.  
(*Aameen*)

I certify that a Thesis Examination Committee has met on the 15<sup>th</sup> February, 2013 to conduct the final examination of Nida Jafri on her M.Sc thesis entitled "Transesterification of palm oil using free and immobilized lipase from *Pseudomonas species* for biodiesel production" in accordance with Universities and University Colleges Act 1971 and the constitution of the Universiti Putra Malaysia [P.U. (A) 106]. The committee recommends that the student be awarded the Master of Science degree.

Members of the Thesis Examination Committee were as follows:

Assoc. Prof. Dr. Siti Aslina Hussain, PhD  
**(Chairman)**

Assoc. Prof. Dr. Rosfarizan binti Mohamad, PhD  
**(Internal Examiner)**

Dr. Dayang Radiah binti Awang Biak, PhD  
**(Internal Examiner)**

Assoc. Prof. Dr. Mashitah Mat Don, PhD  
**(External Examiner)**

---

**SEOW HENG FONG, PhD**

Professor and Deputy Dean  
School of Graduate Studies  
Universiti Putra Malaysia

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirements for the degree of Master of Science. The members of the Supervisory committee were as follows:

**Norhafizah Abdullah, PhD**

Associate Professor  
Faculty of Engineering  
Universiti Putra Malaysia  
(Chairman)

**Tinia Idaty Md. Ghazi, PhD**

Associate Professor  
Faculty of Engineering  
Universiti Putra Malaysia  
(Member)

---

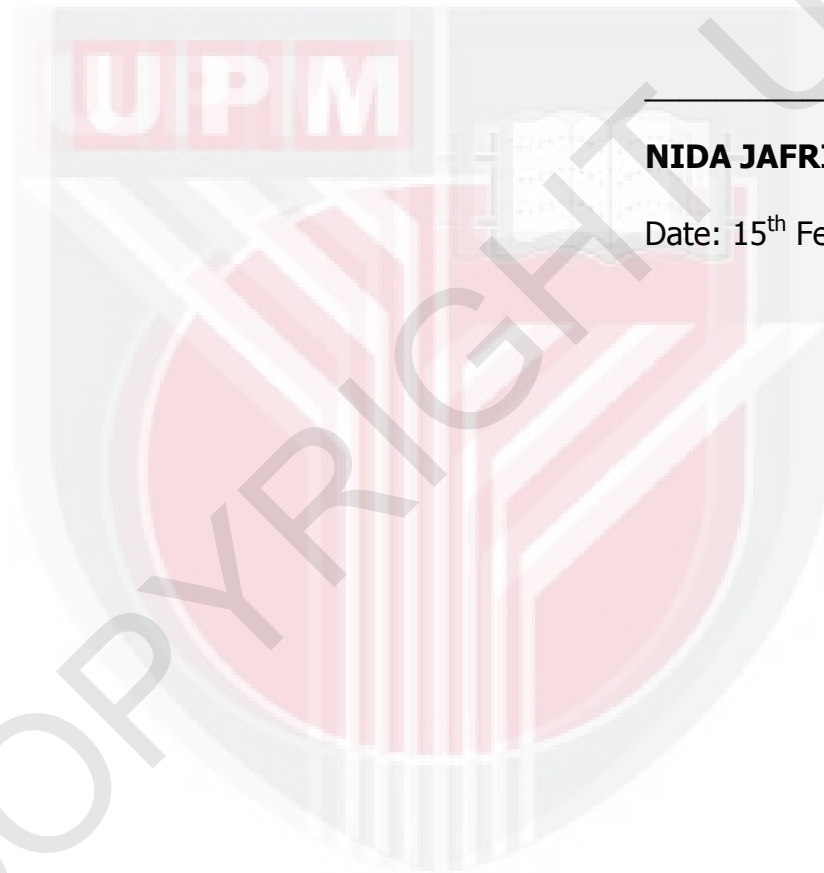
**BUJANG BIN KIM HUAT, PhD**

Professor and Dean  
School of Graduate Studies  
Universiti Putra Malaysia

Date:

## DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.



---

**NIDA JAFRI**

Date: 15<sup>th</sup> February 2013



## TABLE OF CONTENTS

	<b>Page</b>
<b>ABSTRACT</b>	ii
<b>ABSTRAK</b>	v
<b>ACKNOWLEDGEMENTS</b>	viii
<b>APPROVAL</b>	ix
<b>DECLARATION</b>	xi
<b>LIST OF TABLES</b>	xvi
<b>LIST OF FIGURES</b>	xvii
<b>LIST OF ABBREVIATIONS</b>	xix
<b>CHAPTER</b>	
<b>1 INTRODUCTION</b>	<b>1</b>
1.1 Introduction	1
1.2 Background	2
1.3 Problem Statement	3
1.4 Objectives	6
1.5 Significance of Study	7
1.6 Scope of Study	8
1.7 Thesis Organization	9
<b>2 LITERATURE REVIEW</b>	<b>11</b>
2.1 Introduction	11
2.2 Background	11
2.2.1 Vegetable Oils as A Direct Fuel in Engines	12
2.2.2 Biodiesel	14
2.3 Methods for Biodiesel Production	18
2.3.1 Direct Use and Blending	18
2.3.2 Micro-emulsion	19
2.3.3 Pyrolysis (Thermal Cracking)	19
2.3.4 Transesterification (Alcoholysis)	20
2.4 Enzyme-catalysed transesterification	25
2.4.1 Lipases	25
2.4.2 Method of Lipase Mediated transesterification	26

2.5	Effects of Operating Variables on Transesterification Reaction Involving Lipase	29
2.5.1	Alcohol/Oil Molar Ratio	29
2.5.2	Choice of Lipase	30
2.5.3	Temperature	31
2.5.4	Use of Solvent	32
2.6	Transesterification Using Immobilized Lipase	32
2.6.1	Adsorption	34
2.6.2	Covalent Bonding	35
2.6.3	Entrapment	36
2.6.4	Cross-Linking	36
2.7	Applications of Lipase for Biodiesel Synthesis	38
2.8	Economics between Enzyme Catalyzed Transesterification and Chemical Transesterification	39
2.9	Reactors for Transesterification	40
2.10	Concluding Remarks	44
<b>3</b>	<b>METHODOLOGY</b>	<b>46</b>
3.1	Materials and Methods	46
3.2	Preparation of Enzyme Solution	47
3.3	Scouting of Lipase Producing Enzyme	47
3.4	Experimental Procedure	48
3.5	Transesterification of Refined Palm Oil in Stirred Tank Reactor using Free Lipase PS as Catalyst	50
3.6	Optimization of Process Variables in STR for Biodiesel Production	51
3.6.1	Effect of Palm Oil Concentration	52
3.6.2	Effect of Methanol to Oil Molar Ratio	52
3.6.3	Effect of Enzyme Dosage	53
3.6.4	Effect of Reaction Time	53

3.7	Lipase Immobilization	53
3.7.1	Immobilization of Lipase by Entrapment Method	54
3.7.2	Beads Morphology	54
3.7.3	Transesterification in STR using Immobilized Lipase PS	55
3.7.4	Regeneration Efficiency of Lipase Entrapped Calcium Alginate Beads	55
3.7.5	Transesterification in STR using empty Calcium Alginate Beads as Control Experiment	56
3.8	Transesterification Reaction in Oscillatory Flow Reactor using Free Lipase PS	56
3.9	Transesterification Reaction in Oscillatory Flow Reactor using Immobilized Lipase PS	57
3.10	Analytical methods	58
3.10.1	Sample Preparation for GC	58
3.10.2	GC Analysis	58
3.10.3	GC-MS Analysis	59
<b>4</b>	<b>RESULTS AND DISCUSSION</b>	<b>60</b>
4.1	Proximate Analysis of Refined Palm Oil	60
4.2	Scouting Of Lipases For Transesterification Reaction	63
4.3	Optimization of Process Parameters that Affect Transesterification Reaction	64
4.3.1	Effect of Methanol / Oil molar ratio	64
4.3.2	Effect of Palm Oil Concentration	67
4.3.3	Effect of Enzyme Dosage	68
4.3.4	Effect of Reaction Time	70
4.4	Analysis of Transesterification Products	71
4.5	Lipase Immobilization	75
4.5.1	Entrapment in Calcium Alginate beads	75
4.5.2	The choice of Immobilization matrix	75

4.5.3	Characterization and Microstructure of Calcium Alginate Beads	76
4.6	Transesterification Reaction in STR Using Immobilized Lipase	79
4.6.1	Regeneration Efficiency of Lipase	82
4.6.2	Control Experiment Using Empty Calcium Alginate Beads	85
4.7	Transesterification Reaction in Oscillatory Flow Reactor	86
4.7.1	Transesterification Reaction in Oscillatory Flow reactor Using Free Lipase PS	86
4.7.2	Transesterification Reaction in Oscillatory Flow Reactor Using Immobilized Lipase PS	90
<b>5</b>	<b>CONCLUSIONS AND RECOMMENDATIONS</b>	<b>93</b>
5.1	Introduction	93
5.2	Future Work	94
	<b>REFERENCES</b>	<b>95</b>
	<b>APPENDICES</b>	<b>110</b>
	<b>BIODATA OF STUDENT</b>	<b>117</b>
	<b>LIST OF PUBLICATIONS</b>	<b>118</b>