UNIVERSITI PUTRA MALAYSIA

BIOETHANOL PRODUCTION USING LIGNOCELLULOSE BIOMASS HYDROLYSATE FROM INDIGENOUS FUNGI FERMENTATION

AHMADU ALI FAROUQ

IB 2012 12

BIOETHANOL PRODUCTION USING LIGNOCELLULOSE BIOMASS HYDROLYSATE FROM INDIGENOUS FUNGI FERMENTATION

By

AHMADU ALI FAROUQ

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

May 2012

DEDICATION

This dissertation is dedicated to my parents:

Late Alhaji Ahmadu Gwandu and Late Hajiya A'ishatu Yalli Ahmadu Gwandu

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Doctor of Philosophy

BIOETHANOL PRODUCTION USING LIGNOCELLULOSE BIOMASS HYDROLYSATE FROM INDIGENOUS FUNGI FERMENTATION

By

AHMADU ALI FAROUQ

May 2012

Chairman: Professor Dzulkefly Kuang Abdullah, PhD

Faculty: Institute of Bioscience

The use of fossil fuel for energy purposes is becoming increasingly unreliable, skeptical and environmentally unfriendly while energy generated from foods such as corn and sugarcane is generating crisis of food prices inflation globally. Although, lignocellulose provides a better alternative source for bioethanol, pretreatment of the lignocellulose biomass for efficient enzymatic hydrolysis of the substrates is a major challenge. Pretreatment has been viewed as one of the most expensive processing steps within the conversion of biomass to fermentable sugar. The use of naturally sourced native fungi for the pretreatment of lignocellulose biomass materials is thus a viable alternative devoid of toxic chemicals. In this study, eight native fungi were isolated from Asian elephant (*Elephas maximus*) dung sourced locally from the natural environment. The isolated fungi were identified morphologically and through the use of molecular assay. All the fungi isolated were further screened for cellulolytic activities and candidate fungi with best cellulolytic activity, namely, *T. aureoviride* strain UPM 09 and *F. equiseti* strain UPM 09 were selected and used for pretreatment of lignocellulose biomass materials, namely, rice husk (RH), rubber

wood saw dust (RW) and oil palm empty fruit bunch (EFB). Prior to pretreatment, the effect of temperature (30°, 40° and 50°C) on the growth of the fungi was investigated based on optical density (600nm), dry weight and FPase activity. Solid state cultivation (SSC) and submerged cultivation (SMC) methods were used for pretreatment of biomass materials using individual fungi and their consortium.The effect of fungi on pretreatment of biomass materials was analyzed using X-Ray diffraction and scanning electron microscopy. Additionally, cellulase enzymes production was analyzed and the composition of lignocellulose biomass was determined before and after the pretreatment. Following pretreatment, the best pretreated biomass material was selected for bioethanol production using *Saccharomyces cerevisiae* D5A (ATCC 200062) by Simultaneous Saccharification and Fermentation (SSF). Ethanol production was analyzed using Gas Chromatography. The result of the present study shows that *T. aureoviride* strain UPM 09 and *F. equiseti* strain UPM 09 by submerged cultivation (agitated) (SMC) converted about 60-80% of the biomass substrates to glucose thus exhibiting pretreatment and saccharification of the lignocellulose biomaterials simultaneously. GC-MS analysis revealed the presence of volatile hydrocarbons as by-products in the samples of rubber wood saw dust and oil palm empty fruit bunch. *F. equiseti strain* UPM 09 and *T. aureoviride* strain UPM 09 produced the highest amount of glucose, reducing sugar with a correspondingly higher amount of enzymes FPase, CMCase, and beta-glucosidase and protein concentration during submerged cultivation (agitated). The results of statistical analysis of the pretreated biomass substrates showed that there was no significant difference ($P>0.05$) in pretreatment by *T. aureovide* UPM 09 and *Fusarium equiseti strain* UPM 09 either individually or in consortium using either SSC or SMC against RH, RW or EFB. The lignin and

hemicellulose reduction was 19.9% and 21.17%, 11.17% and 24.97%, and 31.93% and 21.77% for RH, RW and EFB, respectively. Therefore, any of the two fungi can be used for pretreatment purposes either individually or in consortium. Likewise, any of the pretreatment methods can be used. There was also no significant difference ($P>0.05$) in cellulase production between the two fungi (individually or in consortium) in either SSC or SMC in the three biomass materials used (RH, RW and EFB). In conclusion, the new native fungi can be used in the pretreatment of the lignocellulose biomass materials by submerged cultivation or solid state cultivation using native *T. aureoviride* strain UPM 09 (JN811061) and *F. equiseti* strain UPM 09 (JN811063) either individually or in consortium may offer good prospects in the bioethanol production industry by being cost-effective and environmentally friendly. This is because the two fungi each individually and in consortium exhibited a potential for achieving simultaneous pretreatment and hydrolysis of biomass thus shortening the steps in bioethanol production from three to two and at the same time the cost of commercial cellulase enzyme will be reduced. In addition, non-volatile hydrocarbon compounds were produced as by-products by the new native fungi during pretreatment using submerged cultivation. This study provides the first use of native fungi isolates of elephant dung for bioethanol production from lignocellulose biomass in Malaysia.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan ijazah Doktor Falsafah

PENGHASILAN BIOETANOL MENGGUNAKAN HIDROLISAT BIOJISIM LIGNOSELULOSA DARIPADA FERMENTASI KULAT ASLI

Oleh

AHMADU ALI FAROUQ

Mei 2012

Pengerusi: Professor Dzulkefly Kuang Abdullah, PhD

Fakulti: Institut Biosains

Pengunaan bahan fosil bagi penjanaan tenaga telah semakin meruncing dan tidak mesra alam. Di samping itu, tenaga yang dihasilkan daripada makanan tumbuhan seperti jagung dan tebu telah menimbulkan krisis inflasi harga makanan dunia. Walaupun lignoselulosa menawarkan alternatif yang lebih baik sebagai satu sumber bioetanol, prarawatan lignoselulosa diperlukan bagi mendapatkan hidrolisis enzim berkesan terhadap substrat tersebut merupakan satu halangan yang utama. Prarawatan telah dikenalpasti sebagai salah satu proses termahal di dalam proses penukaran bahan biojisim kepada gula fermentasi. Penggunaan kulat asli yang diperolehi secara semulajadi bagi prarawatan bahan biojisim lignoselulosa merupakan alternatif sesuai tanpa menggunakan bahan kimia yang memudaratkan. Di dalam penyelidikan ini, lapan kulat asli telah diasingkan daripada najis gajah Asia ((*Elephas maximus*) yang diperolehi secara tempatan di persekitaran semulajadi. Kulat yang diasingkan telah dikenalpasti secara morfologi dan juga menggunakan asai molekul. Kesemua kulat yang diasingkan telah kemudiannya disaring bagi mengenalpasti aktiviti selulolitik dan calon kulat yang mempunyai aktiviti selulolitik

terbaik, iaitu, *T. aureoviride* strain UPM 09 dan *F. equiseti* strain UPM 09 yang telah dipilih dan digunakan bagi tujuan prarawatan bahan biojisim lignoselulosa seperti sekam padi, habuk kayu getah dan buah tandan kosong kelapa sawit. Sebelum prarawatan, kesan suhu (30°, 40° dan 50°C) kepada pertumbuhan lignoselulosa telah dikaji berdasarkan ketumpatan optikal (600nm), berat kering dan aktiviti FPase. Kaedah-kaedah kultivasi keadaan pejal dan kultivasi terendam telah digunakan bagi prarawatan bahan biojisim bagi setiap lignoselulosa dan konsortiumnya. Kesan kulat terhadap prarawatan bahan biojisim telah dianalisa menggunakan pembelauan X-Ray dan mikroskop elektron pengimbas. Di samping itu, penghasilan enzim selulosa telah dianalisa dan komposisi bahan biojisim lignoselulosa telah ditentukan sebelum dan selepas prarawatan. Setelah prarawatan, bahan biojisim diprarawat terbaik telah dipilih bagi penghasilan bioetanol menggunakan *Saccharomyces cerevisiae* D5A (ATCC 200062) melalui Saccharification Serentak dan Fermentasi. Penghasilan bioetanol telah dianalisa menggunakan Gas Kromatografi. Hasil penyelidikan ini menunjukkan bahawa *T. aureoviride strain* UPM 09 dan *F. equiseti* strain UPM 09 melalui kultivasi terendam (diagitasi) menukarkan 60-80% substrat biojisim kepada glukosa seterusnya mempamerkan prarawatan dan *saccharification* bahan biojisim lignoselulosa secara serentak. Analisis GC-MS menunjukkan kehadiran hidrokarbon volatile sebagai produk sampingan di dalam sampel habuk kayu getah dan tandan sawit kosong kelapa sawit. *F. equiseti* strain UPM 09 dan *T. aureoviride* strain UPM 09 menghasilkan jumlah glukosa tertinggi dengan reducing sugar mengandungi jumlah enzim FPase, CMCase, dan beta-glucosidase serta kepekatan protein yang lebih tinggi semasa kultivasi terendam (diagitasi). Hasil analisis statistik terhadap substrat biojisim diprarawat menunjukkan bahawa tiada perubahan ketara ($P>0.05$) di dalam prarawatan oleh *T. aureoviride* strain UPM 09 dan *Fusarium equiseti* strain

UPM 09 sama ada secara inidividu atau konsortium menggunakan sama ada kultivasi keadaan pejal atau kultivasi terendam terhadap sekam padi, habuk kayu getah dan buah tandan kosong kelapa sawit. Pengurangan lignin dan hemiselulosa adalah 19.9% dan 21.17%, 11.17% dan 24.97%, serta 31.93% dan 21.77% bagi sekam padi, habuk kayu getah dan buah tandan kosong kelapa sawit, masing-masing. Justeru itu, mana-mana dari dua kulat boleh digunakan bagi tujuan prarawatan sama ada secara individu atau konsortium. Sehubungan dengan itu, mana-mana kaedah prarawatan boleh digunapakai. Tiada sebarang perubahan ketara ($P>0.05$) di dalam penghasilan selulosa oleh kedua-dua kulat (secara individu atau konsortium) sama ada melalui kultivasi keadaan pejal atau kultivasi terendam terhadap ketiga-tiga bahan biojisim yang digunakan. Secara kesimpulan, kulat asli baru boleh digunapakai bagi tujuan prarawatan bahan biojisim lignoselulosa melalui kultivasi keadaan pejal atau kultivasi terendam menggunakan *T. aureoviride* strain UPM 09 (JN811061) dan *F. equiseti* strain UPM 09 (JN811063) asli secara individu atau konsortium seterusnya mampu menawarkan prospek baik kepada industri penghasilan bioetanol secara lebih ekonomi dan mesra alam. Ini disebabkan kedua-dua kulat masing-masing secara individu dan konsortium mempamerkan potensi meningkatkan prarawatan dan hidrolisis biojisim serentak, justeru memendekkan langkah penghasilan bioetanol dari tiga ke dua, dan sekaligus menjimatkan masa serta kos enzim selulosa komersial. Tambahan pula, sebatian hidrokarbon tidak volatile telah dihasilkan sebagai produk sampingan oleh kulat asli baru semasa prarawatan melalui kaedah kultivasi terendam. Penyelidikan ini menyumbang kegunaan pertama kulat asli yang diasingkan dari najis gajah bagi tujuan penghasilan bioetanol daripada biojisim lignoselulosa di Malaysia.

ACKNOWLEDGEMENTS

All thanks are due to the Almighty Allah in His mercy for making this journey possible. I wish to express my sincere gratitude and appreciation to my supervisor Prof Dr. Dzulkefly Kuang Abdullah for his guidance, patience and invaluable contribution throughout the course of this work. This gratitude and appreciation is extended to my co-supervisors Prof. Madya Dr. Foo Hooi Ling and Prof. Madya Dr. Norhafizah Abdullah for their supervision and contributions in this work.

I am very grateful to Alhassan M. Alhassan for the academic discourse, cooperation, assistance, friendship and support i enjoyed from him in the course of this study. I wish to thank Mr. Mirsasan Mirpour and Miss Forough K. Nazarpour, with whom we worked together in the Laboratory of Industrial Biotechnology at the Institute of Bioscience (IBS), for their cooperation. I am also especially grateful to Pn. Zainura Mat Hussein, Encik Ahmad Adlie Shamsuri and other members of staff of our laboratory and other laboratories in IBS and other faculties: Electron Microscopy Unit and Laboratory of Marine Science and Biotechnology especially Mr. Perumal Kuppan for the cooperation and support I enjoyed from them in the course my experiments at the Institute of Bioscience.

I am very grateful to Abdoulie Ceesay of IBS for his cooperation, assistance, friendship, support and academic discourse I enjoyed from him in the course of this study. I am also very grateful to Dr. Faruk Bande for formatting the thesis.To many others, whose names are too numerous to mention and to avoid the risk of making some important omissions here, I am very grateful. Finally, I am most grateful to my

brother Usman Ahmed for financial, spiritual and moral support; and to my family, particularly my wife Mrs. Fatima Yusuf, my children Hauwa'u Maijidda Farouq, Halimat Sadiya Farouq and Ahmad Aslam Farouq for their understanding, patience and moral support.

I certify that a Thesis Examination Committee has met on 23rd May,2012 to conduct the final examination of Ahmadu Ali Farouq on his thesisentitled "Bioethanol Production Using Lignocellulosic Biomass Hydrolysate from Indigenous Fungi Fermentation '' in accordance with the Universities and University colleges Act 1971 and Constitution of Universiti Putra Malaysia [P.U.(A)106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Y. Bhg. Prof. Dr. Gwendoline Ee Cheng Lian
Chemistry Department
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Assoc. Prof. Dr. Rosfarizan Binti Mohamad
Department of Bioprocess Technology
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Internal Examiner)

Assoc. Prof. Dr. Muskhazli b Mustapha
B iology Department
Faculty of Science
Universiti Putra Malaysia
(Internal Examiner)

Assoc. Prof. Dr. Ratna R. Sharma-Shivappa
Biological and Agricultural Engineering D.S Weavers labs,
North Carolina State University, Campus box 7625,
North Carolina, USA.
(External Examiner)

SEOW HENG FONG, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

.

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Dzulkefly Kuang Abdullah, PhD
Professor
Faculty of Science
Universiti Putra Malaysia
(Chairman)

Foo Hooi-Ling, PhD
Associate Professor
Faculty of Biotechnology and Biomolecular Sciences
Universiti Putra Malaysia
(Member)

Norhafizah Bt. Abdullah, PhD
Associate Professor
Faculty of Engineering
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

_____

AHMADU ALI FAROUQ

Date:

TABLE OF CONTENTS