EXPANDED BED ANION EXCHANGE ADSORPTION FOR THE PURIFICATION OF BACTERIOPHAGE M13

MAYAMARNI SHAMSU ZAMAN

FK 2008 97
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By

MAYAMARNI SHAMSU ZAMAN

MARCH 2009
DEDICATION

To my beloved parents, husband, daughter, siblings and friends.
Abstract of thesis presented to Senate of Universiti Putra Malaysia in fulfilment of the requirements of the degree of Master of Science

EXPANDED BED ANION EXCHANGE ADSORPTION FOR THE PURIFICATION OF BACTERIOPHAGE M13

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MARCH 2009

Chairman : Ling Tau Chuan, PhD
Faculty : Engineering

The filamentous bacteriophage M13 can be used to display foreign peptides and proteins, allowing the construction of therapeutic, vaccine development, diagnostic and technological tools of broad utility. The current technique used to purify bacteriophage M13 by using conventional method but the long processing time and large unit requirement makes this method not economical. The present work focused on the importance of performing an expanded bed anion exchange adsorption experiments for purification of bacteriophage M13 and to identify the fluidization properties of Streamline DEAE. UpFront FastLine™20 (ID = 20 mm) column from UpFront Chromatography was used as a contactor and Streamline™ DEAE (ρ = 1.2 g/cm³) from GE Healthcare Lifesciences was used as the anion exchanger. 50 mM of Tris buffer at pH 7.5 was used as the equilibration buffer while 2.0 M of NaCl in 50 mM of Tris buffer was used as the elution buffer.
The influence of the operational parameters of UpFront Fastline™ 20 was studied and demonstrated that 13.03 mL/min flow rate, bed height $H_0 = 17.5$ cm are optimum conditions for protein released. The dynamic binding capacity of Streamline DEAE for the bacteriophage (M13) was found to be 63 mL adsorbent at velocity of 250 cm h$^{-1}$. Due to higher density, higher flow rates (200 to 350 cm h$^{-1}$) and biomass concentrations (5% to 20% ww/v) could be applied on expanded bed adsorption. The developed breakthrough curve was measured at different bed height and different biomass concentrations. The accuracy of the model predictions was improved by employing information on the axial variations in the bed voidage, liquid phase axial dispersion and dynamic binding capacity for the experiment. Breakthrough curves for bacteriophage M13 were compared and the process was more efficient at a bed expansion degree of 2.0 (bed voidage of 0.7). The performance of two methods were evaluated and analyzed. Purification of the M13 bacteriophage by precipitation, centrifugation and microfiltration yielded a low recovery percentage (36.07%) but with high purity while purification of the M13 bacteriophage by expanded bed anion exchange adsorption yielded a high recovery percentage (82.86%) but with low purity. Additionally, the total processing time of the expanded bed adsorption process has been shortened by 8 times compared to that of the conventional method.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENULENAN DARIPADA BAKTERIOFAJ M13 DENGAN PENGGUNAAN KAEDAH PENJERAPAN LAPISAN TERKEMBANG

Oleh

MAYAMARNI SHAMSU ZAMAN

MAC 2009

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Bakteriofaj (M13) boleh digunakan untuk mempamerkan protin dan peptide asing bagi memudahkan pembangunan bagi terapi, pembinaan vaksin, mengenali penyakit dan alatan teknologi bagi keperluan global. Teknik yang biasa digunakan untuk penjernihan bakteriofaj (M13)ialah dengan menggunakan kaedah tradisional tetapi kaedah ini kurang berhemat kerana mengambil masa yang lama dan keperluan unit yang besar. Kerja yang dilakukan adalah tertumpu pada kepentingan penggunaan penjerapan lapisan terkembang untuk penjernihan bagi bakteriofaj (M13) dan untuk mengenal pasti sifat bendalir bagi penjerap garis arus DEAE. UpFront Fastline™ 20 (diameter 20 mm) dari UpFront Chromatography telah digunakan sebagai penghubung dan penggunaan
penjerap garis arus DEAE ($\rho = 12$ gl$^{-1}$) dari GE Healthcare Lifesciences sebagai penukar anion. Penimbal keseimbangan pada pH 7.5 yang digunakan ialah 50mM Tris penimbal. Sementara 2.0 M NaCl di dalam 50 mM Tris penimbal pada pH 2.2 digunakan sebagai penimbal elutan. Parameter operasi yang mempengaruhi proses penjerapan dengan penggunaan UpFront Fastline telah dikaji dan menunjukkan kadar pengaliran ialah 13.03 mL/min pada ketinggian turus 17.5 cm merupakan parameter operasi yang optimum bagi pelepasan protin. Kapasiti pengikatan dinamik bagi penjerap garis arus DEAE untuk bakteriofaj (M13) telah dikenalpasti pada 63 mL penjerap dengan kadar pengaliran 250 cm/h. Oleh sebab itu kadar pengaliran (200 hingga 300 cm/h) dan bio-jisim (5% sehingga 20%) dapat disesuaikan dalam turus lapisan mengembang. Pembinaan lengkung bulus telah dikaji pada perbezaan ketinggian turus dan perbezaan kepekatan bio-jisim. Lengkung bulus bagi bakteriofaj (M13) telah dibandingkan dan didapati pada tahap peningkatan turus 2.0 (lompangan lapisan 0.7) adalah lebih berkesan berbanding dengan tahap peningkatan yang lain. Pencapaian bagi dua kaedah telah dianalisis dan dikembangkan. Penjernihan bagi bakteriofaj (M13) dengan menggunakan kaedah mendakan, pengemparan dan penurasan mikro mempunyai tahap peratusan pemulihan yang rendah (36.07%) tetapi dengan faktor penulenan yang tinggi manakala bagi kaedah penjerapan lapisan terkembang pula, kadar peratusan pemulihan yang tinggi (82.86%) tetapi dengan faktor penulenan yang rendah. Jumlah masa pemprosesan bagi kaedah penjerapan lapisan terkembang adalah lapan kali lebih cepat berbanding dengan kaedah tradisional.
ACKNOWLEDGEMENTS

First of all, with the humble gratitude, I would like to express my thanks and deepest praise to The Most Gracious and Most Merciful ALLAH Who has given me all strength, faith, confidence and patience to complete this project.

It would be my pleasure to express my most sincere thanks to my supervisor Dr. Ling Tau Chuan and members of my supervisor committee Dr. Tey Beng Ti, Dr. Tan Wen Siang and Dr. Wan Muhamad for their advice, understanding, support, criticism, guidance and co-operation in completing this thesis.

Finally, I would like to thank Universiti Putra Malaysia, my family and all my friends especially my beloved parents, Shamsu Zaman Bin Muhamad and Che Hapsah Binti Seman, my husband, Mohd Shukri Ibrahim and my sweet daughter, Nur Ayuni Qistina for their support. I pray to Allah that He rewards each a beautiful reward.
I certify that an Examination Committee has met on 18 September 2008 to conduct the final examination of Mayamarni Shamsu Zaman on her Master of Science thesis entitled “Expanded Bed Anion Exchange Adsorption for the Purification of Bacteriophage M13” in accordance with 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 except for quotations and citations which have been duly acknowledge. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

__________________________________________
MAYAMARNI SHAMSU ZAMAN

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Relative expansion at different flow velocities (50 to 350 cm hr$^{-1}$) of Streamline DEAE matrix with different biomass concentration, binding buffer (♦), 5% (■), 10% (▲), 15% (●) (w/v) wet weight per volume in Tris-HCl buffer in a Fastline20 column with 13 cm of sedimented bed height.

Relative expansion at different flow velocities (50 to 350 cm hr$^{-1}$) of Streamline DEAE matrix with different biomass concentration, binding buffer (♦), 5% (■), 10% (▲), 15% (●) (w/v) wet weight per volume in Tris-HCl buffer in a Fastline20 column with 10 cm of sedimented bed height.

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Relative voidage at different velocities of Streamline DEAE matrix with different biomass concentration, binding buffer (♦), 5% (■), 10% (▲), 15% (●) (w/v) wet weight per volume in Tris-HCl buffer in Fastline20 column with 13 cm of sedimented bed height.

Relative voidage at different velocities of Streamline DEAE matrix with different biomass concentration, binding buffer (♦), 5% (■), 10% (▲), 15% (●) (w/v) wet weight per volume in Tris-HCl buffer in Fastline20 column with 10 cm of sedimented bed height.

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Effect of the phage concentration at different bed height on expanded bed adsorption onto Streamline DEAE. 17.5 cm (▲), 13 cm (●) and 10 cm (■), (63 mL settled volume corresponding to 17.5 cm settled bed height, 36 mL settled volume, corresponding to 10 cm and 46.8 mL settled volume, corresponding to 13 cm settled bed height) were loaded into the contact and equilibrated with Tris buffer in expanded bed mode. The feedstock application was continued until a desired adsorbent saturation state (C/C₀ = ~1) was reached. The dynamic binding capacity at C/C₀ = 0.1 for various bed height are 2.71x10⁹ pfu/mL (17.5 cm), 1.89x10⁹ pfu/mL (13 cm) and 1.3x10⁹ pfu/mL (10 cm).

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29 Flowchart of purification of M13 bacteriophage. Left hand panel shows the steps involved in precipitated, centrifugation and microfiltration, while right hand panel shows the stages in expanded bed adsorption. Parameters listed are for feedstock volume of 1 litre.
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<td>Bovine Serum Albumin</td>
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<tr>
<td>CIP</td>
<td>Clean-in-place</td>
</tr>
<tr>
<td>$C_m$</td>
<td>M13 concentration at equilibrium, $\text{mg mL}^{-1}$</td>
</tr>
<tr>
<td>DEAE</td>
<td>Diethylaminoethyl</td>
</tr>
<tr>
<td>$dH_2O$</td>
<td>Distilled water</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethyl formamide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
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<tr>
<td>EBA</td>
<td>Expanded bed adsorption</td>
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<tr>
<td>HCl</td>
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<tr>
<td>IPTG</td>
<td>Isopropyl-beta-D-thiogalactopyranoside</td>
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<td>Luria-Bertani Agar</td>
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<tr>
<td>LB Broth</td>
<td>Luria-Bertani Broth</td>
</tr>
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<td>MgCl$_2$.6H$_2$O</td>
<td>Magnesium dichloride hexahydrate</td>
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<td>NaCl</td>
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<tr>
<td>Na$_2$CO$_3$</td>
<td>Sodium Carbonate</td>
</tr>
<tr>
<td>NaOH</td>
<td>Sodium Hydroxide</td>
</tr>
<tr>
<td>PEG</td>
<td>Polyethylene glycol</td>
</tr>
<tr>
<td>pfu</td>
<td>plaque forming unit</td>
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<tr>
<td>SDS-PAGE</td>
<td>Sodium dodecyl sulfate-polyacrylamide gel electrophoresis</td>
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<tr>
<td>v/v</td>
<td>Volume per volume</td>
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<td>w/v</td>
<td>Wet weight per volume</td>
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<td>5-bromo-4-chloro-3-indocyl-β-D-galactopyranoside</td>
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CHAPTER 1

INTRODUCTION

Production of proteins by genetically engineered microorganisms, yeast and animal cells have become a very important technique for the preparation of bio-process and biochemical engineering profession. The protein is necessary to obtain a pure, defined substance of guaranteed purity and potency before it can be used as pharmaceutical. The feedstocks from which proteins are prepared are generally complex, containing solid and dissolved biomass of various sizes and molecular masses. Therefore, the purification process cannot be completed with a single step but it is usually completed by a combination of several unit operations that account for the different separation necessities.

M13 is a filamentous bacteriophage composed of a circular single stranded DNA (ssDNA) which is 6407 nucleotides long encapsulated in approximately 2700 copies of the major coat protein P8 and copped with 5 copies of two different minor coat proteins (P9, P6, P3) on the ends. M13 plasmids are used for many recombinant DNA processes and the virus has also been studied for its uses in nanotechnology.
The filamentous bacteriophage M13 can be used to display foreign peptides and proteins, allowing for the construction of therapeutic, diagnostic and technological tools of broad utility. The relationship between bacteriophages and their bacterial hosts is very important in the context of the food fermentation industry. Other uses of bacteriophage include spray application in horticulture for protecting plants and vegetable produce from decay and the spread of bacterial disease and as biocide for environmental surface and as a preventative treatment for catheters and medical devices prior to use in clinical settings.

Downstream processing is challenging because the products must be concentrated from a very dilute condition in the feedstock and purified from other protein impurities having very similar properties. The feedstock is generally very complex in nature and contains various sizes of dissolved solid, biomass and cell debris. In the conventional way, a final product of guaranteed purity and potency can only be achieved with a combination of several unit operations (Anspach et al., 1999). prior to the concentration and fermentation broth or cell homogenates by centrifugation or filtration. In the large scale process of protein recovery, these clarification methods often show limitations in practice (Lee, 1989).

The traditional primary purification of the target molecule has been addressed by adsorption chromatography using a conventional packed bed of adsorbent. Before further purified by traditional packed bed chromatography, centrifugation and 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 (Johansson et al., 1996). Accordingly, direct adsorption from crude feedstocks potentially offers significant reduction of process time and costs compared to traditional processes (Chase and Drager, 1992; Chase, 1994). Therefore, it is obvious that elimination of the clarification step will significantly simplify and improve the purification process.

The development of molecular biology techniques has enabled researchers to produce large quantities of biologically important molecules from bacteria, plants and animals. Although this ability has revolutionized the production and delivery of pharmaceutical and therapeutic products but one problem has remained. To accomplish the particulates removal, centrifugation and microfiltration are accepted as method in the biotechnology industry, but the long processing time and large unit requirement makes this method not economical.

Expanded bed adsorption (EBA) is one of the suitable methods for the protein separation from the cultivation broth (Kaezmarski et al., 2004). Expanded bed procedures are becoming increasingly popular in bioseparation as a way of avoiding the need for clarification techniques such as centrifugation and filtration (Chang et al., 1995). The performance of protein adsorption in expanded bed is obviously nonuniform and complex along the column since EBA is a special chromatography technique with perfect classification of adsorbent particles in the column (Junxian et al., 2005).
The formation of a perfectly classified fluidized bed of adsorbent particles in the particular feedstock is the key of successful expanded bed adsorption processes, from which the target molecule is to be isolated (Reichert et al., 2001). The efficiency of the adsorption can be represented by an adsorbate breakthrough curve (BTC), or more specifically by the amount of adsorbate loaded onto the column until a defined breakthrough concentration in the column effluent is reached (Bruce and Chase, 2002; Pai et al., 2000; Hansen and Mollerup, 1998). Furthermore, the EBA technique is not only limited at laboratory process scale, it is available for scale-up and potentially offer industrial scale process. One step unit operation of capture target molecules from crude feedstock may reduce products degradation and avoid bio-product handling problem. Expanded bed adsorption has also proved to be a versatile tool that can be applied on all commonly used source materials (Clemmitt and Chase, 2000a).

Successful processing by expanded bed adsorption at large scale for E. coli homogenate has been reported (Clemmitt and Chase, 2000; Tan et al., 2005), yeast cell homogenates (Smith et al., 2002; Chow et al., 2005; Ling et al., 2005; Vergnault et al., 2007), orotic acid from whey (Baumeister et al., 2003), whole mammalian cell culture broth (Batt et al., 1995), milk and animal tissue extracts (Noppe, 1996), plant materials (Bertrand et al., 1998).
The present work is focused on the development of a simplified and rapid technique for the purification of bacteriophage M13. Ion exchange chromatography has been applied in this study. Ion exchange is a versatile technique of separation in which it has been utilized in the separation of ionic materials from each other and also in the separation of completely nonionic mixtures. Ion exchange is a low energy process ideally suited for treating water soluble, ionic molecules. UpFront FastLine™ 20 was used as a contactor to recover protein from bacteriophage M13. The performance of an anion exchanger, Streamline DEAE, ($\rho = 1.2$ g/mL) as adsorbent was studied.
Objectives

The objectives of this study are:

1. To identify the fluidization properties of Streamline DEAE (ρ \approx 1.2 \text{ gmL}^{-1}) and degree of expansion of Streamline DEAE (ρ \approx 1.2 \text{ gmL}^{-1}) in Fastline\textsuperscript{TM} 20 column.

2. To optimize a process capable of directly extracting protein at high purity and yield from an unclarified feedstock using expanded bed adsorption.

3. To investigate and compare the efficiency of ion-exchange chromatography on the capture of bacteriophage M13 by utilizing expanded bed adsorption technique in Fastline\textsuperscript{TM} 20 column to the conventional method.
REFERENCES


Finette, G. M. S., Mao, Q., and Hearn, M. T. W. (1997) *Comparative studies on the isothermal characteristics of proteins adsorbed under batch equilibrium conditions to ion-exchange, immobilized metal ion affinity and dye affinity matrices with different ionic strength and temperature conditions,* Journal of Chromatography A, 763: 71-90


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