

**BIODIESEL PRODUCTION FROM CRUDE
JATROPHA CURCAS OIL USING LIPASE
IMMOBILIZED IN HYBRID MATRIX**

RAHMATH ABDULLA



PERPUSTAKAAN
UNIVERSITI MALAYSIA SABAH

**THESIS SUBMITTED IN FULFILLMENT FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY**

**SCHOOL OF ENGINEERING AND
INFORMATION TECHNOLOGY
UNIVERSITI MALAYSIA SABAH
2013**

UNIVERSITI MALAYSIA SABAH

BORANG PENGESAHAN STATUS TESIS

JUDUL: BODIESEL PRODUCTION FROM CRUDE JATROPHA CURCAS OIL
USING LIPASE IMMOBILIZED IN HYBRID MATRIX

IJAZAH: DR.FALSAFAH

Saya RAHMATH ABDULLA, Sesi Pengajian 2009-2013, mengaku membenarkan tesis ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan syarat syarat kegunaan seperti berikut :

1. Tesis ini adalah hak milik Universiti Malaysia Sabah
2. Perpustakaan Universiti Malaysia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebahai bahan Pertukaran antara institusi pengajian tinggi.
4. Sila tandakan (/)

(Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA RASMI 1972)

(Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)

TIDAK TERHAD


Disahkan oleh ,



(Tandatangan Penulis)

(Tandatangan Pustakawan)

Tarikh: 14 Sep 2013



(PROF DR.POGAKU RAVINDRA)

DR. POGAKU RAVINDRA
Professor
Chemical Engineering Program
School of Engineering and Information Technology
Universiti Malaysia Sabah

DECLARATION

I hereby declare that the materials in this thesis are original except for quotations, excerpts, summaries and references, which have been duly acknowledged

25 August 2013



Rahmath Abdulla

PK2009-9024



UMS
UNIVERSITI MALAYSIA SABAH

CERTIFICATION

NAME : RAHMATH ABDULLA

MATRIC NO. : PK2009-9024

TITLE : BIODIESEL PRODUCTION FROM CRUDE
JATROPHA CURCAS OIL USING LIPASE
IMMOBILIZED IN HYBRID MATRIX

DEGREE : DOCTOR OF PHILOSOPHY
(CHEMICAL ENGINEERING)

VIVA DATE : 29 JULY 2013



UMS
UNIVERSITI MALAYSIA SABAH

DECLARED BY

1. SUPERVISOR

Professor Dr. Pogaku Ravindra

Signature

A handwritten signature in black ink, consisting of stylized, overlapping loops and a long horizontal stroke at the end, positioned above a solid horizontal line.

DR. POGAKU RAVINDRA
Professor
Chemical Engineering Program
School of Engineering and Information Technology
Universiti Malaysia Sabah

ACKNOWLEDGEMENT

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

I take this opportunity to express my heartfelt appreciation to the people who have contributed directly or indirectly to this thesis and stood by my side during this amazing journey through a bed of thorns and roses.

First and foremost, I would like to thank my supervisor, Professor Dr. Pogaku Ravindra for his guidance, enlightening discussions and brainstorming ideas. His deep insights and handful of knowledge on the research field helped me a lot especially during the difficult conceptual stages of PhD pursuit. The joy, enthusiasm and dedication he has for the research was contagious and inspired me throughout my study. I appreciate all his contributions of time, ideas and patience together with trustful guidance to make my PhD experience productive and stimulating.

My full gratitude and appreciation to The Dean - Associate Prof Dr. Rosalam Hj. Sarbatly, friends and all the staff of School of Engineering and Information Technology, UMS for extending facilities, help and togetherness in carrying out my research.

I owe my most sincere gratitude to Dr. Mahyar Sakari, Senior Lecturer, School of Science and Technology, UMS for his help on GC-MS. My special thanks to the members of the Encapsulation Research Group for their help throughout my research especially Chemical Engineer, Ms. Chong Wan Cheng.

I am thankful to all my pals in Universiti Malaysia Sabah as well as abroad for their support and motivation in all these years. I am greatly indebted to my dear friend, Ms. Zarrintaj and her family for her care and never ending love.

Words cannot express the feelings I have for my parents, sister and brother for all their love and encouragement. A big " Thank You" to the most important person in my life, my husband, Muhammad Naseef, for all his love, support, encouragement and patience in all my pursuits. Last but not the least; I would like to express my love for my little daughters Jannah and Junainah by dedicating this thesis to them.

Finally, I acknowledge the financial support provided by FRGS, MOHE for my research.

ABSTRACT

The present world is on the verge of a severe 'global energy crisis' with limited energy reserves exceeding its supply. In such a scenario, biodiesel production using immobilized lipase has immense significance. *Jatropha curcas* oil (CJO) has gained much importance as a non edible biodiesel feedstock in many countries. The objective of this research was to produce biodiesel from CJO using *Burkholderia cepacia* lipase immobilized in hybrid matrix. The research was divided into two main parts. First part incorporated the immobilization of lipase onto hybrid matrix and its stability studies followed by the second part which included optimization of biodiesel production from CJO with the immobilized lipase. At first, the lipase was cross linked with glutaraldehyde prior to entrapment in a hybrid matrix of natural polymers of alginate and κ -carrageenan. The lipase beads were spherical in shape with an average diameter of 3mm. A specific activity yield of 89.26% was obtained following immobilization. Further, a significant reduction of 65.76% enzyme leakage was observed. The immobilized lipase also retained 84.02% of its initial activity upon two weeks of storage. Optimum pH for immobilized lipase was found to be 7 and temperature 40°C. Comparative kinetic parameters K_m and V_{max} values were found to be 3.15 μ M and 12.5 μ mol/min for free lipase and 4.17 μ M and 11.11 μ mol/min for immobilized lipase respectively. Immobilized lipase also retained 75.54% of its initial activity after 10 cycles of reuse.

In the second part of the research, the immobilized lipase in hybrid matrix was employed for biodiesel production from CJO. A 100% yield of biodiesel (FAEE) was obtained with the optimized parameters :10g CJO, 1:10 molar ratio of oil to ethanol, 1 g water, 5.2g immobilized lipase, 35°C, 6 g \times RCF and 24 hour reaction time. The immobilized lipase retained 73% relative transesterification activity after six cycles of reuse. On the other hand, a simple and effective external mass transfer model was established in a recirculated packed bed batch reactor (RPBBR) with immobilized lipase and CJO. Based on different biopolymer material used in immobilized beads, Colburn factor J_D for alginate was 1.674 $Re^{-0.4}$ and for κ -carrageenan was 1.881 $Re^{-0.3}$. In addition, the environmental friendly nature of *Jatropha* biodiesel produced by immobilized lipase was affirmed through LCA. Moreover, it was found out that when the immobilized lipase was reused for 10 times, the cost of *Jatropha* biodiesel production was similar to that of alkali catalyzed one. Thus, in short, biodiesel produced from CJO using immobilized lipase in hybrid matrix can be a sustainable fuel in the near future.

ABSTRAK

PENGHASILAN BIODIESEL DARIPADA MINYAK JATROPHA CURCAS DENGAN MENGGUNAKAN LIPASE STATIK DALAM MATRIKS HIBRID

Dewasa ini, 'Krisis Tenaga Global' kian memuncak apabila tenaga simpanan yang terhad sedang mencapai tahap penggunaannya. Dalam sinario sebegini, penghasilan biodiesel menggunakan lipase statik kian menjadi keutamaan. Minyak *Jatropha curcas* (CJO) sedang menunjukkan potensi yang tinggi untuk menjadi bahan mentah bagi menghasilkan biodiesel di peringkat antarabangsa. Objektif dalam penyelidikan ini adalah untuk menghasilkan biodiesel daripada CJO menggunakan lipase *Burkholderia cepacia* yang statik dalam matriks hibrid. Penyelidikan ini boleh dibahagikan kepada dua bahagian utama. Pertama, ikatan lipase dalam matriks hibrid dan ujian kestabilan lipase. Setelah itu diikuti dengan pengoptimuman penghasilan biodiesel daripada CJO menggunakan lipase statik. Untuk permulaan, ikatan lipase dihasilkan dengan menggunakan glutaraldehyde dalam matriks hibrid polimer semulajadi alginate dan κ -carrageenan. Bijian lipase berbentuk sfera dengan purata ukuran diameter 3mm. Aktiviti ikatan mampu menghasilkan penukaran 89.26%. Pemerhatian kemudiannya mendapati bahawa kebocoran matriks menjatuhkan jumlah penghasilan kepada 65.76%. Lipase statik kekal menghasilkan penukaran sebanyak 84.02% daripada aktiviti asal selepas penyimpanan selama dua minggu. pH yang optimum untuk lipase statik adalah 7 dan pada suhu 40°C. Melalui pembezaan, parameter kinetic K_m dan V_{max} adalah 3.15 μ M dan 12.5 μ mol/min bagi lipase bebas manakala 4.17 μ M dan 11.11 μ mol/min bagi lipase statik. Lipase statik mengekalkan penukaran 75.54% daripada aktiviti asal selepas penggunaan semula sebanyak 10 kali.

Pada bahagian kedua kajian ini, Lipase statik digunakan untuk penghasilan biodiesel daripada CJO. Penghasilan 100% biodiesel (FAEE) boleh dicapai apabila parameter-parameter yang berikut berada di tahap yang optimum: 10g CJO, 1:10 nisbah molar 1:10 minyak kepada etanol, 1g air, 5.2g lipase statik, 35°C, 6 g \times RCF and 24 jam tindakbalas kimia. Lipase statik mengekalkan penukaran sebanyak 73% secara relatif dalam aktiviti transesterifikasi selepas penggunaan sebanyak enam kali. Sementara itu, model pemindahan jisim yang mudah dan efektif ditubuhkan dalam 'recirculated packed bed batch reactor' (RPBBR) dengan lipase statik dan CJO. Berdasarkan bahan biopolymer berbeza dalam bijian statik, JD untuk alginate adalah 1.674 $Re^{-0.4}$ dan untuk κ -carrageenan adalah 1.881 $Re^{-0.3}$. Tambahan pula, sifat mesra alam biodiesel *Jatropha* yang dihasilkan melalui lipase statik telah dinilai melalui LCA. Selain itu, apabila lipase statik digunakan semula melebihi 10 kali, kos penghasilan biodiesel *Jatropha* adalah bersamaan dengan laluan penghasilan menggunakan pemangkin alkali. Ringkasnya, penghasilan biodiesel daripada CJO menggunakan lipase statik dalam matriks hybrid boleh dijadikan sumber bahan api yang mampan pada masa hadapan.

TABLE OF CONTENTS

TITLE	Page
DECLARATION	i
CERTIFICATION	ii
ACKNOWLEDGEMENT	iii
ABSTRACT	iv
ABSTRAK	v
TABLE OF CONTENTS	vi
LIST OF TABLES	vii
LIST OF FIGURES	xi
LIST OF ABBREVIATIONS	xiii
LIST OF SYMBOLS	xviii
APPENDIX	xix
	xxi
CHAPTER 1 : INTRODUCTION	
1.1 General outlook on Energy	1
1.2 Renewable Energy	2
1.3 Biofuels	3
1.4 History of biodiesel	5
1.5 Usage of vegetable oils as fuels	5
1.6 Biodiesel production: Global scenario	6
1.7 Food versus Fuel debate	8
1.8 Non edible oils as biodiesel feedstock	9
1.9 <i>Jatropha curcas</i> oil	11
1.9.1 History	11
1.9.2 Benefits of <i>Jatropha curcas</i> . L	12
1.9.3 Toxicity of <i>Jatropha curcas</i> . L	12
1.9.4 <i>Jatropha</i> plantations: Global scenario	13
1.9.5 <i>Jatropha</i> plantations: Malaysian scenario	15
1.10 Lipase in biodiesel production	16
1.11 Immobilization	19
1.12 Hybrid Matrix for lipase immobilization	19
1.13 Research Background	20
1.13.1 Mass transfer limitation of immobilized lipase	23
1.13.2 Life Cycle Assessment (LCA) of biodiesel production	23
1.13.3 Economic Analysis of biodiesel production	24
1.14 Research Problem	24
1.15 Objectives	26
1.16 Research Approach	27
1.17 Scope	28
1.18 Summary	28
CHAPTER 2 : LITERATURE REVIEW	
2.1 Introduction	30
2.2 Global biofuels scenario	30
2.3 History of Biodiesel	35
2.4 Biodiesel	35
2.5 Feedstock	36

2.6	<i>Jatropha curcas</i> . L – General outlook	38
2.7	<i>Jatropha curcas</i> as a biodiesel feedstock	39
2.8	Process description- Transesterification	41
2.8.1	Alkali catalyst	42
2.8.2	Acid catalyst	42
2.8.3	Enzyme catalyst- Lipase	42
2.9	Enzyme Immobilization	43
2.10	Various lipase Immobilization techniques for biodiesel production	45
2.10.1	Cross-linking	46
2.10.2	Entrapment	47
2.10.3	Hybrid immobilization techniques	48
2.11	Hybrid Matrix	51
2.12	Sodium alginate and κ -carrageenan as hybrid matrix	52
2.12.1	Sodium alginate	53
2.12.2	Calcium-alginate gel structures	54
2.12.3	κ -carrageenan	55
2.12.4	Gelation of κ -carrageenan	56
2.13	Factors affecting enzymatic transesterification using immobilized lipase	57
2.13.1	Feedstock	57
2.13.2	Selection of Alcohol	61
2.13.3	Lipase	62
2.13.4	Pre treatment of Immobilized lipase	65
2.13.5	Water content	65
2.14	Mass transfer effect	66
2.15	Life Cycle Assessment	69
2.16	Economic Analysis	70
2.17	Summary	74

CHAPTER 3 : MATERIALS AND METHODS

3.1	Introduction	75
3.2	Materials	75
3.3	Solution preparations	76
3.4	Methods	77
3.4.1	Lipase immobilization	77
3.4.2	Moisture content	79
3.4.3	Immobilization efficiency	79
3.4.4	Surface and internal morphology of immobilized lipase	79
3.4.5	Interaction between lipase and hybrid matrix	79
3.4.6	Lipase activity	80
3.4.7	Enzyme leakage studies	80
3.4.8	pH stability	81
3.4.9	Thermal stability	81
3.4.10	Solvent stability	81
3.4.11	Storage stability	82
3.4.12	Reusability of immobilized lipase	82
3.4.13	Determination of kinetic constants	82
3.4.14	Olive oil hydrolysis	82
3.4.15	Crude <i>Jatropha curcas</i> oil analysis	83

3.4.16	Optimization of biodiesel production from crude <i>Jatropha curcas</i> oil using immobilized lipase in hybrid matrix	83
3.4.17	Effect of oil to ethanol molar ratio	84
3.4.18	Effect of water content	84
3.4.19	Effect of enzyme loading	84
3.4.20	Effect of temperature	85
3.4.21	Effect of mixing intensity	85
3.4.22	Effect of reaction time	85
3.4.23	Reusability of immobilized lipase	85
3.4.24	Biodiesel sampling and analysis	85
3.4.25	Development of external mass transfer model for hydrolysis of crude <i>Jatropha curcas</i> oil	86
3.4.26	Hydrolysis of <i>Jatropha</i> oil in recirculated packed bed batch reactor (RPBBR)	86
3.4.27	Titration method for fatty acid concentration	87
3.4.28	Life Cycle Assessment	88
3.4.29	Goal and scope of the study	88
3.4.30	Impact assessment	89
3.4.31	Inventory analysis	92
3.4.32	Economic Analysis of Biodiesel production	94
3.4.33	Process Flow Sheets, Time Charts and Costs	94
3.5	Summary	102

CHAPTER 4 : RESULTS AND DISCUSSION

4.1	Introduction	103
	PART I: STABILITY STUDIES OF IMMOBILIZED LIPASE IN HYBRID MATRIX	
4.2	Lipase Immobilization	103
4.3	Moisture content	105
4.4	Immobilization efficiency	105
4.5	Surface and internal morphology of immobilized lipase	106
4.6	Interaction between lipase and hybrid matrix	108
4.7	Enzyme leakage studies	109
4.8	Stability studies	111
	4.8.1 pH stability	112
	4.8.2 Thermal stability	113
	4.8.3 Solvent stability	114
	4.8.4 Storage stability	115
	4.8.5 Reusability of immobilized lipase	116
4.9.	Kinetic parameters	117
4.10	Olive oil hydrolysis	119
	PART II: BIODIESEL PRODUCTION FROM CRUDE JATROPHA CURCAS OIL USING IMMOBILIZED LIPASE IN HYBRID MATRIX	
4.11	Optimization of biodiesel production from crude <i>Jatropha curcas</i> oil	120
	4.11.1 Effect of oil to ethanol molar ratio	120
	4.11.2 Effect of water content	121
	4.11.3 Effect of immobilized enzyme loading	122
	4.11.4 Effect of temperature	123

4.11.5	Effect of mixing intensity	124
4.11.6	Effect of reaction time	125
4.11.7	Reusability of immobilized lipase	126
4.12	Optimization of biodiesel production with Response surface Methodology (RSM)	128
4.13	Fuel properties of <i>Jatropha</i> biodiesel	130
4.14	Development of external mass transfer model for hydrolysis of crude <i>Jatropha curcas</i> oil	131
4.14.1	Apparent reaction rate	131
4.14.2	Combination of mass transfer and biochemical reaction	133
4.14.3	Empirical model	135
4.14.4	Establishment of external mass transfer model	137
4.14.5	Determination of mass transfer coefficient	144
4.15	Life Cycle Assessment (LCA) of biodiesel production	146
4.16	Economic Analysis of biodiesel production	159
4.17	Summary	163

CHAPTER 5 : CONCLUSION AND RECOMMENDATIONS

5.1	Conclusion	164
5.2	Recommendations	165

REFERENCES	167
-------------------	-----

APPENDIX	194
-----------------	-----



UMS
UNIVERSITI MALAYSIA SABAH

LIST OF TABLES

		Page
Table 1.1	Classification of biofuels based on production technologies	3
Table 1.2	Biodiesel specifications according to ASTM D6751 and EN 14214 standards	7
Table 1.3	Potential non edible sources for biodiesel production	10
Table 1.4	Comparison of alkali, acid and enzymatic catalysis in biodiesel production	18
Table 2.1	Global biofuel production in top fifteen countries and EU, 2011	32
Table 2.2	Biofuel blending policies in various countries	33
Table 2.3	Oil content and production of non-edible oil seeds	37
Table 2.4	Fatty acid composition of crude <i>Jatropha curcas</i> oil	40
Table 2.5	Various immobilization techniques employed for biodiesel production from CJO	49
Table 2.6	Hybrid matrices used for lipase immobilization	51
Table 2.7	Biodiesel production with various immobilized lipases	59
Table 2.8	Commercial Microbial lipases	62
Table 2.9	Comparative economic assessment among previous studies for biodiesel production plants	72
Table 3.1	List of materials and manufacturers	75
Table 3.2	Process conditions for biodiesel production	92
Table 3.3	Materials and energy used to produce biodiesel using alkali catalyst	92
Table 3.4	Materials and energy used to produce biodiesel using soluble lipase	93
Table 3.5	Materials and energy used to produce biodiesel using immobilized lipase	93
Table 3.6	Equipment specifications and procurement costs for biodiesel production of 1000 tonne using different catalytic processes	96
Table 3.7	Total plant investment costs for biodiesel production of 1000 tonne using different catalytic processes	99
Table 3.8	Variable costs and fixed costs for biodiesel production of 1 tonne using different catalytic processes	100

Table 4.1	Physical observation of beads produced using different concentrations of alginate and κ -carrageenan	104
Table 4.2	Moisture content of immobilized <i>Burkholderia cepacia</i> lipase	105
Table 4.3	Immobilization efficiency	106
Table 4.4	Comparison of enzyme leakage studies on different hybrid matrices	111
Table 4.5	Solvent stability of immobilized lipase	115
Table 4.6	Kinetic parameters of free and immobilized lipase	119
Table 4.7	Fuel properties of Jatropha biodiesel	130
Table 4.8	Observed first-order reaction rate constant, k_p	140
Table 4.9	The percent deviation of calculated values of k_p from experimental values at different ' n ' for calcium alginate parameter	142
Table 4.10	The percent deviation of calculated values of k_p from experimental values at different ' n ' for κ -carrageenan parameter	143
Table 4.11	Calculation of constant K for (a) calcium alginate (b) κ -carrageenan	144
Table 4.12	Comparison of k_m values for (a) calcium alginate (b) κ -carrageenan	145
Table 4.13	Comparison of concentration difference between the external film (a) calcium alginate (b) κ -carrageenan	146
Table 4.14	Cost of production of biodiesel for varying capacities based on alkali, soluble lipase and immobilized lipase catalyst	161

LIST OF FIGURES

		Page
Figure 1.1	Classification of energy resources	1
Figure 1.2	Cultivation limits of <i>Jatropha curcas</i>	13
Figure 1.3	Major <i>Jatropha curcas</i> projects around the world	14
Figure 1.4	Identified lands in Malaysia suitable for <i>Jatropha curcas</i> cultivation	15
Figure 1.5	Enzymatic transesterification of biodiesel	17
Figure 1.6	Summary of Introduction	29
Figure 2.1	Overview of biomass conversion pathways	31
Figure 2.2	Ethanol and Biodiesel production, 2000-2011	32
Figure 2.3	Potential benefits of <i>Jatropha curcas</i> .L	39
Figure 2.4	Transesterification reaction	41
Figure 2.5	Different approach to immobilization of enzymes	44
Figure 2.6	Basic synthesis methods for Simultaneous Interpenetrating Polymer Networks	52
Figure 2.7	Structural characteristics of alginates (a) chain conformation (b) block distribution	54
Figure 2.8	Structure of alginate G and M blocks and illustration of alginate chains forming ionically crosslinked structures with the addition of calcium ions	55
Figure 2.9	Structure of κ -carrageenan	56
Figure 2.10	Gelling mechanism of κ -carrageenan	57
Figure 2.11	Overall three dimensional structure of a lipase from <i>Psuedomonas</i> strain	63
Figure 2.12	Mechanism of lipase in transesterification reaction	64
Figure 2.13	Schematic diagram of the transfer of substrate from the bulk medium to an entrapped enzyme	67
Figure 2.14	Hydrolysis reaction of oil by lipase	68
Figure 2.15	Summary of literature review	74
Figure 3.1	Schematic diagram of entrapment method	78
Figure 3.2	Stirred tank batch reactor for biodiesel production	84

Figure 3.3	Schematic representation (A) and block diagram (B) of a recirculated packed-bed batch reactor (RPBBR) with boundary conditions	87
Figure 3.4	Flow chart of biodiesel production from CJO using alkali as catalyst	89
Figure 3.5	Flow chart of biodiesel production from CJO using soluble lipase as catalyst	90
Figure 3.6	Flow chart of biodiesel production from CJO using immobilized lipase as catalyst	91
Figure 3.7	Process time chart for the production of biodiesel using alkali catalyst	95
Figure 3.8	Process time chart for the production of biodiesel using soluble lipase catalyst	95
Figure 3.9	Process time chart for the production of biodiesel using immobilized lipase catalyst	95
Figure 3.10	Summary of Materials and Methods	102
Figure 4.1	Immobilized lipase in hybrid matrix	104
Figure 4.2	SEM pictures of crosslinked lipase immobilized in a hybrid matrix of alginate and κ -carrageenan	107
Figure 4.3	Comparison of crosslinking in hybrid matrix	108
Figure 4.4	FTIR spectrum of κ -carrageenan, sodium alginate, lipase and immobilized lipase	109
Figure 4.5	Leakage of protein from immobilized lipase as a function of incubation time	110
Figure 4.6	pH stability of free and immobilized lipase at room temperature	112
Figure 4.7	Temperature stability of free and immobilized lipase at pH 7	114
Figure 4.8	Storage stability of free and immobilized lipase at 4°C in different buffers	116
Figure 4.9	Reusability of immobilized lipase for <i>p</i> -NPP hydrolysis	117
Figure 4.10	Lineweaver – Burk plot of free and immobilized lipase for <i>p</i> -NPP hydrolysis	118
Figure 4.11	Time course of olive oil hydrolysis by immobilized lipase with and without crosslinking	119
Figure 4.12	Effect of alcohol concentration on immobilized lipase catalyzed transesterification of crude <i>Jatropha curcas</i> oil	120

Figure 4.13	Effect of water content on immobilized lipase catalyzed transesterification of crude <i>Jatropha curcas</i> oil	121
Figure 4.14	Effect of immobilized enzyme loading on transesterification of crude <i>Jatropha curcas</i> oil	123
Figure 4.15	Effect of temperature on immobilized lipase catalyzed transesterification of crude <i>Jatropha curcas</i> oil	124
Figure 4.16	Effect of mixing intensity on immobilized lipase catalyzed transesterification of crude <i>Jatropha curcas</i> oil	125
Figure 4.17	Effect of time course on immobilized lipase catalyzed transesterification of crude <i>Jatropha curcas</i> oil	126
Figure 4.18	Reusability of immobilized lipase	127
Figure 4.19	Immobilized lipase in alginate/ κ -carrageenan hybrid matrix before (A) and after (B) 10 cycles of reuse in transesterification of CJO with ethanol at 6g RCF and 35°C	128
Figure 4.20	Contour plot of biodiesel yield with effect of molar ratio and water content	129
Figure 4.21	Contour plot of biodiesel yield with effect of temperature and enzyme loading	129
Figure 4.22	Contour plot of biodiesel yield with effect of mixing intensity and reaction time	130
Figure 4.23	Increment of fatty acid concentration against time by calcium alginate	137
Figure 4.24	Increment of fatty acid concentration against time by κ -carrageenan	138
Figure 4.25	Plot of $\ln(C/C_0)$ vs time to estimate number of transfer units for calcium alginate parameter	139
Figure 4.26	Plot of $\ln(C/C_0)$ vs time to estimate number of transfer units for κ -carrageenan parameter	139
Figure 4.27	Plots of $1/k_p$ vs $1/G^n$ for hydrolysis of <i>Jatropha</i> oil in immobilized lipase (calcium alginate parameter) for various value of n. (a) n = 0.1; (b) n = 0.3; (c) n = 0.6; (d) n = 1.0.	140
Figure 4.28	Plots of $1/k_p$ vs $1/G^n$ for hydrolysis of <i>Jatropha</i> oil in immobilized lipase (κ -carrageenan parameter) for various value of n. (a) n = 0.1; (b) n = 0.4; (c) n = 0.7; (d) n = 1.0	141
Figure 4.29	Comparison of the environmental impacts of the 11 environmental categories due to the production of 1tonne <i>Jatropha</i> biodiesel	149

Figure 4.30	Comparison of the environmental impacts of the 11 environmental categories due to the production of 5 tonne Jatropha biodiesel	150
Figure 4.31	Comparison of the environmental impacts of the 11 environmental categories due to the production of 10 tonne Jatropha biodiesel	151
Figure 4.32	Comparison of the environmental impacts of the 11 environmental categories due to the production of 1 tonne(A), 5 tonne(B) and 10 tonne (C) Jatropha biodiesel	152
Figure 4.33	Comparison of the environmental impacts on each environmental category based on a single cumulative score due to the production of 1 tonne Jatropha biodiesel	153
Figure 4.34	Comparison of the environmental impacts on each environmental category based on a single cumulative score due to the production of 5 tonne Jatropha biodiesel	154
Figure 4.35	Comparison of the environmental impacts on each environmental category based on a single cumulative score due to the production of 10 tonne Jatropha biodiesel	155
Figure 4.36	Comparison of the environmental impacts on human health, ecosystem and resources due to the production of 1 tonne Jatropha biodiesel	156
Figure 4.37	Comparison of the environmental impacts on human health, ecosystem and resources due to the production of 5 tonne Jatropha biodiesel	157
Figure 4.38	Comparison of the environmental impacts on human health, ecosystem and resources due to the production of 10 tonne Jatropha biodiesel	158
Figure 4.39	Plant investment costs for 1000 tonne capacity biodiesel production from CJO	159
Figure 4.40	Manufacturing costs for 1 tonne capacity biodiesel production from CJO	160
Figure 4.41	Manufacturing costs for 1000 tonne capacity biodiesel production from CJO	161
Figure 4.42	Economics of Jatropha biodiesel	162
Figure 4.43	Summary of results and discussion	163

LIST OF ABBREVIATIONS

BSA	Bovine Serum Albumin
CJO	Crude <i>Jatropha curcas</i> oil
FAEE	Fatty Acid Ethyl Ester
FAAE	Fatty acid alkyl ester
FAME	Fatty Acid Methyl Ester
FAO	Food and Agricultural Organization
FFA	Free Fatty Acid
IPNs	Interpenetrating Polymer Networks
LCI	Life cycle inventory
lipase PS	Burkholderia cepacia lipase
LCA	Life Cycle Assessment
MPOB	Malaysian Palm Oil Board
p-NPP	p-nitrophenyl palmitate
PAH	Polycyclic Aromatic Hydrocarbons
rpm	Rotations per minute
RPBBR	Recirculated packed bed batch reactor
T/Ca	Tris- CaCl ₂

LIST OF SYMBOLS

a_m	External surface area for mass transfer ($\text{cm}^2 \text{mg}^{-1}$)
C	Bulk substrate concentration (mg l^{-1})
C_{in}	column inlet substrate (CJO) concentration (mg l^{-1})
C_{out}	column outlet substrate (CJO) concentration (mg l^{-1})
C_s	substrate concentration at surface of the immobilized lipase (mg L^{-1})
C_o	initial CJO concentration in the reservoir
C_1	concentration of CJO (mg l^{-1}) in the reservoir
C_2	concentration (mg l^{-1}) at the outlet of the packed-bed column to be recirculated to the reservoir
D_f	Diffusivity (cm min^{-1})
E_a	Activation energy (KJ/mol)
G	mass flux ($\text{g cm}^{-2} \text{min}^{-1}$)
H	Height of the column
J_D	Colburn factor
k	surface first order reaction rate constant
k_m	external mass transfer coefficient (cm h^{-1})
K_m	Michaelis Menten constant (μM)
k_p	apparent first-order reaction rate constant ($\text{l g}^{-1} \text{h}^{-1}$)
K	mass transfer correlation coefficient
n	exponential factor in mass transfer correlation
N	group of parameters
N_{sc}	Schmidt number
Q	Volumetric flow rate (ml min^{-1})

R	Gas constant (8.314 J mol^{-1})
Re	Reynolds number
r	reaction rate ($\text{mg g}^{-1} \text{ h}^{-1}$)
r_m	external mass transfer rate of substrate ($\text{mg g}^{-1} \text{ h}^{-1}$)
t	time
U	Unit of lipase activity ($\mu\text{mol}/\text{min}$)
V_A	Triglyceride molar volume ($\text{m}^3 \text{ kg mol}^{-1}$)
V_{max}	Maximum reaction velocity ($\mu\text{mol}/\text{min}$)
z	distance from the bottom of the packing in a column
ϵ	Molar extinction coefficient ($15000 \text{ M}^{-1} \text{ cm}^{-1}$)
μ_B	Viscosity of the water (Pa.s)
μ_r	Fluid viscosity ($\text{g cm}^{-1} \text{ min}^{-1}$)
ρ	fluid density
ρ_p	Particle density (mg cm^{-3})
T	Residence time (min) in the reservoir



UMS
UNIVERSITI MALAYSIA SABAH

LIST OF APPENDIX

	Page
APPENDIX A	195
APPENDIX B	197



UMS
UNIVERSITI MALAYSIA SABAH

CHAPTER 1

INTRODUCTION

1.1 General outlook on Energy

The present world is on the verge of a severe 'global energy crisis' with limited energy reserves exceeding its supply. The reasons for this fast depletion of energy resources can be increase in population, economic growth, better standards of living, inappropriate utilization, ageing infrastructure, accidents at refineries and over consumption. One of the main explanations for energy crisis can be attributed to too much dependence on non-renewable resources rather than utilizing renewable energies to the maximum. Almost 90% of the world's energy is met by non-renewable sources such as natural gas, petroleum and coal (Lior, 2008) (Figure 1.1).

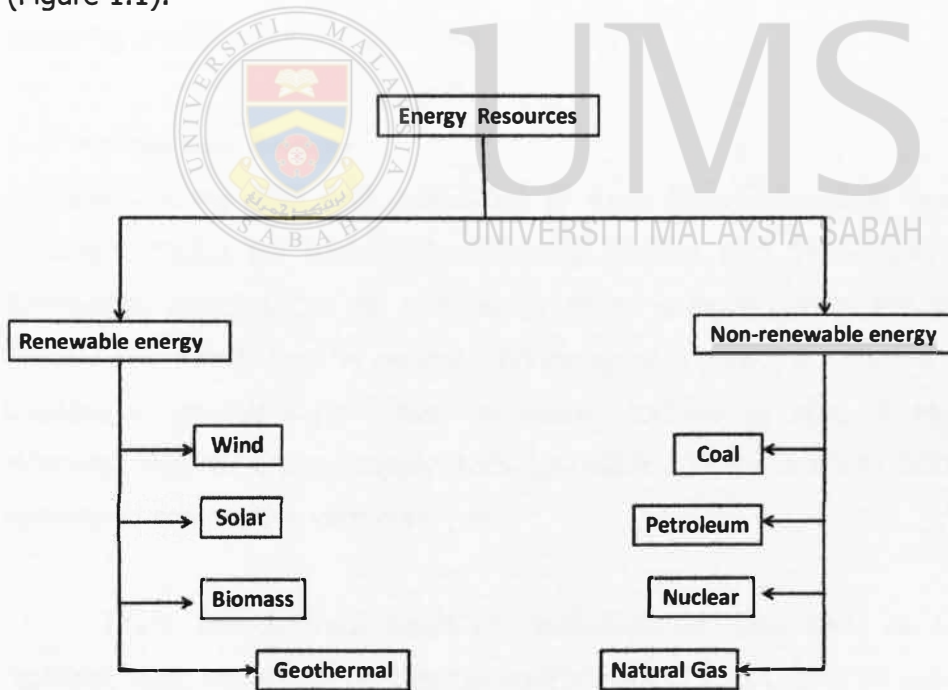


Figure 1.1: Classification of energy resources

Source: Demirbas, 2005

Tremendous exploitation of non renewable energies has resulted in fast depletion of these reserves along with adverse effect on climate mainly due to the emissions from fossil fuel combustion, especially green house gases. In other words, the present population is making the earth a place same as primitive mankind used. This will have adverse effect on our future generations.

The main contribution of green house gases comes from the use of carbon related fossil fuels such as coal and oil (Quadrelli and Peterson, 2007). Increase in green house gases like CO₂ in the atmosphere eventually lead to increase in global temperature which has adverse effects on the earth. In such a scenario, measures have to be taken to curb energy related CO₂ emissions through a number of timely implemented technologies (Ghoniem, 2011). Thus, accelerating the use of renewable energies could replace the shortage of fossil fuels in the near future as well as drive our earth to a safer and cleaner place. So nowadays, renewable energy exploitation has become an interesting area of research with future scope of powering the human activities to the maximum.

1.2 Renewable Energy

In today's world with fast exhaustion of fossil fuels, renewable energy is the inevitable choice for sustainable economic growth and future energy reserve. Renewable energies can be defined as those energies which are environment friendly and which can be recycled (Peidong *et al.*, 2009). There is a significant increase in energy supply from renewable sources as seen in recent years. However, still, the energy supply from renewable sources is a way far to go to be considered competitive with fossil fuels.

There are different types of renewable energies such as solar, wind, biomass, tidal, hydro and geothermal energy. Out of these, biofuels grouped under the biomass category is gaining immense potential mainly because these can be produced from different feedstocks around the world.

1.3 Biofuels

The term 'biofuel' or 'bio-renewable fuel' refers to solid, liquid or gaseous fuels mainly produced from bio-renewable feedstocks. Out of this, liquid biofuels are mostly used as fuel for vehicles in addition to power engines or fuel cells for electricity generation. Liquid biofuels produced from a variety of biomass feedstock include: biodiesel, bioethanol, methanol and Fischer-Tropsch diesel (Demirbas, 2008). It was found that there are two bio-renewable liquid fuels, which can replace gasoline and diesel in the near future, namely bioethanol and biodiesel. However, biodiesel has caught more attention of researchers due to its environmental benefits (Altun, 2011).

Based on the production technologies, biofuels can be classified into first generation; second generation; third generation, fourth and fifth generation biofuels. Table 1.1 shows classification of biofuels based on production technologies.

Table 1.1: Classification of biofuels based on production technologies

Generation	Feedstock	Examples
First-generation biofuels	Sugar, starch, vegetable oils or animal fats	Bioalcohols, vegetable oil, biodiesel, biosyngas, biogas
Second generation biofuels	Non food crops, wheat straw, corn, wood, solid waste, energy crop	Bioalcohols, bio-oil, bio-DMF, biohydrogen, bio-Fischer-Tropsch diesel
Third generation biofuels	Algae	Vegetable oil, biodiesel
Fourth generation biofuels	Vegetable oil, biodiesel	Biogasoline
Fifth generation biofuels	Gaseous raw materials	methane

Source: Demirbas(2011); Porqueras *et al* (2012)