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

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

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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 3.15μM and 12.5μmol/min for free lipase and 4.17μM and 11.11μmol/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 × 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 penghasilkan 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 k-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 3.15μM dan 12.5μmol/min bagi lipase bebas manakala 4.17μM dan 11.11μmol/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 × 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 $1.674Re^{-0.4}$ 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.

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LIST OF ABREVIATIONS

BSA	Bovine Serum Albumin
CJO	Crude *Jatropha curcas* 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 ($cm^2\ mg^{-1}$)
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 ($cm\ min^{-1}$)
Ea	Activation energy (KJ/mol)
G	mass flux ($g\ cm^{-2}\ min^{-1}$)
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 ($l\ g^{-1}\ h^{-1}$)
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 (mg g^{-1} h^{-1})
r_m	external mass transfer rate of substrate (mg g^{-1} h^{-1})
t	time
U	Unit of lipase activity (µmol/min)
V_A	Triglyceride molar volume (m^3 kg mol^{-1})
V_{max}	Maximum reaction velocity (µmol/min)
z	distance from the bottom of the packing in a column
ε	Molar extinction coefficient (15000 M^{-1} cm^{-1})
μ_B	Viscosity of the water (Pa.s)
μ_f	Fluid viscosity (g cm^{-1} min^{-1})
ρ	fluid density
ρ_p	Particle density (mg cm^{-3})
T	Residence time (min) in the reservoir

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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).

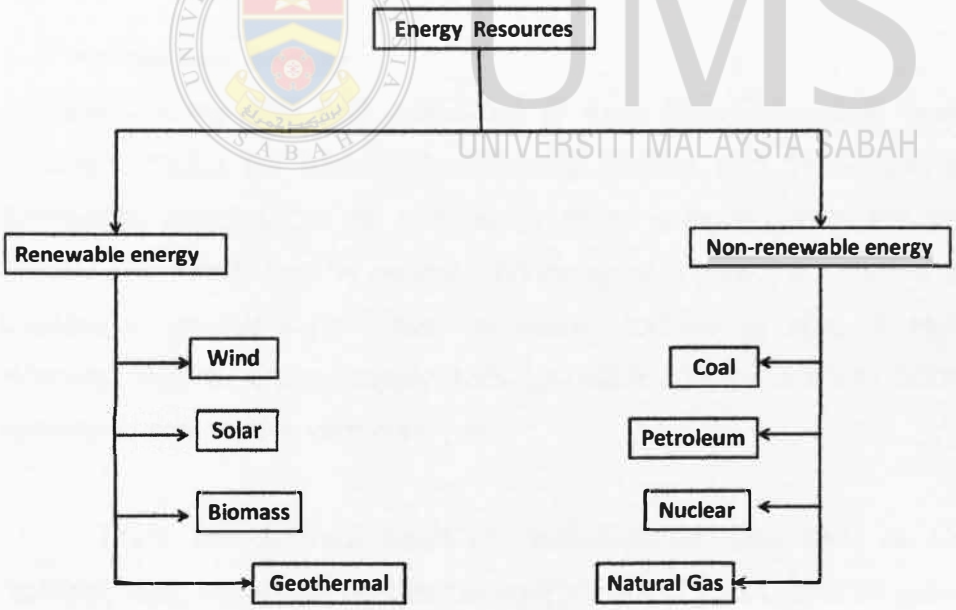

Figure 1.1: Classification of energy resources

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 CO_2 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 CO_2 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 asignificant 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 mainlyproduced frombio-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 intofirst 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)