

**SIMULTANEOUS SACCHARIFICATION AND
FERMENTATION PROCESS OF
BIOETHANOL FROM PALM
OIL EMPTY FRUIT
BUNCHES**

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**PERPUSTAKAAN
UNIVERSITI MALAYSIA SABAH**

**THESIS SUBMITTED IN FULFILLMENT FOR
THE DEGREE OF MASTER OF SCIENCE**

**FACULTY OF SCIENCE AND NATURAL RESOURCES
UNIVERSITI MALAYSIA SABAH**

2018



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BORANG PENGESAHAN STATUS THESIS

JUDUL : **SIMULTANEOUS SACCHARIFICATION AND FERMENTATION
PROCESS OF BIOETHANOL FROM PALM OIL EMPTY FRUIT
BUNCHES**

IJAZAH: **SARJANA SAINS (BIOTEKNOLOGI)**

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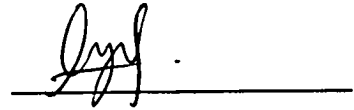


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ACKNOWLEDGEMENT

First of all, I am thankful to Almighty God Allah S.W.T for the countless blessing and infinite mercy, which enabled me to complete this research and dissertation successfully. I also would like to take this opportunity to express my profound appreciation and sincerest gratitude to the people who have generously contributed to my success as a graduate student. I will forever indebted to my supervisor Dr. Rahmath Abdulla for her invaluable guidance and supervision throughout my time at Universiti Malaysia Sabah as a postgraduate student. Her suggestions, ideas and availability during her busyness, helped me a lot towards the development of this thesis. I would like to take this opportunity to convey my sincere regards to my family and friends who were always there for me regardless of the situation and need. Besides, without their blessings this kind of work would not have been possible. My deepest thanks go out to my parents for their never ending encouragement and guidance.

I would also like to mention that I could not have done this without the help of my friends and labmate: Siti Azmah Jambo, Siti Hajar Mohd Azhar and Nor Amirah Syamsuddin. Thank you for always be there for me through thin and thick for this few years. I will always remember the hard time as well as the good time we spent together as it was all a precious memories that can only be experience one time in our life. Not to be forgotten, all my postgraduate friends from different programme and faculty in UMS (Siti Hafizah, Nur Farhana, Asiah).

I would like to express my sincere thanks to all the people which contributed directly or indirectly in this research. I will remain indebted to all the lecturers, lab assistants, UMS Staffs and colleagues for their kind cooperation. Last but not least, I would like to thank the Ministry of Education (KPM) for the MyBrain Scholarship as well as UMS Centre Postgraduate Studies (CPS) for their financial support through the Teaching Assistance Special Scheme (EKPP).

Eryati Binti Derman

9th July 2018



ABSTRACT

Currently, new renewable energy resources are seek out to substitute fossil fuels in the transportation sector in order to tackle the increasing energy demand. Bioethanol emerge as a potential option in replacing transportation fuels of gasoline. Oil palm empty fruit bunches (EFBs) are one of the promising biomass wastes, which can be utilized as a feedstock for the second generation bioethanol production. Optimal conditions are required for a cost-efficient bioethanol fuel processes from EFBs. Thus, this study aims to optimize the process conditions for bioethanol production from EFBs through simultaneous saccharification and fermentation (SSF) process using Response Surface Methodology (RSM). This study can be divided into two main parts which are the screening of the optimum concentration of enzymes and microorganisms and optimization of fermentation parameters. In this study, EFBs were treated using sequential acid and alkali treatment before being used as substrate. Physical morphologies and structures of the EFBs were analyzed using Scanning Electron Microscope (SEM) and Fourier Transform Infrared (FTIR). The findings revealed that the pretreatment has changed the morphology and EFBs structure by removing silica which act as the chemical composition barrier that causes pores formation. In the first part of this study, the optimum combination of enzymes and microbes for bioethanol production was screened. According to the results, co-cultures of *S. cerevisiae* and *T. harzianum* combined with Cellulase and β -glucosidase was selected for further used in the fermentation steps. This combination produced the highest bioethanol concentration determined at 11.76 mg/mL. Under optimal conditions for enzymatic saccharification, 4% (w/v) of pretreated EFB was completely hydrolyzed and produced 21.14 ± 1.49 mg/mL glucose at 50 °C, 150 rpm and 72 hours operating conditions. In the second part in this study, Central Composite Design of RSM was employed to optimize the SSF process including the fermentation time, temperature, inoculum concentration, and pH. It was found that fermentation for 72 hours duration, 30 °C and pH 4.8 of media using 6.79% (v/v) of inoculum concentration could produce up to 9.72 mg/mL of bioethanol and 0.46 g/g glucose of bioethanol yield with 90.63% conversion efficiency. Fermentation conducted under optimum conditions yielded 9.65 mg/mL of bioethanol, 0.46 g/g glucose of bioethanol yield and 89.56% conversion efficiency which were in close agreement with the model suggested. Overall, this study showed better results for bioethanol production as compared to previous research done using EFBs as the feedstocks.



ABSTRAK

SIMULTANEOUS SACCHARIFICATION AND FERMENTATION PROCESS OF BIOETHANOL FROM PALM OIL EMPTY FRUIT BUNCHES

*Pada masa ini, sumber tenaga baru yang boleh diperbaharui untuk menggantikan bahan api fosil dalam sektor pengangkutan dicari untuk menangani permintaan tenaga yang semakin meningkat. Bioetanol muncul sebagai pilihan yang berpotensi untuk menggantikan bahan api pengangkutan petrol. Tandan kosong kelapa sawit (EFBs) adalah salah satu daripada sisa biomas yang berpotensi untuk digunakan sebagai bahan mentah untuk pengeluaran bioetanol generasi kedua. Keadaan optimum diperlukan untuk menghasilkan bioethanol yang kos efektif. Oleh itu, matlamat kajian ini adalah untuk mengoptimumkan pengeluaran bioetanol dari EFBs melalui proses sakarifikasi dan penapaian serentak (SSF) menggunakan Metodologi permukaan tindakbalas (RSM). Kajian ini boleh dibahagikan kepada dua bahagian utama iaitu pemeriksaan optimum enzim dan mikroorganisma serta pengoptimuman parameter penapaian. Dalam kajian ini, EFBs dirawat dengan menggunakan Pra-rawatan alkali asid berturutan sebelum digunakan sebagai substrat. Morfologi dan struktur fizikal EFBs dianalisis menggunakan Mikroskop Pengimbasan Elektron (SEM) dan Inframerah Transformasi Fourier (FTIR). Dapatan kajian menunjukkan bahawa proses pra-rawatan telah mengubah morfologi dan struktur EFBs dengan membuang silika yang bertindak sebagai komposisi kimia yang menyebabkan pembentukan liang-liang. Pada bahagian pertama kajian ini, gabungan optimum enzim dan mikroorganisma bagi pengeluaran bioetanol telah ditapis. Menurut hasil proses penyaringan, kokultur *S. cerevisiae* dan *T. harzianum* digabungkan dengan enzim selulase dan β -glucosidase dipilih untuk digunakan lebih lanjut dalam penapaian proses. Kombinasi ini menghasilkan kepekatan bioetanol tertinggi iaitu 11.76 ± 0.79 g/ L bioethanol. Di bawah keadaan optimum untuk sakarifikasi enzimatik, 4% (w/v) EFB yang sudah dirawat, dihidrolisis sepenuhnya dan menghasilkan 21.14 ± 1.49 mg/mL glukosa pada 50 °C, 150 rpm dan 72 jam operasi. Dalam bahagian kedua kajian ini, CCD dalam kaedah RSM digunakan untuk pengoptimuman bagi SSF proses termasuk masa penapaian, suhu, kepekatan inokulum dan pH. Ia didapati bahawa penapaian selama 72 jam, 30 °C dan pH 4.8 media menggunakan 6.79% (v/v) kepekatan inokulum boleh menghasilkan 9.72 mg/mL bioetanol, 0.46 g/g glukosa hasil bioetanol dengan 90.63% kecekapan penukaran. Proses penapaian yang dilakukan menggunakan keadaan yang dioptimumkan berjaya menghasilkan 9.65 mg/mL bioetanol, 0.46 g/g glukosa hasil bioetanol dan 89.56% kecekapan penukaran bersesuaian dengan jumlah yang diramalkan oleh model CCD. Secara keseluruhannya, kajian ini telah menunjukkan hasil yang lebih baik dalam penghasilan bioetanol berbanding dengan kajian terdahulu menggunakan buah tandan kosong sebagai sumber utama.*



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LIST OF ABBREVIATIONS

ANOVA	-	Analysis of Variance
ATCC	-	American Type Culture Collection
C	-	Carbon
CO₂	-	Carbon Dioxide
CCD	-	Central Composite Design
C.V.	-	Coefficient of Variation
EFBs	-	Empty Fruit Bunches
FTIR	-	Fourier Transform Infrared Spectroscopy
GC-MS	-	Gas Chromatography Mass Spectrophotometer
HCl	-	Hydrochloric Acid
H₂SO₄	-	Sulphuric Acid
HPLC	-	High Performance Liquid Chromatography
PRESS	-	Predicted Residual Sum of Squares
POME	-	Palm Oil Mill Effluent
R²	-	Correlation coefficient
RPM	-	Rotation per Minute
RSM	-	Response Surface Methodology
SD	-	Standard Deviation
SEM	-	Scanning Electron Microscope
SSF	-	Simultaneous Saccharification and Fermentation



LIST OF SYMBOLS

μm	-	Micromolar
μL	-	Microlitre
%	-	Percentage
$^{\circ}\text{C}$	-	Degree celcius
g/g	-	Gram per gram
g/L	-	Gram per litre
g/mL	-	Gram per Millilitre
h	-	Hour
s	-	Second
min	-	Minute
U	-	Enzyme unit
mg/mL	-	Milligram per Millilitre
v/v	-	Volume per volume
w/v	-	Weight per volume



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CHAPTER 1

INTRODUCTION

1.1 Research Overview

More and more research are done to discover new renewable energy resources due to the increasing energy security, global energy demand, industrialized economy and continuous growth of world population (Gaurav *et al.*, 2017; Raman and Gnansounou, 2014). Hence, research on the biofuels production is done to overcome this matter since it can be used as a source of energy to meet the world's increasing energy demand. Biofuels had been one of the candidates among the renewable energy resources in replacing fossil fuels in the transportation sector (Raman and Gnansounou, 2014). However, its feedstock becomes an issue as it is mainly used human-food which causes worldwide dissatisfaction due to a shortage of food mainly in developing nations (Alam *et al.*, 2015). To tackle this food versus fuel conflict, the focus of biofuels research has changed towards manipulating agricultural waste and non-crop resource for example biomass wastes for production of bioethanol, and algae and non-edible oil crops for biodiesel (Raman and Gnansounou, 2014). Biodiesel and bioethanol are two most important liquid biofuels which emerged as a potential option in replacing transportation fuels of gasoline and diesel (Luque *et al.*, 2009).

Bioethanol is a renewable and sustainable liquid biofuels which can be produced locally to meet day to day high-energy sources demand (Sudiyani *et al.*, 2013). It is a promising renewable energy sources, but it requires a suitable technology in order for the conversion is economically feasible (Samsudin and Mat Don, 2015). Its production from cellulosic biomass is also rising as one of the essential technologies for the production of sustainable renewable fuels for



transportation (Sukumaran *et al.*, 2009). In recent years, extensive expansion and swift growth of bioethanol have produced a terrific amount of ethanol by-products from the fermentation process. Sustainable and renewable bioethanol will limit and reduce the usage of fossil fuels. Consequently, bioethanol will soon become fossil gasoline substitution or replacement as an alternative and renewable energy (Hanif *et al.*, 2016). Prior to that, developments of suitable bioprocesses are needed to convert biomass feedstocks into higher value and yield of bioethanol production. It is important to reduce its production cost by reducing the feedstock and operational cost (Zabed *et al.*, 2017).

Agricultural and forestry residues (lignocellulosic biomass), and algae are potential substrates in terms of availability and cost, however, it faces some processing problems as it produce lower yield ethanol with higher production costs (Izmirioglu and Demirci, 2017). Thus, a more valuable feedstock actually comes from a biomass waste as it does not involve in the “food versus fuel” conflict, cheaper and renewable which can benefit the biofuels industry. Bioconversion of the lignocellulosic waste materials to chemical and biofuel also attracts attention as they are renewable, low cost, and widespread in nature (Sudiyani *et al.*, 2013). In Malaysia, agricultural biomass wastes became very promising alternative resources for production of second generation bioethanol (SGB) since agricultural are one of the major industries contributed to the economy. Malaysian market for bioethanol is larger than the biodiesel market as larger proportion of the transportation vehicle runs on gasoline. Thus, promoting and implementing bioethanol production effectively is a tactical move for Malaysia to become a self-sufficient country in the near future (Tye *et al.*, 2011). Hence, Malaysia has the potential to play a major role in the world of biofuels and food market because of its large and growing palm oil industry, plus with a strong global demand for palm oil (Gan and Li, 2014).

Malaysia is one of the growing economies countries in South-East Asia which is important for its oil palm industry (Basri *et al.*, 2015). Oil palm industry is one of the important agricultural sectors in Malaysia. However, the industry contributed to a major biomass waste production, where empty fruit bunches are discarded after the oil extraction from the fruit bunches. Every million tonnes of oil

palm caused another million tonnes of EFBs to be thrown, as each tonne harvested from the palm plantation, 20 % will be the oil while the rest, 80 % became the biomass waste (Hassan *et al.*, 2013). Utilization of sustainable and renewable energy sources, mainly oil palm wastes, has been improved since it is able to lessen the agriculture disposal dilemma in an environmental friendly approach (Al-Zuhair *et al.*, 2011). Oil palm biomass is a promising renewable energy source due to rising price of crude oil. Therefore, converting oil palm biomass into biofuel is not only able to reduce the petrol crisis but also helps to protect the environment by reducing CO₂ and greenhouse gas emission (Shuit *et al.*, 2009). Hence, EFBs is a promising feedstock for bioconversion into bioethanol fuel because it is rich in lignocellulosic content, easily accessible and abundant in Malaysia. Bioethanol production from oil palm industrial wastes has gained attention not only because of the reduced production cost, but also the ethanol production productivity (Izmirliglu and Demirci, 2017).

Bioethanol production from lignocellulosic biomass requires four main steps: physical and chemical pretreatment of the lignocellulosic biomass, enzymatic hydrolysis of cellulose to sugars, fermentation of the resulting sugars and distillation of ethanol (Kamm and Kamm, 2004). Pretreatment processes are key technologies for generating fermentable sugars based on lignocellulosic biomass. It is necessary to remove the lignin and hemicellulose contents in EFBs (Kim *et al.*, 2012). In hydrolysis, enzymes that are mostly employed to degrade the polysaccharides are cellulases which can be categorized into three main types including β -glucosidase, endo-glucanases and exo-glucanases. Research by Nur Atikah *et al.* (2016) treated the EFBs with combination enzymes of Cellic Ctech2 and Cellic Htech2 in the enzymatic hydrolysis. Hydrolysis and fermentation process can be achieved by several process strategies which include separate hydrolysis and fermentation (SHF), and simultaneous saccharification and fermentation (SSF). According to Chen *et al.*, the SSF process has other benefits such as reduced operation costs, lower enzyme requirement and increased productivity (Chen *et al.*, 2007). A great number of microorganisms can be used for bioethanol production. *S. cerevisiae* (baker's yeast) is the most frequently and traditionally used organism in

fermentation (Dahnum *et al.*, 2015; Kim and Kim, 2013; Samsudin and Mat Don, 2015).

Application of microorganism in biofuels production is believed to be the key in solving problems of environmental pollution associated with fossil fuels (Tang *et al.*, 2015). Different microorganisms that are used in the fermentation process can also influence the yield of bioethanol production. Microorganism such as *Saccharomyces cerevisiae* is mainly used for bioethanol production. Kabbashi *et al.*, compared the compatibility of several fungal and yeast to develop direct solid-state bioconversion using the potential mixed culture to produce bioethanol (Kabbashi *et al.*, 2007). Thus, a combination of fungal and yeast such as *Trichoderma harzianum* and *Saccharomyces cerevisiae* can be used if higher ethanol yield is produced than using one strain of microorganisms (Kabbashi *et al.*, 2007). Study by Alam and Al-Khatib (2014) also produced bioethanol from EFBs by using co-culture of *Saccharomyces cerevisiae* and *Aspergillus niger*. Meanwhile, Ali *et al.* (2014) produced higher bioethanol by using co-culture of *Saccharomyces cerevisiae* and *Pichia stipitis*.

To our knowledge, there is no published report yet on the bioethanol production using SSF from empty fruit bunches (EFB) by a combination of two different microorganisms, *Saccharomyces cerevisiae*, and *Trichoderma harzianum*. The aim of this study is to optimize the production of ethanol by two different strains of microorganisms and enzymes during the fermentation process by employing the Response Surface Methodology (RSM). RSM was used in this study, to overcome the limitation of one-at-a-time-parameter optimization.

1.2 Problem Statement

Development of sustainable bioethanol has become a central solution to overcome the issue of fossil fuels demand. However, there are several problems needed to be tackled in order to meet the bioethanol demand. One of the major problems with the bioethanol production is the raw materials availability (Balat *et al.*, 2008). Thus, oil palm empty fruit bunches (EFBs) which is the biomass waste from the oil palm

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