UNIVERSITI PUTRA MALAYSIA

IMPROVEMENT OF RICE STRAW HYDROLYSATE PREPARATION FOR BIOETHANOL PRODUCTION USING Saccharomyces cerevisiae ATCC 24860

AHMAD MUHAIMIN BIN ROSLAN

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

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia
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IMPROVEMENT OF RICE STRAW HYDROLYSATE PREPARATION FOR BIOETHANOL PRODUCTION USING *Saccharomyces cerevisiae* ATCC 24860

By

Ahmad Muhaimin bin Roslan

February 2011

Chairman: **Prof. Mohd Ali Hassan, PhD**

Institute: **Bioscience**

Production of biological ethanol (bioethanol) from biomass waste residues through biotechnological approach (cellulosic bioethanol) is important nowadays as it is a mitigation process towards fossil fuel depletion, energy crisis and greenhouse gasses pollution. It is an environmental friendly process which also facilitates carbon sequestration and provides a carbon neutral fuel for transportation and other applications. It is also an alternative way to utilize biomass waste from agro-industries such as oil palm empty fruit bunches (OPEFB) and rice straw. In this study, cellulosic bioethanol was produced from rice straw through a three-stage system which are pretreatment of the rice straw, enzyme production and cellulosic bioethanol fermentation.

The first stage is pretreatment, where improvements on existing pretreatment technologies were studied, without chemical treatment. Wet disc milling machine was used with the addition of water to the rice straw prior the milling process involving rotating grinding stones. By incorporating thermal treatment (121°C) to the wet disc-milled product, there are improvements in free glucose released prior to enzymatic hydrolysis and reduction in lignocellulosic particle size. It was found that by wet disc milling and thermal treatment, 0.046 g glucose was released per g rice straw as compared to 0.024 g glucose per g rice straw respectively. While for NaOH pretreatment, no glucose release can be detected after pretreatment since the rice straw must be rinsed to remove the chemical.

The second stage involves cellulase production and enzymatic hydrolysis of rice straw. By incorporating 50 mL of palm oil mill effluent (POME) as nutrient in 1 liter Mandel's medium, cellulase production from rice straw by *Aspergillus* sp. at 30°C after 5 days produced remarkable activity, which is 6.3 FPU/g rice straw used. This crude cellulase when used on pretreated rice straw in 50 mL bottle with magnetic stirrer bar at pH 4.8 and temperature of 50°C gave higher glucose compared to non-thermal treated rice straw, with increment from 0.245 g glucose/g rice straw to 0.380 g glucose/g rice straw.

The third stage involves ethanol fermentation by yeast, *Saccharomyces cerevisiae* ATCC 24860. The pH of the hydrolyzed rice straw was adjusted to 6.0 prior to the yeast inoculation. Incubation was carried out in 50 mL stirrer bottle at 37°C. Theoretically, one mole of glucose (180.16 g) will be converted into two moles of

ethanol (92.14 g). In this study, 0.10 g ethanol/g rice straw obtained, which counted for 62.61% of bioethanol produced.

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

PENAMBAHBAIKAN PERSEDIAAN HIDROLISAT JERAMI PADI UNTUK PENGHASILAN BIOETANOL MENGGUNAKAN *Saccharomyces cerevisiae* ATCC 24860

Oleh

AHMAD MUHAIMIN BIN ROSLAN

Februari 2011

Pengerusi: **Prof. Mohd Ali Hassan, PhD**

Institut: **Biosains**

Penghasilan bioetanol daripada bahan buangan biojisim melalui kaedah bioteknologi (bioetanol) adalah penting pada masa kini kerana ia adalah salah satu langkah untuk mengurangkan kesan daripada kehabisan bahanapi fosil, krisis tenaga dan pencemaran gas rumah hijau. Ia adalah proses yang mesra alam yang mana membantu penyerapan karbon dari atmosfera dan juga menghasilkan bahanapi untuk kenderaan dan aplikasi lain yang karbon-neutral. Ia juga adalah langkah alternatif untuk penggunaan bahan buangan biojisim dari industry pertanian seperti hampas kelapa sawit (OPEFB) dan bahan buangan padi (terutamanya jerami padi). Dalam kajian ini, bioetanol dihasilkan daripada jerami padi melalui sistem tiga peringkat iaitu pra-rawatan jerami padi, penghasilan enzim dan fermentasi bioetanol.

Peringkat pertama ialah pra-rawatan, di mana penambahbaikan kepada teknologi pra-rawatan yang sedia ada dikaji, tanpa melibatkan pra-rawatan kimia. Mesin pengisar cakera basah telah digunakan, di mana teknik pengisaran ini melibatkan penambahan air kepada jerami padi sebelum proses kisaran dilakukan melalui cakera batu giling yang berpusing. Dengan melibatkan pra-rawatan terma (121°C) kepada produk kisaran cakera basah, terdapat peningkatan glukosa terbebas sebelum hidrolisis enzim, dan juga pengurangan pada saiz partikel lignosellulosik. Didapati bahawa melalui pra-rawatan kisaran cakera basah dan terma, glukosa bebas yang dihasilkan adalah 0.046 g glukosa/g jerami padi berbanding 0.024 g glukosa/g jerami padi melalui teknik pra-rawatan kisaran cakera basah sahaja. Sementara untuk pra-rawatan menggunakan NaOH, tiada glukosa bebas dikesan kerana jerami mestilah dibilas selepas pra-rawatan untuk membuang bahan kimia.

Peringkat kedua melibatkan penghasilan enzim cellulase dan hidrolisis enzim terhadap jerami padi. Dengan hanya menambah 50 mL efluen kilang kelapa sawit (POME) sebagai nutrien tambahan dalam 1 liter media Mandel , penghasilan enzim cellulase daripada jerami padi menggunakan kulat yang diasingkan dari sumber tempatan, pada suhu 30°C selepas 5 hari didapati mempunyai aktiviti yang baik, iaitu 6.3 FPU/g jerami padi. Apabila enzim ini digunakan dalam bentuk mentah ke atas jerami padi yang telah melalui proses pra-rawatan di dalam dalam botol dengan pengaduk bar bermagnet 50 mL pada pH 4.8 dan suhu 50°C, terdapat peningkatan terhadap glukosa yang dihasilkan, dari 0.245 g glukosa/g jerami padi kepada 0.380 g glukosa/g jerami padi.

Peringkat ketiga melibatkan fermentasi etanol menggunakan yis, *Saccharomyces cerevisiae* ATCC 24860. pH jerami padi terhidrolisis diubah kepada 6.0 sebelum inokulasi yis. Inkubasi dalam botol dengan pengaduk bar bermagnet 50 mL dijalankan pada suhu 37°C. Secara teorinya, satu mol glukosa (180.16 g) boleh menghasilkan 2 mol etanol (92.14 g). Dalam kajian ini, sebanyak 0.10 g etanol/g jerami padi telah diperolehi, di mana ia merupakan perolehan sebanyak 62.61%.

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I certify that an Examination Committee has met on **11th February 2011** to conduct the final examination of **Ahmad Muhaimin bin Roslan** on his **Master** thesis entitled "**Improvement of Rice Straw Hydrolysate Preparation for Bioethanol Production by *Saccharomyces Cerevisiae* ATCC 24860"** in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the student be awarded the **Master of Science.**

Members of the Examination Committee were as follows:

Mohd Arif Syed, PhD
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The thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement to the **Master** degree. The members of the Supervisory Committee were as follows:

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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.

AHMAD MUHAIMIN BIN ROSLAN

Date: 18 February 2011

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