UNIVERSITI PUTRA MALAYSIA

A SIMPLIFIED AND SELECTIVE TECHNIQUE FOR THE DIRECT RECOVERY OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE FROM UNCLARIFIED YEAST FEEDSTOCK

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By

CHOW YEN MEI

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

September 2005

Specially dedicated

To LORD my GOD
"My success is the glory of your name"

To my lovely mother

Chan Tai Thai
"My success is only for you"

To my lovely friend

Michael Ling Tung Lien
"Thanks for your spiritual support and caring"

To my siblings

Yuen Kuan & Lip Hor
"Thanks for your lovely caring and support"

To my dear friends
"Thanks for your support"

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

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September 2005

Chairman : Ling Tau Chuan, PhD

Faculty : Engineering

The development of a simplified and rapid technique for the selective recovery of proteins from Bakers' yeast was undertaken. Purification of Glucose-6-phosphate dehydrogenase (G6PDH) from *Saccharomyces cerevisiae* was chosen demonstrate of this principle. Cell disruption is a mandatory first step in the recovery of intracellular products. The influence of the operational parameters of Dyno bead mill on the release of G6PDH and proteins study were studied, and demonstrated that 45 Lhr^{-1} flow rate, 85% (v/v) bead volume, 10 ms^{-1} tip speed are optimum condition for protein released. The comparative study on expanded beds ion-exchange and affinity adsorbents for the purification of G6PDH from crude feed-stock was conducted. The use of Streamline DEAE (ρ~1.2 gmL^{-1}) and UpFront Cibacron Blue 3GA (ρ~1.5 gmL^{-1}) in adsorption of G6PDH from Bakers' yeast is adapted in this study. Hydrodynamic performance testing indicated that UpFront adsorbent providing a more stable fluidized bed than Streamline adsorbent does. Due to consisting higher density, higher flow rate (225 to 450 $cmhr^{-1}$) and biomass concentration (up to 30% w/v) could be applied on expanded UpFront adsorbent bed. In contrast, Streamline adsorbent only able to afford a range of flow rate, from 164.2 to 248.3 $cmhr^{-1}$ and

biomass concentration up to 20% w/v. For dye affinity system, there is a light reduction on dynamic binding capacity of BSA (11.1% to 27.8%) only as compare with ion-exchange system (43.1% to 68.6%) when the adsorption was conducted in the presence of intact yeast cells. The adsorption characteristics of the affinity system were not greatly altered in the presence of cells in contrast to the results from a less selective ion-exchange system. It was demonstrated that dye affinity chromatography had provided a higher purification factor (3.9 to 8.2) with as compared with ion-exchange chromatography (2.7 to 4.1) in G6PDH recovery.

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

SUATU TEKNIK YANG MUDAH DAN MEMILIH BAGI PROSES PEMULIHAN TERUS GLUCOSE-6-PHOSPHATE DEHYDROGENASE DARIPADA BEKALAN YIS YANG TIDAK DIJELASKAN

Oleh

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Pembinaan suatu teknik yang mudah dan pantas untuk memulihkan protein daripada yis telah diusahakan. Pemulihan Glucose-6-phosphate dehydrogenase (G6PDH) daripada *Saccharomyces cerevisiae* telah dipilih sebagai tunjukan pada dasarnya. Pemecahan sel microorganisma merupakan langkah pertama yang diperlukan dalam proses pemulihan enzim dalaman. Parameter operasi yang mempengaruhi proses pemecahan untuk melepaskan enzim (G6PDH) dan protein dalam loji Dyno telah dikaji, dan menunjukan bahawa kadar pengaliran, 45 Lhr^{-1}, isipadu manik, 85% (v/v), kelajuan penghasut, 10 ms^{-1} merupakan parameter operasi yang optimum bagi pelepasan protein. Kajian perbandingan dalam penggunaan penjerap "ion-exchange" dan "affinity" dalam proses penulenan G6PDH daripada bekalan aslinya telah dijalankan. Penggunaan penjerap Streamline DEAE (ρ~1.2 gmL^{-1}) and UpFront Cibacron Blue 3GA (ρ~1.5 gmL^{-1}) dalam proses penjerapan G6PDH daripada yis telah disesuaikan dalam kajian ini. Pemeriksaan perlaksanaan hidrodinamik telah menunjukkan bahawa penjerap UpFront dapat memberikan lapisan mengembang yang lebih mantap jika dibanding dengan penjerap Streamline. Oleh sebab penjerap UpFront mengandungi ketumpatan yang lebih tinggi, dan seterusnya membolehkan

kadar pengaliran (225 hingga 450 $cmhr^{-1}$) dan bio-jisim (30% w/v) yang lebih tinggi dapat disesuaikan dalam turus lapisan mengembang. Sebaliknya, penjerap Streamline hanya dapat mengatasi kadar pengaliran, dari 164.2 hingga 248.3 $cmhr^{-1}$ dan bio-jisim sebanyak 20% w/v sahaja. Bagi sistem "dye affinity", kapasiti penjerapan dalam lapisan mengembang hanya mengalami pengurangan yang sedikit sahaja (11.1% hingga 27.8%) jika dibanding dengan sistem "ion-exchange" (43.1% hingga 68.6%) apabila penjerapan dijalankan dalam keadaan kehadiran keseluruhan sel yis (tanpa pemecahan). Ciri-ciri penjerapan bagi sistem "affinity" hanya mengalami perbezaan yang minimum dan ia amat berbeza daripada sistem "ion-exchange" yang kurang memilih. Kromatografi "dye affinity" telah menunjukkan faktor penulenan yang lebih tinggi (3.9 hingga 8.2) jika dibandingkan kromatografi "ion-exchange" (2.7 hingga 4.1) dalam proses pemulihan G6PDH.

ACKNOWLEDGEMENTS

I wish to express my gratitude and appreciation to my Supervisory Committee: Dr. Ling Tau Chuan, Prof. Dr. Arbakariya Ariff and Dr. Tey Beng Ti for their patience, constructive suggestions and encouragement throughout the years of my master study. I would like to express my thanks to members and staffs in Unit Fermentation Laboratory, Institute Bioscience, UPM.

My special thanks go to all my friends, especially Hii Siew Ling, Lim Chin Ming, Tan Yan Peng and Liew Shiao Tsui. You all had given me a strong spirit to go through the years. With special recognition to: my sweet mom, sister, brother and Ling Tung Lien. You did a great job of raising me along the years.

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LIST OF ABBREVIATIONS

AMP	Adenine Monophosphate
ATP	Adenine Triphophate
BSA	Bovine Serum Albumin
CB	Cibacron Blue
CIP	Clean-in-place
C_s	Adsorbed mg/mL of BSA per g of adsorbent at equilibrium
C_m	BSA concentration at equilibrium, $mgmL^{-1}$
DEAE	Diethylaminoethyl
DNA	Deoxyribonucleic Acid
EBA	Expanded bed adsorption
G6PDH	Glucose-6-phosphate dehydrogenase
HETP	Height equivalent to a theoretical plate
HCl	Hydroxide chloride
HEPES	N-2-Hydroxyethylpiperazine-N'-2-ethaneslfonic acid
MES	2-(N-Morpholino) ethanesulfonic acid
MOPS	3-(N-Morpholino) propanesulfonic acid
NaCl	Sodium chloride
Na_2CO_3	Sodium Carbonate
NaOH	Sodium Hydroxide
$NaHCO_3$	Sodium Bicarbonate
NAD^+	Ion Nicotinanide Adenine Dinucleotide
NADP	Nicotinanide Adenine Dinucleotide Phosphate
NADPH	Nicotinanide Adenine Dinucleotide Phosphate Dehydrogenase

w/v	Wet weight per volume
v/v	volume per volume

CHAPTER 1

INTRODUCTION

The success in simplifying industrial practice with the latest biotechnology is the ultimate responsibility of the bio-process and bio-chemical engineering profession. The advances in recovery, separation, and purification techniques used in downstream processing may play a crucial role in the development of large scale biotechnology. Downstream operations are crucial stages in terms of maintaining product characteristics and activity aiming at high yield and purity and cost saving. A dominant cost element in the production of the biological origin can be downstream operations. This is particularly true when the application of the product demands a very high level of product purity. For an example the cost ratio between fermentation and product recovery is approximately 60:40 for older antibiotics produced by fermentation. For newer antibiotics, third and fourth generation, the ratio is reversed to 40:60 fermentation to recovery. For recombinant DNA fermentation products such as therapeutic proteins the downstream purification accounts for 80 to 90% of the process costs (Dwyer, 1984). Downstream process unit operations included primary separation, product purification and product isolation.

Disruption of the outer envelope of microbial cells is an essential step in the recovery of microbial products such as intracellular products. Mechanical methods are generally applicable for cell disruption, while the non-mechanical methods may be very effective but are restricted to special cases (Gaver & Huyghebaert, 1990). From an industrial applications point of view, only cell disruption technology based on

mechanical technique has its potential use (Chisti & Moo-Young, 1986). Probably because of the high capital, operating costs and complexity of separation processes (especially disintegrated by chemical mean) for large scale recovery of intracellular products, non-mechanical methods lost its industrial potential use. Cell disruption in bead mill is considered as one of the most efficient techniques for the physical cell disruption (Darbyshire, 1981). A continuous protein production process involving disruption of 10% dry weight bakers' yeast and brewers' yeast in a 5 liter nominal capacity bead mill has been reported (Hedenskog & Morgan, 1973). A wide range of bacteria (*E.coli, Bacillus sphaericus, Lactobacillus confuses, Brevibacterium ammmoniagenes* and *Bacillus subtilis*) and fungus (*S. cerevisiae, S. carlsbergensis, C. boidinii, C. utilis*) have been disrupted in bead mills (Chisti & Moo-Young, 1986).

The traditional primary purification of the target molecule has been addressed by adsorption chromatography using a conventional packed bed of adsorbent. Before being further purified by traditional packed bed chromatography, centrifugation and microfiltration are needed, in order to obtain a particle free solution. However, microfiltration has its drawbacks. The flux of liquid per unit membrane area is often dramatically decreased, even though microfiltration could provide a particle free solution. During the filtration process, fouling of the microfiltration membrane is another critical problem that significantly adds to the operational cost. Normally, the combined use of centrifugation and microfitration may result in long process time and cause significant additional costs for equipment maintenance. It also brought in significant product loss due to product deterioration, especially the intracellular products. Consequently, direct adsorption from crude feed-stocks potentially offers

significant reduction of process time and costs compared to traditional processes (Chase, 1994; Chase & Draeger, 1992).

Expanded bed adsorption (EBA) is a technique which was created to circumvent all the drawbacks of conventional downstream processing. The process steps of clarification, concentration and initial purification can combine into one unit operation by using EBA technique. This leads to providing increased process economy due to a decreased number of process steps, increased yield, shorter overall process time (Suding & Tomusiak, 1993), reduced labor cost (Batt *et al.*, 1995) and reduced running cost and capital expenditure (Schmidt *et al.*, 1993). Further more, EBA technique is not only limited at laboratory process scale, it is available for scale-up and potentially offer industrial scale process.

Expanded bed procedures are becoming increasingly popular in bio-separation as a way of avoiding the need for clarification techniques such as centrifugation and filtration (Chang *et al.*, 1995; McCreath *et al.*, 1995). One step unit operation of capture target molecules from crude feed-stock may reduce products degradation and avoiding bio-product handling problems. Expanded bed adsorption has postulated to be a versatile tool that can be applied on cells commonly used source materials. Successfully processing by expanded bed adsorption has been reported for *E. coli* homogenate (Daniels *et al.*, 1996; Ollivier *et al.*, 1996), *E. coli* lysate (Daniels *et al.*, 1996; Johansson *et al.*, 1996), yeast cell homogenate (Chang *et al.*, 1995; Chang & Chase, 1996), secreted products from yeast (Blomqvist *et al.*, 1996; Gellissen *et al.*, 1996; Zurek *et al.*, 1996), whole hybridoma fermentation broth (Born *et al.*, 1996; Lutkemeyer *et al.*, 1996), myeloma cell culture (Jagersten *et al.*, 1996), whole

mammalian cell culture broth (Beck *et al.*, 1996; Zapata *et al.*, 1996) milk, and animal tissue extracts (Garg *et al.*, 1996).

The present work is focused on the development of a simplified and rapid technique for the selective recovery of intracellular enzyme from bakers' yeast. G6PDH was chosen as reference enzyme due to its high level present in Bakers' yeast and commercial value. The Dyno bead mill had been used in this work for effective release of G6PDH from yeast. Ion exchange and dye affinity chromatography has been applied in this study. The matrices are Streamline DEAE and UpFront Cibacron Blue 3GA. UpFront Fastline20 was used as a contactor to recover glucose 6-phophate dehydrogenase (G6PDH) from Bakers' yeast homogenate. The performance of an anion exchanger, Streamline DEAE, (ρ~1.2 gmL^{-1}) was studied and compared with UpFront adsorbent (ρ~1.5 gmL^{-1}) immobilized with Cibacron Blue 3GA. The applicability and practicability of an innovative contactor characterized with mechanized stirring flow distribution was explored.