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PRODUCTION OF ACETONE-BUTANOL-ETHANOL FROM SAGO PITH RESIDUES

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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

September 2012

DEDICATED TO MY PARENT, SISTER AND BROTHER

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

PRODUCTION OF ACETONE-BUTANOL-ETHANOL FROM SAGO PITH RESIDUES

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SIREN ANAK LINGGANG

September 2012

Chairman : Suraini Abd. Aziz, PhD

Faculty : Biotechnology and Biomolecular Sciences

Sago pith residues are one of the abundant lignocellulosic residues that left behind after starch extraction process and contains significant amount of starch (58%), cellulose (23%), hemicellulose (9.2%) and lignin (3.9%). This residue has a great potential as cheap and affordable substrate for the production of enzymes, fermentable sugars and biofuel due to their high content of cellulose and hemicellulose. This study was carried out to produce fermentable sugars from sago pith residues using crude cellulases produced from local fungal isolates namely *Trichoderma asperellum* UPM1 and *Aspergillus fumigatus* UPM2 either individual or in combination. The fermentable sugars produced were then converted to acetone-butanol-ethanol (ABE) by *Clostridium acetobutylicum* ATCC 824. In this study, crude cellulases produced from *Aspergillus fumigatus* UPM2 exhibited high specific cellulases activity (0.77 U/mg FPase, 47.76 U/mg CMCase and 1.56 U/mg β-glucosidase) as compare to crude cellulases from *Trichoderma asperellum* UPM1 and mixed cultures. Therefore, crude cellulases produced from *Aspergillus fumigatus* UPM2 was used for further study. Enzymatic hydrolysis using crude cellulases

produced by *Aspergillus fumigatus* UPM2 based on 23.4 I.U of β-glucosidase as rate-limiting released approximately 21 g/L fermentable sugars from 5% (w/v) sago pith residues at pH 5.0 and temperature of 50ºC. The hydrolysis yield obtained was 73% which were comparable with the hydrolysis yield of maize straw (66%) and rice straw (70%) as reported in earlier studies by other researchers. High Performance Liquid Chromatography (HPLC) analysis on sago pith residues hydrolysate showed that it consisted mainly of glucose (10 g/L) and cellobiose (4.26 g/L). The total acetone-butanol-ethanol (ABE) production by *Clostridium acetobutylicum* ATCC 824 using concentrated sago pith residues hydrolysate with addition 0.5 g/L yeast extract was 8.84±0.20 g/L (5.41±0.10 g/L of butanol) after 72 hours of fermentation. Yield and productivity of the total acetone-butanol-ethanol were 0.30 g ABE/g glucose and 0.12 g/L/h, respectively. This result was almost comparable to the result obtained using synthetic glucose (40 g/L) for ABE production by *Clostridium acetobutylicum* ATCC 824 which the yield and productivity were 0.32 g ABE/g glucose and 0.15 g/L/h, respectively.

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

PENGHASILAN ASETON-BUTANOL-ETANOL DARIPADA SISA EMPULUR SAGU

Oleh

SIREN ANAK LINGGANG

September 2012

Pengerusi : Suraini Abd. Aziz, PhD

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Sisa empulur sagu atau hampas sagu adalah salah satu daripada sisa lignoselulosa yang banyak terhasil daripada proses pengekstrakan kanji dan mengandungi 58% kanji, 23% selulosa, 9.2% hemiselulosa dan 3.9% lignin. Sisa ini mempunyai potensi yang besar sebagai substrat yang murah dan berpatutan untuk penghasilan enzim, gula terfermentasi dan biotenaga kerana mempunyai kandungan selulosa dan hemiselulosa yang tinggi. Kajian ini telah dijalankan untuk menghasilkan gula terfermentasi daripada sisa empulur sagu dengan menggunakan enzim selulase yang dihasilkan oleh kulat pencilan tempatan iaitu *Trichoderma asperellum* UPM1 dan *Aspergillus fumigatus* UPM2 sama ada secara individu atau gabungan kedua-dua kultur. Gula terfermentasi yang dihasilkan ditukarkan kepada aseton-butanol-etanol (ABE) oleh *Clostridium acetobutylicum* ATCC 824. Di dalam kajian ini, keputusan menunjukkan bahawa enzim selulase yang dihasilkan oleh *Aspergillus fumigatus* UPM2 mempamerkan aktiviti spesifik enzim selulase (0.77 U/mg FPase, 47.76 U/mg CMCase and 1.56 U/mg β-glucosidase) yang lebih tinggi berbanding dengan enzim selulase yang dihasilkan oleh *Trichoderma asperellum* UPM1 dan gabungan kedua-

dua kultur. Oleh itu, enzim selulase yang dihasilkan oleh *Aspergillus fumigatus* UPM2 telah dipilih untuk kajian selanjutnya. Hidrolisis berenzim menggunakan enzim selulase yang dihasilkan oleh *Aspergillus fumigatus* UPM2 berdasarkan aktiviti β-glucosidase sebanyak 23.4 I.U sebagai kadar pengehad, menghasilkan kira-kira 21 g/L gula terfermentasi daripada 5% sisa empulur sagu pada pH 5.0 dan suhu 50ºC. Kadar hasil hidrolisis yang dicapai adalah 73% dimana ianya adalah setanding dengan hasil hidrolisis jerami jagung (66%) dan jerami padi (70%) seperti yang dilaporkan dalam kajian terdahulu oleh penyelidik lain. Analisa menggunakan kromatografi cecair berprestasi tinggi (HPLC) terhadap hidrolisat sisa empulur sagu menunjukkan bahawa ianya terdiri daripada glukosa (10 g/L) dan selobiosa (4.26 g/L). Jumlah penghasilan aseton-butanol-etanol (ABE) oleh *Clostridium acetobutylicum* ATCC 824 menggunakan hidrolisat sisa empulur sagu yang dipekatkan dengan penambahan 0.5 g/L yis ekstrak adalah 8.84±0.20 g/L (5.41±0.10 g/L butanol) selepas 72 jam fermentasi. Hasil dan produktiviti aseton-butanol-etanol masing-masing adalah 0.30 g ABE/g glucose dan 0.12 g/L/h. Keputusan ini adalah setanding dengan keputusan yang diperolehi dengan menggunakan glukosa sintetik (40 g/L) untuk penghasilan ABE oleh *Clostridium acetobutylicum* ATCC 824 iaitu hasil dan produktiviti masing-masing adalah 0.32 g ABE/g glucose dan 0.15 g/L/h.

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I certify that a Thesis Examination Committee has met on 27 September 2012 to conduct the final examination of Siren anak Linggang on her thesis entitled "Production of Acetone-Butanol-Ethanol from Sago Pith Residues" in accordance with the Universities and University College 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 of Science.

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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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Date: 10 January 2013

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 institutions.

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SIREN ANAK LINGGANG

Date: 27 September 2012

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