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OPTIMIZATION OF GLYCEROL FERMENTATION CONDITIONS FOR BIOETHANOL PRODUCTION BY LOCALLY ISOLATED Escherichia coli SS1

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By

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Faculty : Biotechnology and Biomolecular Sciences

The increase of biodiesel production worldwide lead to the increase of glycerol present in environment. Bioconverting glycerol into various valuable products is one of glycerol's promising applications due to its high availability, lower costs and existence of many glycerol-utilizing microorganisms. Bioethanol is one of the interest products generated and its production is depending on the types of microorganism used and fermentation conditions. The effective screening procedure is needed to screen and isolate broad ranges of bacteria from environment. This study aims to isolate a suitable glycerol-fermenting microorganism and able to produce high ethanol production under optimized condition using crude glycerol as a substrate.

The screening method was modified based on enzymatic oxidation of ethanol which is correlated to reduction of 2,6-dichlorophenol-indophenol dye. The formation of decolourized zone was apparent using modified assay containing 5 ml/L of 0.05 M 2,6-dichlorophenol-indophenol, 10 ml of reaction mixture and 500 µl/L of enzyme, respectively. In this study, several isolates were obtained showing capability in producing ethanol. The isolate namely *E. coli* SS1 was obtained after several screening processes. The fermentation of glycerol was carried out in batch fermentation using 120 mL serum bottle, incubated in 37°C with 120 rpm of agitation under anaerobic condition. The maximum ethanol production was achieved at 96 hours with 9.23 g/L, which is corresponding to the yield of 1 mol ethanol per mol glycerol and the productivity of 0.01 mol/molh^{-1}. This isolate also showed a higher affinity to glycerol than glucose for bioethanol production.

Response surface methodology (RSM) employed to obtain optimized condition. Six parameters were subjected to two level factorial design which were initial pH, substrate concentration, sodium chloride content, trace element solution, incubation temperature and the concentration of yeast extract and tryptone. The results showed that only 4 parameters were identified as significant factors, i.e initial pH, substrate concentration, sodium chloride content and the mixture of yeast extract and tryptone. The effect of the significant variables was subsequently evaluated in Central Composite Design (CCD) to determine the optimum value for each variable. The optimized conditions obtained were at initial pH of 7.61, substrate concentration of 34.5 g/L, the mixture of yeast extract and tryptone at 6.42 g/L whereas salt content was identified as a non-significant parameter, with predicted maximum ethanol production of 17.05 g/L (1.0 mol/mol). A validation run was performed using crude glycerol based on optimized conditions and maximum ethanol obtained was 15.72 ± 0.26 g/L with the yield of 1.0 mol/mol and productivity at 0.01 mol/mol.h^{-1} which was comparable to the predicted ethanol concentration. The isolated E.coli SS1 is a potential ethanol producer from glycerol, where the increased ethanol production is observed under optimized fermentation conditions. The crude glycerol also is feasible to be fermented using this isolate for comparable ethanol production using pure glycerol.

Abstrak tesis yang dikemukan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

PENGOPTIMUMAN KEADAAN FERMENTASI GLISEROL UNTUK PENGHASILAN ETANOL MENGGUNAKAN *Escherichia coli* SS1 PENCILAN TEMPATAN

Oleh

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Peningkatan pengeluaran biodiesel di seluruh dunia membawa kepada peningkatan gliserol dalam alam sekitar. Bio-penukaran gliserol kepada pelbagai produk yang bernilai tinggi adalah salah satu cara yang berkesan kerana kuantiti gliserol tersedia yang tinggi, kosnya yang rendah dan pelbagai mikroorganisma boleh menggunakan gliserol. Bioetanol merupakan salah satu produk yang boleh dihasilkam daripada bio-penukaran gliserol dan ia bergantung kepada jenis mikroorganisma yang digunakan dan keadaan fermentasi. Prosedur saringan yang berkesan diperlukan untuk menyaring dan mengasingkan bakteria dari persekitaran. Objektif kajian ini adalah untu medapatkan bakteria yang mampu menghasilkan etanol yang tinggi dalam keadaan yang optima dengan menggunakan gliserol.

Satu kaedah saringan telah diubahsuai berdasarkan pengoksidaan enzim etanol, yang berkait rapat dengan pemudaran pewarna 2,6-dichlorophenol-indophenol. Pembentukan zon warna yang pudar dapat dilihat dengan jelas apabila menggunakan komposisi yang telah diubahsuai iaitu 5 ml/L 0.05 M 2,6-dichlorophenol-indophenol, 10 ml campuran tindak balas dan 500 µl/L enzim. Dalam kajian ini, beberapa bakteria yang boleh menggunakan gliserol untuk penghasilan etanol telah diperolehi. Bakteria pencilan tempatan, *E. coli* SS1 telah diperolehi selepas beberapa proses saringan. Fermentasi gliserol telah dijalankan di dalam botol serum bersaiz 120 mL, dan difermentasi pada suhu 37°C dan emparan pada 120 rpm, di bawah keadaan anerobik. Pengeluaran etanol maksimum telah dicapai pada 96 jam dengan 9.23 g/L, iaitu bersamaan dgn 1 mol etanol bagi setiap mol gliserol dan kadar

pengeluaran pada 0.01 mol/mol.jam $^{-1}$. Bakteria pencilan tempatan ini juga menunjukkan keupayaan yang lebih tinggi menggunakan gliserol berbanding glukosa dalam penghasilan etanol.

Kaedah rangsangan permukaan (RSM) digunakan untuk mendapatkan keadaan optimum. Enam parameter telah tertakluk kepada dua tahap reka bentuk faktorial iaitu pH awal, kepekatan substrat, kandungan garam, kepekatan unsur surih, suhu fermentasi dan campuran ekstrak yis dan tryptone. Keputusan menunjukkan bahawa hanya 4 parameter telah dikenalpasti sebagai faktor penting, iaitu pH awal, kepekatan substrat, kandungan garam dan campuran ekstrak yis dan tryptone. Kesan pembolehubah penting (dua peringkat reka bentuk faktorial) pada pengeluaran etanol kemudiannya dinilai dalam Rekabentuk Pusat Komposit (CCD) untuk membangunkan model empirikal untuk menentukan nilai optimum setiap pemboleh ubah. Parameter yang optimum diperolehi pada pH awal 7.61, kepekatan substrat pada 34.5 g/L, campuran ekstrak yis dan tryptone pada 6.42 g/L manakala kandungan garam tidak menunjukkan parameter penting, dengan ramalan pengeluaran etanol maksimum pada 17.05 g/L. (1.0 mol/mol) Pengesahan dijalankan menggunakan gliserol mentah berdasarkan keadaan optimum dan etanol maksimum yang diperoleh adalah 15.72 ± 0.26 g/L iaitu bersamaan dengan 1.0 mol/mol dan kadar pengeluaran pada 0.01 mol/mol.h^{-1} setanding dengan kepekatan etanol diramalkan. Bakteria E.coli SS1 pencilan tempatan ini adalah pengeluar etanol yang berpotensi dari gliserol, di mana pengeluaran etanol meningkat di bawah keadaan fermentasi yang optimum. Dengan menggunakan bakteria ini juga, gliserol mentah juga dapat difermentasi untuk penghasilan etanol yang setanding dengan gliserol tulen.

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