PRODUCTION OF BIODIESEL FROM WASTE COOKING OIL BY IMMOBILIZED LIPASE IN PVA-ALGINATE-SULFATE BEADS

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DEDICATION

Specially dedicated to my parents, Alhaji Ali Alhaji Deba and Hajiya Hafsat Mahdi, my relatives and all my well wishers whom aspired me to be a better person.

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Certainly all praise be to Allah, whom I praise and seek his forgiveness. I seek refuge with him from the evil of my soul and from the wickedness of my deeds. Surely, Whomever He has guided shall never go astray, and whomever He allows astray shall never be guided. I bear witness that there is no deity of being worshipped except Allah whom partnership shall never be associated with him. I bear witness Muhammad (S.A.W) is his Messenger and slave. May the peace and blessings of Allah be upon Him, his family and Companions and those that follow through his path till the last day.

My unalloyed appreciation goes to my supervisor Dr. Azimah Mohd Zain for her professional guidance and help throughout the research project. Her patience as well as her hospitality remained history worth to be remembered.

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ABSTRACT

With the huge varieties of oil produced worldwide, management and disposal are the major challenges facing the production. This is due to contamination of water bodies and land resources as a result of incessant disposal. Thus embarking on waste oil utilization in biodiesel production by immobilized enzymatic transesterification is a triple step forward towards the waste management. Therefore in these studies, *Candida rugosa* (Type VII, 1176 units/mg) was immobilized in PVA-Alginate-Sulphate beads by entrapment and cross linking method. Transesterification process was conducted using the immobilized lipase. 32 treatment combinations were generated by the Design Expert software version 6.0.4 using 2 level fractional factorial designs. The treatment combination; 1:6 oil to methanol, 2.5 v/v, 60 U at 50 °C under 200 rpm was found to be the optimum condition that gave the highest production of 4.516g/L, 1.65 g/L and 1.6 g/L, corresponding to 45 %, 10.65 %, 10.6 %, oleic acid methyl ester, palmitic acid methyl ester and linoleic acid methyl ester respectively. In another vain the treatment combination used for the optimum production using immobilized lipase was applied to the free lipase resulting to 0.234 g/L, 0.130 g/L, 0.049 g/L, corresponding to 2.34%, 1.30%, 0.49 %, oleic acid methyl ester, palmitic acid methyl ester and linoleic acid methyl ester respectively. Approximately, the immobilized lipase performed 15 folds better than the free lipase. Enzyme assay was conducted and compared between free lipase and immobilized lipase over 3 hours of reaction using spectrophotometer, absorbance was read at 410nm. Immobilized lipase in general maintained their activity after an hour of the reaction. In other higher concentrations, both the free and the immobilized showed a steep fall with time. FESEM-EDX was also carried out to study the immobilization matrix morphology which revealed the native structure of the entrapped *Candida rugosa* lipase. Furthermore, reusability test of the immobilized lipase was conducted and found capable of maintaining a production of five consecutive cycles

ABSTRAK

Penghasilan pelbagai jenis minyak diseluruh dunia mendatangkan cabaran utama bagi mencari penyelesaian terbaik untuk menguruskan sisa sampingan hasil daripada industri tersebut. Ini adalah disebabkan oleh pencemaran air dan alam sekitar akibat pelupusan secara berterusan Oleh itu, kaedah transesterifikasi dengan menggunakan enzim tersekat-gerak merupakan salah satu penyelesaian kepada rawatan sisa sampingan minyak. Dalam eksperimen ini, *Candida rugosa* (jenis VII, 1176 unit/mg) telah disekatgerak pada manik PVA-Alginat-Sulfat dengan menggunakan kaedah pengurungan dan hubung pangkah. Enzim lipase tersekatgerak telah digunakan dalam proses transesterifikasi. Sebanyak 32 kombinasi transesterifikasi telah dihasilkan dengan menggunakan reka bentuk factorial 2- level (2-level fractional factorial) oleh perisian *Design Expert* versi 6.0.4. Penghasilan biodiesel yang optima ialah 4.516g/L, 1.65 g/L dan 1.6 g/L merujuk kepada 45 % asid oleik metil ester, 10.65 % asid palmitik metil ester dan 10.6 % ester asid linoleik metil ester dengan keadaan nisbah minyak kepada metanol (1:6) , 2.5 v/v kandungan air, 60 U jumlah enzim,suhu 50 °C dan kelajuan 200 rpm. Manakala, penghasilan optima biodiesel bagi lipase bebas telah menghasilkan 0.234 g/L, 0.130 g/L dan 0.049 g/L, iaitu 2.34% asid oleik metil ester, 1.30% asid palmitik metil ester dan 0.49 % asid linoleik metil ester pada keadaan yang sama. Secara purata, lipase tersekatgerak menunjukkan prestasi penghasilan biodiesel 15 kali ganda lebih baik berbanding lipase bebas.Ujian enzim dilakukan bagi membandingkan aktiviti enzim antara lipase tersekatgerak dan lipase bebas dalam tindak balas selama tiga jam. Bacaan spektrofotometer ialah 410 nm. Aktiviti enzim lipase tersekatgerak pada 0.1-0.3 M menjadi stabil selepas sejam tindak balas berlaku. Dalam kepekatan yang lebih tinggi, aktiviti enzim bagi lipase bebas dan lipase tersekatgerak menunjukkan penurunan berkadaran dengan masa. FESEM-EDX juga digunakan untuk mengkaji morfologi sekatgerak matriks dimana ia menonjolkan struktur natif lipase *Candida rugosa* yang terperangkap.Selain itu, lipase sekatgerak telah dibuktikan dalam eksperimen ini boleh digunakan bagi penghasilan biodiesel sebanyak lima kitaran secara berterusan.

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GLOSSARY OF TERMS

ANOVA	Analysis of variance
ASTM	American society of testing materials
COD	Chemical oxygen demand
EDX	Energy dispersive x-ray spectrometer
FAME	Fatty acid methyl ester
FE-SEM	Field emission scanning electron microscope
FFA	Free fatty acids
KOH	Potassium hydroxide
MGs	Monoglycerides
PBR	Packed bed reactor
p-NP	p-nitrophenol
p-NPP	p-nitrophenol palmitate
PUFAs	Poly unsaturated fatty acids
PVA	Poly vinyl alcohol
RSM	Response surface methodology
TGs	Triglycerides
WCO	Waste cooking oil

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CHAPTER 1

INTRODUCTION

1.0 Background of the study

Vegetable oils have become a focus of attention as a potential renewable source for the production of an alternative source of fuel for petroleum based diesel. This is owing to continuous world-wide consumption of petroleum reserves in addition to environmental consequences attached to the emission of exhaust gases from diesel engines (Noureddini *et al*., 2001). Biodiesel is a renewable fuel that can be synthesized from edible, non-edible and waste oils. Vast varieties of starting materials come from edible oil, such as; soybean oil, rapeseed oil, cotton seed oil, sunflower oil, palm oil and restaurant kitchen wastes. In countries, such as India, such oils are not in abundance, choice of other starting materials like jatropha can be appropriate (Gayatri *et al*., 2010).

The Methyl and ethyl esters of fatty acids, also well-known as biodiesel, are nontoxic, non-polluting and biodegradable fuel, and therefore considered as a good substitute for fossil petroleum diesel. Viscosity, cetane number, energy content, and other fuel characteristics of biodiesel are also similar to those of petroleum-based diesel fuel (Mittelback and Tritthart, 1988). Furthermore, biodiesel is basically sulfur free and is considered ultra-sulfur fuel quality and the diesel engines fueled with the such fuel emit significantly fewer hydrocarbons, particulate, carbon monoxide and carbon dioxide than those operating on conventional fossil fuel diesel. NOx emissions, however, are slightly higher than those of diesel engines operating on conventional diesel fuel (Schumacher *et al.*, 1996 and Ali *et al.*, 1995).

Transesterification of vegetable oils and animal fat can be catalyzed by chemical and/or biological catalysts, such as acid, base and enzymes such as lipase. However, the biodiesel produced by chemical catalyst has several drawbacks such as difficulty in recovery of glycerol, removal of acid or base catalysts from the product and the treatment of wastewater (Freeman *et al*., 1984; Mittelbach, 1990; Basri *et al*., 1997; Fukuda *et al*., 2001). On the contrary, Enzymatic transesterification of triglycerides using immobilized lipase is a good substitute to chemical process owing to its; continuous operation, improved stability, reuse, chance of better control of reaction, high purity and product yields and hence more positive economic factors can be expected (Ruckenstein and Wang, 1993).

Therefore this study focuses on the production of biodiesel from waste cooking oil (WCO) by immobilized *Candida rugosa* lipase in PVA-alginate-sulfate beads. The immobilization would enhance the stability and re-usage of the enzyme while making the whole production process an eco friendly one.

1.3 Statement of the problem

Due to the fact that there are a lot of environmental problems associated with the use of fossil fuels, biodiesel can be expected as a replacement for conventional fuel. At present, biodiesel has been produced chemically using vegetable oil in USA and Europe (Mamoru *et al.*, 2001). However, requirement of removal of catalyst and extreme energy requirement are the key drawbacks of such chemical process (Mamoru *et al.,* 2001).

Because the enzymatic method may overcome the problems for the reaction, numerous researches have been carried out using lipase (Nelson *et al.,* 1996; Selmi *et al.*, 1998; Shimada *et al.*, 1999; Charuchinda *et al.,* 2011). However the production of biodiesel fuel by enzymatic means has not yet been adopted industrially due to the high cost of enzymes (Mamoru *et al.,* 2001), hence the need for additional researches using immobilized enzymes to come up with improved production process that is cost reduced.

1.1 Objectives of the research

- To immobilize lipase from *Candida rugosa* on PVA-alginate-sulfate beads.
- To study the optimum conditions of transesterification process with the immobilized lipase by using 2-Level fractional factorial design.
- To study the performance of free and immobilized lipase in the enhancement of biodiesel production.

1.2 Scope of the research

In this study, the type of lipase choosen is *Candida rugosa* (Type VII, 1176 units/mg). The lipase was made more stable by entrapment and cross linking in

PVA-alginate sulfate beads. With this development biodiesel is produced by enzymatic transesterification using waste cooking oil as substrate. The optimum condition of this production is only considered based on the parameters selected for this study, namely, oil to methanol molar ratio, temperature, agitation, water content and enzyme concentration. The activity of the lipase would be determined by the degradation of p-NNP. Figure 1.1 illustrates the summary of the scope.

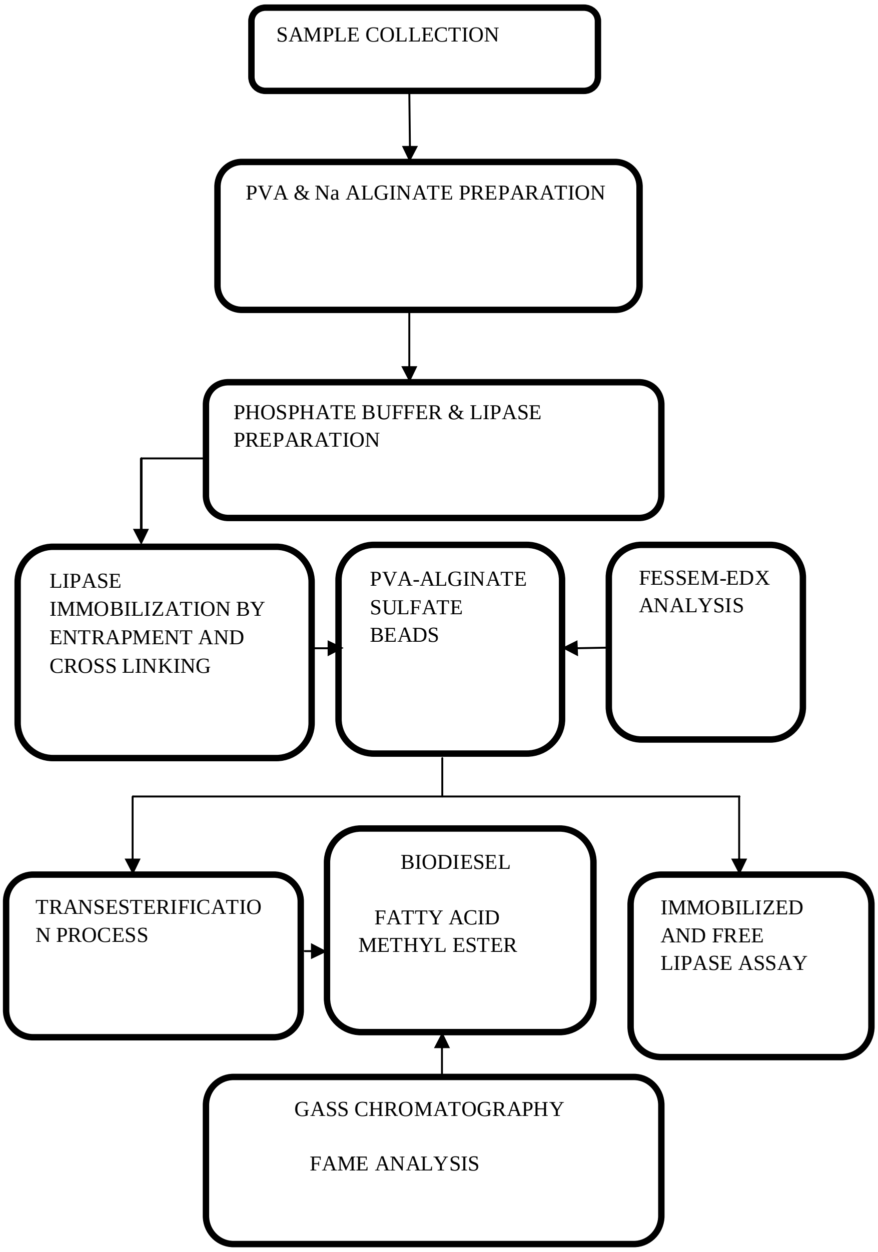

Figure 1.1 Scope of the research in summary

1.4 Research significance

From the invention of PVA-alginate-sulfate method to date no attempt had been made to transesterify waste cooking oil to produce biofuel. Therefore, this study emerges to establish the effectiveness of immobilized *Candida rugosa* lipase by this technique towards biodiesel production. Explored parameters from other researchers such as oil to methanol molar ratio, temperature, water content, agitation and enzyme concentration besides activity and reusability would also be studied.

The recycling process of waste cooking in order to produce biodiesel is of paramount importance considering the environmental concerned in the management of such oils as a result of pollution and contamination problems associated with its disposal (Arjun *et al*., 2008), therefore embarking on the use of the waste cooking oil would serve as a good step towards management of such oil and at the same time attaining biodiesel production.

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