UNIVERSITI PUTRA MALAYSIA

THE INFLUENCE OF M13 BACTERIOPHAGE ON PROTEIN ADSORPTION USING ANION EXCHANGE EXPANDED BED CHROMATOGRAPHY

MASNIZA BT MOHAMED @ MAHMOOD

THE INFLUENCE OF M13 BACTERIOPHAGE ON PROTEIN ADSORPTION USING ANION EXCHANGE EXPANDED BED CHROMATOGRAPHY

By

MASNIZA BT MOHAMED @ MAHMOOD

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

May 2006

KESAN KEHADIRAN BAKTERIOFAJ M13 DALAM PENJERAPAN PROTEIN MENGGUNAKAN KROMATOGRAFI PENJERAPAN LAPISAN TERKEMBANG

Oleh

MASNIZA MOHAMED @ MAHMOOD

Tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

Mei 2006

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

THE INFLUENCE OF M13 BACTERIOPHAGE ON PROTEIN ADSORPTION USING ANION EXCHANGE EXPANDED BED CHROMATOGRAPHY

By

MASNIZA BT MOHAMED @ MAHMOOD

May 2006

Chairman : Ling Tau Chuan, PhD

Faculty : Engineering

The adsorption of a model protein Bovine Serum Albumin (BSA) from unclarified feedstock containing M13 bacteriophage and its host cells has been explored. The matrix used was Streamline Diethylaminoethyl (DEAE) as an anion exchanger (ρ=1.2 g/cm^3) supplied by Amersham Pharmacia Biotechnology. The UpFront Chromatography column (20 mm i.d) was used as the Expanded Bed Adsorption (EBA) contactor. In this study, M13 bacteriophage carrying an insert (C-WSFFSNI-C) was propagated in bacteria *Escherichia coli* ER2738.

Batch adsorption experiment method using Streamline DEAE as adsorbate was also done to determine the influence in the presence of M13 Bacteriophage and its host cell (*E. coli*) concentrations at various biomass (0%-15% w/v). The maximum protein adsorption of Streamline DEAE was investigated using batch adsorption. The maximum protein adsorption capacity, q_{max}, and the dissociation constant, K_d were determined. Moreover, the operating parameters of EBA operation such as the

degree of the bed expansion and dynamic binding capacity of different settled bed heights under various biomass concentrations were investigated. In this study, the maximum protein binding capacity of Streamline DEAE, q_{max} was achieved at 230.03 mg/ml adsorbed. At the lowest M13 bacteriophage and its host cells concentration (5% w/v), the dissociation constant, $K_{d,}$ of this operation is 36.87 mg/ml. For the batch binding experiment using 10% biomass concentration, q_{max} of 130.01 mg/ml and a K_d of 16.12 mg/ml was determined. Further increase of the biomass concentration to 15% has caused the q_{max} of the adsorbent reduced to half of that 5% (w/v) biomass concentration, and the K_d determined is only 9.45 mg/ml. It can be concluded that the higher percentage of M13 bacteriophage and its host cells present in the feedstock, the lower q_{max} can be achieved. From the analysis of breakthrough curve at 10% which C/Co= 0.1, the dynamic binding capacities of various settled bed height under various biomass concentrations were determined. The value varies between the three models (10 cm, 13 cm and 15 cm settled bed height column) and the highest dynamic binding capacity was obtained at the lowest percentage of M13 bacteriophage and its host cells in the feedstock at the highest settled bed height (15 cm).

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

KESAN KEHADIRAN BAKTERIOFAJ M13 DALAM PENJERAPAN PROTEIN MENGGUNAKAN KROMATOGRAFI PENJERAPAN LAPISAN TERKEMBANG

Oleh

MASNIZA BT MOHAMED @ MAHMOOD

Mei 2006

Pengerusi : Ling Tau Chuan, PhD

Fakulti : Kejuruteraan

Penjerapan model protein, Bovine Serum Albumin (BSA) daripada bahan utama yang tercemar mengandungi Bakteriofaj M13 dan juga sel perumahnya telah dikaji. Matrik yang digunakan ialah Streamline Diethylaminoethyl (DEAE), (ρ=1.2 g/m^3) yang boleh diperolehi dari Amersham Pharmacia Biotechnology. Kolum UpFront (20 mm i.d) digunakan sebagai kolum untuk Penjerapan Lapisan Terkembang. Dalam kajian ini, M13 bakteriofaj yang membawa sisipan (C-WSFFSNI-C) telah dibiakkan di dalam bakteria *Escherichia coli* ER2738.

Ujikaji penjerapan berkumpulan juga dilakukan menggunakan Streamline DEAE untuk mengesan pengaruh kepekatan bakteriofaj M13 dan sel perumahnya (*E. coli*) di dalam kepekatan biomas yang berbeza (0%-15% w/v). Penjerapan maksima protein dilakukan menggunakan pejerapan berkelompok. Kapasiti penjerapan maksima, q_{max} dan juga nilai tetap pelekangan, K_d dikenalpasti. Selain daripada itu,

parameter berfungsi untuk operasi EBA (Penjerapan Lapisan Terkembang) seperti darjah pengembangan lapisan dan juga kapasiti ikatan dinamik pada kepekatan biomas dan tinggi lapisan termendak yang berbeza. Dalam kajian ini, ikatan protein maksima untuk Streamline DEAE, diperolehi ialah 230.03 mg/ml. Pada kepekatan bakteriofaj M13 dan sel perumah yang paling rendah, nilai tetap pelekangan, K_d ialah 36.87 mg/ml. Untuk penjerapan berkelompok meggunakan 10% kepekatan biomas, q_{max} ialah 130.1 mg/ml dan nilai K_d ialah 16.12 mg/ml diperolehi. Dengan menaikkan nilai kepekatan biomas kepada 15% telah menyebabkan q_{max} penjerap berkurang separuh daripada 5% kepekatan biomas, nilai K_d yang kecil iaitu 9.45 mg/ml. Dapat disimpulkan apabila terdapat semakin tinggi peratus bakteriofaj M13 dan sel perumahnya di dalam stok, semakin kecil nilai q_{max} yang diperolehi. Analisa lengkung kemajuan pada 10% iaitu pada C/Co=0.1, kapasiti ikatan dinamik dari kepelbagaian kepekatan biomas diperolehi. Nilainya berubah di antara ketiga-tiga model (10 cm, 13 cm dan 15 cm tinggi lapisan termendak) dan nilai kapasiti dinamik yang tertinggi diperolehi apabila peratusan terkecil bakteriofaj M13 dan sel perumahnya berada di dalam kolum 15 cm tinggi lapisan termendak.

ACKNOWLEDGEMENTS

In the name of Allah, the Most Gracious, the Dispenser of Grace. All praise due to God alone, the sustainers of all words, as without the help, this project will never be completed.

I wish to express my gratitude and appreciation to my Supervisors: Dr. Ling Tau Chuan, Assoc. Prof. Dr. Tey Beng Ti, Assoc. Prof. Dr. Wan Muhammad Wan Abdullah and Assoc. Prof. Dr. Tan Wen Siang for their encouragements, constructive comments and guidance through out these years of my master study.

My special thanks and my heartfelt gratitude goes to Miss Siti Salwa, Miss Chow Yan Mei, Mrs. Mayamarni and Mr. Termizi for their valuable suggestion and valuable information to share. I also wish to express my appreciation to all staff members in the Bioprocess Laboratory and Biochemical Laboratory for their cooperation in so many ways.

Last but not least, to my family and friends, you all had given me a strong spiritual to go through the years. Thanks for the loves, constant encouragements, inspirations and my achievement is sincerely dedicated to all of you.

I certify that an Examination Committee has met on_____ to conduct the final examination of Masniza Mohamed @ Mahmood on her Master of Science thesis entitled "The Influence of M13 Bacteriophage in Protein Adsorption in Anion Exchange Expanded Bed Chromatography" 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:

RUSSLY BIN ABD RAHMAN, PhD
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

SURAYA BINTI ABDUL RASHID, PhD
Lecturer
Faculty of Engineering
Universiti Putra Malaysia
(Internal Examiner)

ROSFARIZAN MOHAMAD, PhD
Lecturer
Faculty of Biotechnology and Molecular Science
Universiti Putra Malaysia
(Internal Examiner)

SHARLIZA IBRAHIM, PhD
Associate Professor
Universiti Malaya
(External Examiner)

_____
HASANAH MOHD GHAZALI, PhD
Professor/Deputy Dean
School of Graduate Studies
University Putra Malaysia

Date:

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. The members of the Supervisory Committee are as follows:

Ling Tau Chuan, Phd
Lecturer
Faculty of Engineering
Universiti Putra Malaysia
(Chairman)

Tey Beng Ti, Phd
Associate Professor
Faculty of Engineering
Universiti Putra Malaysia
(Member)

Wan Muhammad Wan Abdullah, Phd
Associate Professor
Universiti Kuala Lumpur
(Member)

Tan Wen Siang, Phd
Associate Professor
Faculty of Biotechnology and Biomolecular Science
Universiti Putra Malaysia
(Member)

AINI IDERIS, PhD
Professor/Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 10 MAY 2007

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.

_____

MASNIZA BT MOHAMED @ MAHMOOD

Date:

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BSA	Bovine serum albumin
CIP	Clean In Place
C_s	Adsorbed mg/mL of BSA per g of adsorbent at equilibrium
C_m	BSA concentration at equilibrium, mg/mL
DEAE	Diethylaminoethyl
DMF	Dimethylformamide
DNA	Deoxyribonucleic acid
HA	Hyaluronic acid
EBA	Expanded bed adsorption
HCl	Hydroxide chloride
IPTG	Isopropyl-ß-D-thiogalactopyranoside
LB	Luria Bertani
$MgCl_2.6H2O$	Magnesium chloride hexahydrate
NaCl	Sodium chloride
NaOH	Sodium hydroxide
PEG	Poly ethyl glycerol
TBS	Tris base solution
v/v	Volume per volume
w/v	Weight per volume
X-Gal	5-Bromo-4-Chloro-3-Indoyl-ß-D-Galactopyranoside

CHAPTER 1

INTRODUCTION

1.1 Downstream Processing in Biotechnology

The completion of the human genome will lead to an unprecedented demand for the production of human proteins for diagnosing and treating disease and deficiencies. Recombinant DNA technology can provide for the large-scale production of human proteins using various host organisms (bioreactors) ranging from *E. coli* bacterial cells to transgenic farm animals (Harvey *et al*., 2002). The exciting revolution within the field of genetic engineering and recombinant DNA technology has continued to extent the variety of the protein products which has generated new challenges in the design and development of novel purification (Chi, 2000).

Hence, the downstream processing of these recombinant proteins from various sources is gaining importance. Downstream processing is the general term used to describe the separation process for recovery of biological products (Liddell, 1994).

1.1.1 Problems Associated With Conventional Downstream Processing

Proteins vary from each other in size, shape, charge, hydrophobicity, solubility and biological activity. These differences of the protein itself were used to separate it in complex solution. Conventional methods of downstream processing involve a number of unit operations performed in a defined sequence. Generally, the sequences of conventional downstream processing are clarification, concentration, purification and final product polishing. In the clarification step, the common methods used to separate the solids in soluble components from the cultivation broth or the cell homogenate are microfiltration (MF) and centrifugation (CF). However, both of the techniques show limitations in practice (Anspach *et al.*, 1999).

The cell and cell debris form a particle layer on top of the membrane surface that not only leads to decrease of transmembrane flux but also to a partial rejection of proteins in micro filtration techniques. The extent of the rejection depends on the molecular mass of the proteins which is related to the dynamic membranes and also due to the ultrafiltration type characteristic (Datar and Rosen, 1996; Anspach *et al.*, 1999). The high shear forces which may influence the shear sensitive cells generates by recirculation of the feedstock needed a high input of energy pumping (Chi, 2000).

Furthermore, the continuous centrifuges employed in the large scale clarification processes are not as effective as batch centrifuges that used in the laboratory scale. The broth usually needs to be centrifuged twice or an additional microfiltration step is incorporated (Anspach *et al.*, 1999). The shear sensitive cells like mammalian cells may

be damage by the shear stress induced by the turbulence during the feedstock application into the centrifuge and the strong centrifugal force generated during the operation (Chi, 2000). The damage of these cells may release protease or other contaminant proteins that may cause the product degradation or process complication in the subsequent separation steps.

Most of the products of biotechnology are proteins and these proteins must be prepared in a very pure form. The degree of purity required by the injectable protein is set by the Food and Drug Administration (FDA). In general, any contaminants can be detected must be removed from the recovered protein.

The process time for the conventional recovery process is typically very long which may adversely affect the stability of the labile proteins. The stability of the proteins is normally gained when it was adsorbed onto a solid support and it is become an advantage to position the primary adsorption process as early as possible in a recovery process (Morton and Lydiatt, 1994; Chi, 2000).

The direct adsorption of the target protein from a particulate-containing feedstream is an approach to achieve this objective. Over the years the method of expanded bed chromatography has emerged to address this issue. By expanding an adsorbent bed with upward flow of the feedstream, bioparticulates (cells, cell debris, organelles etc.) can pass relatively unimpeded through the enhanced bed voidage without seriously

constraining the adsorption of target products to the stationary phase (Hjorth, 1997; Ling *et al.,* 2005).

Typically the purification of compounds from particle-containing crude liquids involves different pre-treatment steps such as centrifugation and/or microfiltration prior to chromatography. The direct adsorption from untreated crude liquids offers a significant decrease in time and costs due to reduction in the overall number of purification steps. In this case, expanded bed adsorption (EBA) is the method of choice compared to traditional processes.

EBA is an integrated technology which can be used to minimize the separation step by combining clarification, concentration and initial purification into one unit operation. The combination of these steps into one unit operation of capturing target molecules from crude feed-stock may reduce product degradation and avoid bio-product handling problems. EBA is postulated to be a versatile tool that can be applied on fermentation broth which is commonly used as source materials.

1.2 Project Aims and Objectives

The present study was undertaken to investigate the effect of M13 bacteriophage and *E. coli* in adsorption of bovine serum albumin (BSA) in expanded bed chromatography. Moreover, this study was also carried out to investigate the operating parameters such as the expansion bed height, the increase of biomass concentration and the flow rate of buffer distribution by using the EBA column. The study was carried out to investigate the performance of UpFront Column (20 mm i.d) from Amersham Pharmacia Biotechnology in protein purification. Batch adsorption experiment method using Streamline Diethylaminoethyl (DEAE) as adsorbent was also done to investigate the influence of *E. coli* biomass concentrations (0-15% w/v biomass) on BSA adsorption capacity. Besides, the maximum protein adsorption capacity and dissociation constant of DEAE adsorbent were determined in this present study.

The analysis of batch adsorption was done using Langmuir's adsorption isotherm. The stability of fluidized beds was determined by measuring the degree of expansion in EBA chromatography system and the dynamic binding capacity was obtained from the breakthrough curve study.

1.2.1 Objectives

The main objectives of this project were:

1. To investigate the performance of Streamline Diethylaminoethyl (DEAE) as anion exchanger in adsorption of a model BSA protein from unclarified *E. coli* feedstock containing M13 bacteriophage.
2. To investigate the effect of settled bed height on the performance of UpFront Column (20 mm i.d) in protein purification process.
3. To investigate the effect of increasing biomass concentrations on the protein adsorption performance in EBA direct recovery process.

CHAPTER 2

LITERATURE REVIEW

2.1 Bacteriophage

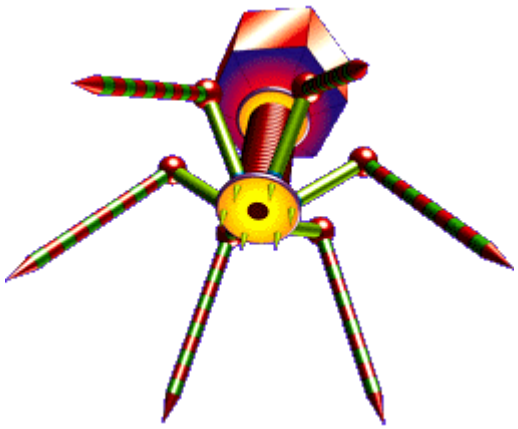

Figure 2.1: Illustration of a bacteriophage (www.phage.com)

In 1921, Frederick Twort and Felix d'Herelle discovered the viruses that infect bacteria, and they named it as bacteriophages (eaters of bacteria) (Adams, 1959). In the 1930s and subsequent decades, pioneering virologists such as Luria, Delbruck and many others utilized these viruses as model systems to investigate many aspects of virology, including virus structure, genetics, replication, etc. Bacteriophages, viruses that prey upon bacteria, typically attack only a single bacterial strain. Bacteriophage virus infects bacteria and sometimes destroys them by causing cell lysis, or dissolution of the cell. Bacteriophages, or phages, have a head composed of protein, an inner core