OPTIMIZATION OF CONDITIONS FOR HIGH CELL DENSITY CULTIVATION OF *BACILLUS SUBTILIS*

By

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

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Chairman: Rosfarizan Mohamad, PhD

Institute: Bioscience

The objectives of this study are (i) To investigate the effect of using different types of carbon source and their concentration on the growth of *B. subtilis*, (ii) To optimize the pH condition for improvement of growth of *B. subtilis* through the different pH control strategies and (iii) To develop high cell density cultivation methods for *B. subtilis* based on exponential fed-batch fermentation. Preliminary, the effects of different carbon sources such as glucose, sucrose, starch hydrolysate and sago starch on growth performance of *B. subtilis* was investigated using batch fermentation technique. Subsequently, the effects of different initial glucose concentrations, which is the preferred carbon source for growth of this bacterium, was studied in batch fermentation. The experiment was designed to find the repression effect of glucose on growth of *B. subtilis*. The culture pH condition for growth of *B. subtilis* was also optimized using different pH control strategies. The exponential fed-batch fermentation aimed at production of high cell density of *B. subtilis* was developed using kinetic parameter values and information generated from the preliminary batch fermentation data. Fed-batch fermentations were carried

out at different required specific growth rates and their effects on final cell concentration attained, yield and productivity were analyzed.

The performance of fed-batch fermentation with controlled glucose concentration at certain level in the culture for improvement of *B. subtilis* cultivation was also investigated.

Among the different carbon sources investigated, the highest growth was obtained when glucose was used as the carbon source. *B. subtilis* was also capable to grow in sucrose and sago starch, indicating that *B. subtilis* was able to secrete invertase and amylolytic enzymes. Final cell concentration obtained in cultivation using glucose, starch hydrolysate, sucrose and sago starch all at a concentration of 20 g/L was 15.66 g/L, 9.71 g/L, 9.16 g/L and 5.72 g/L, respectively. This gave the cell yield ($Y_{x/s}$) of 1.08, 0.85, 0.50 and 0.32 g/g based on glucose, starch hydrolysate, sucrose and sago starch consumed, respectively.

Initial glucose concentration was found to have inhibitory effect on growth of *B. subtilis*. Growth increased with increasing initial glucose concentration from 15 to 20 g/L. A slight reduction in growth was observed at 35 g/L glucose and a drastic reduction on growth was observed at high glucose concentration (50 g/L). Growth of *B. subtilis* was also greatly influenced by the culture pH. The highest growth, in term of viable cell, was obtained in cultivation where the culture pH was controlled at 7 throughout the process. Reduction in growth was observed when the culture pH was controlled at lower and higher than pH 7.

In exponential fed-batch fermentation with controlled specific growth rate (μ), higher final viable cell (1.93 x 10^{10} cfu/mL) was obtained in fermentation where μ was controlled at 0.30 h^{-1} as compared to cultivation when μ was controlled at 0.20 h^{-1}, where final viable cell obtained was only 3.52 x 10^{9} cfu/mL. Further improvement of the cultivation performance was obtained in fed-batch fermentation with controlled glucose level in the culture throughout the process. The highest final viable cell at 3.50 x 10^{10} cfu/mL was obtained when glucose in the culture was maintained at the very low level of 0.20 g/L. Reduction in growth was observed when glucose in the culture was maintained at high levels, above 1 g/L, suggesting that glucose has repression effect on growth of *B. subtilis*.

As a conclusion, among the techniques tested in this study, the best fermentation technique to achieve high cell density cultivation of *B. subtilis* was fed-batch fermentation where glucose in the culture was maintained at very low level (0.20 g/L), while the culture pH was maintained at 7 throughout the process. Using this cultivation technique, improvement in term of final viable cell was increased up to 324% compared to batch fermentation. This method of cultivation may be used for industrial application to produce large quantity of *B. subtilis* for subsequent use as a probiotic in the aquaculture industry, especially in controlling the water quality of the shrimp pond.

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

PENGOPTIMAAN KEADAAN UNTUK PENGKULTURAN SEL *BACILLUS SUBTILIS* BERKETUMPATAN TINGGI

Oleh

LEW KOK CHEONG

Mac 2006

Pengerusi: **Rosfarizan Mohamad, PhD**

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Tujuan-tujuan bagi pembelajaran ini adalah seperti berikut, (i) Untuk mengkaji kesan daripada penggunaan jenis dan kepekatan sumber karbon yang berlainan terhadap pertumbuhan *B. subtilis*, (ii) Untuk mengoptimumkan syarat pH melalui strategi pengawaln pH yang berlainan bagi memajukan pertumbuhan *B. subtilis* dan (iii) Untuk membangunkan teknik pengkulturan sel *B. subtilis* yang berketumpatan tinggi berpandukan fermentasi suapan sesekelompok secara eksponen. Pada peringkat awal, kesan daripada sumber karbon yang berlainan seperti glukosa, sukrosa, kanji terhidrolisis dan kanji sago terhadap prestasi pertumbuhan *B. subtilis* telah dikaji dengan menggunakan kaedah fermentasi sesekelompok. Kemudian, kesan kepekatan awal glukosa, yang mana ia merupakan sumber karbon yang lebih digemari oleh bakteria ini telah dikaji dalam fermentasi sesekelompok. Eksperimen ini bertujuan untuk mencari kesan penekanan glukosa terhadap pertumbuhan bakteria tersebut. Keadaan pH kultur untuk pertumbuhan bakteria ini juga telah dioptimumkan dalam eksperimen ini. Fermentasi suapan sesekelompok yang tertumpu pada penghasilan sel *B. subtilis* yang berketumpatan tinggi telah dibangunkan dengan menggunakan nilai kinetik parameter dan maklumat yang

dijanakan daripada data fermentasi sesekelompok sebelumnya. Fermentasi suapan sesekelompok telah dijalankan mengikut kadar pertumbuhan spesifik berlainan yang diperlukan dan kesannya kepada kepekatan akhir sel yang diperolehi, hasilan dan produktiviti telah dianalisa. Prestasi fermentasi suapan sesekelompok dengan kawalan kepekatan glukosa kultur pada tahap tertentu untuk peningkatan pengkulturan *B. subtilis* juga telah dikaji.

Di antara kajian sumber karbon yang berlainan, kadar pertumbuhan yang paling tinggi telah diperolehi apabila glukosa digunakan sebagai sumber karbon. *B. subtilis* juga berupaya hidup dalam sukrosa dan kanji sago, ini menunjukkan bakteria ini berkebolehan untuk merembeskan enzim invertase dan enzim amilolitik. Kepekatan akhir sel yang diperolehi dalam pengkulturan yang menggunakan glukosa, kanji terhidrolisis, sukrosa dan kanji sago semuanya pada kepekatan 20 g/L ialah 15.66 g/L, 9.71 g/L, 9.16 g/L dan 5.72 g/L masing-masing. Ini memberikan hasilan sel ($Y_{x/s}$) masing-masing iaitu 1.08 g/g, 0.85 g/g, 0.50 g/g dan 0.32 g/g berdasarkan penggunaan glukosa, kanji terhidrolisis, sukrosa dan kanji sago.

Kepekatan awal sukrosa didapati mempunyai kesan perencatan terhadap pertumbuhan *B. subtilis*. Pertumbuhan meningkat dengan peningkatan kepekatan awal glukosa kultur dari 15 g/L hingga 20 g/L. Penurunan sedikit dalam pertumbuhan bakteria diperhatikan pada 35 g/L glukosa dan penurunan yang ketara dalam pertumbuhan bakteria ini berlaku pada kepekatan glukosa yang tinggi (50 g/L). Pertumbuhan *B. subtilis* juga amat dipengaruhi oleh pH kultur. Pertumbuhan yang paling tinggi dari segi sel hidup telah diperolehi apabila kawalan pH kultur pada 7 sepanjang proses pengkulturan dijalankan.

Dalam fermentasi suapan sesekelompok yang kadar pertumbuhan spesifiknya, (μ), dikawal, kepekatan akhir sel yang lebih tinggi (1.93×10^{10} cfu/mL) telah diperoleh dalam fermentasi yang mana μ dikawal pada 0.30 j^{-1} berbanding dengan pengkulturan μ pada 0.20 j^{-1} dengan kepekatan akhir sel yang diperolehi hanyalah 3.52×10^{9} cfu/mL. Peningkatan seterusnya bagi prestasi pengkulturan telah diperoleh dalam fermentasi suapan sesekelompok dengan kawalan tahap glukosa dalam kultur sepanjang proses fermentasi. Kepekatan akhir sel (3.50×10^{10} cfu/mL) telah diperoleh apabila glukosa dalam kultur dikekalkan pada tahap yang sangat rendah (0.20 g/L). Penurunan dalam pertumbuhan diperhatikan apabila glukosa dalam kultur dikekalkan pada tahap yang tinggi, lebih daripada 1 g/L, mencadangkan bahawa glukosa mempunyai kesan penekanan terhadap pertumbuhan *B. subtilis*.

Kesimpulannya, teknik fermentasi yang terbaik untuk mencapai pengkulturan sel *B. subtilis* yang berketumpatan tinggi ialah fermentasi suapan sesekelompok yang mana glukosa dalam kultur dikekalkan pada tahap yang sangat rendah (0.2 g/L), manakala pH kultur dikekalkan pada 7 sepanjang proses fermentasi. Dengan menggunakan teknik pengkulturan ini, sel hidup menunjukkan peningkatan sebanyak 324% berbanding dengan fermentasi sesekelompok. Kaedah pengkulturan ini mungkin boleh digunakan dalam aplikasi perindustrian untuk menghasilkan *B. subtilis* yang berkuantiti besar untuk digunakan sebagai bahan probiotik dalam industri akuakultur terutamanya dalam mengawal kualiti air kolam udang.

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I certify that an Examination Committee met on 24th March 2006 to conduct the final examination of Lew Kok Cheong on his Master of Science thesis entitled "Optimization of Conditions for High Cell Density Cultivation of *Bacillus subtilis*" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regilations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science.
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DECLARATION

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

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LEW KOK CHEONG

Date:

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