SIMULTANEOUS SACCHARIFICATION AND FERMENTATION WITH DELAYED YEAST EXTRACT FEEDING AND IN-SITU RECOVERY FOR BIOBUTANOL PRODUCTION FROM OIL PALM EMPTY FRUIT BUNCH

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By

MUHAMMAD SIDDIQ BIN MOHAMED SALLEH

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

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Dedicated to my Wife, Mama, Ayah and family

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Abstract of a thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Master of Science

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November 2018

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Oil palm empty fruit bunch (OPEFB) is an abundant waste generated from palm oil mill processing after extraction of palm oil from fresh fruit bunch (FFB). OPEFB contributes about a quarter of the oil palm biomass generated equivalent to 23 million tonnes per year. This abundant and sustainably produced lignocellulosic biomass could be one of the potential biomass feedstocks for biofuel production such as biobutanol. Biobutanol is appealing to researchers as it has higher energy content and lower volatility as compared to bioethanol and biomethanol. However, utilising OPEFB as raw material for biobutanol production has several challenges including multiple processing steps, low biobutanol concentration and yield which lead to inefficient biobutanol production and recovery. In order to overcome these problems, several bioprocessing strategies were evaluated in this study. Three types of impeller have been used with and without baffle in order to obtain a good OPEFB homogeneity in the 2-L bioreactor during saccharification. It shows that, pitched turbine impeller without baffle shows better efficiency of homogeneity with 31.98 g/L reducing sugar. Simultaneous saccharification and fermentation (SSF) process was applied in order to reduce the processing steps by combining saccharification and fermentation simultaneously in a single operation in the same reactor. From this study. approximately 2.88 g/L of biobutanol produced from SSF as compared to 2.86 g/L of biobutanol produced from separate hydrolysis and fermentation (SHF). Although the biobutanol concentrations are almost similar, SSF shows better performance in term of process duration, reducing the apparatus and labour needed. However, the biobutanol concentration is still considered low, which is due to acid accumulation caused by slow acid reassimilation for solventogenic phase. Therefore, delayed yeast extract feeding (DYEF) was introduced in the SSF to reduce acids and enhance the biobutanol concentration. DYEF was conducted by introducing yeast extract after 39 h of SSF operation instead of adding the yeast extract at the beginning of the fermentation, resulted with an increase of 46% of biobutanol titre. The process was further enhanced up to 26% by implementing *in-situ* recovery using a gas stripping to reduce the solvents inhibition.

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The *in-situ* recovery using gas stripping had successfully recovered 20 g/L of biobutanol with 83% purity. In overall, this study had improved the biobutanol production with 72% increment (0.16 biobutanol yield, 0.056 g/L/h of productivity), by conducting SSF with DYEF and *in-situ* recovery.

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Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Sarjana Sains

SAKARIFIKASI DAN FERMENTASI SERENTAK DENGAN SUAPAN EKSTRAK YIS SECARA TERTANGGUH DAN PEROLEH SEMULA SECARA *IN-SITU* UNTUK PENGHASILAN BIOBUTANOL DARIPADA TANDAN KOSONG KELAPA SAWIT

Oleh

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Tandan kosong kelapa sawit (TKKS) adalah sisa buangan yang dihasilkan daripada pemprosesan kilang kelapa sawit selepas perolehan minyak kelapa sawit dari tandan buah segar (TBS). TKKS menyumbang kira-kira suku daripada keseluruhan biomas kelapa sawit yang dihasilkan bersamaan dengan 23 juta tan setahun. Biomas lignoselulosa ini dihasilkan dengan banyak dan secara berterusan yang boleh menjadi salah satu daripada bahan biomas yang berpotensi untuk penghasilan biofuel seperti biobutanol. Biobutanol memberi tarikan terhadap para penyelidik kerana ia mempunyai kandungan tenaga yang lebih tinggi dan kadar kemeruapan lebih rendah berbanding dengan bioethanol dan biometanol. Walau bagaimanapun, penggunaan TKKS sebagai bahan mentah untuk pengeluaran biobutanol berhadapan dengan beberapa cabaran termasuk langkah pemprosesan yang pelbagai, kepekatan biobutanol yang rendah yang menyebabkan penghasilan dan perolehan biobutanol yang tidak cekap. Untuk mengatasi masalah ini, beberapa strategi biopemprosesan telah dinilai di dalam kajian ini. Tiga jenis impeller yang dipasang dan tidak dipasang sesekat digunakan untuk mendapatkan kehomogenan OPEFB yang baik di dalam 2-L bioreactor semasa proses sakarifikasi. Ia menunjukkan,. Pic impeler turbin tanpa sesekat lebih efisien dalam kehomogenan dengan 31.98 g/L penegeluaran gula. Proses sakarifikasi dan fermentasi serentak (SFS) digunakan untuk mengurangkan langkah-langkah pemprosesan dengan menggabungkan proses sakarifikasi dan fermentasi di dalam satu operasi tunggal dan di dalam reaktor yang sama. Dari kajian ini. kira-kira 2.88 g/L biobutanol yang dihasilkan daripada SFS berbanding dengan 2.86 g/L biobutanol yang dihasilkan daripada sakarifikasi dan fermentasi berasingan (SFB). Walaupun kepekatan biobutanol hampir sama, SFS memperlihatkan prestasi yang lebih baik dari segi tempoh masa pemprosesan dan mengurangkan kos bahan, radas, serta buruh. Walau bagaimanapun, kepekatan biobutanol masih dianggap rendah disebabkan oleh pengumpulan asid akibat assimilasi semula asid yang perlahan untuk ke fasa penghasilan pelarut. Oleh itu, suapan ekstrak yis secara tertangguh (SEYT) diperkenalkan dalam SFS untuk mengurangkan asid dan meningkatkan kepekatan biobutanol. SEYT

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telah dijalankan dengan memasukkan ekstrak yis selepas 39 jam SFS dikendalikan dan bukannya menambahkan ekstrak yis pada awal fermentasi, menghasilkan peningkatan sebanyak 46% biobutanol titer. Proses ini dipertingkatkan sehingga 26% dengan menerapkan perolehan semula secara *in-situ* menggunakan pelucutan gas untuk mengurangkan perencatan pelarut. Perolehan semula secara *in-situ* yang menggunakan pelucutan gas telah berjaya memperolehi 20 g/L biobutanol, dengan 83% ketulenan. Keseluruhannya, kajian ini telah menambahbaik penghasilan biobutanol dengan peningkatan 72% (0.16 biobutanol yield, 0.056 g/L/h produktiviti) dengan menjalankan SFS bersama SEYT dan perolehan semula secara *in-situ*.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

ABE	Acetone Butanol Ethanol
Abs	Absorbance
ATCC	American Type Culture Collection
Conc.	Concentration
CPO	Crude Palm Oil
DCW	Dry Cell Weight
DNS	Dinitrosalicylic Acid
DYEF	Delayed Yeast Extract Feeding
OPEFB	Oil Palm Empty Fruit Bunch
FFB	Fresh Fruit Bunch
FPU	Filter Paper Unit
G	Gram
g/L	Gram per litre
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
M	Molar
H	Hour
Min	Minute
Rpm	Rotation per minute
SSF	Simultaneous saccharification and fermentation
SHF	Separate hydrolysis and fermentation
USD	US Dollar
Vol	Volume
w/v	weight/volume
w/w	weight/weight

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CHAPTER 1

INTRODUCTION

Human population shows an increasing trend and it is projected to increase to 8 billion in 2024 (United Nations, 2013). The increasing numbers of the human population had caused a high demand for energy especially for transportation and industrial activities. For more than two centuries after petroleum was found, now it supplies 90% of world energy (Chew and Bhatia, 2008). Unfortunately, the petroleum is non-renewable resource and utilization of petroleum had caused the negative consequences to the environment including direct impact to the global warming due to the release of uncontrollable greenhouse gases. Besides, due to its unrenewable resource, the world also faces the insecure energy source due to the depletion of fossil fuels reserves (Adams *et al.*, 2013). Therefore, the alternative energy is highlighted in this few decades, as the scientific community continuously reporting and exploring the possible alternative energy source to overcome this major problem.

Biobutanol is one of the promising alternative energy and can substitute both bioethanol and biodiesel sources with the estimated fuel market around $247 billion USD by 2020 (Green, 2011). As compared to other bioenergy, biobutanol has a lower vapour pressure, less volatile and explosive, less hygroscopic, easily mix with gasoline, and can be transported in the existing pipeline (García *et al.*, 2011). Apart from that, biobutanol can reduce hydrocarbon emissions by 95%; and oxides of nitrogen by 37% (Bellido *et al.*, 2014). Interestingly biobutanol can be produced through acetone-butanol-ethanol (ABE) fermentation which has been produced for several decades after world war II. Due to advance in petroleum technology few decades backs, biobutanol was not ready to be commercialized. Recently, researchers are focusing on ABE fermentation using lignocellulosic biomass as substrate. The lignocellulosic biomass is cheap (at current price rate is RM 50 per tonne of OPEFB), abundant and readily available that can be obtained from most agricultural and forestry industry. The utilisation of lignocellulosic biomass will be feasible in the future as several reported studies that used whey permeate (Setlhaku *et al.*, 2013), corn fiber (Guo *et al.*, 2013), wood pulp (Lu *et al.*, 2013), and other agriculture wastes shown promising aspects to produce biobutanol beside it benefit in reducing the pollution from the burning of wastes.

In Malaysia, oil palm empty fruit bunch (OPEFB) is one of the most abundant lignocellulosic biomass being produced with 6.61 million tonnes in 2016 (Noorshamsiana *et al.*, 2017). This value is estimated to be increased by up to 8 million tonnes in 2020 (National Innovation Agency of Malaysia, 2012). The OPEFB composed of 39% of cellulose, 21% of hemicellulose and 19% of lignin (Ibrahim *et al.*, 2012). It could be reached up to 84% of total potential sugars (cellulose + hemicellulose) after pretreatment (Ibrahim *et al.*, 2017). However, utilizing OPEFB as raw material for biobutanol production has several challenges including multiple processing steps, low biobutanol concentration and yield (Ibrahim *et al.*, 2015). In order to overcome these problems, several bioprocessing strategies of ABE fermentation were evaluated in this study.

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Simultaneous saccharification and fermentation (SSF) process can be applied to combine the process of saccharification and fermentation in a single reactor and at the same processing time, thus reducing the cost of materials, apparatus, time and labour and subsequently improve the whole productivity (Ibrahim *et al.*, 2015). In addition, biobutanol production through SSF is comparable to separate hydrolysis and fermentation (SHF) as reported by Ibrahim *et al.*, (2015). However, one of the major problems of conducting ABE fermentation through SSF is the production of acids which was produced double to the production of biobutanol due to slow phase conversion from acidogenesis to solventogenesis phase, and slow acid re-assimilation (Shah and Lee, 1994). It was reported that, manipulation the feeding of yeast extract during fermentation able to shorten phase conversion from acidogenesis to solventogenesis and enhances the acid re-assimilation towards butanol production (Li *et al.*, 2012). Therefore, introducing delayed yeast extract feeding (DYEF) is expected to enhance the biobutanol production in SSF process. In addition, the *in-situ* recovery using gas stripping was conducted to reduce the solvent toxicity in the fermentation and improve the biobutanol production. It was reported that the necessary to keep biobutanol concentration below the toxic level 2 g/L, thus biobutanol recovery is needed to overcome this drawback (Taya *et al.*, 1985). The research scope of this study is focus on improvement of biobutanol production through simultaneous saccharification and fermentation in 2-L bioreactor and employ several fermentation strategies.

Therefore, the objectives of this study were:

1. To investigate the effects of using different types of impeller in 2-L bioreactor on biomass homogeneity through simultaneous saccharification and fermentation for biobutanol production.
2. To enhance biobutanol production in simultaneous saccharification and fermentation by conducting delayed yeast extract feeding and *in-situ* recovery using gas stripping.

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