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EXPANDED BED ANION EXCHANGE ADSORPTION FOR THE PURIFICATION OF BACTERIOPHAGE M13

MAYAMARNI SHAMSU ZAMAN

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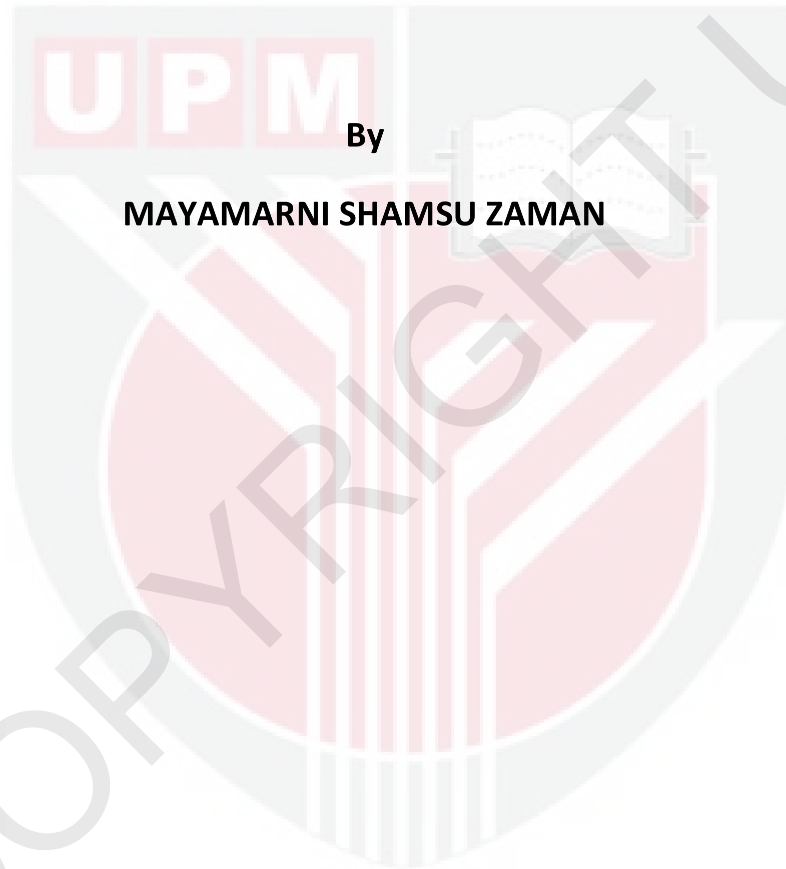

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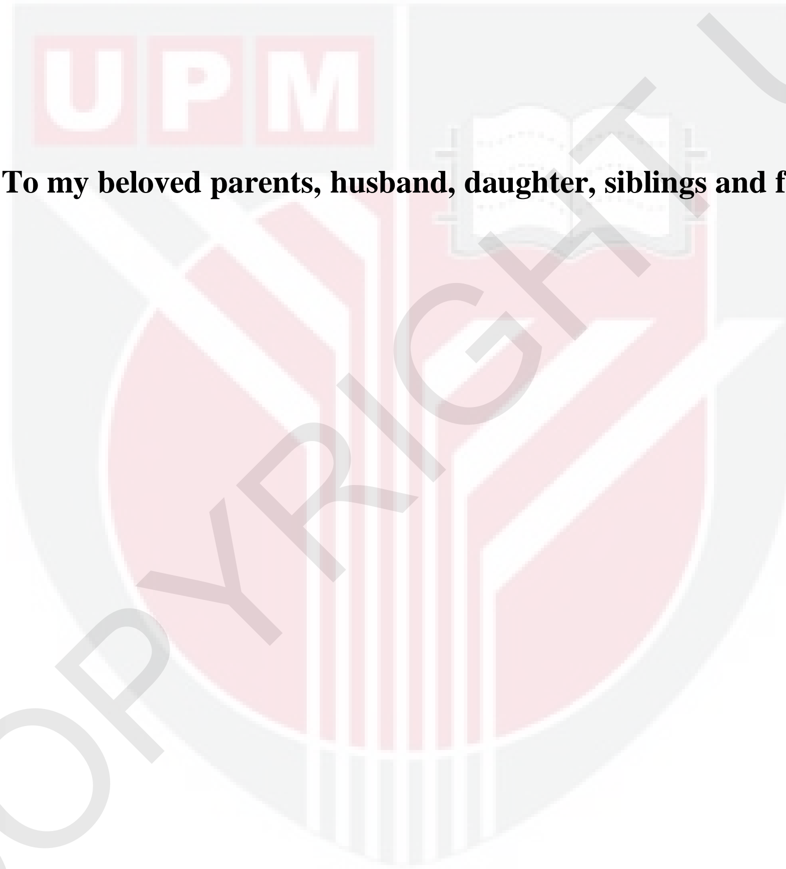

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To my beloved parents, husband, daughter, siblings and friends.

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

By

MAYAMARNI SHAMSU ZAMAN

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 theurapeutic, 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 ($\rho = 1.2$ g/cm^3) 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.

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The influence of the operational parameters of UpFront Fastline^{TM} 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 $cmhr^{-1}$) 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.

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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

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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^{TM} 20 (diameter 20 mm) dari UpFront Chromatography telah digunakan sebagai penghubung dan penggunaan

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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 hinga 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.

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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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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.

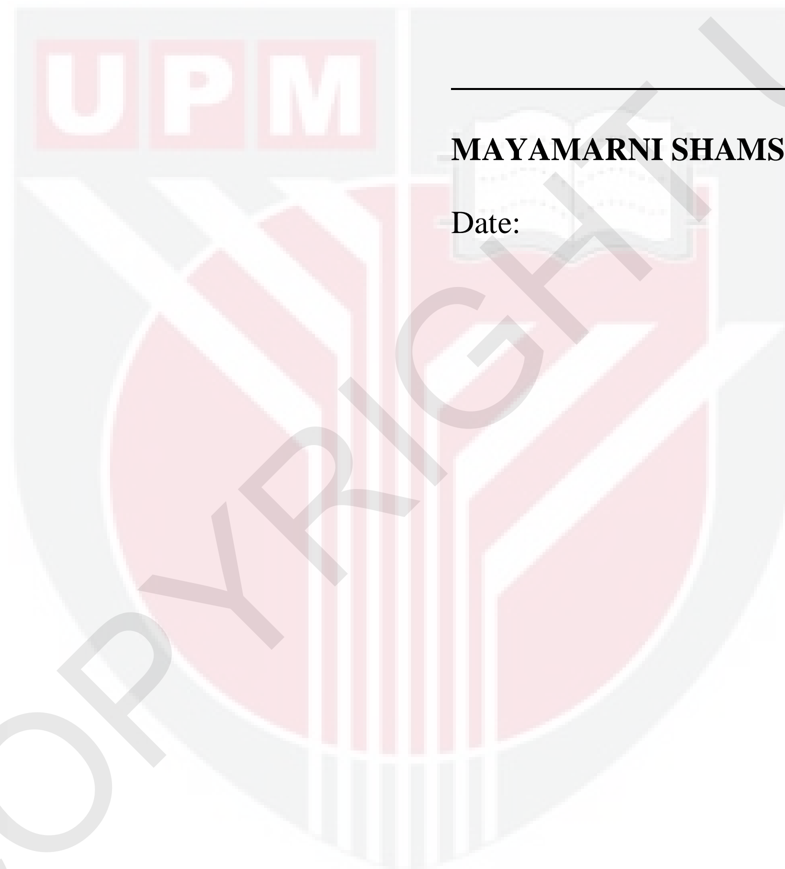

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TABLE OF CONTENT

	Page
DEDICATION	iii
ABSTRACT	iv
ABSTRAK	vi
ACKNOWLEDGEMENTS	viii
APPROVAL	ix
DECLARATION	xi
LIST OF TABLES	xv
LIST OF FIGURES	xvi
LIST OF ABBREVIATIONS	xx
CHAPTER	
1. INTRODUCTION	
Objectives	6
2. LITERATURE REVIEW	
Bacteriophage	7
Filamentous Bacteriophage	8
Composition and Structure of Bacteriophage	9
Structure of Bacteriophage (M13)	11
Application of Bacteriophage (M13)	13
Purification of Protein	14
Conventional method for purification of protein	15
2.3.1 Problem Associated with the conventional method	18
Expanded Bed Adsorption (EBA)	18
Principles of EBA	20
Design features	
The adsorbent	22
Streamline Column	25
Characteristics of Expanded Bed Adsorption	
Breakthrough curves	26
Bed expansion	27
Elution of Adsorbed Protein	30
Biomass concentration	31
Ion Exchange Chromatography	31
Interactions of Protein Between Ion Exchanger	34
Buffer pH and Concentration	35
Ion Exchange Batch Binding	36
Equilibrium Adsorption Isotherm	37
The pH Parameters	40

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3. GENERAL MATERIAL AND METHOD

Feedstock preparation

Top agar	41
LB Broth	41
IPTG and X-Gal in DMF	42
Tris Buffer Saline	42
PEG/NaCl (20% w/v PEG 8000/2.5 M NaCl)	42
Tris buffer for adsorption	43
Tris buffer for elution	43

Cell Culture

Agar plate for plating *E. coli* ER 2738	43
Agar plate for plating bacteriophage	44
Plating *E. coli* ER 2738	44
Preparation of log phase *E. coli* ER2738	45
Propagation of bacteriophage	45
3.3 Large Scale preparation and purification of M13	46
3.4 Phage titration	48
3.4.1 Calculation for phage concentration (pfu/mL)	48
3.5 Total protein determination	49
3.5.1 The Bradford Assay	49
3.6 Protein separation	50
3.6.1 SDS-PAGE	50
3.7 Batch Binding	51
3.7.1 Effect of pH on the binding of protein	52
3.7.2 Equilibrium adsorption isotherm	53
3.8 Expanded Bed Adsorption	55
3.8.1 Streamline Adsorbent	56
3.8.2 Hydrodynamic characteristics	56
3.8.3 Bed Expansion measurement	58
3.8.4 Equilibration of Bed	58
3.8.5 Feedstock application	59
3.8.6 Wash	59
3.8.7 Elution	60
3.8.8 CIP/Regeneration	60

4. RESULTS AND DISCUSSIONS

Purification of bacteriophage by ion exchange batch binding	62
The Total Plaque Forming Units (pfu)	62
Equilibrium Adsorption Isotherms	63
Effect of pH	67
Purification of bacteriophage by EBA	69
Phage titration	69
Effect of degree of bed expansion	70
Fluidization properties of Streamline DEAE	81
Breakthrough curve	83
Effect of flow velocity	88
Effect of biomass concentration	91
Purification of M13 phage using expanded bed adsorption	95

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Purification of bacteriophage by Conventional Method 97
4.3.1 Analysis of M13 protein by SDS-PAGE 98
Comparison of EBA and Conventional method 99

5. CONCLUSIONS
5.1 Ion Exchange Batch Binding 103
5.2 Expanded bed adsorption 103
5.3 Future Suggestions 106

REFERENCES 107
APPENDIXE I 116
APPENDIX II 117
BIODATA OF THE AUTHOR 118

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LIST OF TABLES

Table		Page
1	The phage coat contains several thousand copies of P8, but only fives copies each of the four minor coat proteins	13
2	Precipitation Agents	15
3	Properties of the expanded bed adsorption anion exchanger matrices	24
4	Typical functional groups used in ion exchangers	35
5	Technical specifications of the Upfront Fastline^{TM}20 EBA column	55
6	Protocol for cleaning-in-place	61
7	Influence of pH on the binding of M13 protein from unclarified feedstock.	67
8	Fluidization properties of Streamline DEAE in a 20 mm diameter FastLine column.	83
9	Experimental values applied to Richardson-Zaki correlation to determine the terminal velocity at different biomass concentration in feedstock (5% -15% w/v concentration of M13 bacteriophage cells).	83
10	Summary of effect of superficial flow velocity upon M13 adsorption onto Streamline DEAE adsorbent at 17.5 cm bed height.	89
11	Purification of M13 using expanded bed adsorption from unclarified feedstock. Streamline DEAE (63 mL settled volume, corresponding to 17.5 cm settled bed height) was loaded into the contactor and equilibrated with Tris buffer in expanded bed mode.	95
12	Phage concentration and total pfu in propagated and purified phage using conventional method	97
13	Phage concentration and total pfu in various stage of expanded bed adsorption	98
14	Comparison of the operational conditions and processing time for conventional methods, and expanded bed adsorption.	102

LIST OF FIGURES

Figure		Page
1	Diagram of bacteriophage structure	10
2	The structure of bacteriophage M13. The M13 virus particle is made up of the single-stranded circular viral genome, a large number of copies of the major coat protein and five copies each of four minor structural proteins.	11
3	Schematic representative of Ff phage. The ssDNA is surrounded by 2700 copies of gp8 proteins but the number varies with genome length. The minor coat proteins (gp7, gp9, gp6 and gp3) consist of 4 or 5 copies	12
4	General purification schemes of extracellular and intracellular proteins using traditional approach. Using the expanded bed, when the product is extracellular, the fermentation broth is pumped directly on the column, when the product is intracellular, the suspension of distrupted cells is pumped directly on the column.	17
5	The principle of operation of expanded bed adsorption.	21
6	Structure of coupled ligand in Streamline DEAE ion exchange adsorbent	25
7	Simplified filtration curve of a protein	33
8	Some common types of adsorption isotherm	38
9	The graph plotted exploiting Langmuir isothermal equation in order to obtain the dissociation value, Kd. The slope represent the value of the Kd/q_{max}	65
10	Adsorption isotherm of M13 from unclarified feedstocks at different biomas concentration on loaded Streamline DEAE.	66
11	The M13 protein binding with different pH values in 50mM Tris-HCl	68

12	The bacteriophage (blue plaques) were randomly picked from a plate less than 100 plaques.	70
13	Relative expansion at different flow velocities (50 to 350 $cmhr^{-1}$) 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 17.5 cm of sedimented bed height.	72
14	Relative expansion at different flow velocities (50 to 350 $cmhr^{-1}$) 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.	73
15	Relative expansion at different flow velocities (50 to 350 $cmhr^{-1}$) 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.	74
16	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 17.5 cm of sedimented bed height.	75
17	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.	76
18	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.	77
19	Comparison of data for bed expansion at 17.5 cm of sedimented bed height versus superficial linear fluid velocity determined using the Richardson-Zaki as fluidized by the presence of M13 bacteriophage cells, binding buffer (♦) 5% w/v (■); 10% w/v (▲) and 15% w/v (•)	78

20 Comparison of data for bed expansion at 13 cm of sedimented bed height versus superficial linear fluid velocity determined using the Richardson-Zaki as fluidized by the presence of M13 bacteriophage cells, binding buffer (♦) 5% w/v (■); 10% w/v (▲) and 15% w/v (●) 79

21 Comparison of data for bed expansion at 10 cm of sedimented bed height versus superficial linear fluid velocity determined using the Richardson-Zaki as fluidized by the presence of M13 bacteriophage cells, binding buffer (♦) 5% w/v (■); 10% w/v (▲) and 15% w/v (●) 80

22 Effect of the 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/C_0 = ~1) was reached. The dynamic binding capacity at $C/C_0 = 0.1$ for various bed height are 2.71×10^9 pfu/mL (17.5 cm), 1.89×10^9 pfu/mL(13 cm) and 1.3×10^9 pfu/mL(10 cm). 86

23 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/C_0 = ~1) was reached. The dynamic binding capacity at $C/C_0 = 0.1$ for various bed height are 2.71×10^9 pfu/mL (17.5 cm), 1.89×10^9 pfu/mL(13 cm) and 1.3×10^9 pfu/mL(10 cm). 87

24 Comparison of breakthrough curves in the expanded bed adsorption of M13 onto Streamline DEAE in different expansion degree, expansion degree 2.0 (♦), expansion degree 2.5 (■) and expansion degree 3.0 (▲).63 mL settled volume corresponding to 17.5 cm settled bed height was 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/C_0 = ~1) was reached. The dynamic binding capacity at $C/C_0 = 0.1$ for various bed expansion are 2.05×10^9 pfu/mL (2.0), 8.4×10^8 pfu/mL(2.5) and 5.7×10^8 pfu/mL(3.0). 90

25 The effect of different biomass concentration on Streamline DEAE applied to expanded bed adsorption containing M13 bacteriophages cells; binding buffer (♦), 5% (▲); 10% (■) and 15% (●) w/v in Tris HCl buffer. The sedimented bed height was 17.5 cm. The feedstock application was continued until a desired adsorbent saturation state (C/C_0 = ~1) was reached. The dynamic binding capacity at C/C_0 = 0.1 for various biomass concentration are 3.1x10^9 pfu/mL (binding buffer), 7.9x10^8 pfu/mL (5% w/v), 1.3 x10^8 pfu/mL(10% w/v) and 9.5x10^7 pfu/mL (15% w/v). 92

26 Purification of M13 bacteriophage on an expanded bed of Streamline DEAE. The eluents were 2.0 M NaCl in 50 mM Tris HCl at pH 2.2 in a Fastline 20 column with 17.5 cm of sedimented bed height. Sample collected during chromatography were analyzed for total protein content (♦). The various stages of the procedure were performed at a constant flow rates. 94

27 Chromatographic profiles of M13 bacteriophage on Streamline DEAE in a FastLine 20 column. Both systems utilized 63 mL adsorbent respectively which are corresponding to 17.5 cm of settlement bed height. 96

28 SDS-PAGE of purification of M13 from an unclarified *E. Coli* using an expanded bed adsorption onto Streamline DEAE as a adsorbent. Where: Lane M, molecular mass standards; lane 1-4, conventional method; lane 5, elution 2; lane 6, elution 1; lane 7-8, wash; lane 9, feedstock. 99

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. 101

LIST OF ABBREVIATIONS

BSA	Bovine Serum Albumin
CIP	Clean-in-place
C_m	M13 concentration at equilibrium, $mgmL^{-1}$
DEAE	Diethylaminoethyl
dH_2O	Distilled water
DMF	Dimethyl formamide
DNA	Deoxyribonucleic Acid
EBA	Expanded bed adsorption
HCl	Hydroxide chloride
IPTG	Isopropyl-beta-D-thiogalactopyranoside
KCl	Kalium Cloride
K_d	Equilibrium Dissociation constant
LB Agar	Luria-Bertani Agar
LB Broth	Luria-Bertani Broth
$Mg.Cl_2.6H_2O$	Magnesium dichloride hexahydrate
NaCl	Sodium Chloride
Na_2CO_3	Sodium Carbonate
NaOH	Sodium Hydroxide
PEG	Polyethylene glycol
pfu	plaque forming unit
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

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v/v	Volume per volume
w/v	Wet weight per volume
X-Gal	5-bromo-4-chloro-3-indocyl-β-D-galactopyranoside

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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 bio-chemical 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.

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The filamentous bacteriophage M13 can be used to display foreign peptides and proteins, allowing for the construction of theurapeutic, 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.

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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).

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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). Further more, 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).

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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^{TM} 20 was used as a contactor to recover protein from bacteriophage M13. The performance of an anion exchanger, Streamline DEAE, (ρ =1.2 gmL^{-1}) as adsorbent was studied.

Objectives

The objectives of this study are:

1. To identify the fluidization properties of Streamline DEAE (ρ~1.2 gmL^{-1}) and degree of expansion of Streamline DEAE (ρ~1.2 gmL^{-1}) in Fastline^{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^{TM} 20 column to the conventional method.

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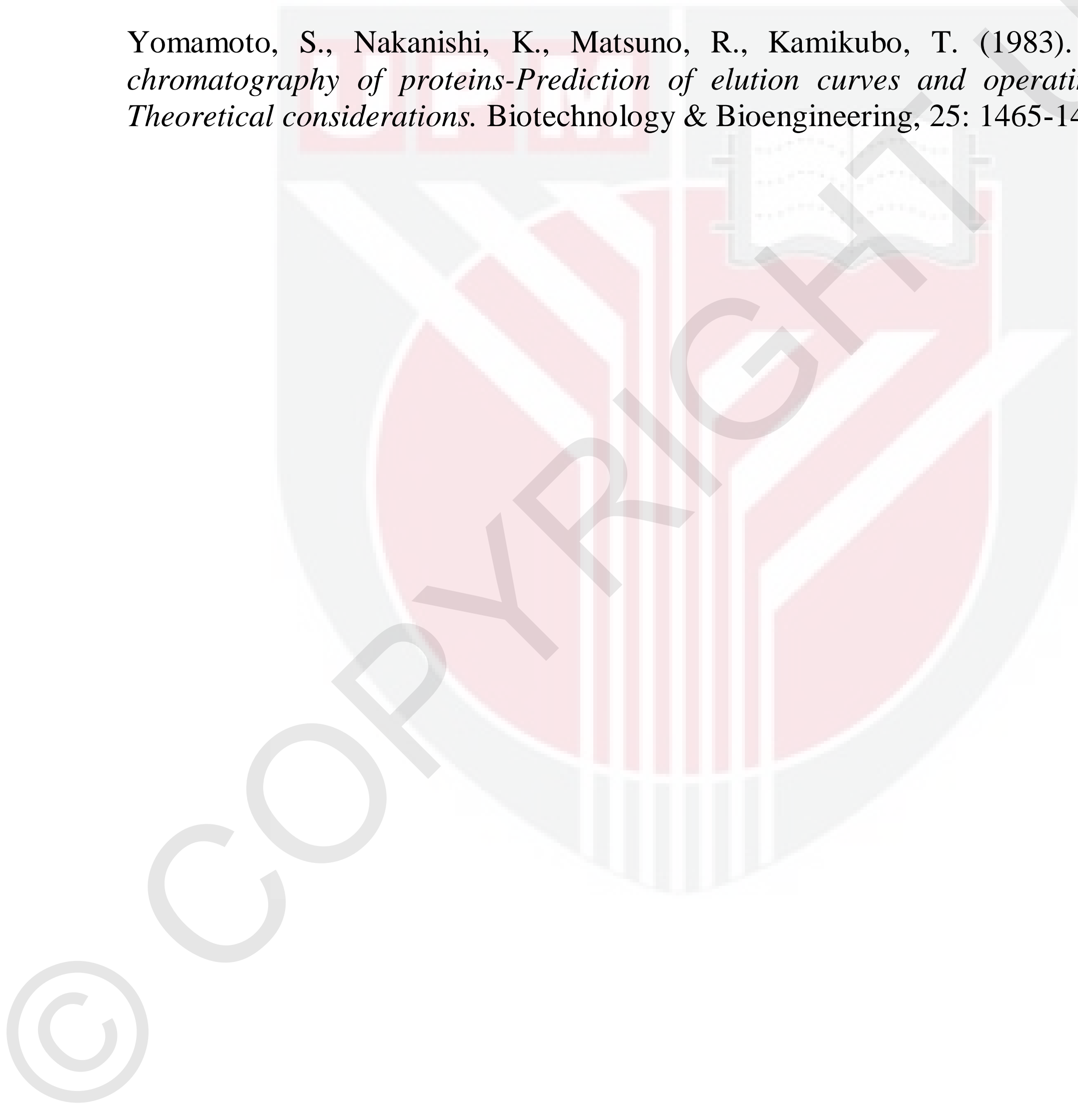

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