UNIVERSITI PUTRA MALAYSIA

PERFORMANCE OF SOLVENT (ACETONE-BUTANOL-ETHANOL) FERMENTATION BY CLOSTRIDWMSACCHAROBUTYL/CUM STRAIN P262 AND NCIMB8052 USING FREE AND IMMOBILIZED CELLS SYSTEM

By

NOR SUHAILA BINTI YAACOB

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

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Specially dedicated to my most beloved parents

Yaacob Othman and Dzaharah Faridah.

Your care, support and endless love
"My success is only for you"

I never know what the future brings
but
I know you are here with me
We'll make it through....

To all my Family

With you
I have the strength to stand at all....

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

PERFORMANCE OF SOLVENT (ACETONE-BUTANOL-ETHANOL) FERMENTATION BY *CLOSTRIDIUM SACCHAROBUTYLICUM* STRAIN P262 AND NCIMB8052 USING FREE AND IMMOBILIZED CELLS SYSTEM

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June 2003

Chairman: Associates Profesor Arbakariya Ariff, Ph.D.

Institute: Bioscience

The performance of solvent (Acetone-butanol-ethanol) fermentation by two strains of *Clostridium saccharobutylicum* (P262 and NCIMB8052) were studied using different sizes of bioreactor (28 mL McCartney bottle, 0.5 L and 2 L stirred tank fermenter). The fermentations were carried out with batch process using freely suspended cells and immobilized cells system. Immobilization of cells was carried out, passively, using cubes of polyurethane foam as biomass support particles (BSP). To study the efficiency of cell immobilization, the variables investigated include pore size of BSP, BSP number and BSP size. The effect of chemical pretreatment on the efficiency of cell immobilization using BSP was investigated using activated carbon (charcoal) and glutaraldehyde. Among the chemical pretreatment applied to the BSP were 4% activated charcoal and 25% glutaraldehyde.

The size of bioreactor gave a significance influence on solvent fermentation performance by both solvent-producing strains. The highest production of total solvent (10.86 g/L) and (8.23 g/L) by strain P262 and NCIMB8052 was obtained in 2 L fermenter using 50 g/L glucose, respectively. In 0.5 L fermenter, production of total solvent was reduced to 7.99 g/L and 7.17 g/L for strain P262 and NCIMB8052, respectively. Further reduction of total solvent was observed in fermentation using 28 mL McCartney bottle. Reduction of solvent production was found to be associated with the activity of proteolytic enzyme (protease) detected in the culture during the fermentation. This proteolytic enzyme may be responsible in the degradation of enzyme involved in solvent fermentation, which in turn reduced solvent production significantly.

Among the different types of starch investigated, corn starch was the most susceptible to solvent production (15.67 g/L) by strain P262 followed by sago starch (14.54 g/L), rice (10.21 g/L), tapioca (8.84 g/L) and potato (8.66 g/L). In subsequent experiment to investigate the effect of sago starch concentration (10-80 g/L) on the performance of solvent fermentation, it was found that the highest solvent production (13.81 g/L) was obtained at 50 g/L sago starch. For fermentation using sago starch, the use of 2 L fermenter enhanced the solvent production by about 1.5 times higher as compared to 0.5 L fermenter (13.16 g/L), though the activity of amylolytic enzyme secreted did not significantly differ. When sago starch was used as a carbon source, very low solvent production (12.49 g/L-16.87 g/L) was obtained in all fermentations using strain NCIMB8052.

The highest efficiency of immobilization of cells of *C. saccharobutylicum* strain P262 and NCIMB8052 was achieved by using 15 units/28 ml culture medium of 1 cm^3 BSP having 60 ppi. The BSP pretreated with 4% activated charcoal increased the efficiency of cell immobilization significantly as compared to untreated BSP. On the other hand, BSP treated with glutaraldehyde was not singnificantly different as compared to untreated BSP. Fermentation using immobilized cells of strain P262 gave higher solvent production (19.86 g/L) than that obtained in fermentation using free cell (8.851 g/L). In addition, solvent production in fermentation using immobilized cells of strain P262 was higher as compared to fermentation using immobilized cells of NCIMB8052.

Abstrak tesis yang dikemukan kepada Senat Universiti Putra Malaysia sebagai memunuhi keperluan untuk ijazah Master Sains

PERLAKSANAAN FERMENTASI PELARUT (ACETON-BUTANOL-ETHANOL) OLEH *CLOSTRIDIUM SACCHAROBUTYLICUM* STRAIN P262 DAN NCIMB8052 MENGGUNAKAN SEL BEBAS DAN SISTEM SEL YANG DISEKATGERAK

Oleh

NOR SUHAILA BINTI YAACOB

Jun 2003

Pengerusi: Profesor Madya Arbakariya Ariff, Ph.D.

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Perlaksanaan fermentasi pelarut (Acetone-butanol-ethanol) oleh dua strain *C. saccharobutylicum* (P262 and NCIMB8052) dikaji menggunakan saiz bioreaktor yang berlainan (28 mL, 0.5 L dan 2 L fermenter yang berpengaduk). Fermentasi ini dijalankan secara proses sesekelompok menggunakan sel bebas dan sistem sel yang disekat gerak. Sel disekat gerak secara pasif menggunakan kiub span polyurethane sebagai partikel penyokong biojisim (BSP). Untuk mengkaji kecekapan sel yang disekat gerak, pembolehubah yang dikaji termasuklah saiz liang BSP, bil BSP dan saiz BSP. Kesan prarawatan kimia ke atas kecekapan sel disekat gerak menggunakan BSP dikaji menggunakan karbon yang diaktifkan (arang) dan glutaraldehyde. Di antara prarawatan kimia yang digunakan ke atas BSP adalah 4% arang yang diaktifkan dan 25% glutaraldehyde.

Saiz bioreactor memberikan pengaruh yang penting ke atas perlaksanaan fermentasi pelarut oleh kedua-dua strain penghasil pelarut. Penghasilan jumlah pelarut yang paling tinggi (10.86 g/L) dan (8.23 g/L) oleh strain P262 and NCIMB8052 didapati di dalam fermenter 2 L menggunakan 50 g/L glukosa, masing-masing. Di dalam fermenter 0.5 L, penghasilan jumlah pelarut berkurang kepada 7.99 g/L and 7.17 g/L bagi strain P262 and NCIMB8052, masing-masing. Penurunan jumlah pelarut seterusnya didapati di dalam fermentasi menggunakan 28 mL botol McCartney. Penurunan penghasilan pelarut didapati bergantung kepada aktiviti enzim proteolitik (protease) yang dikesan di dalam kultur semasa fermentasi. Enzim proteolitik mungkin bertanggungjawab di dalam penguraian enzim yang terlibat di dalam fermentasi pelarut, yang seterusnya akan menurunkan kadar penghasilan pelarut secara bekadaran.

Diantara jenis kanji berlainan yang dikaji. Kanji jagung adalah yang paling diterima untuk penghasilan pelarut (15.67 g/L) oleh strain P262 diikuti dengan kanji sagu (14.54 g/L), nasi (10.21 g/L), ubikayu (8.84 g/L) dan kentang (8.66 g/L). Di dalam ujikaji yang seterusnya untuk mengkaji kesan kepekatan kanji sago (10-80 g/L) di dalam perlaksanaan fermentasi pelarut, adalah didapati penghasilan pelarut yang paling tinggi (13.81 g/L) adalah didapati apabila 50 g/L kanji sagu digunakan. Untuk fermentasi menggunakan kanji sago, penggunaan 2 L fermenter menggalakkan penghasilan pelarut sehingga 1.5 kali lebih tinggi berbanding 0.5 L fermenter (13.16 g/L), meskipun begitu aktiviti enzim amilolitik yang direkodkan adalah tidak banyak berbeza. Apabila kanji sago digunakan sebagai sumber karbon, penghasilan pelarut adalah sangat rendah (12.49 g/L-16.87 g/L) di dapati di dalam semua proses fermentasi menggunakan strain NCIMB8052.

Kecekapan *C. saccharobutylicum* strain P262 dan NCIMB8052 yang disekat gerak adalah paling tinggi apabila menggunakan 15 unit/28 mL kultur medium, dengan saiz BSP 1 cm^3 dan mempunyai 60 ppi. Prarawatan BSP dengan 4% arang yang diaktifkan meningkatkan kecekapan kadar sekat gerak sel berbanding dengan BSP yang tidak dirawat. Dengan kata lain, BSP yang dirawat dengan glutaraldehyde adalah tidak jauh berbeza berbanding BSP yang tidak dirawat. Fermentasi menggunakan sel P262 yang disekat gerak menghasilkan jumlah pelarut yang tinggi (19.86 g/L) berbanding yang diperolehi di dalam fermentasi menggunakan sel bebas (8.851 g/L). Sebagai tambahan, penghasilan pelarut di dalam fermentasi menggunakan strain P262 yang disekat gerak adalah lebih tinggi berbanding fermentasi menggunakan strain NCIMB8052 yang disekat gerak.

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I certify that an Examination Committee met on 12th June 2003 to conduct the final examination of Nor Suhaila binti Yaacob on her Master of Science thesis entitled "Performance of Solvent (Acetone-Butanol-Ethanol) Fermentation by *Clostridium saccharobutylicum* Strain P262 and NCIMB8052 Using Free and Immobilized Cells System" in accordance with the Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work expect for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other at UPM or other institutions.

Nor Suhaila Yaacob

Date : 25 AUG 2003

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LIST OF ABBREVIATIONS

ABE	:	Acetone-Butanol-Ethanol
RCM	:	Reinforced Clostridial Media
rpm	:	Rotation per minute
ppi	:	Pore Per Inch
BSP	:	Biomass Support Particles
M	:	Molar
μ	:	Specific growth rate
D	:	Dilution rate
X	:	Cell concentration
h	:	Hour
s	:	Substrate
P	:	Product