PROTEIN FRACTIONATION USING ULTRAFILTRATION SYSTEM FOR DIFFERENTS MODULE CONFIGURATIONS

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A thesis submitted in fulfillment of the requirements for the award of the degree of Bachelor of Chemical Engineering

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"I declare that this thesis is the result of my own research except as cited references. The thesis has not been accepted for any degree and is concurrently submitted in candidature of any degree."

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DEDICATION

This thesis is a symbol of appreciation for my beloved parents, Hamid @ Osman bin Daii and Rohana binti Hashim, my encouraging siblings Syamsul Azhar and Dalyana together with my soulmate, Hazlan bin Haris.

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Bismillahirrahmanirrahim,

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ABSTRACT

Protein bioseparation is an important yet expensive activity especially in biotechnology industry. The production of protein is growth along with rapid commercialization and demand. Unfortunately, the industry having problem in term of cost and the production as the cost is extremely high but the production rate is low. The most effective way to solve this problem is by using membrane as the medium of separation. In this study, the effects for different mode of operations of ultrafiltration membranes – batch, single stage and two stages forward cascade. Amersham Quick Lab cross flow system was used to separate lysozyme by using 30 kDa polyethersulfone ultrafiltration membranes. For this research, the lysozyme is obtained from the cheapest source which is chicken egg white solution. Lysozyme is a very important protein in pharmaceutical field in order to produce a vaccine to prevent bacteria that inhabit the intestinal tract. In order to determine the most efficient configurations, the constant parameters are set for all configurations. The value of pH must be 10, the transmembrane pressure (TMP) is 1.0 bar, and the volume feed is 960 ml with the ratio of chicken egg white to sodium chloride 0.2 M is 1:31. The effective configuration can be determined by comparing the permeate flux, concentration of lysozyme in both permeate and retentate solution at 5 minutes after the separation process occurred and measurement of water flux. The analysis procedure is done using the UV-VIS spectrometer at wave length equal to 595 nm. The most effective configuration is two stages forward cascade which has the highest average permeate flux of 74.4 $L/m^2.h$. Moreover, it also has percentage for concentration of lysozyme in permeate solution equal to 84% which is the biggest percentage of all configurations and the lowest value of water flux measurement after separation process compare to the benchmark value at TMP equal to 1.0 bar. The second configuration then is single stage and followed by batch at third place.

ABSTRAK

Dalam perkembangan industri bioteknology dewasa ini, pembuatan protein adalah satu proses yang penting walaupun kosnya mahal. Perkembangan pembuatan protein pada masa ini juga selari dengan permintaan yang semakin meluas. Malangnya, industri ini menghadapi masalah disebabkan kosnya yang mahal dan seterusnya menghadkan perkembangan industri ini. Salah satu cara yang paling efektif ialah dengan menggunakan membran sebagai medium pemisah. Untuk kajian ini, kesan untuk modul-modul yang berbeza – batch, single stage dan two stages forward cascade dikaji. Peruses pemisahan ini dijalankan menggunakan Amersham Quick Lab cross flow system dan membran 30 kDa ultrafiltration untuk memisahkan sejenis protein iaitu lysozyme. Bagi kajian ini juga, protein lysozyme diperoleh daripada sumber protein termurah iaitu putih telur. Protein lysozyme merupakan sejenis protein yang penting dalam pembuatan vaksin bagi membasmi bakteria yang mendiami saluran-saluran seperti tekak, telinga, hidung dan sebagainya. Bagi mengetahui modul yang paling efektif, beberapa parameter untuk proses pengasingan hendaklah ditetapkan. Parameter yang ditetapkan ialah nilai pH hendaklah bernilai 10, tekanan trans membrane pula bersamaan dengan 1.0 bar dan isipadu sampel ialah 960 ml dengan kadar larutan putih telur kepada larutan natrium klorida 1:31. Modul yang paling berkesan diukur dengan berdasarkan nilai permeate flux, kepekatan lysozyme dalam kedua-dua larutan permeate dan retentate serta nilai water flux yang dibanding selepas proses pemisahan berlaku.. Prosedur analisis dilakukan menggunakan UV-VIS Spectrometer pada gelombang bersamaan 595 nm. Modul yang paling efektif ialah two stages forward cascade yang mempunyai nilai purata permeate flux bersamaan dengan 74.4 $L/m^2.h$, nilai kepekatan lysozyme dalam larutan permeate sebanyak 84% berbanding kepekatan dalam larutan asal dan mempunyai nilai yang water flux yang paling hampir dengan nilai benchmark pada TMP bersamaan 1.0 bar. Kesimpulan bagi modul yang paling efektif ber pihak kepada modul two stages forward cascade diikuti dengan modul single stage dan batch.

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SYMBOLS	DESCRIPTION
TMP	Trans-membrane pressure
MWCO	Molecular weight cut off
kDa	kiloDalton
M	Molar
R	Rejection
P	Pressure
ΔP	Difference of pressure
Cp	Concentration of permeate
Co	Concentration of original/feed
A	Absorbance

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CHAPTER 1

INTRODUCTION

1.1 Overview

Proteins are biological macromolecules which having a wide range of applications which are composed of basic building blocks called amino acids [1]. Bioseparation especially in proteins is an important yet expensive activity in food, pharmaceutical and biotechnology industry. The definition can be refers as the recovery or the process of separating components which is protein products from various biological feed of streams [2].

Ultrafiltration can be defined as a pressure-driven (1010 bar) of separation process in which membranes having pore sizes ranging from 10 – 10000 Å are used for the concentration, diafiltration, clarification and fractionation of macromolecules such as proteins [2]. Ultrafiltration has evolved significantly since it was introduced in 1970s, and it will continue to evolve as life science research progresses. Compared to non-membrane processes (chromatography, dialysis, solvent extraction, or centrifugation), ultrafiltration is more preferable for laboratory scale and even pilot scale because [2, 12, 13]:

- does not require an organic extraction which may denature labile proteins
- maintains the ionic and pH value
- is far gentler to the molecules being processed
- fast and relatively inexpensive

- is very efficient and can simultaneously concentrate and purify molecules.
- can be performed either at room temperature or in a cold room (at low temperature)
- high throughout of products
- relative ease to scale up
- ease of equipment for cleaning

Protein bioseparation quite often becomes the limiting factors in the successful development of protein based products as the major cost can be an incentive for developing cost effective processes [2]. Therefore, much more dedicated experimental work with real biological streams is essential in promoting the wide acceptance of ultrafiltration in fractionation of protein although ultrafiltration already known as an efficient and scalable bioseparation technique [3, 4]. Recently, researchers have demonstrated the potentials for using ultrafiltration in bioseparation process such as in protein concentration, protein fractionation, protein clarification and diafiltration of protein solution.

In this study, different mode of operations of ultrafiltration membranes will be tested in order to identify the most efficient module configuration in biosepation process involving protein. Protein fractionation is used to separate proteins using ultrafiltration is much more demanding and significant research work in this area has only been done in recent years. Therefore, a substantial amount of work still needs to be done in order to ‘perfect the technology’ and achieve the satisfactory level of proteins separation.

Module of configurations or mode of operations can be a factor that can effect protein fractionation. In this study, effect on permeate flux versus time will be discussed by the different configurations of protein fractionation. The chicken egg white will be the source of protein and will be separated into lysozyme from chicken egg white.

1.2 Problem Statement

Protein bioseparation in any type either in concentration, clarification, diafiltration or even in fractionation was the main reason why bioseparation caused a fortune that limits the industry to develop the technology. The challenge for the future will be to develop and adapt the further current technology or to create innovative separation technology to meet the bioprocess requirements.

Therefore it's compulsory for the industry to choose the best method in order to achieve the satisfactory in bioseparation. According to the recent research and studies, ultrafiltration is preferred in carrying this task based on the advantages of this type of membrane compare to the others. According to the industry needs, market for ultrafiltration will increase at average annual growth rate at 9.7% from $US 458 million in 2002 to $US 727 million in 2007. This can proved the importance of ultrafiltration in bioseparation process nowadays.

Furthermore, the industries have to be detailed in all figures, calculations and operation in order to capitalize on the production at the lower budget using the most efficient operation. So, based on the industries involved, the module configuration or mode of the operation in ultrafiltration can also be the factor that can be adjusted in order to have the perfect operation to produce maximum product and profits at the minimum cost.

1.3 Objective

The purpose of this thesis is to study the effect of module configurations in ultrafiltration membrane separation process for protein fractionation precisely to separate lysozyme from chicken egg white.

1.4 Scope of Study

In order to achieve the goal in this study, extra effort and focus have to be done to:

i. determine the effective configurations for separate lysozyme from chicken egg white using ultrafiltration membrane separation process.
ii. determine the value of permeate flux at a constant time
iii. analysis the concentration of lysozyme in filtrate/permeate solution
iv. measure the water flux after separation process and make comparison to the benchmark value

CHAPTER 2

LITERATURE REVIEW

2.1 Protein Bioseparation

Protein bioseparation is a process of separating component by passing a solution through certain medium or column so that each component of the protein can be separated, recovered and purified from various biological feed streams is an important unit operation in food, pharmaceutical and biotechnology industry [1, 2].

The biotechnology industry is poised for rapid growth and implementation in diverse areas – protein bioseparation. However one major constant problem that limits the developments in previous years is the need for a more complete understanding of protein bioseparation process itself [6]. But the understandings among the researchers on fundamental and importance of protein separation is increasing and protein separation is at the present moment more important in the bioprocess industry than at any time before. This is largely due to the phenomenal developments in recent years in the field of modern biotechnology. More and more proteins products need to be separated and purified in larger quantities.

2.2 Economic Aspects of Protein Bioseparation

The isolation and purification of proteins is widely recognized to be technically and economically challenging. The main problem is the cost of the process. Table 2.1.2 shows the protein bioseparation cost as approximate proportion of cost of production for certain protein based products [2]. As clearly indicated by these figures, protein bioseparation cost is the major cost of protein production and because of this factor, the industry always welcome ideas for developing cost-effective isolation and purification process.

Table 2.1: Cost of protein bioseparation

PRODUCT	APPROXIMATE RELATIVE PRICE (US DOLLAR)	PROTEIN BIOSEPARATION COST AS % OF TOTAL COST OF PRODUCTION
Food/additives	1	10-30
Nutraceuticals	2-10	30-50
Industrial Enzymes	5-10	30-50
Diagnostic Enzymes	50-100	50-70
Therapeutic Enzymes	50-500	60-80

2.3 Methods for Protein Bioseparation

In producing useful protein, there been a myriad of protein bioseparation technique is available. Some of the most common methods being used in biotechnology industry are:

i. Precipitation and centrifugation
ii. Column chromatography
iii. Electrophoresis
iv. Membrane separation

2.3.1 Precipitation and Centrifugation

Proteins can be partially isolate and purified using precipitation by salting out salts (e.g. ammonium sulfate, sodium chloride), by solvents (e.g. ethanol, acetone) or by concentrated alkali or acids [2]. Then, the progress is continued by separated the precipitants using centrifugation. Centrifugation is a device for isolate the precipitated proteins from a solution by spinning the sample at rotation speed, either using the analytical centrifuges for small scale separation or preparative centrifuges for larger sample volumes. Although the process is generally favored at low temperature and at very large process volumes, the purity of the protein obtained can hardly be pure.

2.3.2 Column Chromatography

Chromatography relies on the distribution of component to be separated between two phases; a stationary or binding phase and a mobile phase which carries these components through the stationary phase. The mixture of components enters a chromatographic column and each individual component is flushed through the system at a different rate [2].

This traditionally technique is used in research laboratories are excellently suited for purifying small quantities of proteins however it requires complex and difficult to scale up. Besides, by using chromatography, the cost structure of the different component involved include the operating cost (solvent cost), and the capital cost (columns, pump etc) which make the equipment expensive [6].

2.3.3 Electrophoresis

Electrophoresis is another technique for protein bioseparation. Electrophoresis can be defined as separation of components by employing their electrophoretic mobility. The application is basically like fuel cell where positively charge component will migrate to negative electrode and oppositely. This method can be done in two type either gel electrophoresis and liquid phase electrophoresis based on their medium in which the separation is carried out.

However, like chromatography, these processes are extremely difficult to scale-up and this factor restricts the scale of production. In addition to scale-up problems these techniques require complex instrumentation support to run efficiently, and give low throughput of product at an extremely high cost [14].

2.3.4 Membrane Separation

In 1998, membrane for separation is only use at laboratory scale and this technique is not ready as yet for application at the commercial level [6]. But, nowadays membrane is presently established part of several industry process that can be commercialized [7]. Membrane technology has gained a huge importance in the previous years, competing with established technology for water desalination, food processing or even in medical applications. Figuring on the fields to which membranes are already serving as important tools but also to which the membrane technology could add new solution in the near future, the chemical industry certainly one of the most interesting.

Generally, membrane separation is depends on the size of the particle and the size of the membrane pores. As showed in Figure 2.1, the protein sample will be put in the feed tank and been pumped through the membrane. The particles that can go through the membrane are smaller than the membrane pores which known as permeate. Meanwhile, for the larger particle that being blocked by the membrane is called

retentate. The product can be obtained either in permeate or the retentate based on the production.

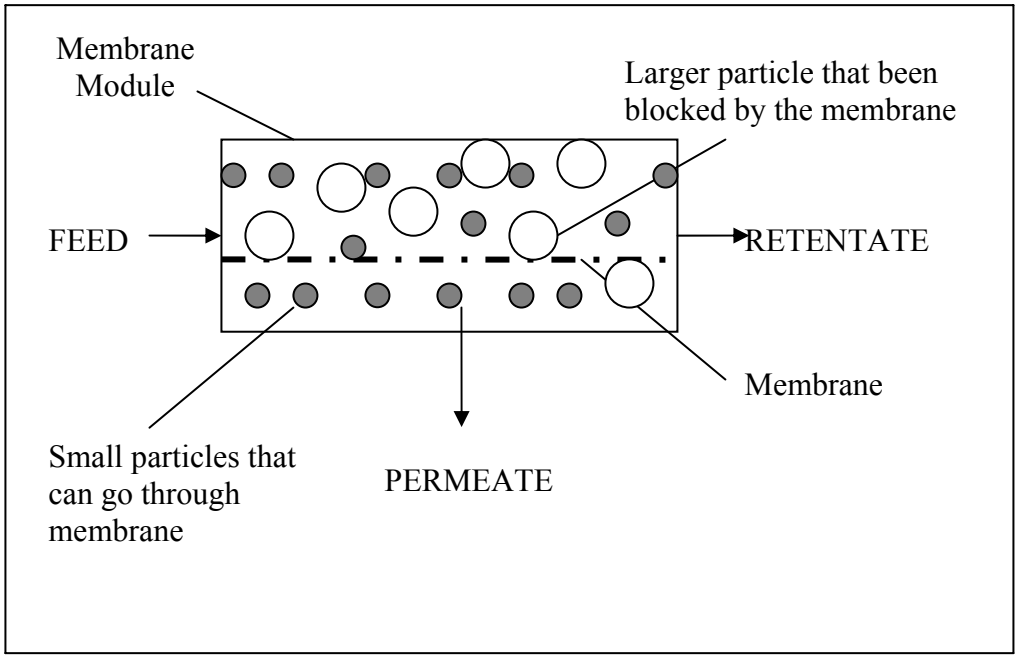

Figure 2.1 Schematic representation of membrane process

Membrane technology is an emerging technology and because of its multi-disciplinary character it can be used in a large number of separation processes. The benefits of membrane technology can be summarized as follows [12]:

- Separation can be carried out continuously and under mild conditions
- Energy consumption is generally low
- Up-scaling is easy
- Membrane properties are variable and can be adjusted
- No additives is required

Unfortunately, every process has its disadvantages. Same with membrane process which are membrane fouling and low membrane lifetime. But these disadvantages are not as huge as other methods faced. The four developed industrial membrane separation process are microfiltration, ultrafiltration, reverse osmosis and electrodialysis that are well established for their applications.

2.4 Categories for Protein Bioseparation

Most conventional protein bioseparation processes rely on a scheme, which is best described as RIPP (Removal, Isolation, Purification and Polishing) [8]. Biological feed streams are generally dilute with the respect to the target proteins, which need to be separated from a large number of impurities in protein solution.

The separate protein is valued by the rate of the productivity and resolution. Productivity is amount of the product by time. In the meantime, resolution is the purity of the protein produced. Protein bioseparation can be classified into three broad categories [2]:

i. high productivity low resolution
ii. low productivity high resolution
iii. high productivity high resolution

2.4.1 High Productivity Low Resolution

This category is the first category that manages to reduce the volume and overall concentration of the process stream. Although, the production of protein is highly obtained but the proteins produced have low purity. Examples of bioseparation methods under this category are precipitation, centrifugation, liquid-liquid extraction, cell disruption.

2.4.2 Low Productivity High Resolution

Proteins that produced under this category are pure protein and commonly used in pharmaceutical fields. Unfortunately, the production required high cost at low production. This factor had leads to the development of bioseparation technology to search for other method that more cost effective as pure protein is very demanding in the

market. Among the bioseparation methods for this category are ultracentrifugation, electrophoresis, packed bed chromatography, affinity separation.

2.4.3 High Productivity High Resolution

This is the high demand category in the industry nowadays as it can produce pure protein at the high rate and most importantly at affordable cost. The suitable bioseparation methods and widely used for this category are ultrafiltration and membrane chromatography.

2.5 Chicken Egg White

Proteins are biological macromolecules which having a wide range of applications which are composed of basic building blocks called amino acids [1, 9]. Naturally occurring proteins are made up of up to 20 different amino acids [2]. Proteins also have a diverse array of applications such as in:

- food and nutritional products
- pharmaceutical products
- industrial catalysts
- diagnostic products

Because of the wide range of applications, protein bioseparation is a challenging process in producing protein based products.

Chicken egg white is easy to get and it is the most affordable protein sample in the market. As been mentioned in previous section, protein bioseparation is categorized as expensive process in biotechnology; a cheap protein source such as chicken egg white is always welcomed. Besides being the cheapest protein source, chicken egg white contains many kinds of proteins that were very important and valuable.

Moreover, chicken egg white has been study widely using different methods in order to separate the proteins. Table 2.2 lists all the proteins that contain in chicken egg white [10].

Table 2.2: Properties of chicken egg white

PROTEIN	% OF TOTAL	MOLECULAR WEIGHT CUT OFF
Ovalbumin	54	45 000
Ovotransferrin	12	77 700
Ovomucoid	11	28 000
Lysozyme	3.4	14 300
Ovomucin	3	220 000
G3 Ovoglobulin	1	50 000
G2 Ovoglobulin	1	47 000
Ovoglycoprotein	1	24 400
Ovoflavoprotein	0.8	66 500
Ovomacroglobin	0.5	32 000
Avidin	0.05	900 000
Cystatin	0.05	68 300
Thiamin-binding protein	} 12.2	12 700
Glutamul aminopeptidase		320 000
Minor glycoprotein		52 000
Human serum albumin		66 500

2.5.1 Lysozyme

Lysozyme is a commercially valuable enzyme and is used for different applications. Lysozyme occurs naturally in chicken egg white (CEW) [11], which is a mixture of proteins (see Table 2.2). The structure of lysozyme is showed in Figure 2.2.

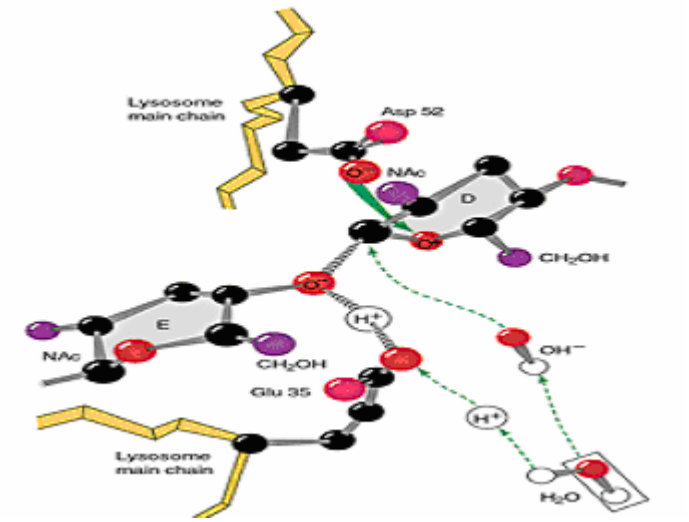

Figure 2.2 Structure of lysozyme

Using chicken egg white as the sample protein, it is the cheapest way to obtain proteins particularly lysozyme. The varieties of use for lysozyme are [5, 14, 15]:

- as a cell-disrupting agent for extraction of bacterial intracellular products by attacking the protective cell walls of bacteria and destroy bacteria on contact which has a strong influence on the type of bacteria that inhabit the intestinal tract like in the ears, nose and throat.
- for biological functions which include antiviral action by forming an insoluble complex with acidic viruses, enhanced antibiotic effects, anti-inflammatory and antihistaminic actions, direct activation of immune cells, and antitumor action.
- enhance immunoglobulin production.
- as a microbial agent in various food, either as preservative to control microbial in cheese and anti-bacterial food packaging films.

- keeps both immune and growth factors from deterioration within the digestive system generally for kids.
- as a drug for treatment of ulcers and infections.
- as a food additive in milk products.

The low content of lysozyme in CEW (about 3.4%) makes its separation and purification a challenge, since a large amount of raw material has to be processed in order to get even small amounts of lysozyme.

2.6 Ultrafiltration Separation Process

The separation of proteins is an important part of the rapidly growing bioprocess industry. Many bioproducts are proteins and there is a great demand for their separation. Techniques used today such as chromatography, affinity separation and electrophoresis can produce small quantities of very pure proteins. In many cases, large amounts of particular proteins are needed, making the classical separation methods too expensive.

Over the last 20 years, the ultrafiltration industry has grown steadily. Ultrafiltration is known to be a very effective alternative operation process and is already widely used in the production of many bioproducts in the pharmaceutical sector and the food industry [16] as it is easy to scale up, high throughput of product and ease of equipment for cleaning and sanitization.

Ultrafiltration is a pressure-driven separation process in which membranes having pores ranging from 10-1000 Å are used for the concentration, diafiltration, clarification and fractionation of macromolecules (e.g. protein, nuclei acids) [2]. Membrane based separation process for ultrafiltration, microfiltration, nanofiltration or

reverse osmosis are generally classified on the basis of the membrane pore size (see Figure 2.3) or the type of material being separated.

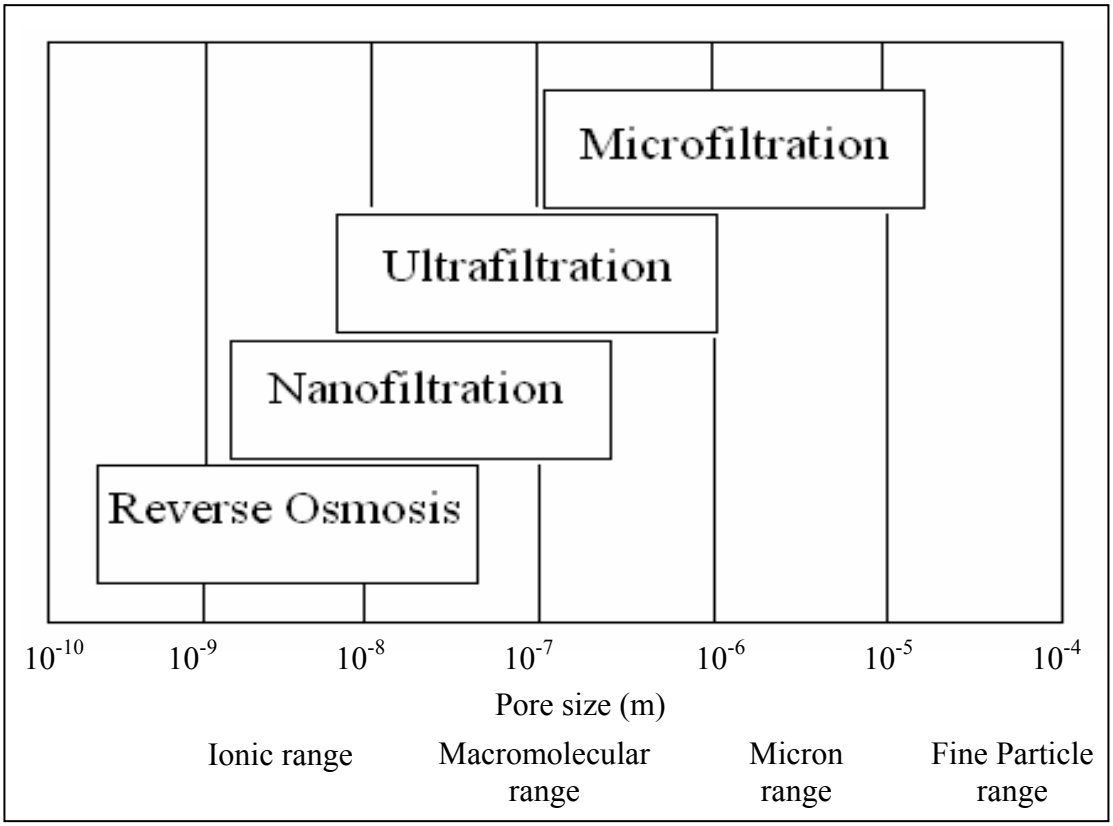

Figure 2.3 Classification of membrane based on pore size

However, it must be emphasized that membrane pore size is not the sole basis for separation in ultrafiltration processes. Others are include pH, salt concentration, feed concentration, permeate flux and even interaction between the macromolecules [4].

Ultrafiltration has a broad variety of applications. Some of the applications are listed below [2, 13]:

- fractionation of macromolecules e.g. proteins, nuclei acids
- concentration of macromolecules - removal of solvent from solutions of these macromolecules
- diafiltration – removal of salts and other low molecular weight compounds from solutions of macromolecules.

- virus removal from therapeutic products
- membrane reactors
- food industry e.g. in cheese production
- automotive industry e.g. electro coat paint that being used in cars manufacturing sectors

For economy aspects, ultrafiltration has evolved significantly since it was introduced to commercial sectors in 1970s. Processing of biological macromolecules such as proteins had assumed significant importance in the bioprocess industry, where the impact of downstream processing on the overall process economics is now being appreciated.

Ultrafiltration membranes have brought about US$ 200 million in sales in 1998 with an expected growing rate at 10% a year [6]. Ultrafiltration membranes are in large scale responsible for supplying pure water for semiconductor industry besides growing demands of ultra high purity chemicals. The ultrafiltration is surely at demand in market as market for ultrafiltration will increase at average annual growth rate at 9.7% from US$458 million in 2002 to US$ 727 million in 2007 [7].

2.7 Ultrafiltration for Protein Bioseparation

When it comes to processing proteins, ultrafiltration can hold up to 4 types of separations. Every type is based on what particle need to be separated from the protein sample/solution. The types of separations are:

i. protein concentration
ii. diafiltration
iii. protein clarification
iv. protein fractionation

2.7.1 Protein Concentration

Protein concentration involves the removal of solvent, mostly water from the protein solution. Among the purposes for this process are to increase or adjust the concentration of a therapeutic protein in a formulation for example vaccines or monoclonal antibody besides as a pre-treat protein solutions for polishing steps such as crystallization or freeze drying.

On a small scale of laboratory, different method can also be used for protein concentration like vacuum evaporation, centrifugal ultrafiltration or ultrafiltration itself. Vacuum evaporation is very slow and it is only feasible for very small amounts of protein solution which is less than 10ml. Therefore, this method is absolutely impossible for commercialized.

For centrifugal ultrafiltration, it is a small scale variant of ultrafiltration with protein sample less than 50 ml. The protein is loaded in the upper chamber of the centrifuge tube and the filtrate/permeate is collected in the lower chamber. Meanwhile for ultrafiltration, the system is simple, easy to scale up and can hold up to larger volume for commercialize purpose.

2.7.2 Diafiltration

Diafiltration is a method to removed low molecular particles such as salts from a protein solution through ultrafiltration membrane. Some of major applications for diafiltration are:

- removal of precipitating salts-ammonium sulfate, sodium chloride from protein solution
- removal of precipitating solvents-ethanol, acetone from protein solution
- removal of toxic metabolites from blood-hemodiafiltration
- removal of inhibitors from enzyme solution

2.7.3 Protein Clarification

Protein clarification is removal of particulate matter from protein solution. The objective of a membrane based protein clarification process is the efficient removal of particulate matter, along with high protein recovery. The use of ultrafiltration for clarification is primarily in the area of pharmaceutical like virus removal. As the increasing risk of contamination of therapeutic products, the use of ultrafiltration for removal of these substances from protein solutions might be an option worth considering [2].

2.7.4 Protein Fractionation

Protein fractionation process is separation proteins from protein solution. For example that been study widely and more seriously now is separation protein like lysozyme from chicken egg white [3, 5, 10, 14, 15]. Concentration, diafiltration and clarification process have been widely used in bioprocess industry for quite some time. Protein fractionation using ultrafiltration is considerably as more challenging and is more recent development.

Start from the middle of 90's, industry has taken protein fractionation seriously. Protein fractionation using ultrafiltration is strongly influenced by operating and physicochemical parameters and hence the process need to be precisely fine tuned to achieve satisfactory level of separation. The parameters that need to be considered include the optimization of pH, salt concentration, ionic strength between protein molecules, permeate flux, and the most important is membrane pores size and the molecules size.

Ultrafiltration has been used for a wide variety of protein separations and these studies are invaluable for developing the ultrafiltration function. Regrettably, there are

relatively fewer papers done on protein fractionation compare to other method-concentration, diafiltration, and clarification.

On the other hand, because of the demand of the industry to separate protein using cost effective method has gain attentions from the researchers to study on protein fractionation using ultrafiltration for the sack of the technology. And these days, protein fractionation is much more demanding compare to other types of protein bioseparation.

2.8 Mode of Separation in Ultrafiltration

In general, for membrane separation process, there are two basic type of filtration involved:

i. dead end filtration
ii. cross flow filtration

2.8.1 Dead End Filtration

This type flow pattern where the feed flows towards the membrane in a normal direction while the permeate flows away from the membrane also in a normal direction, but the retentate is remains blocked at the membrane surface and form the cake. This type has an obvious weakness whereby the separation will be at complete stop when the fouling happened.

The mechanism starts as all the feed is forced through the membrane. Thus, the concentration of rejected components in the feed increases and the quality of permeate decreases with time. The filtration cake grows with time and the flux decrease with time. Figure 2.4 showed the mechanism for dead end filtration while Figure 2.5 is a graph

showed the reduction of flux versus time since the filter cake being formed at the membrane surface.

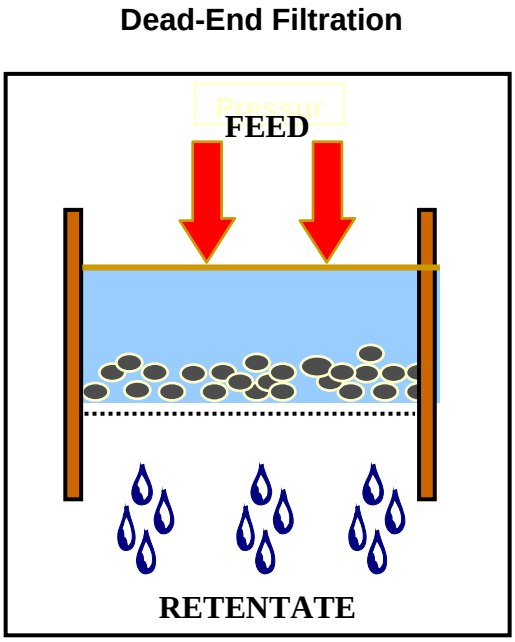

Figure 2.4 Mechanism of dead end filtration

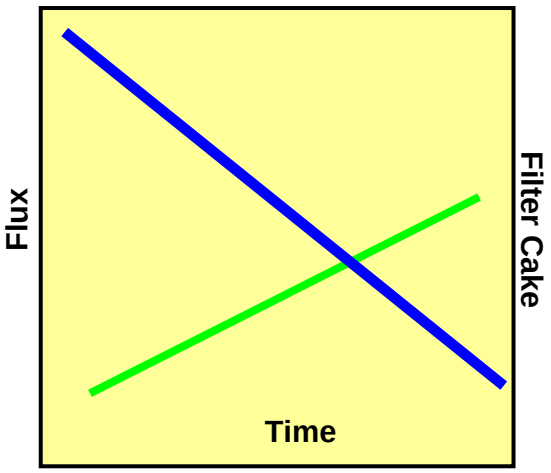

Figure 2.5 Graph flux and filter cake versus time for dead end filtration

2.8.2 Cross Flow Filtration

This flow pattern in which the feed moves parallel with the membrane surface while the retentate moves using the normal way. The mechanism for this type is all the feed flows parallel to the membrane surface. Then, the feed stream is separated into permeate and retentate stream.

For industry sectors, mostly cross flow is preferable as the process can be done continuously. Besides that, the percentage to cause fouling is low as the filter cake will formed mush more slowly compare to dead end filtration. Cross flow velocity is proportional to retentate flow rate. In practical terms the cross flow velocity is equivalent to difference of pressure, ΔP. ΔP is the value for pressure of inlet stream (feed) minus pressure of outlet stream (retentate) in unit bar or Psi.

$$\Delta P = P_{feed \ / \ inlet} - P_{retentate \ / \ outlet} \tag{2.1}$$

The velocity for cross flow can be obtained, not like dead end filtration which the feed is force to go through the membrane. The mechanism on cross flow and the graph against time for flux and filter cake are shown in Figure 2.6 and Figure 2.7.

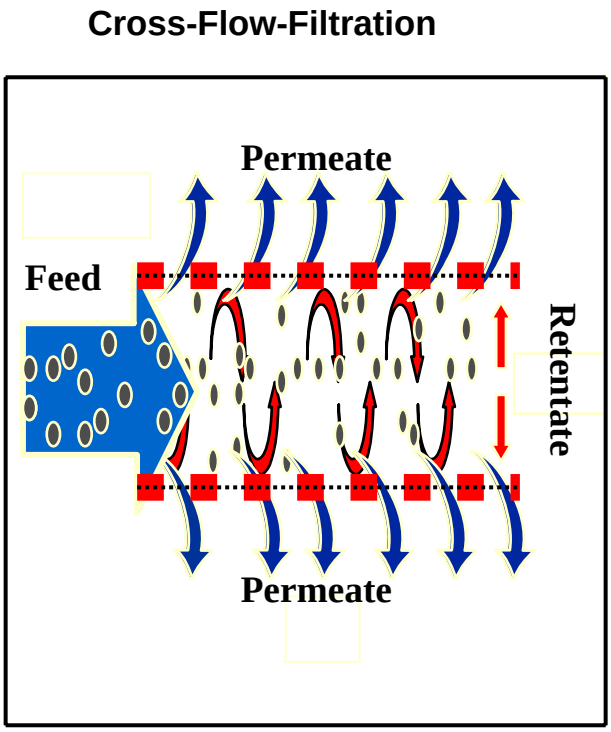

Figure 2.6 Mechanism of cross flow filtration

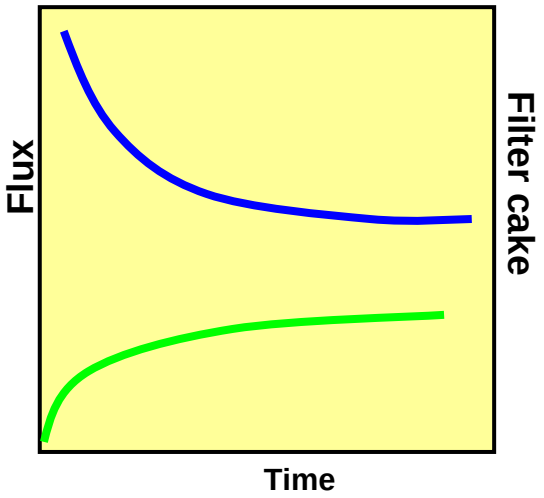

Figure 2.7 Graph flux and filter cake versus time for cross flow filtration

2.9 Operating Factors in Ultrafiltration Separation Process

In ultrafiltration separation process, there are operating factors that need extra concern before the separation occurs. These factors are:

(a) driving force (trans-membrane pressure)
(b) flux
(c) rejection

2.9.1 Driving force

In all membrane separation neglecting any types, driving force for process is depending on trans-membrane pressure (TMP). TMP can be defined as the applied driving force in bar or Psi for the materials to transport through the membrane [2].

$$TMP = \frac{P_{feed} + P_{retentate}}{2} - P_{permeate} \qquad (2.2)$$

TMP for membrane for ultrafiltration is between range 1-10 bar but low TMP is preferable to avoid fouling like showed in Figure 2.8. Pressure for permeate is always equal to zero as there is no permeate pressure at atmosphere pressure. When feed is pumped through the membrane, the TMP will be higher if the pressure is high. But this action will create fouling at the membrane surface earlier that expected. Therefore, it is better to have low TMP around 1.5 bar to avoid fouling and exceed the membrane lifetime as the pore size is extremely small which can easily cause fouling.

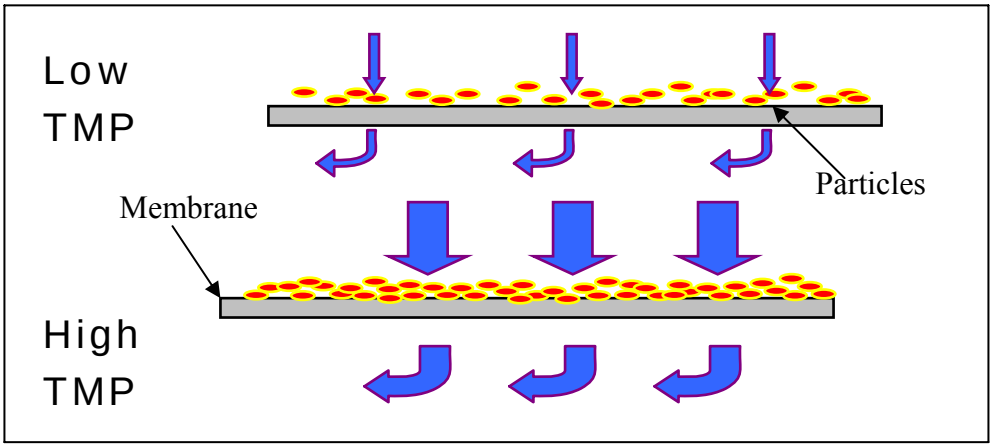

Figure 2.8 The difference between high TMP and low TMP in Ultrafiltration Separation Process

2.9.2 Flux

Flux is the most important parameter and it can describe the performance for membrane separation process. Flux is obtained for permeate, retentate or both based on which stream is needed. Flux has a strong connection with flow rate. Equation below can simplify the connection.

$$Flowrate = \frac{volume}{time} = \frac{litres}{hour} \tag{2.3}$$

$$Flux = \frac{volume}{(area)(time)} = \frac{litres}{(meter)^2 (hours)} \tag{2.4}$$

High value of flux can definitely describe a good membrane separation process but flux can be influenced by factors such as TMP, cross flow rate, temperature, membrane material, configurations, ionic strength, pH, membrane pore size and membrane porosity.