UNIVERSITI PUTRA MALAYSIA

GLYCEROL AS ALTERNATIVE SUBSTRATE FOR BIOETHANOL PRODUCTION USING FREE AND IMMOBILIZED *Escherichia coli* SS1

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By

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

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Chairman : Phang Lai Yee, PhD
Faculty : Biotechnology and Biomolecular Sciences

Increase in the crude oil price and the concern about climatic change has resulted in the rapid increase of biodiesel production. In the production of this biofuel, glycerol will essentially generate as by-product. As a result, the production of glycerol has also increased. Biofuel from glycerol can be categorized as second generation of biofuels where it can be used to replace the first generation of biofuel including glucose and vegetable oils as feedstock to reduce the competition between biofuel and food production. Thus the aim of this study is to carry out bioethanol production using glycerol in batch and continuous fermentation by free and immobilized *Escherichia coli* SS1. Bioethanol production was carried out using both free and immobilized cells in 2 liter bioreactor with 800 mL working volume. Glycerol concentration of 20 g/L, 35 g/L and 45 g/L were used as initial substrate concentration in batch free cells fermentations. Dilution rate of 0.1/h and 0.2/h were used and was selected based on the maximum specific growth rate from batch fermentation. Immobilization of *E.coli* SS1 was done by using sodium alginate and calcium chloride as crossed link agent. Optimization study was done to determine the stability and rigidity of alginate beads. Parameters involved in this optimization study were sodium alginate concentration, calcium chloride concentration, beads diameter and initial pH of medium. The optimum conditions for cell immobilization were 0.2 M calcium chloride, at pH 7 with 3% concentration of sodium alginate and beads diameter of 3 mm. Results showed that high glycerol concentration did not affect the yield of ethanol with the yield was closed to theoretical yield; 1 mol ethanol per 1 mol glycerol. Dilution rate of 0.1/h was the optimum dilution rate to be used in this fermentation where glycerol consumption and ethanol production was similar to batch fermentation by yielding 1 mol ethanol per mol glycerol. Continuous fermentation of immobilized *E. coli* SS1 was done by using the optimized beads with the dilution rate of 0.2/h and 20 g/L. The results showed that immobilized cells can last up to 3rd cycle of continuous fermentation. Ethanol production obtained was 6.17 g/L by utilizing approximately 19 g/L glycerol. The yield achieved was 0.65 mol ethanol per

mol glycerol. Compare with other studies, the result in this experiment was slightly lower where other managed to obtain about 0.6 to 0.8 mol ethanol per mol glycerol, respectively.

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

GLISEROL SEBAGAI SUBSTRAT ALTERNATIF BAGI PENGHASILAN BIOETANOL MENGGUNAKAN SEL *Escherichia coli* SS1 BEBAS DAN TERSEKAT-GERAK

Oleh

NUR AMELIA AZREEN BINTI ADNAN

Jun 2014

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Kenaikan harga minyak mentah dan kesedaran terhadap perubahan iklim telah mengakibatkan peningkatan dalam penghasilan biodiesel. Dalam penghasilan bahan api biologi ini, gliserol akan dijana sebagai bahan sampingan. Oleh sebab itu, kadar pengeluaran gliserol juga meningkat. Bahan api biologi dari gliserol boleh dikategorikan sebagai generasi kedua bahan api biologi di mana ia boleh digunakan untuk menggantikan generasi pertama bahan api biologi termasuk glukosa dan minyak sayur-sayuran sebagai bahan mentah untuk mengurangkan persaingan antara bahan api biologi dan pengeluaran makanan. Oleh itu, tujuan kajian ini adalah untuk menjalankan pengeluaran bioetanol daripada gliserol dalam fermentasi sesekelompok dan selanjar dengan menggunakan *Escherichia coli* SS1 bebas dan tersekat-gerak. Pengeluaran bioetanol telah dijalankan dengan menggunakan kedua-dua sel bebas dan tersekat-gerak dalam bioreaktor 2 liter dengan 800 mL isipadu kerja. Kepekatan gliserol 20 g/L, 35 g/L dan 45 g/L telah digunakan sebagai kepekatan substrat awal dalam fermentasi sesekelompok dengan menggunakan sel bebas. Kadar pencairan 0.1/h dan 0.2/h telah digunakan di mana ia dipilih berdasarkan kadar pertumbuhan spesifik maksimum daripada fermentasi sesekelompok. Penyekat-gerak sel *E. coli* SS1 telah dilakukan dengan menggunakan natrium alginat dan kalsium klorida sebagai ejen pautan bersilang. Kajian pengoptimuman telah dijalankan untuk menentukan kestabilan dan ketegaran manik alginat. Parameter yang terlibat dalam kajian pengoptimuman ini adalah kepekatan natrium alginat, kepekatan kalsium klorida, diameter manik dan pH awal medium. Keadaan optimum untuk penyekat-gerak sel adalah 0.2 M kalsium klorida, pada pH 7 dengan kepekatan 3% natrium alginat dan diameter manik 3 mm. Keputusan menunjukkan bahawa kepekatan gliserol tinggi tidak menjejaskan hasil bioetanol dimana nilainya berdekatan dengan nilai teori. Kadar pencairan sebanyak 0.1/h ialah kadar pencairan optimum untuk digunakan dalam fermentasi ini di mana penggunaan gliserol dan pengeluaran etanol adalah sama dengan fermentasi sesekelompok dengan menghasilkan 1 mol etanol kepada mol gliserol. Fermentasi selanjar sel pegun *E. coli* SS1 telah dilakukan dengan menggunakan manik yang telah

dioptimumkan dengan kadar pencairan 0.2/h dan 20 g/L. Hasil kajian menunjukkan bahawa sel-sel pegun boleh bertahan sehingga kitaran ke-3 di dalam fermentasi selanjar. Pengeluaran bioetanol yang diperolehi ialah 6.17 g/L dengan menggunakan kira-kira 19 g/L gliserol. Hasil yang diperolehi adalah 0.65 mol etanol kepada mol gliserol. Jika dibandingkan dengan kajian-kajian lain, hasil eksperimen ini adalah lebih rendah sedikit di mana penyelidik lain berjaya menghasilkan antara 0.6-0.8 mol etanol kepada mol gliserol, masing-masing.

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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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DECLARATION

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

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