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IMPROVEMENT OF XYLANASE PRODUCTION BY RECOMBINANT *Escherichia coli* DH5α USING FED-BATCH FERMENTATION

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By

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Master of Science

IMPROVEMENT OF XYLANASE PRODUCTION BY RECOMBINANT *Escherichia coli* DH5α USING FED-BATCH FERMENTATION

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Chair: **Professor Arbakariya Ariff, PhD**

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

The past few decades of the twentieth century have witnessed spectacular advances and improvement of living standards due to the beneficial integration of novel and brilliant ideas with scientific progress and rapid translation of laboratory findings into practical technologies and commercial-scale manufacturing processes. In the field of chemical technology, the need for safer and 'environmental friendly' technologies has become imminent where manufacture of a variety of products on large scale has resulted in serious effluent and hazardous waste disposal problems. In recent years, there has been an increasing demand of xylanase enzyme production regarding their wide applications in various industries, especially in paper and pulp industry. The production of xylanase

from wild microorganisms (bacterial, fungal and yeast) were commonly associated with other enzymes such as cellulases and mannases, where purification of xylanases increase the production cost. The xylanase producing-fungi normally have low growth rate thus correspond to lower enzyme productivity which limits its application in commercial fermentation processes. In addition, the metabolic enzymes of xylanase producer such as proteases and transglycosides also affect the actual yield of xylanase. Alternatively, enhanced enzyme production by recombinant strain such as *Escherichia coli* may be used to overcome these problems. Recombinant enzyme production by *E. coli* generates higher productivity and yield due to faster growth rate compared to fungal producers.

The bacterium, *E. coli* DH5α harbouring phagemid pTP510 was used throughout this study for the production of xylanase. The genes encoding exo-xylanase (exo-xyn), α-L-arabinofuranosidase (abfa), and β-xylosidase (xyl) were isolated from a thermophilic bacterium *Geobacillus thermoleovorans* IT-08. Preliminary, the nutrients requirement by the recombinant *E. coli* DH5α for improvement of intracellular xylanase production was investigated in shake flask culture. The effect of the different levels of dissolved oxygen tension on growth of *E. coli* DH5α and xylanase production was carried out in 2 L stirred tank fermenter using optimal medium. The bulk of batch fermentation data were analyzed to generate kinetic parameter values and to develop kinetic models of the fermentation process. Fed-batch fermentation modes were developed using kinetic parameter values and information generated from the preliminary batch fermentation data. Constant and exponential fed-batch fermentations were carried out using 2 L

stirred tank fermenter equipped with Multi Fermenter Control System (MFCS) to control the feeding rate of feeding medium into the culture according to the proposed algorithm.

Among the two basal media tested, defined mineral medium gave higher growth and xylanase production as compared to complex medium of Luria Bertani. The optimal glucose and $(NH_4)_2SO_4$ concentration added to the basal medium for xylanase production was obtained at 10 g L^{-1} and 2 g L^{-1}, respectively. Growth of *E. coli* DH5α and xylanase production was inhibited in oxygen limited fermentation, where dissolved oxygen tension level was controlled at 0% saturation. On the other hand, xylanase production was enhanced at DOT level controlled at 20% saturation, though growth was not significantly improved. The production of xylanase in batch fermentation using optimal medium composition and DOT level was 1784.57 U mL^{-1}. This gave the overall xylanase productivity and yield of 91.43 U $mL.h^{-1}$ and 0.1190 U xylanase g $glucose^{-1}$, respectively. The proposed models based on logistic and Luedeking-Piret equations were found sufficient to describe growth of *E. coli* DH5α in different medium formulation and DOT levels.

Among the different feeding rates (0.025, 0.050 and 0.075 L h^{-1}) employed in constant fed-batch fermentation, the highest cell concentration (10.82 g L^{-1}) xylanase activity (1920.56 U mL^{-1}) was obtained at a feeding rate of 0.050 L h^{-1}. In this fermentation, low concentration of residual glucose (0.028 g L^{-1}) was observed, which was associated with very small quantity of acetic acid accumulated in the culture. In exponential fed-

batch fermentation, the highest cell concentration (10.33 g L^{-1}) and xylanase activity (1939.99 U mL^{-1}) was obtained at specific growth rate (μ) of 0.15 h^{-1}. Very low residual glucose (0.004 g L^{-1}) and very small quantity of acetic acid was detected throughout this fermentation run.

The final cell concentration obtained in fed-batch fermentation (10.33 g L^{-1}) was about 6.5% higher than that obtained in optimal batch fermentation (6.26 g L^{-1}). On the other hand, the production of xylanase (1939.99 U mL^{-1}) in optimal exponential fed-batch fermentation was about 8.7% higher than that obtained in optimal batch fermentation (1784.57 U mL^{-1}) in 2 L stirred tank fermenter. However, the overall xylanase productivity obtained in exponential fed-batch fermentation (58.71 U mL^{-1} h^{-1}) was very much reduced as compared to that obtained in batch fermentation (91.43 U $mL.h^{-1}$). This is very much dependent on the ability of *E. coli* DH5α to express xylanase enzyme based on medium composition and growth morphology. These results indicate that efficient process control strategy is important for the improvement of xylanase production by *E. coli* DH5α.

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

PENINGKATAN PENGHASILAN XILANASE OLEH REKOMBINAN *Escherichia coli* DH5α MENGGUNAKAN FERMENTASI SUAPAN SESEKELOMPOK

Oleh

BAZILAH BINTI BASAR

OGOS 2011

Pengerusi: **Professor Arbakariya Ariff, PhD**

Fakulti: **Fakulti Bioteknologi dan Sains Biomolekul**

Beberapa dekad yang lalu dalam abad kedua puluh telah menyaksikan kemajuan yang menakjubkan dan peningkatan taraf hidup kerana integrasi idea-idea baru yang berfaedah dan cemerlang dalam kemajuan sains dan penterjemahan yang pesat terhadap penemuan makmal kepada teknologi praktikal dan proses pembuatan berskala komersil. Dalam bidang teknologi kimia, keperluan untuk teknologi yang lebih selamat dan mesra alam telah menjadi satu daya tarikan di mana pembuatan pelbagai produk pada skala yang besar telah menyebabkan masalah kumbahan dan pelupusan sisa berbahaya yang

serius. Dalam tahun-tahun kebelakangan ini, terdapat permintaan yang semakin meningkat terhadap pengeluaran enzim xilanase dalam pelbagai industri, terutamanya dalam industri kertas dan pulpa. Penghasilan xilanase dari mikroorganisma liar (bakteria, kulat dan yis) yang sering dikaitkan dengan enzim lain seperti cellulases dan mannases, di mana proses penulenan enzim xilanase akan meningkatkan kos pengeluaran. Xilanase yang dihasilkan oleh kulat biasanya mempunyai kadar pertumbuhan yang rendah dan sekaligus menghasilkan produktiviti enzim yang lebih rendah yang menghadkan aplikasi dalam proses fermentasi secara komersial. Di samping itu, enzim metabolik pengeluar xilanase seperti proteases dan transglikosida juga menjejaskan ketulenan enzim xilanase. Secara alternatifnya, untuk mengatasi masalah ini, pengeluaran enzim dapat dipertingkatkan oleh strain rekombinan seperti *Escherichia coli*. Pengeluaran enzim secara rekombinan oleh *E. coli* akan menghasilkan produktiviti dan hasil enzim yang lebih tinggi disebabkan oleh kadar pertumbuhan yang lebih cepat berbanding dengan kulat.

Bakteria, *Escherichia coli* DH5α yang mengandungi pTP510 phagemid digunakan di seluruh kajian ini untuk pengeluaran xilanase. Gen-gen exo-xilanase (exo-xyn), α-L-arabinofuranosidase (abfa), dan β-xylosidase (xyl) telah diasingkan daripada bakteria termofilik *Geobacillus thermoleovorans* IT-08. Pada peringkat saringan, keperluan nutrisi oleh *E. coli* rekombinan DH5α untuk peningkatan pengeluaran xilanase secara intraselular diselidiki dalam fermentasi sekelompok dalam kelalang bergoncang. Pengaruh tahap optimum ketegangan oksigen terlarut untuk pertumbuhan *E. coli* DH5α dan pengeluaran xilanase dilakukan di dalam fermenter berpengaduk

mekanikal 2 Liter menggunakan media yang optimum. Sebahagian besar data fermentasi sekelompok dianalisa untuk menghasilkan nilai parameter kinetik dan mengembangkan model kinetik dari proses fermentasi. Fermentasi suapan sesekelompok dijalankan menggunakan nilai parameter kinetik dan maklumat yang dihasilkan dari data fermentasi sekelompok. Fermentasi sesekelompok secara konstan dan eksponen dilakukan dengan menggunakan fermenter yang dilengkapi dengan Sistem Multi Kawalan Fermenter (SMKF) untuk mengawal kadar suapan glukosa ke dalam kultur mengikut algoritma.

Di antara dua media dasar yang diuji, media mineral memberikan pertumbuhan dan pengeluaran xilanase yang lebih tinggi berbanding dengan media kompleks, Luria Bertani. Kepekatan glukosa dan $(NH_4)_2SO_4$ yang optimum untuk pengeluaran xilanase diperolehi pada 10 g L^{-1} dan 2 g L^{-1}, masing-masing. Pertumbuhan *E. coli* DH5α dan penghasilan xilanase dihalang oleh oksigen yang terhad semasa proses fermentasi, di mana kadar ketegangan oksigen terlarut dikendalikan pada ketumpatan 0 % . Selain itu, pengeluaran xilanase telah berjaya dipertingkatkan pada KOT dikawal pada ketumpatan 20%, walaupun pertumbuhan *E. coli* DH5α tidak signifikan. Penghasilan xilanase dalam fermentasi sekelompok menggunakan komposisi media dan tahap DOT yang optimum adalah 1784.57 U mL^{-1}. Hal ini memberikan produktiviti xilanase secara keseluruhan dan hasil 91.43 U mL.h^{-1} dan 0.1190 U xilanase g glukosa^{-1}, masing-masing. Model yang dicadangkan berdasarkan kepada persamaan logistik dan Luedeking-Piret adalah memadai untuk menggambarkan pertumbuhan *E. coli* DH5α dalam formulasi media dan tahap DOT yang berbeza.

Di antara tahap kelajuan kadar suapan yang berbeza (0.025, 0.050 dan 0.075 L h^{-1}) yang digunakan dalam kajian ini untuk fermentasi sesekelompok secara konstan, kepekatan sel tertinggi (10.82 g L^{-1}) didapati pada kadar suapan 0.050 L h^{-1}. Sisa kepekatan glukosa yang sangat rendah (0.028 g L^{-1}) didapati dalam media, dikaitkan dengan jumlah akumulasi asid asetik yang sangat kecil semasa proses fermentasi. Dalam fermentasi sesekelompok secara eksponen, kepekatan sel tertinggi (10.33 g L^{-1}) dan aktiviti xilanase (1939.99 U mL^{-1}) didapati pada kadar pertumbuhan spesifik 0.15 h^{-1}. Kepekatan sisa glukosa (0.004 g L^{-1}) dan kuantiti asid asetik yang sangat rendah dan sangat kecil dikesan semasa menjalan fermentasi ini.

Kepekatan sel akhir yang diperolehi dalam fermentasi sesekelompok (10.33 g L^{-1}) adalah sekitar 6.5% lebih tinggi berbanding yang diperolehi pada fermentasi sekelompok yang optimum (6.26 g / L). Selain itu, pengeluaran xilanase (1939.99 U mL^{-1}) dalam fermentasi sesekelompok secara eksponen yang optimum adalah sekitar 8.7% lebih tinggi berbanding yang diperolehi pada fermentasi sekelompok yang optimum (1784.57 U mL^{-1}) dalam fermenter berpengaduk mekanikal 2 Liter. Walau bagaimanapun, produktiviti keseluruhan xilanase yang diperolehi dalam fermentasi sesekelompok secara eksponen (58.71 U $mL.h^{-1}$) sangat jauh berkurangan berbanding dengan yang diperolehi pada fermentasi sekelompok (91.43 U $mL.h^{-1}$). Ini adalah sangat bergantung kepada keupayaan *E. coli* DH5α untuk mengekspresikan enzim xylanase berdasarkan komposisi media dan morfologi pertumbuhan. Ini menunjukkan bahawa strategi kawalan proses yang cekap adalah penting untuk peningkatan pengeluaran xilanase oleh *E. coli* DH5α.

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Special appreciation and gratitude to all my family members especially to my parents and husband for their understanding, caring and moral support. My deepest appreciation

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I certify that a Thesis Examination Committee has met on 10 August 2011 to conduct the final examination of Bazilah binti Basar on her thesis entitled "**Improvement of Xylanase Production by Recombinant *Escherichia coli* DH5α Using Fed-Batch Fermentation**" in accordance with the Universities and University Colleges 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 (with Thesis).

Members of the Thesis Examination Committee were as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the Master of Science. The members of the Supervisory Committee were as follows:

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Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declared that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

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BAZILAH BINTI BASAR

Date: 10 August 2011

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