UNIVERSITI PUTRA MALAYSIA

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By

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

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OPTIMIZATION OF BIOETHANOL PRODUCTION FROM YEAST ISOLATED FROM NYPA SAP USING RESPONSE SURFACE METHODOLOGY

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SHARMILA DEWI NATARAJAN

JULY 2012

Chair: **Dr Nor' Aini Abdul Rahman, Ph.D**

Faculty: **Faculty of Biotechnology and Biomolecular Sciences**

Bioethanol has emerged as an alternative energy to the fossil fuel as it is produced from a renewable source and is environmental friendly. Bioethanol production from economical carbon source with a cost-effective fermentation process and the suitable microorganism with high fermentation ability are the key factors for bioethanol development. This study focused on the production of bioethanol from nypa sap which Malaysia. Nypa sap is obtained from *Nypa fruticans* and it is rich in various types of simple sugars that can readily be fermented into ethanol by yeast. In spite of such usefulness, there is a lack of scientific reports on nypa sap for bioethanol production compared to other substrates. Thus, nypa sap was selected to investigate its potential to produce bioethanol. Besides that, selection of suitable bacteria which can withstand high concentration of sugars in nypa sap and can ferment the sugars effectively is a crucial step

for a successful fermentation. Therefore, isolation of wild yeast strain from the nypa sap itself ensures the high adaptability and genetic stability of the strain during fermentation of nypa sap. Furthermore, in order to attain high ethanol production, optimum fermentation conditions for the wild strain also should be studied as the requirements of microorganism for growth and ethanol production are specific to individual species. Thus, the objectives of this study were to isolate yeast from nypa sap which is capable of producing high ethanol and optimize fermentation conditions for the production of bioethanol by the selected yeast. Six yeast strains were isolated from nypa sap and were identified as *Saccharomyces cerevisiae, Candida tropicalis, Candida parapsilosis, Lachancea fermentati, Candida krusei and Kloeckera sp.* using API 20C AUX kit. *C. krusei* and *Kloeckera sp*. were not selected for further experiments since they do not possess the ability to utilize sucrose which is the main sugar component in nypa sap. Ethanol fermentation was carried out in shake flasks incubated at 30ºC, 200 rpm using 50 g/l nypa sap as carbon source by the isolated *S. cerevisiae, C. tropicalis, C. parapsilosis, L. fermentati* and Baker's *L. fermentati* produced the highest ethanol (18.7 g/l) with 74.7% of theoretical ethanol value. Thus it was selected for further optimization process. In order to attain a higher ethanol production, response surface methodology was employed using temperature, pH, substrate concentrations and fermentation time as independent variables. Fermentation was carried out in 250 ml shake flasks with agitation speed of 200 rpm. Results showed that the optimum conditions for bioethanol production were temperature of 30°C, pH 5.4, substrate concentrations of

110 g/l and fermentation time of 20 hours. The model predicted that the maximum concentration of ethanol under the optimum conditions was 46.91 g/l. The verification experiment produced 46.4 g/l of ethanol which is in close agreement with the model prediction. The knowledge obtained from this research can be used in bioethanol development by carrying out scale up of this fermentation process using the optimized condition in order to apply in industrial scale.

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

PENGOPTIMUMAN PENGHASILAN BIOETANOL OLEH YIS YANG DIPENCILKAN DARI NIRA NIPAH MENGGUNAKAN KAEDAH RANGSANGAN PERMUKAAN

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Bioetanol telah muncul sebagai tenaga alternatif kepada bahan api fosil kerana ia dihasilkan dari sumber yang boleh diperbaharui dan mesra alam. Pengeluaran bioetanol dari sumber karbon yang jimat dengan kos proses penapaian yang berkesan dan mikroorganisma yang sesuai dengan keupayaan penapaian tinggi adalah faktor-faktor utama bagi pembangunan bioetanol. Kajian ini memberi tumpuan kepada penghasilan bioetanol dari sap nipah yang juga dikenali sebagai 'nira nipah' di Malaysia. Sap nipah diperolehi dari *Nypa fruticans* dan ia kaya dengan pelbagai jenis gula ringkas yang boleh ditapai terus kepada etanol oleh yis. Di sebalik kegunaan itu, laporan saintifik mengenai sap nipah untuk pengeluaran bioethanol adalah kurang berbanding dengan substrat lain. Oleh itu, nypa sap telah dipilih untuk menyiasat potensinya untuk menghasilkan bioethanol. Selain itu, pemilihan bakteria yang sesuai yang boleh menahan kepekatan gula

yang tinggi dalam nypa sap dan boleh menapai gula dengan berkesan adalah satu langkah yang penting untuk penapaian yang berjaya. Oleh itu, pengasingan strain yis liar dari sap nipah memastikan penyesuaian dan kestabilan genetik strain yang tinggi semasa penapaian sap nipah. Selain itu, dalam usaha untuk mencapai pengeluaran etanol tinggi, keadaan penapaian optimum untuk strain liar juga perlu dikaji kerana keperluan mikroorganisma untuk pertumbuhan dan pengeluaran etanol adalah khusus kepada spesis individu. Objektif kajian ini adalah untuk memencilkan yis dari sap nipah yang mampu menghasilkan etanol yang tinggi dan mengoptimumkan proses penapaian untuk penghasilan bioetanol oleh yis yang terpilih. Enam strain yis telah dipencilkankan daripada sap nipah dan dikenalpasti sebagai *Saccharomyces cerevisiae, Candida tropicalis, Candida parapsilosis, Lachancea fermentati, Candida krusei* dan *Kloeckera sp*. menggunakan kit API 20C AUX. *C. krusei* dan *Kloeckera sp.* tidak dipilih untuk kajian selanjutnya kerana mereka tidak memiliki keupayaan untuk menggunakan sukrosa yang merupakan komponen utama gula dalam sap nipah. Fermentasi etanol telah dijalankan di dalam kelalang bergoncang dieramkan pada 30ºC, 200 rpm menggunakan 50 g/l sap nipah sebagai sumber karbon oleh *S. cerevisiae*, *C. tropicalis, C. parapsilosis, L. fermentati* terpencil dan yis Baker. *L. fermentati* menghasilkan etanol tertinggi (18.7 g/l) dengan 74.7% nilai etanol teori, oleh itu ia telah dipilih untuk proses pengoptimuman selanjutnya. Dalam usaha mencapai penghasilan etanol yang lebih tinggi, kaedah rangsangan permukaan diaplikasikan menggunakan suhu, pH, kepekatan substrat dan masa fermentasi sebagai

pembolehubah tak bersandar. Penapaian telah dijalankan di dalam kelalang bergoncang 250 ml dengan kelajuan goncangan 200 rpm. Keputusan menunjukkan bahawa keadaan optimum untuk penghasilan bioetanol adalah suhu 30 °C, pH 5.4, kepekatan substrat 110 g/l dan masa penapaian 20 jam. Model meramalkan bahawa kepekatan etanol maksimum di bawah keadaan optimum adalah 46.91 g/l. Uji kaji pengesahan menghasilkan 46.4 g/l etanol yang rapat dengan ramalan model. Pengetahuan yang diperoleh daripada kajian ini boleh digunakan dalam pembangunan bioetanol dengan menjalankan penaikan skala proses penapaian ini menggunakan keadaan yang optimum untuk mengaplikasi dalam skala industri.

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I certify that a Thesis Examination Committee has met on 20 July 2012 to conduct the final examination of Sharmila Dewi A/P Natarajan on her thesis entitled "Optimization of Bioethanol Production from Yeast Isolated from Nypa Sap Using Response Surface Methodology" 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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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.

SHARMILA DEWI NATARAJAN

Date: July 2012

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