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BIOCONVERSION OF RICE STRAW INTO ACETONE-BUTANOL-ETHANOL BY Clostridium sporogenes A3

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By

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

January 2014

DEDICATED TO MY MOTHER, HUSBAND, SISTERS AND BROTHERS

Abstract of 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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January 2014

Chairman : Associate Professor Umi Kalsom Md Shah, PhD

Faculty : Biotechnology and Biomolecular Sciences

The inevitable depletion of the world's energy supply and unstable oil market have renewed the interest of society in searching for alternative fuels. In addition to depletion of petroleum fuels, environmental issues like greenhouse effect, global warming and climate change, are also the issues to be resolved worldwide. Biobutanol has been considered as a suitable alternative to be a source of fuel. Abundant biomass from various agriculture sectors could be a source for biobutanol production. Rice straw is one of the most abundant lignocellulosic biomass that has a great potential as a cheap and affordable substrate for the production of reducing sugars and biofuel such as biobutanol. The feasibility of rice straw as a source of sugar production was evaluated in this study. The effectiveness of alkaline pretreatment on rice straw was assessed taking into consideration the yield of reducing sugars and changes in the morphological and chemical composition of rice straw. Pretreatment of rice straw by 2% (w/v) NaOH and KOH with autoclaving at 121°C, 15 psi (10 min) could be promising pretreatment methods for sugar production. Alkaline pretreatments with 2% (w/v) KOH and NaOH followed by thermal pretreatment at 121°C, 15 psi (20 min) resulted in 58.5 to 64.5% higher conversion rate of reducing sugars production than untreated rice straw showing that alkaline pretreatments were effective even when a higher temperature was used. FTIR and SEM investigations showed that alkaline pretreatments caused chemical and morphological changes in the rice straw. The peaks of the cellulose and lignin materials were decreased after alkaline pretreatment, indicating that some cellulose and lignin were degraded. The reducing sugars obtained were then converted to acetone-butanol-ethanol (ABE) by *Clostridium sporogenes* A3. The total acetone-butanol-ethanol (ABE) production by locally isolated *C. sporogenes* A3 using rice straw hydrolysate was 1.58±0.11 g/L in which 0.73±0.05 g/L was butanol after 120 h of fermentation. Higher ABE yield was obtained from rice straw hydrolysate when compared to using commercial glucose as a carbon source. Increasing concentration of sugars in the rice straw hydrolysate to 40 g/L did not showed any improvements to the total ABE obtained. A higher level of ABE was obtained (1.72±0.39 g/L) at initial culture pH 5.5 (37ºC), in which 0.93±0.22 g/L was butanol. In comparison, higher yield of ABE was obtained when using *C. acetobutylicum* ATCC824. The ABE yield obtained was 0.33 which corresponds to 64% of ABE increment compared to fermentation using C. *sporogenes* A3. These results suggested that the reducing sugars obtained from pretreated rice straw could be used as a substrate for ABE fermentation by *C. sporogenes* A3 and *C. acetobutylicum* ATCC824. This will reduce carbon emission and our dependency on fossil fuel, and at the same time makes butanol as one of our future energy for many applications.

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

PENUKARAN JERAMI PADI KEPADA ASETON-BUTANOL-ETANOL OLEH *Clostridium sporogenes* A3

Oleh

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Pengurangan bekalan tenaga dunia yang tidak dapat dielakkan dan ketidakstabilan pasaran minyak telah mendorong masyarakat mencari bahan api alternatif. Di samping kekurangan bahan api petroleum, isu-isu alam sekitar seperti kesan rumah hijau, pemanasan global dan perubahan iklim, adalah isu-isu yang perlu diselesaikan di seluruh dunia. Biobutanol dianggap sebagai alternatif yang lebih sesuai untuk menjadi bahan api. Biomas yang terhasil daripada pelbagai sektor pertanian boleh menjadi sumber pengeluaran biobutanol. Jerami padi merupakan salah satu daripada biomas yang mengandungi banyak lignoselulosa dan mempunyai potensi yang besar sebagai substrat yang murah dan berpatutan untuk pengeluaran gula penurun dan biofuel seperti biobutanol. Kebolehgunaan jerami padi untuk pengeluaran gula telah dikenalpasti dalam kajian ini. Keberkesanan prarawatan beralkali pada jerami padi telah dinilai dengan mengambil kira hasil gula penurun dan perubahan dalam komposisi morfologi dan kimia dalam jerami padi. Prarawatan jerami padi dengan 2% (w/v) NaOH dan KOH beserta autoklaf pada suhu 121°C, 15 psi (10 min) boleh menjadi kaedah rawatan awal yang

boleh menghasilkan gula. Prarawatan jerami padi dengan alkali 2% (w/v) KOH dan NaOH diikuti oleh prarawatan haba pada suhu 121˚C, 15 psi (20 min) telah meningkatkan kadar penukaran gula penurun sebanyak 58.5 hingga 64.5% berbanding jerami padi yang tidak dirawat. Ini menunjukkan bahawa prarawatan alkali adalah berkesan walaupun suhu yang lebih tinggi telah digunakan. Kajian FTIR dan SEM menunjukkan bahawa prarawatan alkali menyebabkan perubahan kimia dan morfologi dalam jerami padi. Puncak selulosa dan lignin telah menurun selepas prarawatan alkali, menunjukkan bahawa beberapa selulosa dan lignin telah dihuraikan. Gula penurun yang diperolehi kemudiannya ditukarkan menjadi aseton-butanol-etanol (ABE) oleh *Clostridium sporogenes* A3. Jumlah penghasilan aseton-butanol-etanol jumlah (ABE) oleh *C. sporogenes* A3 menggunakan hidrolisat jerami padi adalah sebanyak 1.58 ± 0.11 g/L di mana 0.73 ± 0.05 g/L adalah butanol selepas 120 jam penapaian. Penghasilan ABE daripada hidrolisat jerami padi adalah lebih tinggi jika dibandingkan dengan menggunakan glukosa komersial sebagai sumber karbon. Peningkatan kepekatan gula dalam hidrolisat jerami padi hingga 40 g / L tidak menunjukkan apa-apa peningkatan kepada jumlah ABE yang diperoleh. Penghasilan ABE yang lebih tinggi telah diperolehi (1.72 ± 0.39 g/L) pada pH awal kultur 5.5 (37ºC), di mana 0.93 ± 0.22 g/L adalah butanol. Sebagai perbandingan, ABE yang diperoleh adalah lebih tinggi apabila menggunakan *C. acetobutylicum* ATCC824. Hasil ABE yang diperolehi ialah 0.33 bersamaan dengan kenaikan sebanyak 64% berbanding fermentasi menggunakan *C. sporogenes* A3. Kajian ini menunjukkan bahawa gula penurun yang diperoleh daripada jerami padi yang telah dirawat dapat digunakan sebagai substrat untuk penghasilan ABE oleh C. *sporogenes* A3 dan *C. acetobutylicum* ATCC824. Ini akan mengurangkan pelepasan karbon dan pergantungan kepada bahan api fosil, dan pada masa yang sama menjadikan butanol sebagai salah satu bahan api untuk pelbagai aplikasi pada masa depan.

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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
ADF	Acid detergent fiber
ADL	Acid detergent lignin
AFEX	Ammonia fiber explosion
ATCC	American Type Culture Collection
ATR	Attenuate total reflectance
bp	boiling point
$CaC1.2H_2O$	Calcium chloride dehydrate
CBM	Carbohydrate binding module
$CoC1_2$	Cobalt chloride
DNA	Deoxyribonucleic acid
DNS	Dinitrosalicyclic acid
FAO	Food and agriculture organization
FAOSTAT	The statistics division of food and agriculture organization
$FeSO_4$	Ferrous sulfate
$FeSO_4.7H_2O$	Ferrous sulfate heptahydrate
FID	Flame ionization detector
FPU	Filter paper unit
FPU/mL	Filter paper unit per milliliter
FTIR	Fourier Transform Infrared
g/g	Gram per gram substrate
g/L	Gram per liter
g/L/h	Gram per liter per hour
GNI	Gross national income
H_2SO_4	Sulphuric acid

HCl	Hydrochloric acid
HPLC	High performance liquid chromatography
IU	International unit
LHW	Liquid hot water
Kg/ha	Kilogram per hectare
KH_2PO_4	Potassium dihydrogen phosphate
K_2HPO_4	Potassium hydrogen phosphate
kV	kiloVolt
$MgC1_2$	Magnesium chloride
$MgSO_4$	Magnesium sulphate
$MgSO_4.7H_2O$	Magnesium sulphate heptahydrate
$MnSO_4.H_2O$	Magnesium sulphate hydrate
MOA	Ministry of agriculture
mp	melting point
NaOCl	Sodium hypochlorite
NaOH	Sodium hydroxide
NCBI	National center for biotechnology information
NDF	Neutral detergent fiber
OPEFB	Oil palm empty fruit bunch
PCR	Polymerase chain reaction
POME	Palm oil mill effluent
RCM	Reinforced clostridial medium
rRNA	Ribosomal ribonucleic acid
SEM	Scanning electron microscope
$T\ ha^{-1}$	Tons per hectare
TEM	Transmission electron microscope
TYA	Tryptone-yeast extract-acetate medium

v/v	volume per volume
UV	Ultraviolet
w/v	weight per volume
$ZnSO_4$	Zinc sulphate hydrate

CHAPTER 1

INTRODUCTION

1.1 Background

Rising prices of crude oil and continuous depletion of existing fossil fuel reserves, combined with concerns over global climate change, have created the need for alternative biofuels to replace fossil fuels. Among alternative fuels, biobutanol is considered as the most potential biofuels as it has many chemical and physical features that are particularly attractive for application as a liquid fuel. Various properties of biobutanol have proved its potential of being alternative fuel over ethanol such as giving higher energy, less sensitivity to temperature, less corrosivity, and the absence of any requires modification in combustion engines (Jang *et al.*, 2012; Lee *et al.*, 2008)

Like ethanol, butanol can be produced petrochemically or fermentatively. Biobutanol is produced by acetone-butanol-ethanol (ABE) fermentation using a variety type of bacteria, particularly clostridia. The most commonly used strains for ABE fermentation are *C. acetobutylicum* and *C. beijerinckii* (Badr *et al*., 2001; Qureshi *et al*., 2010). The process was known as ABE fermentation since acetone and ethanol were also produced during the fermentation. The main steps of the fermentation are acidogenesis and solventogenesis phase. ABE fermentation ranked second only to ethanol fermentation in the first part of the 20th century, but disappeared in the second part due to the rise of the petrochemical industry. With the depletion of fossil fuels ABE fermentation becomes interesting again.

Fermentation substrate is the most important factor influencing the cost of biobutanol production (Qureshi and Blaschek, 2000). In order to produce a relatively cheap biobutanol, use of more economical substrates must be identified and evaluated. In this case, lignocelluloses have been identified as the most suitable candidate to expand the biobutanol production capacity with low capital since it is the most abundant renewable resource on the planet and not fully exploited (Qureshi and Ezeji, 2008; Zhang and Shahbazi, 2011).

Malaysia has a lot of agricultural waste that has not been exploited for its usage as a substrate for fermentation. Rice straw is particularly attractive as feedstock for developing a cost-competitive cellulosic biobutanol conversion process since it is available in significant quantities from the rice processing industries. It was estimated about 1.3 million tonnes of rice straw is generated annually from the 684000 hectares of rice fields (MOA, 2004). Commonly, the practice of agricultural wastes are left in the field to rot or discarded through open burning. Since large amount of rice straw produced is disposed by burning which is not only causing environmental hazards but also can cause severe impacts on human health (Gadde *et al.*, 2009; Nori *et al.*, 2008), utilizing the rice straw as substrate for alternative biofuels can help in solving environmental problems associated with its disposal (Binod *et al*., 2010).

Bioconversion of rice straw into biobutanol is a multi-step process consisting of pretreatment, enzymatic hydrolysis, and fermentation (Mosier *et al*., 2005; Zhang and Cai, 2008; Lee *et al*., 2008). To initiate the production of biobutanol from cellulosic biomass, bioconversion of the cellulosic components into reducing sugars is necessary (Kumar *et al*., 2008). The key obstacle for reducing sugars production is the nature recalcitrant of the raw biomass and therefore pretreatment is particularly crucial to alter the cellulosic biomass by physical, thermal or chemical mean to improve the efficiency of enzymatic hydrolysis of carbohydrates to fermentable sugars (Chang and Holtzapple, 2000).

Despite being considered as a crucial step in the biological conversion to biofuels, biomass pretreatment is one of the main economic costs in the process. In fact, it has been described as the second largest expense in the conversion of lignocellulosic biofuels after the feedstock cost (Mosier *et al*., 2005).Therefore, developing a cost-effective and efficient biomass pretreatment technology is required to improve the production efficiencies and reduce the costs of lignocellulosic biofuels production (Yang and Wyman, 2008).

In order to reduce the cost of lignocellulosic biofuels, the present study was focused on the feasibility of rice straw as a substrate for bioconversion to biobutanol. Thus, the objectives of the study were:

i) To evaluate the effects of alkaline pretreatment on the lignocellulosic structure and chemical composition of rice straw in enhancement of reducing sugar production from rice straw.
ii) To screen, isolate and identify acetone-butanol-ethanol (ABE) producing strain.
iii) To optimize ABE production from rice straw hydrolysate by a locally isolated *Clostridium sporogenes* A3.

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