OPTIMIZATION OF BIODIESEL PRODUCTION FROM POME USING LIPASE IMMOBILIZED IN PVA-ALGINATE-SULFATE BEADS

IDRIS ADAMU MATINJA

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Universiti Teknologi Malaysia

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This dissertation is dedicated to my late father Alhaji Idris Matinja Hardawa

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ABSTRACT

Palm oil mill effluent (POME) is an agro-industrial wastewater from palm oil processing. It is characterized by presence high organic compounds that cause serious air, soil and water pollutions. Both large and laboratory scale technologies are used to solve the problems that evolved from the discharge of POME. Previously, biodiesel was obtained from POME using immobilized *Candida rugosa* lipase in Polyvinyl alcohol (PVA) alginate-sulfate beads, which prior that study, was seen as a good substrate for biodiesel production due to its high amount of oil and grease. In this study, biodiesel production from POME using immobilized *Candida rugosa* lipase in PVA-alginate-sulfate beads was optimized using Box-Behnken design (BBD) of response surface methodology (RSM). Four parameters responsible for biodiesel production from POME were adopted from the previous study; these are methanol/POME ratio, reaction time, weight of the immobilized beads and agitation speed. The highest conversion (91%) and biodiesel yield of oleic methyl esters (65%) and palmitic methyl esters (45%) were obtained at the following optimum conditions; agitation speed (300rpm), POME/methanol ratio (1:6), incubation period (5hours) and weight of the immobilized beads weight (2g). Michaelis-Menten kinetic parameters of the immobilized and free lipase were determined through the hydrolysis of olive oil. Lineweaver-Burk plot indicated the corresponding values of K_m and V_{max} were 0.0506 g/ml and 21.09 U/ml for free lipase and 0.0686 g/ml and 15.95 U/ml for immobilized lipase. The important fuel properties of the biodiesel such as flash point, kinematic viscosity, water and sediment and copper strip corrosion were evaluated according to the American Society for Testing of Materials (ASTM D6751) and European Standard (EN 14214) and were found to be in good agreement with the standard quality and specification.

ABSTRAK

Sisa buangan kelapa sawit (POME) adalah air sisa industri pertanian yang terhasil daripada pemprosesan minyak sawit, dimana ianya dicirikan dengan kewujudan bahan organik yang tinggi yang boleh mengakibatkan pencemaran udara, tanah dan air yang serius. Kedua-dua teknologi berskala besar dan skala makmal telah digunakan bagi menyelesaikan masalah yang berpunca daripada POME. Sebelum ini, biodiesel yang terhasil daripada POME menggunakan *Candida rugosa* lipase yang telah disekat gerak ke dalam manik PVA-alginate-sulfat dimana ianya dilihat sebagai substrat yang baik bagi menghasilkan biodiesel kerana ianya mengandugi minyak dan gris yang banyak. Dalam kajian ini, penghasilan optimum biodiesel daripada POME menggunakan *Candida rugosa* lipase yang telah disekat gerak ke dalam manik PVA-alginate-sulfat menggunakan kaedah Box-Behnken (BBD) daripada metodologi permukaan balas (RSM). Empat parameter yang mempengaruhi penghasilan biodiesel daripada POME telah diterima pakai daripada kajian yang terdahulu iaitu nisbah metanol/POME, masa tindak balas, berat manik yang telah disekat gerak dan kelajuan putaran. Penukaran tertinggi (91.02%) dan biodiesel oleik metil ester (65.06%) dan palmitik metil ester (44.57%) telah diperolehi pada keadaan optimum yang berikut; kelajuan putaran (300rpm), nisbah POME / metanol (1:6), tempoh tindak balas (5 jam) dan berat manik sekat gerak (2g). Michaelis-Menten kinetik parameter lipase yang disekat gerak dan bebas ditentukan melalui hidrolisis minyak zaitun. Lineweaver-Burk plot menunjukkan nilai yang berkaitan daripada K_m dan V_{max} iaitu 0.0506 g/ml dan 21.09 U/ml untuk lipase yang bebas dan 0.0686 g/ml dan 15.95 U/ml untuk lipase disekat gerak. Sifat-sifat bahan api yang penting dalam biodiesel seperti titik kilat, kinematik kelikatan, air dan sedimen dan hakisan jalur tembaga didapati memenuhi kehendak piawaian Persatuan Amerika bagi Ujian Bahan (ASTM).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Adj	-	Adjusted
ANOVA	-	Analysis of Variance
APEC	-	Asia-Pacific Economic Cooperation
ASTM	-	American Society for Testing of Materials
AOCS	-	American Oil Chemist's Society
AV	-	Acid value
BBD	-	Box-Behnken Design
BOD	-	Biochemical Oxygen Design
C	-	*Candida*
CRD	-	Complete Randomized Design
CCRD	-	Central Composite Rotatory Design
COD	-	Chemical Oxygen Demand
DOE	-	Design of Experiment
EN	-	European Standard
ERIA	-	Economic Research Institute for ASEAN and East Asia
FAME	-	Fatty Acid Methyl Ester
FAEE	-	Fatty Acid Ethyl Ester
FFA	-	Free Fatty Acids
FESEM	-	Field Emission Scanning Electron Microscope
FID	-	Flame Ionization Detector
GC	-	Gas Chromatography
ISO	-	International Organization for Standardization
KOH	-	Potassium Hydroxide
K_m	-	Michaelis-Menten Constant
Max	-	Maximum
Min	-	Minimum

LIST OF ABBREVIATIONS (Continuation)

OAME	-	Oleic Acid Methyl Ester
PAME	-	Palmitic Acid Methyl Ester
POME	-	Palm Oil Mill Effluent
Pred	-	Predicted
PVA	-	Poly vinyl alcohol
RBD	-	Randomized Block Design
RSM	-	Response Surface Methodology
V_{max}	-	Maximum Velocity
wt.	-	Weight

LIST OF SYMBOLS

%	-	Percentage
µ	-	Micron
™	-	Trade Mark
∑	-	Summation
°C	-	Degree Celsius

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background of the Research

Palm oil mill effluent (POME) is an agro-industrial wastewater from palm oil processing, it has a very high suspended organic components such as nitrogenous compounds, simple sugars, free organic acids as well as lipids. Common sewage is hundred times less polluting than the POME, treatment of this organic pollutant fulfill the demand for sustainable palm oil production (Jeong *et al.*, 2014; Liew *et al.*, 2015). Several technologies for the treatment of POME have been reported, the system requires further exploration which gives a sustainable solution to the polluted water, by taking the waste and using it to produce value added products (Liew, *et al.*, 2015). There are around 3000-6000 mg/l of oil and grease in POME (Ahmad *et al.*, 2008; Ujang *et al.*, 2010), that shows similar characteristics to that of Jatropha oil and commercial palm oil that can be converted into biodiesel (Primandari *et al.*, 2013).

A number of enzymes and cells had been immobilized as biocatalyst in PVA and its derivatives. Some example include lactase, lipase (Bonine *et al.*, 2014; Dave and Madamwar, 2006), amylase (Renmei and Danian, 2009), invertase (Jusoh *et al.*, 2014) and *ochrobactrum* sp. DaVK1 cells (Sanjeev Kumar *et al.*, 2012), lipase play a key role and applied in biotechnology in wider spectrum (Pierozan *et al.*, 2011). The production of biodiesel from POME by incorporating lipases in carrier material like PVA-alginate-sulfate beads has been reported (Zulkifli, 2015).

Poly vinyl alcohol (PVA) is a non-toxic, high strength synthetic polymer, with the ability to stabilize and preserve protein activity that has been extensively applied in biotechnology for immobilization of enzymes as well as complete cell (Bonine, *et al.*, 2014; Zarei *et al.*, 2014). PVA-alginate-sulfate beads are stable microspheres of high quality, rubber like elasticity and strength both mechanically and chemically. The beads do not agglomerate in aqueous solution (Seker and Mohd Zain, 2014).

Lipase is an enzyme that catalyse the synthesis or esterification of free fatty acids esters (biodiesel) from useful or waste oil such as POME (Ranganathan *et al.*, 2008). In recent years the biodiesel production has gained importance because of its ability to replace fossil fuels by blending with conventional fossil diesel. The two-level factorial design in biodiesel production from POME using immobilized commercial lipase in PVA-alginate-sulfate matrix shows the effect of variables including methanol/POME mass ratio, temperature, agitation rate, reaction time, and beads weight (Zulkifli, 2015).

Despite the carrier material (beads) allow continuous process, controlled over the product formation, recovery, reusability and increase in their economic viability as compared with expensive free enzymes which are discarded after single use (Bonine, *et al.*, 2014; Cao *et al.*, 2003), the biodiesel yield from the POME using immobilized lipase in PVA-alginate-sulfate beads is low (Zulkifli, 2015). In this present study, parameters that are involve in the production of biodiesel from POME will be optimized using Response surface methodology (RSM).

1.2 Statement of Problem

One of the most important crop in Malaysia is the oil palm which its plantation reaches 5 million hectares in 2011 as compared to 400 hectares of land in 1920 (Tan *et al.*, 2009). According to Azmi and Yunos (2014), crude oil productions (CPO) have increases drastically up to 18.3 million tonnes in 2014 when compared to only 1.3 million tonnes in 1975. However, increase in capacity of the palm oil industries produce large amount of wastes. These wastes include oil palm fronds, oil palm shell, oil palm trunks, empty fruit bunches, fibres and POME (Azmi and Yunos, 2014).

The POME is a liquid effluent from the wet milling process performed in the palm oil mill industry in which its estimates discharge from the industry is around 55 million m^3 per annum (Ujang, *et al.*, 2010). Therefore, the disposal of this effluent is a critical issue to the environment. That is why Malaysian Department of Environment (DOE) obliged to satisfy the threshold values before being discharged into the watercourses.

Biodiesel as an important biofuel on the other hand had attracted intensive attention because they are renewable product with less CO_2 emmission that is capable of substituting the fossil diesel. The raw materials used in biodiesel production are vegetable oils, short chain alcohols and animal fats. Although there are variations in the type of oil used in biodiesel production across the world, the most widely used are rapeseed oil, soybean oil, sunflower oil and palm oil. However, despite it high free fatty acid contents, the production of biodiesel from the POME waste is less.

Based on the previous work by Zulkifli (2015), two-level factorial design was applied as a tool for screening the parameters that are involve in optimum biodiesel production from POME using immobilized commercial *Candida rugosa* lipase in PVA-alginate sulfate beads. Although there was a significant biodiesel yield of both oleic methyl ester and palmitic methyl ester, this approach is however time consuming as more experimental runs are required. Some of the optimal conditions might be missed in the process and the interactions between the variables selected are unpredictable. The cost of production in the industry would be higher due to the usage of the substances such as enzymes and methanol. Thus, box-behnken design (BBD) of experiments was further used to study the optimization of enzymatic transesterification of POME to biodiesel.

1.3 Objectives of the Research

The objectives are as follows:

1. To immobilize lipase in PVA-alginate-sulfate beads.
2. To optimize the variables that are involved in the efficiency of biodiesel production from the POME (methanol/POME ratio, agitation rate, reaction time and immobilized beads weight).
3. To characterize the biodiesel according to Economic Research Institute for ASEAN and East Asia (ERIA) biodiesel standards.

1.4 Scope of Research

In this optimization study, statistical tool RSM using Design Expert® software (State-Ease Inc. version 7.1.6) was employed in designing of experiments for the biodiesel production from POME. The factors that were optimized include; methanol/POME molar ratio, agitation rate, reaction time, and immobilized beads weight. Commercial lipase from *Candida rugosa* was used for the enzymatic transesterification and the biodiesel was analysed using volumetric and gas chromatography flame ionization detector (GC-FID). The physicochemical properties of the biodiesel were determined in accordance with Economic Research Institute for ASEAN and East Asia (ERIA) standards which applies both American Society for Testing of Material (ASTM D6751) standards and European (EN ISO) standards and specifications, the property and test method used include; flash point (ISO 3679), water and sediment (D2709), corrosiveness (ISO 2160), total glycerol (EN 14105) and kinematic viscosity (ISO 3104).

1.5 Significance of Research

This study would increase the production of biodiesel from POME as well as treating the pollution cause by the agro-industrial wastewater. Apart from this, the study may be able to reduce the usage of fossils fuel which is toxic to the environment as compared to the biodiesel which has less effect to the system.

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