PRODUCTION OF BIODIESEL FROM PALM OIL USING ENCAPSULATED LIPASE DERIVED FROM K-CARRAGEENAN

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

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CERTIFICATION

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DEGREE : **DOCTOR OF PHILOSOPHY (CHEMICAL ENGINEERING)**

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DECLARED BY

1. SUPERVISOR

Professor Dr. Pogaku Ravindra Signature

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Dr. Chan Eng Seng Signature

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ABSTRACT

PRODUCTION OF BIODIESEL FROM PALM OIL USING ENCAPSULATED LIPASE DERIVED FROM Κ-CARRAGEENAN

The objective of this research was to produce biodiesel using encapsulated lipase in an immobilized bioreactor. κ-carrageenan was used as a matrix for encapsulating lipase PS from *Burkholderia cepacia* and the coextrusion technique was adopted to immobilize lipase. This study has been undertaken due to the low cost, non toxic, environmentally benign characteristics of κ-carrageenan and the novelty of co-extrusion technique. The physicochemical studies were conducted by using Microscope, Spectrophotometer SEM, FTIR etc. The results showed the diameter of the encapsulated lipase was in the range of 1.3-1.8 mm with an average membrane thickness of 200 μm. The encapsulation efficiency was found to be 42.6 percent. The optimum stability was observed at pH 7 and at temperature 40°C. The Immobilized lipase retained 72.3 percent of its original activity after using it for 5 cycles of reuse in hydrolysis of ρ-NPP.

Immobilized encapsulated lipase was taken in stirred tank batch immobilized bioreactor (STIBR) and packed bed bioreactor (PBBR). The studies were carried out in a batch mode of operation and various process parameters were optimized for biodiesel production. HPLC was used for analyzing the biodiesel. The optimum conditions for processing palm oil in a stirred tank immobilized bioreactor (STIBR) were 30°C, 72 h reaction time and 23.7 x g relative centrifugal force. Similarly, the optimal conditions for processing palm oil in a PBBR were 1.5 ml/min and 264 h reaction time. STIBR showed conversion of up to 100 percent and the PBR has shown conversion up to 82 percent. Since the STIBR has higher conversion rate, the kinetic parameters K_m and V_{max} were evaluated and found to be 600 $mol.m^{-3}$ and 0.84 $mol.m^{-3}min^{-1}$ respectively. The kinetic parameter values were substituted into Michaelis–Menten empirical equation and the batch time was found to be the same as experimental value of 72 h. The encapsulated lipase retained 82 percent relative conversion after 5 cycles of reuse. The economic assessment of biodiesel production using immobilized enzyme catalyst process was challenging compared to the current alkali process. The Life Cycle Analysis (LCA) studies showed that biodiesel production using immobilized enzyme catalyst has lesser impact on the environment compared to the alkali catalyst and soluble enzyme catalyst. Based on the experimentation and the results, it is concluded that biodiesel production using encapsulated lipase in an immobilized bioreactor open new vistas for the scale up studies of this technology in near future.

ABSTRAK

Objektif kajian ini adalah untuk menghasilkan biodiesel menggunakan kaedah pengkapsulan lipase dalam bioreaktor amobil. κ-carrageenan digunakan sebagai median pengkapsulan Lipase PS daripada Burkholderia cepacia dan aplikasi kaedah pembentukan (coextrusion) untuk menghasilkan lipase amobil. Kaedah ini dipilih kerana ianya memerlukan kos yang rendah berbanding kaedah lain, tidak bertoksik penggunaaan Karragenan yang bercirikian mersa alam dan novelti dalam teknik pembentukannya. Kajian kimia-fizikal dijalankan mengunakan Mikroskop Spektometer (SEM), dan FTIR. Keputusannya menunjukkan bahawa diameter lipase yang pengkapsulan berada dalam linkungan 1.3mm hingga 1.8mm dengan purata ketebalan membran setebal 200 μm. Kepersisan proses kapsulasi adalah sebanyak 42.6 peratus. kestabilan optimum telah diperhatikan pada nilai pH 7, pada suhu 40^oC. Kecekapan aktivitinya sebanyak 72.3 peratus berbanding kecekapan asal diperhatikan dalam lipase amobil walaupun selepas 5 kitaran guna semula menggunakan hidrolisis ρ-NPP

Kapsul lipase amobil dikeluarkan daripada siri bioreaktor yang dikacau (STIBR) dan batas bioreaktor berbungkus (PBBR). Kajian dijalankan dalam beberapa siri operasi dan melibatkan pengoptimum proses bagi pelbagai parameter untuk menghasilkan biodiesel. HPLC digunakan untuk menganalisis biodiesel. Persekitaran optimum bagi pemprosesan minyak kelapa sawit dalam siri bioreaktor yang dikacau (STIBR) adalah pada 30^oC selama 72 jam pada 23.7 x g kelajuan relatif penghemparan. Keadaan optimum bagi pemprosesan minyak kelapa sawit dalam PBBR adalah 1.5 ml/min dan 264 jam untuk masa reaksi. STIBR menunjukkan 100 peratus penukaran berbanding 82 peratus menggunakan PBR. Memandangkan STIBR menunjukkan kadar pertukaran yang lebih tinggi, parameter kinetik K_m dan V_{max} dievaluasi dimana 600 $mol.m^{-3}$ dan 0.84 $mol.m^{-3}min^{-1}$ diperolehi. Parameter kinetik yang dikira digantikan dalam persamaan impirik Michaelis-Menten, di dapati siri masa adalah sama dengan nilai rekod semasa ekperimen dijalankan iaitu 72 jam. Lipase yang pengkapsulan masih mengekalkan 82 peratus daripada penukaran relatif asalnya walaupun selepas 5 kitaran guna semula. Penilaian ekonomi dalam penghasilan biodiesel menggunakan proses katalisis enzim amobil merupakan kaedah yange lebih mencabar berbanding kaedah proses alkali seperti yang digunakan kini. Kajian analisis kitaran hidup (LCA) telah menunjukkan bahawa penghasilan biodiesel menggunakan katalisis enzim membawa impak yang lebih rendah kepada alam sekitar berbanding kaedah katalisis alkali dan katalisis enzim berlarut. Berdasarkan kajian ini dan keputusan yang diperolehi, dapat disimpulkan bahawa penghasilan biodiesel menggunakan kapsul lipase dengan kaedah pembentukan dalam bioreaktor amobil merupakan pendekatan baru untuk diterokai dengan lebih mendalam dalam bidang ini.

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Symbols

K	Boltzman constant, kg m^2/s^2
T	Temperature, ^oC
t	Time, h
E	Activation energy, J/mol
R	Gas constant, 8.314 J/mol ^oK
V	Reaction rate constant or velocity constant
D_e	Diffusivity, m^2/s
r	Particle radius, m
r_1	Capsule outer core radius, m
r_2	Capsule inner core radius, m
V_{max}	Maximum reaction rate, mol/m^3 sec (transeserification)
V'_{max}	Maximum reaction rate, U/mg-protein (hydrolysis)
V''_m	Apparent Maximum reaction rate, mol/m^3 (catalyst).sec
K_m	Michalis Menten constant, mol/m^3 (transeserification)
K'_m	Michalis Menten constant, mmol (hydrolysis)
C_S	Concentration of the substrate at time t, mol/m^3
C_{SO}	Concentration of the substrate at time t =0, mol/m^3
V_o	Frequency factor or pre-exponential factor
$+r_p$	Rate of product formation, mol/m^3 (methyl esters)
$-r_s$	Rate of disappearance of substrate, mol/m^3 (triglycerides)
A_{410nm}	Absorbance , 410 nm
$\Delta\varepsilon$	Molar extinction of nitrophenol
η	Effectiveness factor
Φ	Thiele modulus
β	Michalis constant dimensionless number

CHAPTER 1

INTRODUCTION

The availability and environmental impact of energy resources will play a critical role in the progress of the world's societies and the physical future of our planet. Worldwide energy consumption is increasing exponentially (Figure 1.1) and at present usage rates, these sources will soon be exhausted (Srivastava and Prasad, 2000), contributed to soaring fossil fuel prices. The majority of human energy needs are currently met using petrochemical sources, coal and natural gases. As the demand for energy has grown, so have the adverse environmental effects of its production.

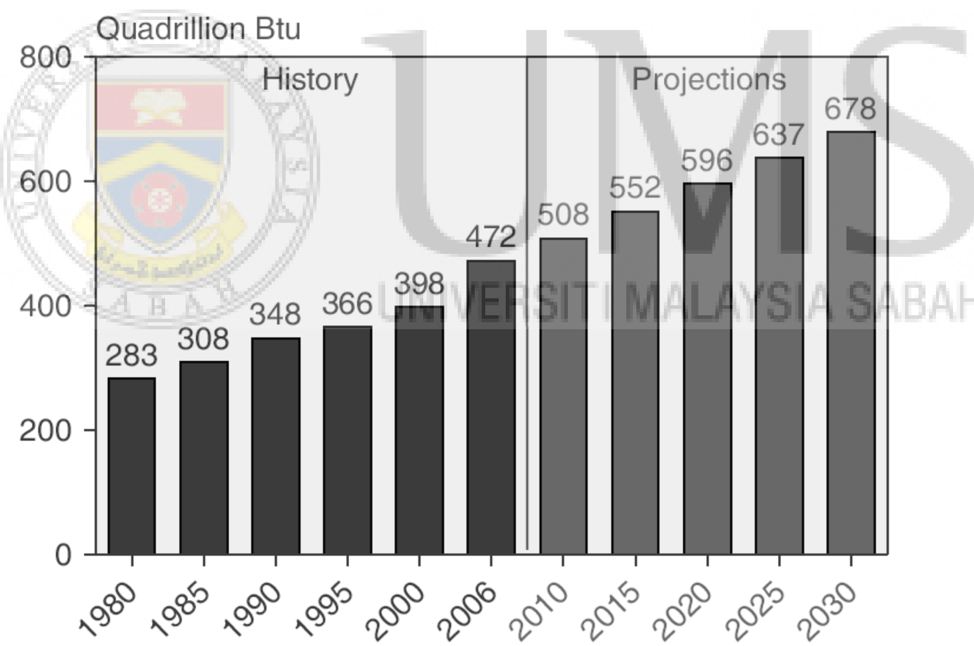

Figure 1.1: World marketed energy consumption.
Source : IEA outlook (2007)

Emissions of CO_2 (Figure 1.2), SO_2 and NO_x from fossil fuel combustion are the primary causes of adverse environmental effects (Ture *et al.*, 1997). The accumulation of carbon dioxide and other greenhouse gases in the atmosphere is thought to be responsible for climate change, which is predicted to have disastrous global consequences for life on this planet (Sheehan *et al.*, 1998). Renewable energy may offer an excellent alternative to the fossil fuels, representing a cornerstone to steer our energy system in the direction of sustainability and supply security. Hence, Renewable energy sources have become a high priority in the energy policy strategies at national level as well as at a global scale.

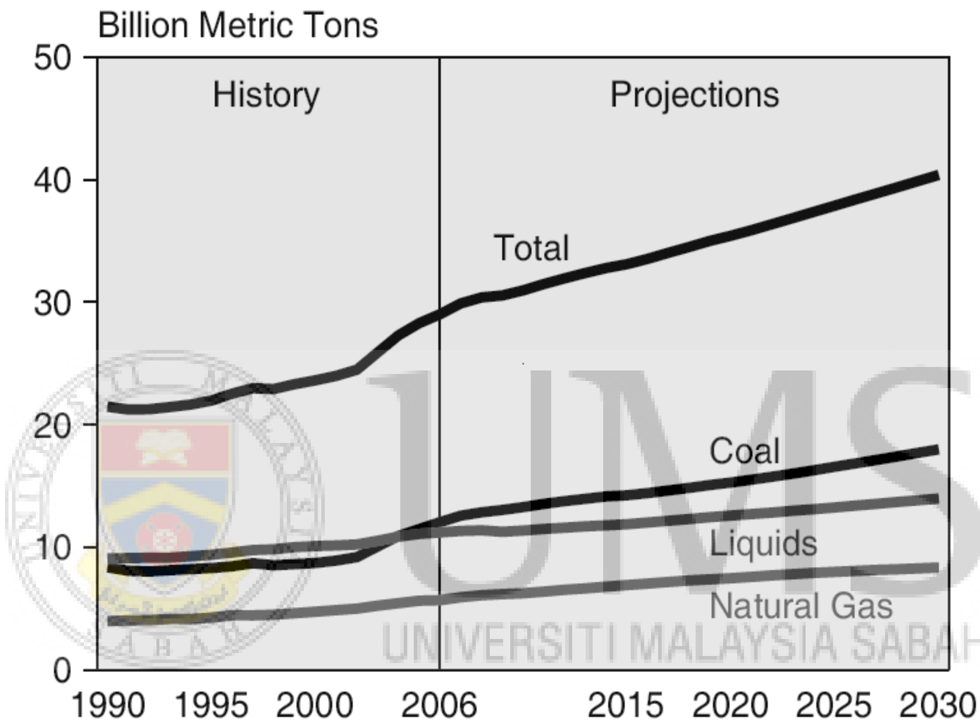

Figure 1.2: World energy-related carbon dioxide emissions by fuel type
Source : IEA outlook (2007)

1.1 Renewable Energy

Renewable energy sources are indigenous, and can therefore contribute to reduce dependency on oil imports, increasing security of supply and environmental benefit. Due to these reasons, the investment towards renewable energy is drastically increasing around the world (Figure 1.3). Renewable energy can be classified into various types (Figure 1.4), among which biofuels could be considered as a major energy source promoted and produced in most of the countries around the world.

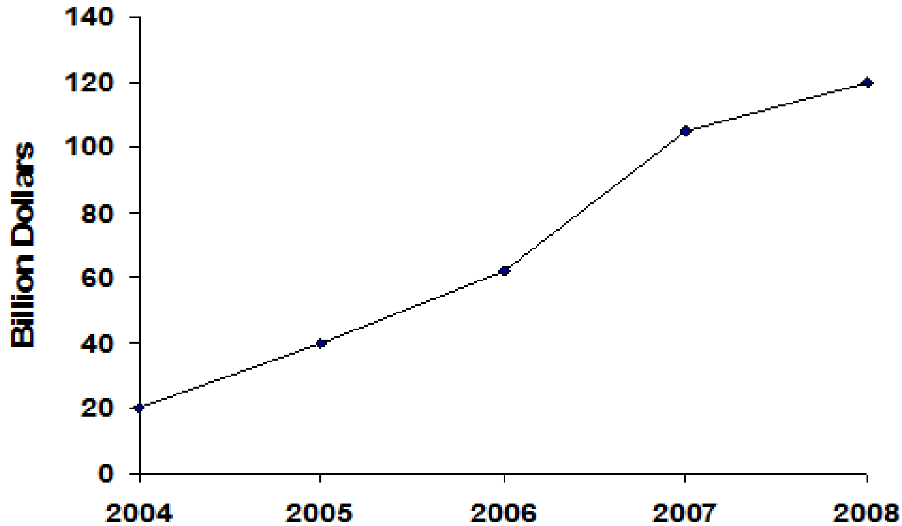

Figure 1.3: Global investment in renewable energy.
Source : REN21 (2009)

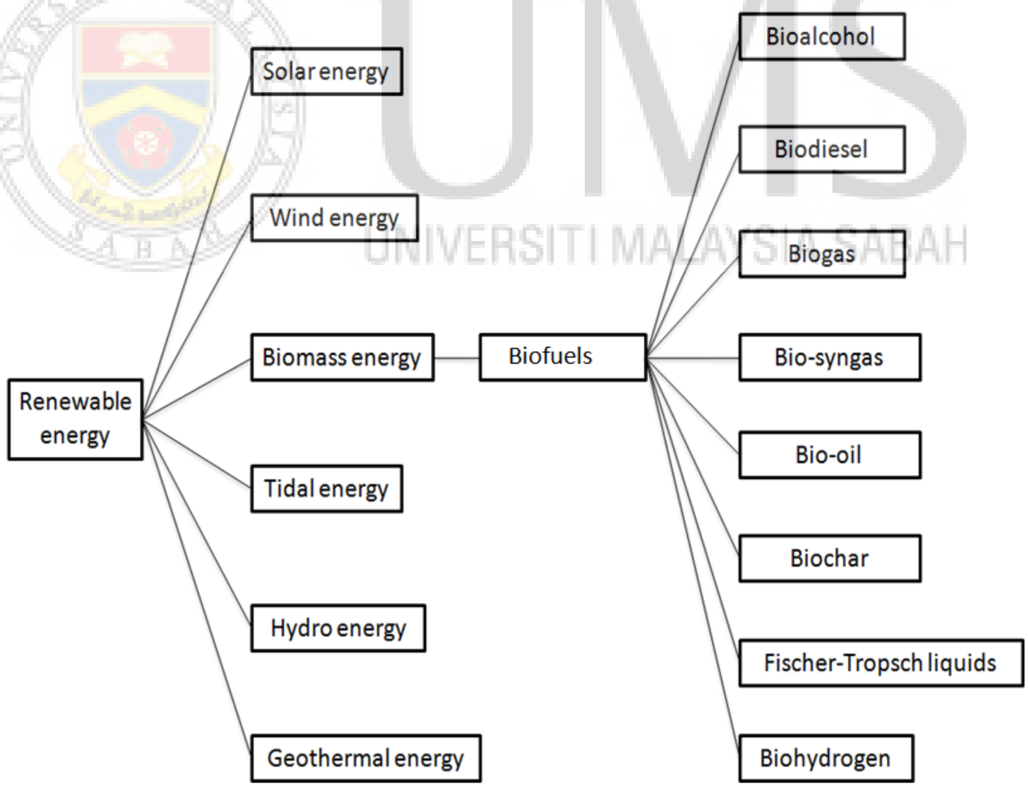

Figure 1.4: Classification of renewable energy.

1.2 Biofuels

Biofuels provide the prospect of new economic opportunities for people in rural areas, concerning job creation, greater efficiency in the general business, and protection of the environment (Demirbas, 2008)(Figure 1.5). Biofuels – liquid or gaseous fuels derived predominantly from biomass may be able to provide an alternative source of energy that could be both sustainable and without serious environmental impact. Biofuels are produced from plant oils, algal oil, animal fats, sugar beets, cereals, organic waste and the processing of biomass. The extent to which biofuels can ultimately replace fossil fuels depends on the efficiency with which they can be produced (Malcaa and Freire, 2006). Biofuel research and deployment has intensified in all countries as an alternative to fossil fuel.

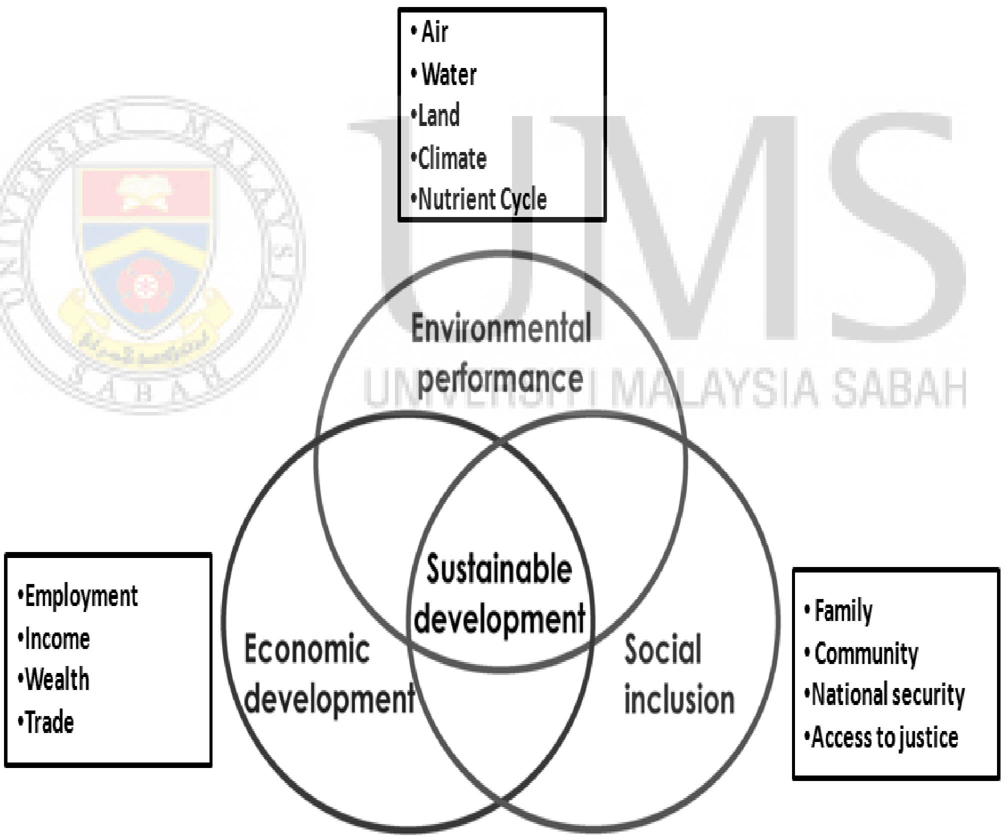

Figure 1.5: Benefits of biofuels.
Source: Demirbas, 2008

Global biofuel production has tripled from 4.8 billion gallons in 2000 to about 16.0 billion in 2007 with the US and Brazil contributing 75% of world production. Biofuels include bioethanol, biodiesel, biogas, bio-synthetic gas (bio-syngas), bio-oil, bio-char, Fischer-Tropsch liquids, and biohydrogen. Among these biodiesel is predominant and the biodiesel production is booming worldwide, with Europe accounting for the by far largest share of the global biodiesel production (Bacovsky *et al.*, 2007).

1.3 History of Biodiesel

A relatively common literature statement on the early use of vegetable oils as diesel fuels is that of Rudolf Diesel, the inventor of the engine that bears his name tested "his" engine on peanut oil at the 1900 World's Fair in Paris (Knothe *et al.,* 2005). He quotes,

"In any case, they make it certain
that motor-power can still be produced
from the heat of the sun,
which is always available for agricultural purposes,
even when all our natural stores
of solid and liquid fuels are exhausted."

—Diesel, 1900

Initially, Rudolf Diesel was interested in running his engine on either coal or vegetable-based fuels. But, Petroleum-based fuels became the main source due to lower cost over the past century (Caye *et al.*, 2008).

Vegetable oils were also used as emergency fuel during World War II. For example, Brazil prohibited the export of cottonseed oil in order to substitute it for imported diesel fuel. Reduced imports of liquid fuel were also reported in Argentina, necessitating the commercial exploitation of vegetable oils. China produced diesel fuel, lubricating oils from tung and other vegetable oils. However, the exigencies of the war caused hasty installation of cracking plants based on fragmentary data. Researchers in India, prompted by the events of World War II, extended their investigations on ten vegetable oils for development as a domestic fuel. Later, Work on vegetable oils as diesel fuel ceased in India when petroleum- based diesel