UNIVERSITI PUTRA MALAYSIA

CONVERSION OF LIGNOCELLULOSIC MATERIALS FROM LOCAL GRASS TO BIOETHANOL

LIONG YAN YEE

FH 2012 13

CONVERSION OF LIGNOCELLULOSIC MATERIALS FROM LOCAL GRASS TO BIOETHANOL

By

LIONG YAN YEE

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

April 2012

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

CONVERSION OF LIGNOCELLULOSIC MATERIAL FROM LOCAL GRASSES TO BIOETHANOL

By

LIONG YAN YEE

April 2012

Chair: Rozi Mohamed, PhD

Faculty: Faculty of Forestry

Grass is a candidate biomass producer because it is fibrous and it thrives growing on poor soil. The chemical properties of two grass species growing naturally and abundantly in idle lands in Malaysia were investigated. Grass selected were 'Lalang' (*Imperata cylindrica*) and Napier grass (*Pennisetum purpureum*). For the analysis, Napier grass was further divided into male and female plants, and stem and leaves. Lignin, hemicellulose and cellulose contents were determined using the TAPPI standard methods. 'Lalang' appeared not to be an attractive biomass producer because of its high lignin content (22%). On the contrary, Napier grass, particularly the female stem had low lignin content (13%) and a favorably high level of cellulose (46%). In female leaf, lignin content was higher (20.7%) while cellulose (30.4%) was lower, when compared to the stem. Although cellulose content in the male stem (51%) was slightly higher than the female, its lignin was two-fold above that of the female stem, making it a less desirable biomass producer. With the results obtained it was concluded that female Napier grass in Malaysia has a good potential of becoming a biomass producer.

Female Napier grass, the best grass candidate was selected to undergo two different pretreatments, alkali pretreatment and biological pretreatment. Alkali pretreatment was carried out at four different NaOH concentrations: 1%, 5%, 7% and 10%. Alkali pretreated materials were subjected to *Trichoderma reesei* ATCC 26921 enzyme hydrolysis; several hydrolysis parameters were tested to optimize glucose yield including temperature and agitation, by applying the Response Surface Method (RSM). HPLC revealed that samples pretreated with 5% NaOH had glucose content of 7.47g/L and 7 % NaOH yielded glucose content of 7.4g/L. There was no difference between 5% and 7% NaOH pretreated material. However between the two, 7 % NaOH can be considered as a better pretreatment because the glucose yield was consistent throughout the parameters of temperature and agitation; these two parameters can affect the optimum activity of cellulase in converting cellulose to glucose. From RSM analysis, glucose yield was optimal at 38.5˚C and 175rpm. When using the white-rot fungus, *Phanerochate chrysosporium* as biological pretreatment, followed by *Trichoderma reesei* ATCC 2692 enzyme hydrolysis at constant enzyme loading of 1ml with 1g of material, samples pretreated for three weeks gave the highest glucose yield (4.5g/L). However, the yield was lower than the alkali pretreated grass. On looking at the efficiency of these two pretreatments, alkali pretreatment was a better pretreatment as it yielded higher glucose content compared to biological pretreatment, despite the ease of handling and time consuming of the experiment conducted.

Hydrolysates from the pretreatments were fermented using the ethanol insensitive strain *Escherichia coli* K011 at 35˚C and 100rpm, and the ethanol content was detected by Gas Chromatography (GC). After 24 hours of fermentation, alkali pretreated material yielded 37.7% ethanol while biological pretreated material yielded 24.4% ethanol. It was observed that alkali pretreated grass material gave out higher ethanol yield. These results indicated that

Malaysia's female Napier grass is capable of becoming an important biomass for producing bioethanol.

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

PENUKARAN BAHAN LIGNOSELULOSA DI DALAM RUMPUT TEMPATAN KEPADA BIOETANOL

Oleh

LIONG YAN YEE

April 2012

Pengerusi: Rozi Mohamed, PhD

Fakulti: Fakulti Perhutanan

Rumput merupakan salah satu tumbuhan biomass yang berpotensi bukan sahaja kerana rumput ialah tumbuhan berurat, tetapi juga kerana ia dapat hidup di atas tanah yang tidak subur. Dalam kajian ini, penyelidikan telah dijalankan ke atas komponen kimia yang terdapat di dalam rumput *Imperata cylindrica* (Lalang) dan *Pennisetum purpureum* (rumput 'Napier'). Kedua-dua jantina rumput 'Napier' telah dikutip dan dibahagikan kepada batang dan daun untuk kegunaan analisis. Selulosa, holoselulosa dan lignin ditentukan dengan kaedah standard TAPPI. Rumput 'Napier' terutamanya bahagian batang rumput betina mempunyai 46% selulosa dan 13% lignin, manakala daunnya mempunyai 30.4% selulosa dan 20.7% lignin. Walaupun selulosa dalam batang rumput Napier jantan (51%) melebihi sedikit daripada batang betina, lignin batang Napier jantan mencatatkan lignin sebanyak dua kali ganda daripada betina. Lignin yang tinggi menyebabkan rumput 'Napier' jantan kurang baik berbanding dengan betina. Lalang kurang dianggap sebagai sumber bahan mentah untuk tenaga alternatif oleh sebab kandungan lignin yang lebih tinggi (22%) daripada rumput 'Napier'. Oleh sebab itu rumput Malaysia, terutamanya rumput 'Napier' betina, mempunyai potensi yang tinggi untuk dijadikan sumber bahan mentah tenaga alternatif.

Dua jenis rawatan telah dijalankan ke atas rumput ‘ Napier’ betina ialah rawatan alkali dan rawatan fungi. Rawatan alkali dilaksanakan dengan empat jenis kepekatan NaOH, iaitu 1%, 5%, 7% dan 10%. Setelah dirawat, bahan rumput diteruskan dengan experimen hydrolisis dengan menggunakan enzim *Trichoderma reesei* ATCC 26921. Kaedah ‘Response Surface Method’ (RSM) diguna untuk mendapatkan kombinasi parameter suhu dan putaran/minit (rpm) untuk mendapatkan hasil glukosa yang terbaik. Cecair hydrolysis dianalysis dengan HPLC dan bahan yang telah dirawat dengan 5% NaOH mngadungi kandungan glukos sebanyak 7.47g/L dan 7% NaOH pula mengandungi kandungan glukos sebanyak 7.4g/L. Antara kedua-dua rawatan, 7% NaOH boleh dianggap sebagai rawatan yang lebih baik kerana ia mencatatkan keputusan yang selaras. Melalui analisis RSM, kesimpulannya kombinasi yang optimum bagi enzim *Trichoderma reesei* ATCC 2692 ialah 38.5˚C dan 175 rpm. Sebaliknya, bahan yang dirawat dengan fungi *Phanerochate chrysosporium* (fungi reput putih) mencatatkan kandungan glukosa yang paling tinggi (4.5g/L) pada minggu ketiga rawatan tersebut. Keputusan glukosa rawatan biologi adalah lebih baik daripada rawatan kimia.

Hydrolysate kedua-dua rawatan akan ditapai pada 35˚C, 100 rpm dengan mengggunakan bacteria jenis tidak peka terhadap etanol, *Escherichia coli* K011. Kandungan etanol dalam proses penapaian dikesan dengan Gas Kromatografi. Pada jam ke 24, bahan rawatan alkali menghasilkan 37.7% ethanol manakala bahan rawatan biologi menghasilkan 24.4% etanol. Dengan berdasarkan keputusan, bahan rawatan alkali didapati mengadungi kandungan etanol yang lebih tinggi. Kesimpulannya rumput Malaysia mempunyai keupayaan untuk dijadikan salah satu biomas yang penting dan menghasilkan bioethanol.

ACKNOWLEDGEMENT

I would like to express my thankfulness to those who have taught me well in my academic research and have gone through some of the life experience together.

I would like to thank especially to my parents who have been supporting me through the good and bad, and also for friends who always stand by me and help me through the hard times.

Lastly, I would like to express my gratitude to all of my supervisors and other lecturers who have helped me in completing the research and thesis.

I certify that a Thesis Examination Committee has met on (the date of viva voce) to conduct the final examination of Liong Yan Yee on her thesis entitled "Conversion of Lignocellulosic Material from Local Grass to Bioethanol" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master Degree.

Members of the Thesis Examination Committee were as follows:

MohamadAzani bin Alias, PhD
Prof. Madya Dr.
Faculty of Forestry
Universiti Putra Malaysia

Zaidon b Ashaari. PhD
Prof. Madya Dr.
Faculty of Forestry
Universiti Putra Malaysia

Wan ZuhainisbintiSaad, PhD
Dr.
Faculty of Biotechnology and BiomolecularScience
Universiti Putra Malaysia

Lee KeatTeong, PhD
Prof. Madya Dr.
Chemical Engineering Research Centre
Universiti Sains Malaysia
Engineering Campus
Malaysia

_____

ZULKARNAIN ZAINAL,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 the degree of Master. The members of the Supervisory Committee were as follows:

Rozi Mohamed, PhD
Senior Lecturer
Faculty of Forestry
Universiti Putra Malaysia
(Chairman)

Lai Oi Ming, PhD
Professor
Faculty of Biotechnology
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.

_____

LIONG YAN YEE

Date: 30.4.2012

TABLE OF CONTENTS