UNIVERSITI PUTRA MALAYSIA

OPTIMISATION OF SIMULTANEOUS SACCHARIFICATION AND FERMENTATION FOR BIOBUTANOL PRODUCTION USING OIL PALM EMPTY FRUIT BUNCH

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By

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Malaysia has a vast amount of tropical agricultural land that suitable for various agricultural activities. A lot of biomass generated from the agricultural activities such as rubber, paddy, fruit and oil palm biomass. Oil palm empty fruit bunch (OPEFB) has been recorded as one of the largest oil palm biomass (18–19 million tonnes/year) produced in palm oil processing mill. OPEFB has the potential as substrate for biobutanol production due to its abundance, cheap and high holocellulose content, thus provide renewability and environmentally friendly biobutanol compared to fossil fuels. Process for biobutanol production from OPEFB can be classified into two major processes: (i) separate saccharification and fermentation (SHF) and (ii) simultaneous saccharification and fermentation (SSF). SSF is a process where the enzymatic saccharification of OPEFB and ABE fermentation are carried out simultaneously in a flask. SSF process has recently gained attention and was proven to be more feasible than SHF, as it reduces the needs for additional equipments therefore lowering in capital, operational costs and time. The main disadvantage of the SSF process is the optimum temperature for fermenting Clostridia (37°C) does not coincide with the optimum temperature for cellulase (50°C). Thus, the objective of this study was to improve the biobutanol production through SSF using OPEFB. The optimisation study consisted of two main parts that employed; one factor at a time (OFAT) approach and using Central Composite Design (CCD) by Response Surface Methodology (RSM).

The SSF process successfully produced maximum biobutanol concentration of 1.74 g/L and biobutanol yield of 0.070 g/g at 96 h. The SSF biobutanol yield was comparable to the SHF biobutanol yield of 0.078 g/g. The SSF process was further enhanced by studying the preliminary investigation by OFAT approach. The results generated maximum biobutanol concentration of 2.91

g/L and biobutanol yield 0.12 g/g. The percentage of biobutanol increment was 40.21% and 1.71 fold. The biobutanol production was further statistically optimised using CCD. The analysis of variance (ANOVA) showed that the model was very significant (p<0.0010) for the biobutanol production. The optimum fermentation conditions obtained the highest biobutanol production were at temperature of 35°C, initial pH of 5.5, cellulase loading of 15 FPU/g of substrate and 5% (w/v) substrate concentration. From the validation study, the statistical optimisation resulted in a significant increment of biobutanol production of 3.97 g/L with biobutanol yield of 0.16 g/g with 55.95% increment (2.14 fold). The model and optimisation design obtained in this study helps to improve the biobutanol production in which was comparable to other studies of SSF processes using Clostridia species.

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

PENGOTIMUMAN PROSES PENSAKARIDAAN DAN FERMENTASI SERENTAK BAGI PENGHASILAN BIOBUTANOL MENGGUNAKAN TANDAN KOSONG KELAPA SAWIT

Oleh

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Malaysia mempunyai sejumlah besar tanah pertanian tropika yang sesuai untuk dijadikan pelbagai aktiviti pertanian. Biojisim yang dihasilkan daripada aktiviti pertanian ini amat banyak antaranya biojisim getah, padi, buah–buahan dan kelapa sawit. Tandan kosong kelapa sawit (TKKS) telah direkodkan sebagai salah satu daripada biojisim kelapa sawit yang terbesar (18-19 juta tan/tahun) dihasilkan di kilang pemprosesan minyak kelapa sawit. TKKS merupakan substrat berpotensi untuk penghasilan biobutanol kerana kuantitinya yang banyak, murah dan kandungan holoselulosa yang tinggi malah menghasilkan biobutanol bersifat lebih mesra alam berbanding dengan bahan api fosil. Proses penghasilan biobutanol dari TKKS boleh diklasifikasikan kepada dua proses utama: (i) pensakaridaan dan penapaian berasingan (PPB) dan (ii) pensakaridaan dan penapaian serentak (PPS). PPS menggabungkan proses pensakaridaan TKKS dan penapaian ABE serentak di dalam bekas yang sama. Proses PPS telah mendapat perhatian dan terbukti lebih baik berbanding daripada proses PPB. Ini kerana proses PPS ini dapat mengurangkan keperluan untuk peralatan tambahan serta bermanfaat dalam menurunkan modal, kos operasi dan masa. Kelemahan utama proses PPS ini ialah suhu optimum untuk penapaian Clostridia (37°C) tidak setara dengan suhu optimum pensakaridaan selulase (50°C). Oleh yang demikian, objektif utama kajian ini adalah untuk meningkatkan pengeluaran biobutanol menerusi proses PPS menggunakan TKKS. Kajian pengoptimuman proses PPS terdiri daripada dua bahagian utama iaitu melalui satu faktor satu masa (SFSM) dan rekaan komposit pusat (RKP) yang dijalankan melalui kaedah permukaan tindakbalas (KPT).

Proses PPS telah berjaya menghasilan pengeluaran maksimum biobutanol pada 96 jam dengan kepekatan 1.74 g/L hasil 0.07 g/g. Hasil biobutanol dalam

PPS adalah setara dengan hasil biobutanol PPB sebanyak 0.078 g/g. Proses PPS ini boleh dipertingkatkan dengan mengkaji pencirian awal yang dinilai oleh pendekatan SFSM. Maximum kepekatan biobutanol yang dihasilkan adalah 2.91 g/L dan 0.12 g/g hasil biobutanol. Peratusan kenaikan biobutanol adalah kira-kira 40.21% dan 1.71 kali ganda. Pengeluaran biobutanol boleh dilanjutkan pengoptimumannya menggunakan RKP. Menerusi analisis varians (ANOVA) model yang dihasilkan adalah sangat signifikan (p<0.0010) untuk pengeluaran biobutanol. Keadaan optimum yang diperolehi untuk pengeluaran biobutanol yang tertinggi adalah pada tahap suhu 35°C, pH awal 5.5, pemuatan selulase sebanyak 15 FPU/g substrat dan 5% (w/v) kepekatan substrat. Pengoptimuman statistik telah merekodkan kenaikan ketara pengeluaran biobutanol kepada 3.97 g/L dengan hasil 0.15 g/g dengan 55.95% kadar kenaikan (2.14 kali ganda). Reka bentuk model dan pengoptimuman yang diperolehi daripada kajian ini telah dapat membantu meningkatkan pengeluaran biobutanol setanding dengan kerja-kerja pengkajian PSS yang menggunakan spesis Clostridia yang lain.

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LIST OF ABBREVIATIONS

ABE	Acetone – butanol – ethanol
ANN	Artificial neural network
ATCC	American type culture collection
CICC	China center of industrial culture collection
CCD	Central composite design
CPO	Crude palm oil
DCW	Dry cell weight
FFB	Fresh fruit bunch
FPU	Filter paper unit
GA	Genetic algorithm
GHG	Greenhouse gas
k/Pa	Kilo per pascal
KJ/kg	Kilojoule per kilogram
LCB	Lignocellulosic biomass
MJ/L	Megajoule per litre
mM	milliMolar
MON	Motor octane number
NCIMB	National collection of industrial and marine bacteria, Aberdeen, UK
OFAT	One factor at a time
OPEFB	Oil palm empty fruit bunch
OPF	Oil palm frond
OPT	Oil palm trunk
POME	Palm oil mill effluent
PKO	Palm kernel oil
RON	Research octane number
RSM	Response surface methodology
SHF	Separate hydrolysis and fermentation
SSF	Simultaneous saccharification and fermentation
SO	Saccharification only
sp	Species
VOCs	Volatile organic compounds

CHAPTER 1

INTRODUCTION

1.1 Research Background

Gasoline derived from petrochemical route is mainly used for various mobilisations in the worldwide transport sector. However, in consideration of the probable declination of petrochemical reserves and environmental issues upon combustion of gasoline, renewable biofuels are among the accessible alternative that can partially or fully substitute the gasoline (Shao and Chen, 2015). Biofuels additional benefits are energy security, environmentally sustain as it derived from renewable substrates and mitigating climate change (Kumar and Gayen, 2012). Among other types of biofuels e.g., bioethanol, biodiesel, biohydrogen and biomethane, biobutanol possesses attractive characteristics almost similar to gasoline (Begum and Dahman, 2015). Biobutanol also has a good blending ability, which can be blend with gasoline at any ratio and used in the engine system without modification (Lee et al., 2008; Uyttebroek et al., 2013). In addition to having comparable octane number to gasoline, biobutanol also has higher energy density, lower Reid vapour pressure, lower water miscibility and is less hydroscopic than bioethanol. The demand on biobutanol is predicted to grow at a rate of 4.7% annually with the total consumption reaching about 2.9 million tonnes per year mainly in China, Europe and United States (Mascal, 2012). The biobutanol production is mainly impacted by substrates cost and improvement on the fermentation technology (Morone and Pandey, 2014).

Lignocellulosic biomass offers better selection as compared to sugar and starch substrates as it is cheaper, highly available and does not compete with food production or animal feed (Xue et al., 2013). Lignocellulosic biomass from agricultural biomass, forest residues, energy crops (switchgrass, yellow poplar, *Miscanthus* and etc.) and municipal solid wastes including food wastes, can become a potential substrate for biobutanol production (Salehi and Taherzadeh, 2015). In Malaysia, agricultural biomass is generated mainly from the palm oil industry with the current production reaching nearly 83 million tonnes and projected to increase to 85 – 110 million tonnes by 2020 (AIM, 2013). There are six types of oil palm biomass produced namely oil palm fronds (OPF), oil palm trunks (OPT), oil palm empty fruit bunch (OPEFB), kernel shells, mesocarp fibre and palm oil mill effluent (POME). Among these, OPEFB is the most abundant biomass produced at the palm oil mill with an annual production of 69,000 dry tonnes per year (Yoshizaki et al., 2013). The low cost of OPEFB is due to its unutilised capacity whereby the conventional management of OPEFB only involves dumping at the mill or mulching at the plantation (Ibrahim et al., 2013). OPEFB was also extensively studied as compost, composite materials and substrates for mushroom cultivation and fermentation (Cai et al., 2013; Sklavounos et al., 2013; Tan et al., 2010).

Harnessing OPEFB as substrate for biobutanol production has gained interest because of its high holocellulose content (Ibrahim et al., 2012; Sklavounos et al., 2013). The OPEFB high cellulose (54 – 59%) and hemicellulose (22 – 28%) content (Geng, 2013; Umikalsom et al., 1997) can be converted into fermentable sugars which comprise of hexoses and pentoses. Biobutanol producing *Clostridium* species are known to metabolised these hexoses and pentoses, therefore offer an economical strategies for biobutanol production (Jang et al., 2012).

A typical biobutanol production using OPEFB requires two major steps: (1) enzymatic saccharification of OPEFB for fermentable sugars production and (2) acetone-butanol-ethanol (ABE) fermentation for biobutanol production. These two steps are done separately and known as separate saccharification and fermentation (SHF) process. Studies have been carried out by combining those two major steps within a single reaction vessel known as simultaneous saccharification and fermentation (SSF) process (Sasaki et al., 2014). The SSF process can reduce the number of step involved in conversion of OPEFB to biobutanol as compared to SHF process (Ibrahim et al., 2015a). Besides, it can also decrease the inhibition of sugars on enzyme as the sugars released is simultaneously being consumed by the fermenting microorganisms (Su et al., 2015). The major challenges faced in SSF is the difference of optimum operating temperature for saccharification which is 40 – 50°C and ABE fermentation which occur at 30 – 37°C (Salehi and Taherzadeh, 2015). The cellulase in saccharification works optimally at higher temperature than fermenting bacteria that produce biobutanol. Besides temperature, pH and cellulase loading also are generally different (Oberoi et al., 2011b). Furthermore, the biobutanol production also required an optimum process control on various fermentation factors including initial pH, substrate concentration and inoculum concentration (Al-Shorgani et al., 2015). Due to the advantages and challenges of the biobutanol production through SSF process, further optimisation studies to improve the biobutanol production are valuable to pursue. Optimisation tools such as one factor at a time (OFAT) and response surface methodology (RSM) are among the widely implemented tools used for seeking optimum conditions for biobutanol production (Al-Shorgani et al., 2015; Razak et al., 2013).

1.2 Problem Statement

The SSF operating conditions were not study in depth, due to the difference in the optimum operating conditions of enzymatic saccharification and ABE fermentation if performed individually. Therefore, the SSF operating conditions needs to be optimised by investigating the factors such as temperature, initial pH, cellulase loading, substrate concentration and inoculum concentration towards biobutanol production.

1.3 Research Objectives

The overall objective of this study is to improve biobutanol production through simultaneous saccharification and fermentation (SSF) using oil palm empty fruit bunch (OPEFB). The specific objectives are:

1. To investigate the effect of environmental factors using one factor at a time (OFAT) approach on biobutanol production through simultaneous saccharification and fermentation (SSF) from OPEFB.

2. To optimise the biobutanol production through simultaneous saccharification and fermentation (SSF) from OPEFB using Central Composite Design (CCD) of Response Surface Methodology (RSM).

1.4 Scope of Study

This thesis is focuses on optimisation of simultaneous saccharification and fermentation for biobutanol production using oil palm empty fruit bunch. It is divided into five chapters. Chapter 1 contained a research background, problem statement, research objectives and scope of study. Chapter 2 reviewed on the literature concerning biobutanol which includes characteristics of biobutanol, microorganisms producing biobutanol, substrates used for biobutanol production and process involved with biobutanol production. Besides, factors affecting biobutanol production in SSF and optimisation of biobutanol production were also reviewed thoroughly. Chapter 3 described the materials used and methods of experimental conditions and equipment employed in this study. Results obtained in this study are presented in Chapter 4 which can be divided in five major sections: characteristics of OPEFB, biobutanol production from SSF, one factor at a time (OFAT) investigation, optimisation by Central Composite Design (CCD) and comparison studies. Chapter 5 includes the conclusion and recommendation of this study.

1.5 Limitation of Study

The limitation of this study occurred on the optimisation study as it can only be conducted in 100 mL laboratory scale operation only. This is because at 2 L bioreactor laboratory scale the operating systems are different especially the temperature control, sparging unit control, stirring/agitation unit and bigger fermentation volume. These factors application would vary in the amount of materials and the operation of the bioreactor system.

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