Membrane Processes in Biotechnology

Final report

By,

Woril Dudley Turner (s041491)

Vijaya Krishna Bodla (s041492)

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1. Introduction

Fermentation broths are complex mixtures of biomass, dissolved macromolecules and electrolytes. Separation and concentration of these materials is made difficult, because the desired products are usually in dilute solution, may have similar physical and chemical properties, and may be sensitive to shear stresses. Membrane processes provides a means of separation and concentration at the molecular

and fine-particle level. Various pressure driven membrane processes can be used to concentrate or purify aqueous or non-aqueous solutions. The characteristics of these processes are that the solvent is in the continuous phase and the concentration of the solute is relatively low. The particle or molecular size and chemical properties of the solute determine the structure, i.e. pore size and pore size distribution, necessary for the membranes employed.

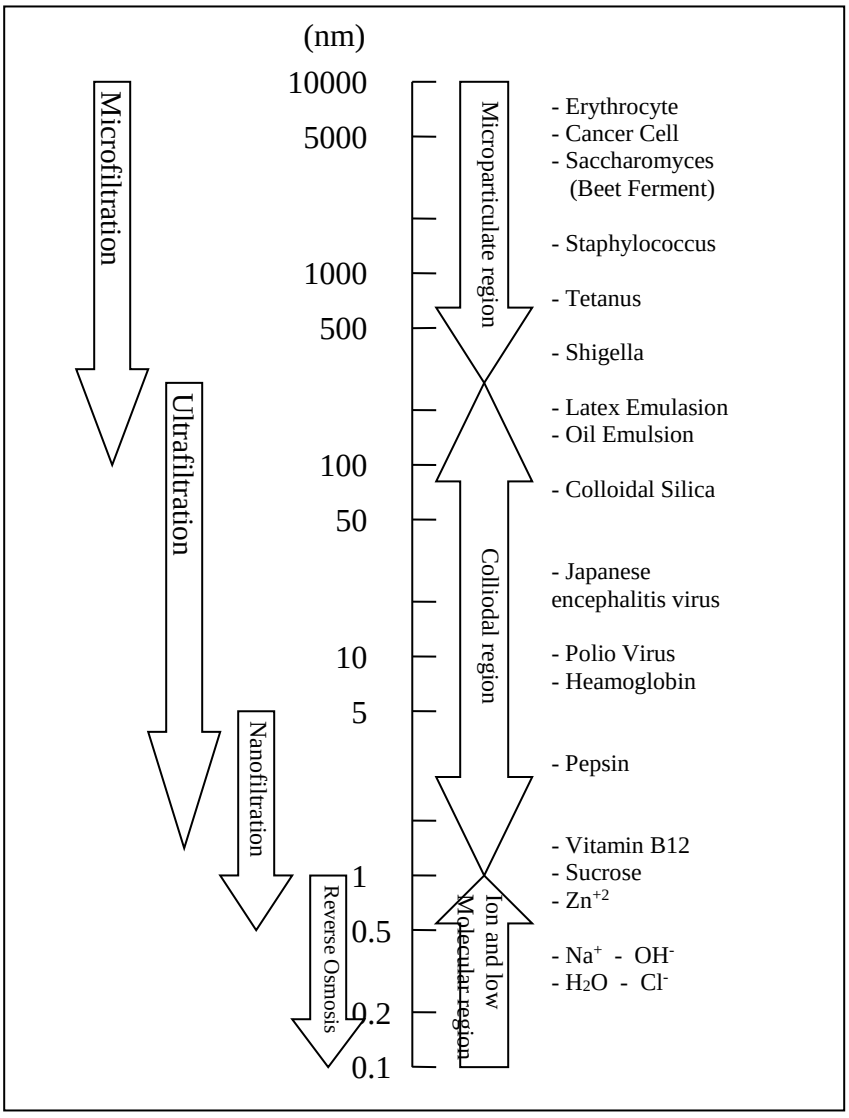

Figure 1. Application of the pressure driven membrane processes in relation to particle or solute size

Various processes can be distinguished related to the particle size of the solute and consequently to membrane structure. The following membrane processes are used for product purification and concentration in the biotechnological industries:

1) Reverse Osmosis

2) Ultrafiltration
3) Microfiltration
4) Dialysis
5) Gas separation
6) Pervaporation
7) Membrane Distillation

In addition a combination of membrane separation and fermentation in a membrane can be done as membrane bioreactors which can use one of the above membrane systems.

First three of these are pressure driven processes i.e. an applied pressure provides the driving force and because of this driving force, the solvent and various solute molecules permeate through the membrane, whereas other molecules or particles are rejected to various extents dependent on the structure of the membrane. Last four of these are activity driven processes. Gas separation depends on permeability and Pervaporation depends both on solubility and diffusivity. Gas separation and Pervaporation require nonporous membranes. Dialysis depends on diffusion and membrane distillation depends on vapor pressure. These processes offer the following advantages for recovering bioproducts:

1) Processing can be at modest, even low temperatures
2) Chemical and mechanical stresses can be minimized
3) No phase change is involved, therefore energy demand is modest.
4) Separation, i.e. purification and concentration may be achieved in one step
5) Equipment for membrane processes is easily scaled up, and is flexible, the process can be batch or continuous process
6) Membrane processes also provides a closed system, i.e. effective containment, which is generally required for most biotechnological processes
7) Membrane Properties are variable and can be adjusted
8) There is a possibility of hybrid processing i.e., combination of different separation techniques

Drawbacks:

1) Strong Fouling tendency
2) Low selectivity for the recovery of a specified product

The membrane processes of interest in the biotechnological industries are described briefly below:

1.1 Reverse Osmosis

Reverse osmosis uses membranes that are permeable to water but not to salts and larger molecular weight species. The term reverse osmosis refers to the fact that applied pressures must exceed the osmotic pressure of the feed before water is forced through the membrane. Reverse osmosis membranes have a dense nonporous skin layer that allows water transport through microvoids, or spaces, between polymer chains. Salt transport is impeded because the ions cannot find “free” water for solvation within the membrane. Other solvents, particularly alcohols, may pass through reverse osmosis membranes.

1.2 Ultrafiltration

The membranes used for ultrafiltration are finely microporous, and in many cases they are asymmetric. In asymmetric membranes the resistance to permeation is concentrated in a very thin layer at the retentate side of the membrane. Water transport is by viscous flow through the pores, driven by a moderate applied pressure. Small solutes may also pass through the membranes, but macrosolutes, colloids and some charge species are retained.

1.3 Microfiltration

This is a pressure driven process similar to ultrafiltration, but the membranes have a larger pore size. Macrosolutes are passed, but larger colloids and micron sized particles such as cells are retained. Transport of solvent and solute through the membranes occurs by convective flow through the micro pores. This convective transport is pressure-driven

1.4 Dialysis

The membranes for dialysis are very fine microporous membranes, although less microporous than ultrafiltration membranes. Dialysis membranes often has the nonporous characteristics of reverse osmosis membranes and the finely microporous characteristics of ultrafiltration membranes. Dialysis separates solute mixtures on the basis of molecular size and, possible molecular conformation and net charge. The driving force is concentration difference. For the removal of ionic species, it is more common to use electrodialysis. Electrodialysis uses ion-exchange membranes and a voltage gradient as the driving force.

1.5 Gas Separation

The membranes used are nonporous membranes. The separation depends on the differences in the permeabilities of various gases thorough the given membrane. The permeability coefficient is a constant intrinsic parameter specific to the gas and membrane defined as the product of the diffusion coefficient and the solubility coefficient. The ideal selectivity is defined as the ratio of the permeabilities. The flux through the membrane is proportional to the permeability coefficient and the pressure difference over the membrane. Permeation rate varies inversely with membrane thickness and thus the permeation properties are optimized by minimizing the effective membrane thickness. The normal membranes used for gas separation are asymmetric membranes and composite membranes. These processes have a high flux and high selectivity but high fluxes and high permeabilities are related to low selectivities. Thus it needs to be optimized.

1.6 Pervaporation

Pervaporation is a membrane process in which a pure liquid or liquid mixture is in contact with the membrane on the feed or upstream side at atmospheric pressure and where the permeate is removed as a vapor because of the low vapor pressure existing on the permeate or downstream side. The low vapor pressure can be achieved by employing a carrier gas or a vacuum pump. The downstream pressure must

be lower than the saturation pressure at least. Both mass and heat transfer occur in this process as the phase transition occurs in the membrane as going from feed to permeate. Thus the heat of vaporization of the permeating components must be supplied. The vapor-liquid equilibrium effects the separation as it affects the driving force. It is very advantageous to use this process for mixtures exhibiting an azeotropic composition where ordinary distillation cannot separate the mixtures. Asymmetric membranes and composite membranes can be used for this process with a dense top layer and an open porous sub layer.

1.7 Membrane Distillation

Membrane distillation is a process in which two liquids or solutions at different temperatures are separated by a porous membrane. The liquids or solutions must not wet the membrane otherwise the pores will be filled immediately as a result of capillary forces. Thus non-wettable hydrophobic membranes must be used in the case of aqueous solutions. When the temperature difference which exists across the membrane results in a vapor pressure difference, the vapor molecules transport through the pores of the membrane from the high vapor pressure side to the low vapor pressure side. This is one of the processes in which the membrane is not directly involved in separation. Selectivity is completely determined by the vapor-liquid equilibrium. The membranes used should normally have a low surface energy. High flux through the membrane can be obtained from high porosity of the membrane but increasing pore size favors wettability. Thus it needs to be optimized. It is also important that membranes should be as thin as possible.

One of the important applications is the removal of volatile bioproducts. It can have a distinct advantage over distillation because of the larger surface are per volume using hallow fiber and capillary modules.

1.8 Membrane Bioreactors

Since fermentation processes sometimes involves inhibitory substances, a combination of a fermenter with a membrane separation system allows the specific removal of an inhibitory component. It can be run both as a batch and continuous process with a cell recycle setup where the broth is pumped through the membrane unit to remove the products and to retain the microorganisms or enzymes. If the substrate and nutrients are added and the products removed then the fermentation can be carried out continuously at much higher concentrations of the biocatalyst. The choice of the membrane system depends on the product that has been prepared.

1.9 Membranes and Modules

In order to implement membrane processes industrially, large membrane areas are normally required. The smallest unit into which the membrane area is packed is called a module. Membrane module design may have to satisfy several criteria, including physical support, packing density, effective fluid management, and suspended solids capability, in situ cleaning and ease of maintenance and replacement. The choice of module design is generally a compromise between capital costs, operating costs, availability and performance of a particular membrane. The simplest design is one in which a single module is used. A number of module designs are possible and all are based on two types of membrane configuration: flat and tubular. The difference between the types of modules generally lies in the

dimensions of the tubes employed.

The size or molecular weights of the particles or molecules separated diminishes moving from microfiltration through ultrafiltration and nanofiltration to reverse osmosis and as a result the pore sizes in the membrane must become smaller, (see figure 1). This implies that the resistance of the membranes to mass transfer increases and hence the applied pressure or driving has to be increased to obtain the same flux. Although no sharp distinction can be drawn between the various processes, it is however possible to distinguish between the various processes in terms of membrane structure. In the case of microfiltration, the complete membrane thickness may contribute towards transport resistance, when a symmetrical porous structure is involved. The membrane thickness can extend from 10 µm to more than 150 µm. However, most microfiltration membranes possess an asymmetric structure build up with a top layer thickness in the order of 1 µm. Ultrafiltration, nanofiltration and reverse osmosis membranes have an asymmetric structure as well with a thin, relatively dense top layer, with a thickness ranging from 0.1 µm to 1.0 µm supported by a porous substructure with a thickness ranging from 50 µm to 150 µm. The hydraulic resistance is almost completely located in the top layer, the sub layer having only a supporting function.

The flux through these and other membranes is inversely proportional to the effective thickness, and because they possess an asymmetrical structure with top layer thicknesses less than 1 µm, membranes of this type are widely used. Biotechnological applications of microfltration and ultrafiltration will be studied in detail in this report.

2. Microfiltration

The rapid growth in the field of biotechnology has led to an increase in the demand for efficient, large scale purification processes. Proteins comprise the largest category of commercially useful bioproducts. Biotechnological processes generally yield products at very low concentrations in the product streams. The product of interest needs to be separated from a large number of impurities, some of which have physical and chemical properties not too different from the product and some are unknown impurities. The ideal bioseparation process must therefore combine high throughput along with high selectivity of separation.

Microfiltration is used extensively in the purification of a variety of bioprocess streams that contain large amount of proteins, e.g. the separation of plasma proteins from blood cells, the sterile filtration of therapeutic proteins prior to final formulation, the harvesting of bacterial, yeast or mammalian cells from protein containing culture medium.

Microfiltration is a membrane process that can be used to concentrate or purify a dilute aqueous or non-aqueous solution. The pore sizes of micro filtration membranes range from 10 to 0.05 µm, making the process suitable for retaining suspensions and emulsions.

2.1 Membranes for microfiltration

Membranes used for microfiltration are porous membranes. Porous membranes contain fixed pores in

the range of 0.1 μm to 10 μm for microfiltration. The selectivity of the membrane is mainly determined by the dimensions of the pores but the choice of the material affects phenomena such as adsorption and chemical stability under condition of actual application and membrane cleaning. The main problem in microfiltration is flux decline because of concentration polarization and fouling and choice of membrane material is primarily based on preventing fouling and how to clean the membranes after fouling.

Microfiltration membranes may be prepared from a large number of different materials, based on either organic materials or inorganic materials. Inorganic membranes are more often used than organic membranes, because of their outstanding chemical and thermal resistance. These membranes also offers the added advantage in that the pore sizes in these membranes can be better controlled. As a result, the pore size distribution is very narrow. Four different types of inorganic materials frequently used may be distinguished:

1) Ceramic membranes
2) Glass membranes
3) Metallic membranes

The large pore structure permits the manufacture of the membrane by a range of processes, examples are:

- Sintering,
- Stretch cracking,
- Track etching.

Sintering can be used to create both polymeric and inorganic membranes. Sintering tends to produce membranes with low porosity and poorly defined pores. This production technique allows the use of materials that have high chemical and thermal stability. The technique has also being used to create metallic membranes.

A modification of the sintering process is the sol/gel process. This produces membranes with an asymmetric structure and potentially lower membrane resistance. Membranes with well defined pore structure are made by this technique.

Polymeric materials can be transformed into microfiltration membranes by a process of stretching. This creates membranes with a ladder like pore structure. The porosity of the membrane can exceed 40%. The majority of the polymers that are suitable for this process are hydrophobic semi crystalline polymers and therefore surface treatment or pre-wetting of the membrane is required if the membrane is to be used for aqueous systems.

2.2 Transport in Microfiltration Membranes

Transport occurs through the pores of the membranes rather than the dense matrix. A large variety of pore geometry is possible, which means that different models have been developed to describe transport adequately, depending on pore geometry. These transport models may be helpful in determining which structural parameters are important and how membrane performance can be improved by varying some

specific parameters.

The simplest representation is one in which the membrane is considered as a number of parallel cylindrical pores perpendicular or oblique to the membrane surface. The length of each cylindrical pore is equal or almost equal to the membrane thickness. The volume flow through the membranes can be described by Darcy's law, the flux through the membrane being directly proportional to the applied pressure:

$$J = A \cdot \Delta P \qquad 1$$

Where A is the permeability constant and contains structural factors such as porosity and pore size. Furthermore the viscocity of the permeating liquid is also included in this constant.

For laminar convective flow through a porous system both the Hagen Poiseuille and the Kozeny-Carman equations can be applied. If the membrane consists of straight capillaries, the Hagen-Poiseuille relationship can be used with $A \approx \varepsilon r^2$. One can therefore write,

$$J = (\varepsilon r^2 \Delta P)/ (8 \eta \tau \Delta x) \qquad 2$$

Here r is the pore radius, Δx is the membrane thickness, η is the dynamic viscocity and τ is the tortuosity factor which is unity in the case of cylindrical pores. However, very few membranes possess such a structure in practice.

When a modular structure exists, i.e. an assembly of spherical particles, the Kozeny-Carman equation can be employed,

$$J = (\varepsilon^3 \Delta P)/ (K \eta S^2 \Delta x) \qquad 3$$

K is a dimensionless constant which depends on the pore geometry; S is the surface area of the spherical particles per unit volume and ε is the porosity.

In order to optimize microfiltration membranes, it is essential to ensure that the structural parameters are such that the surface porosity is as high as possible with the pore size distribution as narrow as possible. The convective flow as described by these equations only involves membrane related parameters and none which apply to the solutes.

Another approach used to describe transport through a porous membrane is the friction model. This considers that passage through the porous membrane occurs both by viscous flow and diffusion. This implies that the pore sizes are so small that the solute molecules cannot pass freely through the pore, and that friction occurs between the solute and the pore wall, between the solvent and the pore wall and between the solute and solvent. The frictional force F per mole is related linearly to the velocity difference or relative velocity. The proportionality factor is called the friction coefficient f.

On considering permeation of the solvent and solute through a membrane and taking the membrane as a frame of reference, the following frictional forces can be distinguished,

$$F_{sm} = f_{sm} (V_s - V_m) \quad (4)$$

$V_m = 0$, so we get,

$$F_{sm} = - f_{sm} V_s \quad (5)$$

$$F_{wm} = -f_{wm} (V_w - V_m) = -f_{wm} V_w \quad (6)$$

$$F_{sw} = -fsw (V_s - V_w) \quad (7)$$

$$F_{ws} = -f_{ws} (V_w - V_s) \quad (8)$$

Subscripts s, w and m refer to solute, water (solvent) and membrane respectively. The proportionality factor f_{sm} denotes interaction between the solute and the polymer i.e. the pore wall.

Using linear relationships between the fluxes and forces in accordance with the concept of irreversible thermodynamics and assuming isothermal conditions, the forces can be described as the gradient of the chemical potential, that is,

$$X_i = - \partial \mu_i / \partial x \quad (9)$$

However, including the friction force acting on component i, the following equation is obtained,

$$X_i = -\partial \mu_i / \partial x + F_i \quad (10)$$

The diffusive solute flux can be written as the product of the mobility, concentration and driving force. The mobility m may be defined as follows,

$$m = D/RT \quad (11)$$

So that the flux now becomes,

$$J_s = m_{ws} c_{sm} (-\partial \mu_s / \partial x + F_{sm}) \quad (12)$$

Where, csm is the concentration of solute inside the pore. The above expression describes the solute flux as a combination of diffusion and viscous flow. Assuming ideal solution then the following expression can be written,

$$(\partial \mu_s / \partial x)_{P, T} = \partial \mu_s / \partial C_{sm} (\partial C_{sm} / \partial x) \quad (13)$$

For dilute ideal solutions, $(\partial \mu_s / \partial x)_{P, T} = RT/C_{sm}$

The frictional force per mole of solute is given by,

$$F_{sm} = - f_{sm} V_s = - f_{sm} J_s/C_{sm} \quad 14$$

Relating the mobility of the solute in water to the frictional coefficient between the solute and water, then,

$$m_{sw} = 1/f_{sw} \quad 15$$

A parameter b can be defined that relating the frictional coefficient fsm, between the solute and the membrane, to f_{sw}, between the solute and water, then,

$$b = (f_{sw} + f_{sm})/f_{sw} = 1 + f_{sm}/f_{sw} \quad 16$$

Combining the equations, the solute flux can then be written as,

$$J_s = - RT/ (f_{sw}\, b) \cdot dC_{sm}/dx + C_{sm}\, V_s/b \quad 17$$

The coefficient of distribution of solute between the bulk and the pore is given by,

$$K = C_{sm}/C \quad 18$$

The frictional coefficient fsw between the solute and water mat be written as,

$$D_{sw} = RT/f_{sw} \quad 19$$

D_{sw} is the diffusion coefficient for the solute in dilute solutions.

$$J_v = \varepsilon \cdot v \quad 20$$

$$J_i = J_s \cdot \varepsilon$$

$$\xi = \tau \cdot x$$

Then equation 17 becomes,

$$J_i = - (KD_{sw}/b\tau)\, dc/dx + KcJ_v/b \quad 21$$

Now because Cp = Js/Vs, integration of equation 21 with the boundary conditions,

$$X = 0 \rightarrow c1,\ sm = KC_f$$

$$X = l \rightarrow c2,\ sm = KC_p$$

Where C_f and C_p are the solute concentrations in the feed and permeate respectively. This yields the following equation,

$$C_f/C_p = b/K + (1 - b/K) \exp(- \tau l J_v/\varepsilon D_{sw}) \quad 22$$

The friction factor b is large when the friction between the solute and membrane, f_{sm} is greater than the friction between the solute and the solvent, fsw. The parameter K is small when the uptake of solute by the membrane from the feed is small compared to the solvent uptake, i.e. when the solute distribution coefficient is small.

An important fact for porous membranes of the type used for microfiltration, is that both the distribution coefficient, which is an equilibrium thermodynamic parameter, and the frictional forces, a kinetic parameter, determine selectivity.

Solute rejection is given by,

$$R = (C_f - C_p)/C_f \quad 23$$

$$= 1 - C_p/C_f \quad 24$$

The maximum rejection, R_{max}, occurs when $Jv \rightarrow \infty$ and is given by,

$$R_{max} = 1 - K/b \quad 25$$

$$= 1 - K/(1 + f_{sm}/f_{sw}) \quad 26$$

This equation shows how the rejection is related to a kinetic term, the friction factor b, and to a thermodynamic equilibrium term, the parameter K. It should therefore be noted that selectivity is considered in terms of a solution-diffusion mechanism, with the exclusion term being equivalent to the solution part and the kinetic term to the diffusion part.

Another equation to describe transport in porous membranes has being proposed by Spiegler and Kedem, "Spiegler, K.S., and Kedem, O., Desalination, 1 (1966)".

$$\delta = 1 - K_s/K_w [f_{sw} + f_{wm} (V_s/V_w)]/ (f_{sw} + f_{sm}) \quad 27$$

Again a thermodynamic term, given by the following expression in the above equation, K_s/K_w, can be distinguished. This term is also known as the exclusion term and is the ratio of solute to water uptake. For a highly selective membrane, this term must be as small as possible. That is the solubility of the solute in the membrane must be as low as possible. This can be achieved by selecting the appropriate polymer.

2.3 Microfiltration of Fermentation broths

The flux in microfiltration systems is largely influenced by factors external to the membrane. Microfiltration membranes can be operated in dead-end mode or cross flow mode. Microfiltration membranes retain particulate matter and in dead end mode the material does not tend to diffuse away

from the membrane surface once carried to the surface. This gives rise to the build up of a layer of material that increases over time and it is this layer that presents the greatest resistance to solvent transport.

If the membrane unit is operated in cross flow mode, the retained layer at the membrane surface reaches a steady state thickness that is a function of the velocity of the retentate phase parallel to the membrane surface. Increasing the velocity of the retentate can reduce the build up of the retentate layer. The majority of microfiltration processes in the biotechnological industries are conducted in this manner if complete separation of retentate and permeate is not a requirement.

Microfiltration is used mainly to harvest and concentrate cells and to recover enzymes or proteins from fermentation broths, or cell lysates. For example Griffith et al (1980) reported that 100% of the scleroglucans were recovered in the permeate of a cross flow microfiltration system, using a 5 μm microporous membrane to filter the Sclerotium glucanicum fermentation broth. Flux was typically 1.0 $mL/min \cdot cm^2$.

Although microfiltration is one of the oldest pressure-driven membrane processes, it is probably the least understood when it comes to the filtration of suspensions and macromolecules. Microfiltration is characterized by operation at low pressures, high permeation fluxes and by cross flow operation mode in flat or cylindrical geometries. The major limitation of microfiltration is membrane fouling due to the deposition of macromolecules on the membrane surfaces and the intrusion of these molecules into the pores of the membrane. As a result, the flux through the membrane decreases with time. The flux decline can be very severe with the process flux often being less than 5% of the pure water flux.

Flux decline can be caused by several factors such as concentration polarization, adsorption, gel layer formation and plugging of the pores. The flux in microfiltration systems is largely influenced by factors external to the membrane. Microfiltration membranes can be operated in dead-end mode or cross flow mode. Microfiltration membranes retain particulate matter and in dead end mode the material does not tend to diffuse away from the membrane surface once carried to the surface. This gives rise to the build up of a layer of material that increases over time and it is this layer that presents the greatest resistance to solvent transport.

The flux can be defined by the following relationship,

Flux = driving force/ (viscosity · total resistance)

Various forms of resistances can be defined which contributes to the total resistance. They are:

1) Resistance due to pore blocking, denoted as R_p
2) Resistance due to adsorption, denoted as R_a. Adsorption can take place upon the surface of the membrane surface as well as within the pores themselves.
3) Resistance due to the membrane itself, which can be denoted as R_m. In the ideal case, only membrane resistance is involved.
4) The membranes retain the solutes to a certain extent; there will be an accumulation of retained molecules near the membrane surface. This result in a highly concentrated layer near the

membrane and this layer exerts a resistance to mass transfer, i.e. a concentration polarization resistance which can be denoted as R_{cp}.

5) The concentration of the accumulated solute molecules may become so high that a gel layer can be formed which exerts the gel layer resistance, which can be denoted by R_g. Gel layer formation is more pronounced in biotechnical applications where the solution contains proteins.

2.3.1 Dead End Microfiltration

When a suspension contains suspended macromolecules that are too large to enter the membrane pores, then the surface filtration mechanism of sieving occurs. The retained particles accumulate on the membrane surface in a growing cake layer. The cake layer forms an additional increasing resistance to filtration, so that the permeate flux declines with time. For unstirred dead end filtration, in which the fluid motion is perpendicular to the membrane surface, the cake continues to grow until the process is stopped.

Dead end filtration is normally used if the microfiltration process is being operated in batch mode. Processes such as sterilization, where bacterial cells are being separated from a solution, often requires complete separation of the retentate and permeate, thus microfiltration is conducted in the dead end mode.

Particles retained by the membrane does not tend to diffuse away from the membrane surface once carried to the surface. The flux can be expressed in terms of the transmembrane pressure difference and the sum of the cake resistance and the membrane resistance,

$$J = \Delta P / (R_c + R_m) \qquad 28$$

R_c = cake resistance
R_m = membrane resistance

In many processes flux will be maintained constant by increasing the pressure differential across the membrane until some limiting pressure is reached in which case the process is stopped and the membrane cleaned or replaced.

The build up of a cake in dead end microfiltration presents a problem that may be minimized by adopting some of the following strategies:

1) Installation of a depth filter upstream of the microfiltration module. This will reduce the load on a dead end microfiltration module. The depth filter removes particles by entrapment and adsorption and has a greater capacity to retain particles but retention is not absolute. Microfiltration membranes provide an absolute cutoff.
2) Regular backwashing of the filter. Regular backwashing will remove the cake buildup.
3) Employing microfiltration, when complete separation of permeate from retentate is not required and continuous mode or semi-continuous mode of operation improves process economics. The use of cross flow helps maintain the cake at reasonable levels and permits continuous operation

provided not all the filtrate is required to be recovered.

2.3.2 Cross Flow Microfiltration

If the membrane unit is operated in cross flow mode, the retained layer at the membrane surface reaches a steady state thickness that is a function of the velocity of the retentate phase parallel to the membrane surface. Increasing the velocity of the retentate can reduce the build up of the retentate layer. This mode of operation means that the outlet concentration of the retentate phase must be such that the phase can still be pumped so complete recovery is not possible.

Although concentration polarization can occur in all membrane processes, its effect are most pronounced in microfiltration and ultrafiltration. The accumulation of retained species at the membrane surface adds resistance of transport of molecules through the membrane and therefore has a major impact on flux.

Microfiltration membranes in cross flow modes also suffer from fouling of the surface. The effects are similar to that of concentration polarization in that fouling reduces flux, but it is differentiated from concentration polarization because the effects are not immediately reversible if the flow conditions are changed. The fouling process follows several different patterns: adsorption of the protein molecules on the membrane surface and in the pores and clogging of the pores of the membrane. Generally fouling materials have to be removed by chemical cleaning of the membrane when it is out of service.

The effect of fouling on transmembrane flux and rejection characteristics is one of the most serious problems affecting pressure driven membrane applications. Flux decline has a negative influence on the economics of a given membrane. Proteins are a class of macromolecules particularly susceptible to adsorption at polymeric membrane surfaces. Solution properties such as pH, ionic strength, temperature, solute type, concentration, viscosity and membrane properties such as hydrophobicity, porosity, surface charge and module configuration leading to hydrodynamic conditions all affects fouling.

The pH and ionic strength affect protein membrane interactions, therefore affecting the transmembrane flux and rejection. Organic non-biological membranes immersed in aqueous solution are charged. This attracts a layer of oppositely charged ions from the solution which forms a cloud or layer of counter-ions which extends out from the surface for some distance. With the surface of the membrane negatively charged, the layer of counter-ions will be positively charged and will tend to repel positively charged colloidal particles away from the surface of the membrane. The interaction between the electrical double layer and charged protein ions being filtered can impact on protein adsorption on the membrane, thus having a major impact on the membrane flux performance.

The interaction and thus adsorption and rejection are influenced by changing the ionic strength and pH. At a pH not close to the isoelectric point, the charged protein may be repelled by the electrical field within the pores of the membrane surface. Protein transmission will then be at its lowest, whilst the flux will be enhanced because pore blockage is less likely to occur. This type of behavior is more likely to occur at low pH, around 3.5. At an alkaline pH, at around 8.5 or greater, the proteins ions are oppositely charged and attraction occurs followed by pore blockage. As a result the protein transmission and flux will be at their lowest. At the isoelectric point the proteins has no net charge and there is reduced

electrokinetics with the membrane, resulting in constant protein transmission but reduced flux.

Under conditions in which the proteins can be denatured, protein multilayers can form in the pores, causing a dramatic decline in performance. Studies of protein fouling indicate that the initial flux decline during the microfiltration of protein containing solutions is primarily due to the deposition of protein aggregates, with these aggregates either blocking or constricting the membrane pores. These protein aggregates can be formed during the initial preparation of the protein solutions, or they may be generated during the filtration process, either by the pumps employed in most cross flow filtration systems or by the high shear rates that exist in the vicinity of the membrane surface or in the membrane pores. Studies have demonstrated that any processing steps typically associated with an increase in protein denaturation will also cause an increase in aggregation and in turn protein deposition.

2.3.2.1 Reducing Concentration Polarization and Fouling

Several approaches are used to counteract fouling and concentration polarization. These include modifying the surface chemistry of the membrane so as to reduce the attractive forces or increase the repulsive forces between the solute and the membrane. Direct chemical techniques for modifying the membrane surfaces are used commercially such as heterogeneous chemical modification, adsorption of hydrophilic polymers, irradiation methods and low temperature plasma activation.

Surface treatment has little effect on the behavior of suspended particles once a secondary cake has been established. In this case, fluid mechanical approaches such as steady and unsteady flows are paramount. Steady flows generally requires cross flow velocities in the turbulent regime, whilst unsteady flows can be effective both in the laminar and the turbulent regimes. Rough channels have been used to induce fluid mixing at the membrane-solution interface. Reversal of axial cross flow and permeate flow have also been used to increase fluxes. Fluid instabilities due to flow in curved ducts have been used to disturb the flux limiting effects of concentration polarization.

Although steady cross flow can be effective in reducing fouling, unsteady flow conditions provides a much more effective tool for accomplishing this goal. There are various ways to induce instabilities in bulk flow across a membrane surface. These include designing membrane surfaces with organized roughness, pulsation of axial and lateral flow, and the use of curvilinear flow under conditions that promotes instabilities or vortices.

Placing protuberances directly onto the membrane surface at defined separation distances induces periodic unsteady flows. These instabilities are produced at the solution-membrane interface, where they depolarize the solute build up. Although rough membrane surfaces do give improved performance over a smooth surface, it has its disadvantages:

a. There are increased axial pressure drops and as a result diminished active surface area results.
b. In order to increase the intensity of the vortices, protuberances are placed at some defined distances away from the membrane surface with pulsatile bulk flow. These techniques are difficult to scale up.

Membrane type seems to have a big impact in fouling of membranes used for protein flirtation. Protein adsorption does appear to be reduced on more hydrophilic membranes. In addition, it appears that intermolecular sulfhydryl-mediated interactions between proteins may be the molecular basis for the aggregation of these proteins, and this may provide a chemical framework for the analysis and possible control of protein fouling during microfiltration.

Hydraulic cleaning methods, i.e. back flushing can be employed. This is done by alternately pressuring and depressuring, and changing the flow direction at a given frequency. After a given period of time the feed pressure is released and the direction of the permeate reverse from the permeate side to the feed side in order to remove the fouling layer within the membrane, or at the membrane surface. A variant of this method known as the back shock method has been developed. Here the time interval of back-flushing has been reduced to seconds, which ensures that the cake resistance remains low since it has no time to build up a layer. The net permeate flux for a membrane filtration with back pulsing is compromised by the permeate flow reversal as it takes time to recover the permeate rate during forward filtration. The overall flux is the weighted average of the fluxes from these two distinct stages. It can be calculated by measuring the difference between the permeate volume collected during the forward filtration and that lost during reverse filtration, and then divided by the membrane area and total filtration time.

Back pulsing frequency, is an important parameter in optimizing membrane performance. High back pulse frequencies are found to be necessary for the most effective back pulsing operation and to maintain high sieving coefficients. However, in order to achieve acceptable cycle times for manufacturing, sufficiently net permeate fluxes must be maintained. Optimizing cycle time requires balancing conditions to achieve sufficient sieving coefficients and sufficiently large permeate flux.

Early models assumed that the flux declines during forward filtration according to the dead-end filtration theory, and the gel layer is assumed to be instantly and completely removed during each back pulse. Later models were introduced which accounted for incomplete cleaning of the membranes.

Other cleaning methods are essential for maintaining adequate long term operation of all pressure-driven membrane processes. These include mechanical, chemical and electrical cleaning methods. The choice of cleaning method depends on the module configuration, the type of membrane and the type of foulant encountered.

Mechanical cleaning can only be applied in tubular systems using oversized sponge balls.
Chemical cleaning is the most important method for reducing fouling, with a number of chemicals being used separately or in combination. The concentration of the chemical and the cleaning time are very important parameters, relative to the chemical resistance of the membrane. The use of chemical cleaning is however, limited with membranes used for protein filtration, where chemical residues can irreversibly denature proteins, reducing protein activity.
Electric cleaning is a very special method of cleaning. By applying an electric field across a membrane charged particles or molecules will migrate in the direction of the electric field. This method offers the advantage in that it can be done without interrupting the process and the electric field is applied at certain time intervals. A disadvantage though, is that electric conducting membranes are required and special module arrangements with electrodes.

2.4 Examples of Microfiltration in biotechnical applications

1) A Microfiltration bioreactor is used to achieve higher cell yield in Sulfolobus solfataricus fermentation.

The accumulation of toxic compounds is thought to be responsible for low biomass yields; a bioreactor was designed from the literature, based on a Microfiltration hollow-fiber module located inside the traditional fermentation vessel. The Microfiltration module proved highly resistant to the extreme working conditions typical of thermoacidophile fermentation. High cell densities were reached during the process, but repeated back flushing was able to maintain the transmembrane flux at an average value of 75% of the theoretical maximum. This was considered to be a very large improvement when compared to the usual cross flow filtration where flux can decline to 20% maximum.

The assembled membrane bioreactor allowed a cell production that was 15 to 20 folds greater than the traditional process. The results also confirmed the active metabolism of the biomass produced. Results also validate the Microfiltration technique as a sound approach for industrial scale production of biomass related product. From experimental data it was shown that 18 batch processes should be carried out to produce an amount of biomass equal to that produced in Microfiltration experiments. The most important difference that was observed is the number of working hours needed to reach the same amount of biomass. Repeated batches would require more than 2000 hours without considering time for cleaning and sterilization of the fermenter between processes, while Microfiltration needs only 310 hours.

2) Inclusion body purification and protein refolding using Microfiltration

The production of recombinant proteins from E. coli is limited by the expression of the cloned gene product as inclusion bodies. These aggregates have no biological activity and there is a need to solubilise the inclusion bodies and refold the protein into its native structure. Aggregation of folding intermediates is the dominating unwanted reaction that occurs during refolding. The impurities present in inclusion bodies may affect aggregation and will therefore influence the final refolding yields. Contaminants include nucleic acids, proteins and phosphor lipids.

The presence of inclusion body impurities can affect the refolding yield of recombinant proteins, thus there is a need to purify inclusion bodies prior to refolding. A comparison was done of centrifugation and membrane filtration for the washing and recovery of inclusion bodies of recombinant egg white lysozyme. It was found that the most significant purification occurred during the removal of cell debris.

With Microfiltration, use of a 0.45 μm membrane gave higher solvent fluxes, purer inclusion bodies and greater protein yield as compared with a 0.1 μm membrane. Purified inclusion bodies gave rise to higher refolding yields. Significant flux decline was observed for both membranes.

Previously, centrifugation was the main separation technique employed for inclusion body purification. However, centrifugation is very sensitive to inclusion body size, which can vary depending on the fermentation conditions and often overlaps with the cell debris size distribution. This leads to inclusion bodies sedimenting, with the cell debris being removed in the supernatant. However, it is impossible to

separate the solid particles quantitatively and thus some of the cell debris will co-sediment with the inclusion bodies. A disadvantage is that proteases can be associated with the cell debris and may lead to subsequent degradation of the product.

More recently cross flow Microfiltration is being used as an alternative to centrifugation. The membrane process is considered a more robust process to deal with the differences in inclusion body characteristics that arise from the upstream process. For cases where density differences between inclusion bodies and cell debris is very small, variations in the process fluid will result in changed centrifugation performance and require re-optimization of the centrifuge operation. This problem does not occur with the use of cross flow microfiltration, because the process is not based on density differences. Membrane pore size however has a large effect on the process. Larger pore size allows more solvent to pass through the membrane at the same pressure as that with smaller pore sizes.

3. Ultrafiltration

The Ultrafiltration membranes have smaller pores compared to microfiltration and are able to reject macro-molecular solutes in the molecular weight range of about 10^3-10^6 Da. Typical pore diameters are in the range of 0.05 µm to 1 nm. Rejection is mainly determined by the size and the shape of the solutes relative to the pore size in the membrane and the transport is directly proportional to the applied pressure.

Applications of ultrafiltration are found in the areas of plasma products, vaccines, diagnostic enzymes, fermentation products and depyrogenation of parenterals. Ultrafiltration has allowed economical, efficient concentration and purification of the biologicals and simple scale-up of production processes.

Ultrafiltartion membranes are produced in a wide variety of polymers that exhibit high resistance to acids, bases, alcohols and temperature allowing effective membrane cleaning. In this way ultrafilters can be reused without deterioration of flow rates and without cross contamination. In most cases, filter lifetime lasts for one to four years. The anisotropic structure of these membranes allows retention of retained substances to take place on the membrane surface rather than within the filter structure.

The concentration of the dissolved species can create some processing problems. If the membrane retains a solute, the solute concentration adjacent to the membrane will increase, resulting in a phenomenon called concentration polarization. Polarization will cause the filtration flux to decline logarithmically with increasing concentration of the bulk solute. The problems can be controlled by various methods. The principle behind them centers on maximizing the movement of solute away from the membrane. This is usually accomplished by cross-flow filtration where retained fluid is recirculated over the membrane surface. Other approaches involving mechanical agitation, with stir bars and vibrators, are limited to small scale equipment. The rate of filtration flux will increase the efficiency of polarization control. When cross-flow methods are used, flux will generally improve with increased velocity across the membrane surface.

3.1 Membrane Configurations

There are two basic configurations of ultrafiltration membranes – hallow fibers and flat sheets. Filtration equipment with these membranes utilize a variety of operational modules. Hallow fibers are generally supplied in a self-contained cartridge housings. Fibers are easy to clean and allow good product recovery. They are somewhat limited in pressure capability. Larger diameter membranes tubes are basically a variant of hallow fibers. Due to their large hold-up volume and high space requirements, tubular membranes are not well suited for biological purification.

Flat sheet membranes have been fabricated in several types of modules. Spiral wound cartridges use a rolled membrane design with spacer screens separating the filters. Certain plate-and-frame type devices also use separator screens except that the membranes are stacked vertically. These devices allow high pressure operation but have cleaning and product recovery problems due to the clogging of the screens. There are some true plate-and-frame devices available that are easier to clean, but these tend to require more space.

3.1.1 System Operation

In the cross-flow filtration operation, the recirculating flow creates a pressure differential between the inlet and the outlet of the system on the retentate side of the filter. The relative recirculation velocity can be measured by the pressure drop from the cartridge inlet to outlet. The recirculation velocity is directly proportional to ΔP in laminar flow and square root of ΔP in turbulent flow.

The filtration driving force is determined by difference between the retentate pressure and that of permeate. Since the retentate pressure decreases from inlet to outlet, an average value is used to determine the transmembrane pressure. In most cases, permeate pressure is negligible.

In system operation, flux rates are optimized by variation of the recirculation rate and outlet pressure, inlet pressure is usually maintained at maximum allowed by the system. It should be noted that the optimum operating conditions are a strong function of product concentration, a higher ΔP required at elevated solute concentrations. At a certain point, outlet pressures of zero are generally used.

Another consideration in system operation is that of maintaining flux rates and cleaning. Most systems are cleaned following operation by flushing with sodium hydroxide, sodium hypo chlorite or other suitable chemical agent. This is normally done on standard ultrafiltration modes of operation but in cases where severe membrane fouling has occurred, membranes can be backflushed with cleaning solution.

A method used for cleaning during product processing is that of recycling. In this case, permeate outlets are closed while the cartridge is filled with ultrafiltrate. This causes back flushing on the outlet end of the cartridge, where the permeate pressure now exceeds the retentate pressure. By then reversing the direction, the other end of the filter can be similarly cleaned. This procedure requires only a few minutes to perform and has proven very useful in regenerating flux rates in process. Recycling is particularly effective in dealing with process streams with high-suspended solids, such as bacterial cells and protein precipitates.

3.1.2 Process Consideration

The operation of ultrafiltration can be broadly categorized into: Concentration, Diafiltration or Purification.

1) Concentration of suspended particles or macromolecules is where retained stream is the product.

In the batch system, the so called concentration factor is merely the ratio of the starting volume to the final volume. The concentration of the completely permeable species such as a salt will not effected by ultrafiltration.

2) Diafiltration of suspended particles or macromolecules is where the retained stream contains the product and low molecular weight solutes such as solutes, sugars and alcohols, pass through the membrane by the addition and removal of water. The permeate that leaves the system is replaced with deionized water, ideally through a level controller at the same rate that permeate is removed. A buffer may be used instead of water if salt exchange is desired.

In processes where diafiltration is combined with concentration, the desalting can be performed before or after concentration. After concentration, smaller diafiltrate volumes are needed but flux rates are slower due to higher protein levels. The optimum point for diafiltration may lie between the desired initial and final protein concentrations.

3) The third type of ultrafiltration process involves the purification of solvents and solutions of low molecular solutes where the permeate stream contains the product, the retained product may also contain product of interest.

Processes where low molecular weight solute is the product of interest generally combine concentration and diafiltration. The retained stream is first concentrated to near its maximum level, while allowing purified solutes to pass through the membrane. A diafiltration step then washes out additional solute from the retentate. In this way maximum recovery of product is obtained with minimal dilution. The retentate is also highly purified, this is important in some fractionation processes where the macromolecules may also be a product of interest.

3.1.3 Bacteria Concentration or Cell Harvesting

The recovery of bacteria and cells after fermentation and culture by centrifugation may be tedious, inefficient and costly due to problems such as aerosol formation, poor product recovery and slow processing. These difficulties are more pronounced when dealing with large batch volumes. Ultrafiltration has been demonstrated to be an attractive alternative to centrifugation for cell harvesting. The unique membrane structure rejects bacteria at the filter surface and prevents clogging. Highly porous microfilters permit penetration of bacteria into the filter matrix. This can lead to irreversible clogging even when used in a cross-flow arrangement. Clogging problems can also be experienced in devices with separator screens, particularly at high solids levels.

Hallow fiber devices can be particularly effective in concentration of bacteria and cells due to their high resistance to clogging. *E.coli* suspensions are said to be concentrated to small volumes achieving high cell densities – over 80-90% by volume. Other micro organisms can also be included – *B.subtilus, Brucella, Pseudomonas, Streptomyces, yeasts, mold and algae*.

Ultrafiltration of such cells behaves differently that a typical protein solution in terms of flux/concentration profiles. Most bacteria suspensions will exhibit relatively little decline in flux at lower cell densities. However when cells approach a near packed condition, filtration rates will drop very sharply. This behavior results from two actors: (1) suspension viscosity rises markedly at higher cell densities (2) the cells are discrete particles, not dissolved molecules. The membrane does not see a build up of macrosolute on the surface that causes true polarization.

Cells may also be washed out without a number of pelleting and suspension steps. Broth, medium, and extracellular products can be recovered while washing solution is used in a diafiltration mode. Hallow fiber systems have been used successfully in several such applications, including the recovery of interferon from cell suspensions.

In cell washing processes, the choice of concentration at which diafiltration is performed is critical. If cell density exceeds the critical point where rapid flux decay takes place, the overall process time can be much longer.

If the product is extracellular such as an antibiotic, minimizing process time may not be the only consideration. Initial washing is the more rapid method but uses more permeate volume. This would further dilute the extracellular product and may not be desirable. This choice would depend on the cost of recovering this product.

Use of ultrafiltration can solve many of the problems associated with centrifugation. First of all equal processing rates can be achieved with a fraction of capital expense. Systems can be easily scaled up. Aerosol problems can be eliminated as discussed before.

3.1.4 Product Concentration or Desalting

Aside from bacteria concentration, ultrafiltration has been important in concentration and diafiltration of many other biological products, such as interferon, insulin, lymphokines, peptides, plasma proteins, growth hormones, monoclonal antibodies and enzymes.

Protein adsorption to the membrane can present a problem in product recovery, particularly with very dilute solutions. In most cases, adsorbed product can be recovered by backflushing with buffers or similar washing techniques. Pretreatment of the membrane with a protein such as albumin has also been effective. In these situations, process trials on a small scale are critical in determining optimum membrane type and procedure.

3.1.5 Purification

Major product purification applications of ultrafiltration are: pyrogen removal, deproteinization of growth medium and cell debris removal.

Products of fermentation, such as antibiotics, often contain pyrogen levels unacceptably high for injectable drugs. Pyrogens are generally lipopolysaccharides derived from fragments of bacterial cell walls. Removal of pyrogens has been successfully carried out by ultrafiltration with successful purification of water as well as low molecular weight parenteral products.

Intracellular products of cell culture must also be free of cellular debris for further purification. The same diafiltration techniques used in cell washing may be applied to solutions containing lysed cell fragments. Cell debris has been successfully removed from such products as interferon with hallow fiber filters. The typical yields are about 80-100%.

Fermentation broths frequently incorporate hydrolysates of soy, casein and other proteins. Such broths contain high molecular weight contaminants before fermentation. This simplifies the later product recovery steps. The filtration step also removes bacteria from the broth before use.

3.1.6 Continuous Fermentation

Research on the use of membranes in small scale dialysis fermentation systems shows substantial removal inhibitory by-products in cell mass and total product yield. Continuous removal of inhibitory by-products allows growth to continue for weeks.

Hallow fiber membranes offer the same advantage as dialysis fermentation but allow easy use in industrial environment. They have proven successful in increasing total cell densities to substantially higher levels than those found in normal batch fermentations. These bacteria can be withdrawn continuously to extract intracellular products or to test cell viability. Where extracellular products are of interest, filtrates are removed for further purification.

The use of hallow fiber ultrafiltration cartridges in a high-solid fermentation system has been reported. The membrane cartridge was connected to a fermenter to remove waste effluent, while fresh substrate was added to the broth. Production efficiency was increased by 100%. It was also determined that at high cell density, lactic acid biomass production was controlled by substrate limitations rather than by product inhibition.

3.1.7 Continuous Cell Culture

Large scale growth of animal or plant cells in culture can be difficult with conventional techniques. Approaches examined for suspension cultures as well as anchorage-dependant cell growth include roller bottles, micro-carrier beads, stacked plate systems and multiple tubes.

Hallow fiber cartridges have been evaluated extensively for growth of animal cell lines. Cells grow to tissue like densities as opposed to monolayers in roller bottles and on microcarriers. Typical densities are

about 10 times higher than those achieved in roller bottles. Cultures can be maintained for as long as months at a time and have been scaled for production purposes with specialized cartridges and systems.

3.2 Biotechnology applications

There is a range of applications within the field of biotechnology, concerned with the transformation of components by biocatalysts. These include microorganisms, enzymes and plant or animal cells. The range of substrates and products is vast and the desired product may be either the cells themselves or their metabolic by-products, which may be intracellular or extracellular. UF membranes have a role, both in the primary production process, for example as a reactor for fermentation processes or enzymatic reactions, or in downstream processing for recovering the products.

3.2.1 Membrane-based bioreactors

Membrane-based bioreactors appear to be a very promising application for the production of ethanol, lactic acid, acetone and butanol, starch hydrolysates and protein hydrolysates; further refinements include the introduction of electrodialysis and pervaporation, into selective processes. The two main types of membrane reactor are the enzyme reactor and the fermentation reactor.

3.2.1.1 Enzyme reactors

Many industrial processes involve the use of enzymes to break down molecules, for example the hydrolysis of starch or proteins, or of simpler molecules such as sucrose or lactose. Most such reactions involving enzymes are of a batch nature and the enzyme needs to be inactivated or is lost at the end of the process because it is not easy to separate it from the product. The processing costs of batch processes are also high. There is considerable interest in continuous processes, where the enzyme is either immobilised or retained within the reaction vessel and reutilised, whereas the products are removed and reutilised. Ultra-thin membranes have been investigated for both these types of application, in the form of enzymatic reactors. Immobilisation of enzymes provides an alternative method to overcome some of the disadvantages of using free enzymes. One system which has been much investigated is the immobilisation of the enzyme by adsorption and entrapment onto the outside of tubular membranes. Hollow fibre systems have been found useful for this purpose, with the enzyme being immobilised on the shell side of the reactor. This is useful in situations where the product and reactant are normally much smaller than the enzyme and both diffuse into the membrane from the feed. One example is the hydrolysis of lactose. These types of reactors are usually operated in plug flow mode. A variation on this theme is to immobilise the enzyme in the tubes and to pass the reactant through the shell; this is less common. The enzyme may also be immobilised within the membrane by incorporating it into the casting solution.

The second variant is where the enzyme is incorporated into the reaction vessel and the membrane not only physically retains the enzyme, but also allows a continuous removal of the product. This is not immobilisation in the strict sense of the word, but it does provide a mechanism for long-term usage. This arrangement is useful for hydrolysis of macromolecules, where the required end-product is often much smaller in molecular weight than the reactant or the enzyme. Dead-end or flow-through systems have

been used. The dead-end system uses the membrane unit both as the reaction unit and for separation and has been more widely investigated Flow-through systems use a cross-flow filtration unit connected in series with the reaction vessel. This arrangement has two major advantages over the dead-end system, namely better induced turbulence to reduce concentration polarisation, which leads to poor activities in dead-end reactors after only a few hours operation and a separate location for the reaction and separation vessels, thereby permitting each to be operated at its optimum set of working conditions, rather than at a compromise set of conditions.

Both systems operate essentially as a continuous stirred tank reactor (CSTR) and feed is supplied to replace the permeate removed. CSTRs are characterised by a wide distribution of residence times and are usually considered to be well mixed, i.e., the concentration of the feed to the membrane is the same as that in the reactor vessel. Thus at high conversions, the substrate concentration would be low; they are particularly useful for reactions which may be inhibited by substrate concentration; at first sight they may also appear useful because it helps reduce end-product inhibition. However, this is not necessarily the case because the end-product concentration in the permeate will be the same as that in the feed A further feature for first- and second-order reactions is that a large volume is required for a CSTR compared to a plug flow reactor The major advantages offered by this type of reactor are a high throughput and productivity, governed by a high enzyme concentration attainable and a constant and long-term use of the enzyme.

Retained activity over a long period is an important function for membrane reactors. Shear damage to enzymes was once thought to be important, although there was little experimental evidence to suggest that it is a major cause of reduced activity over the short term.

Vegetable proteins are more difficult to hydrolyse than animal proteins. The higher the levels of non-hydrolysable protein, the shorter will be the operational period for the reactor, due to the build-up of protein within the system.

Starch hydrolysis for the production of glucose syrups has also received some attention. There are two stages to the process, namely liquefaction and saccharification. Ideally, it would be desirable to perform both stages simultaneously. Liquefaction is difficult to achieve with enzymes at appreciable starch concentrations, because of the high viscosities involved. Saccharification, which makes use of glucoamylase enzymes, is more suited to an enzyme membrane process as the batch process can take up to 48 h and the substrate is less susceptible to concentration polarisation and fouling, due to its lower viscosity.

Other enzymic reactions which have been investigated include the hydrolysis of cellulose, soy protein, fish protein and casein. A semi-continuous process for lignocellulose hydrolysis has also been described.

In these long-term continuous processes it is important to minimise enzyme losses and in some processes, to retain, recover or regenerate important enzyme cofactors. Cofactors are required by many enzymes for activity and are classified as prosthetic groups, coenzymes and metal ions. They include compounds such as NAD+, FAD+, ATP, CoA. Prosthetic groups are distinguished from coenzymes by their tighter binding to the enzyme, although there is considerable overlap in binding affinities of these two groups.

The cofactors may be immobilised or entrapped with the enzyme or retained within the system by selecting a membrane with a low molecular weight cut-off, which will totally reject them. Another approach is to use negatively charged membranes, which also give rise to higher rejections due to electrostatic interactions. Those which have mainly been investigated are the nicotinamide (NAD and NADP) cofactors.

3.2.1.2 Membrane Fermenters

Fermentation processes can also be batch or continuous. Even with continuous processes, the conversion rate may be limited by end-product inhibition and the dilution rate must be less than the specific growth rate of cells, to avoid cell washout. Therefore, another application of membrane reactors is as part of a fermentation vessel, with containment of microorganisms and continuous removal of products. Again the microorganisms can be entrapped or immobilised, typically in the hollow fibre system, or simply allowed to circulate freely with the recycle broth. The freely circulating systems seem to perform more effectively than the immobilised systems. This system operates like a continuous system, with the additional feature that the cells are returned to the fermenter and retained within the system. It is possible to achieve high cell densities and high dilution rates, without the worry of washing out the cells. However, they have not been as thoroughly investigated as enzyme reactors.

One of the most popular applications is the production of alcohol. Cheryan (1986) compares the performance of batch fermentation processes, with continuous culture, immobilised cells and membrane recycle. The membrane recycle gives the biggest productivity in terms of mass of ethanol per unit volume and time. He has also described the use of a membrane reactor to produce alcohol from concentrated whey permeate, using a very high cell density which is retained by the membrane. Such a system has shown a high productivity and has been operated continuously for up to 10 days.

Crespo ***et*** *al.* (1990) studied the effects of recirculation rate for a propionic acid bacterium fermentation process, linked to a tubular UF module, on permeate flux, fermenter homogeneity, energy consumption, temperature rise and cell damage. High circulation rates improved flux and the degree of homogeneity, but increased cell damage. Energy input was also estimated to determine the degree of cooling required.

Minier ***et*** *al.* (1990) combined the membrane process with distillation, and found that the combination was useful to further concentrate the alcohols and to remove the low molecular weight inhibitors. A mineral ultrafiltration membrane has been used to separate lytic enzymes produced extracellularly during an acetonobutylic fermentation process. The membrane retained the cells but allowed the lytic enzymes to permeate. The permeate was heat treated to inactivate the enzymes, before being returned to the fermenter. However, although lytic enzyme activity was decreased by over 6 times, there was no marked increase in the yield of butanol compared to the control.

A novel method of immobilisation involves sandwiching the cells between a reverse osmosis and ultrafiltration membrane. The UF membrane allows free passage of the nutrients to the cells. The RO membrane helps immobilise the cells and permits separation of the alcohol from the sugars and salts. This system has been investigated experimentally and modelled by Jeong ***et*** *al.* (1991).

There also exists the possibility of cultivation of animal and plant cells in bioreactors. The performance of a hollow fibre immobilised system has been investigated for the production of antibodies. Procedures which reduced cell and protein distribution on the shell side of the hollow fibre membrane, led to an improvement in productivity. Ultrafiltration membranes provide a number of advantages, including the retention of growth factors and the selective concentration of high molecular weight protein products.

3.2.2 Recovery of components and downstream processing

Ultrafiltration is now widely used for the recovery of cells, enzymes and other metabolic products. Ultrafiltration and microfiltration can be used for concentrating and harvesting cells but will be in direct competition with continuous centrifugation and to a lesser extent, conventional filtration. The cells may also be shear sensitive, so this factor needs some consideration. Factors such as membrane geometry, pore size, chemical nature of the membrane and ultrastructure should be investigated. Fouling is not necessarily related only to pore size, but to the distribution of pore size, the relationship between pore size and particle size, and whether fouling occurs on the surface or within the pores.

Ultrafiltration is widely used commercially for concentrating enzyme solutions. There are also many diafiltration operations, for washing out low molecular weight contaminants. For example, Olsen *et al.* (1990) describe the recovery of four enzymes, alkaline phosphatase, hyaluronoglucuronidase, chitinase and beta-N-acetylglucosaminidase, from shrimp waste. Flocculation was induced by ferric chloride at pH 7. The supernatant was then ultrafiltered to recover 99% of the enzyme activity. Pacheco-Oliver *et al* (1990) used diafiltration to remove coloured impurities from a lipase enzyme, produced by fermentation.

There has been much less success for fractionating enzymes or other components, based solely on size difference, unless there is at least a tenfold difference in their molecular weights. Again the selection of membranes is very important, to minimise excessive enzyme loss in the permeate.

However, there has been some success at partial fractionation and purification, by using compounds which may either bind with one of the fractions and alter its molecular weight and hence its rejection characteristic, or by contacting it with a specific component with which it has a strong affinity. This other component could be introduced in the feed or immobilised within hollow fibre membrane systems. Thus, in conjunction with affinity or ion exchange gels or resins, a specific component in the crude extract can be bound and separated, and subsequently eluted. Examples are given for the purification of horse serum cholinesterase and cattle liver carboxylesterase by these types of method, using an affinity gel and DEAE sephadex, respectively (Molinari *et al.,* 1990). Trypsin has been purified by affinity chromatography and ultrafiltration, using a water-soluble ligand-bound polymer. This was incubated with the crude extract, forming a soluble polymer-trypsin complex. Unbound enzymes were removed by ultrafiltration, whereas the complex was retained.

A French patent (anon) (1991) describes the isolation and recovery of a wide range of enzymes from waste water treatment sludge. Ultrafiltration is used to concentrate the liquid phase, which results from the sludge treated by a number of methods.

Another development is the attachment of bacteria with outer layers rich in glycoproteins to microfiltration membranes. These are then used as a support for protein immobilisation, or as an ultrafiltration membrane (Sara *et al.,* 1990).

It is also possible to separate components such as dissolved organic materials or metal ions by micellar enhanced ultrafiltration (MEUF). The compounds are solubilised within micelles by the addition of surfactants and subsequently removed by ultrafiltration.

3.2.3 Medical applications: serum fractionation

One important medical application is for treatment of patients suffering kidney failure, which leads to an accumulation of toxic components, such as urea and creatine within the blood. There are several treatment choices available: peritoneal dialysis, haemodialysis, haemofiltration and haemodiafiltration (de Burgh, 1992). Peritoneal dialysis makes use of the natural membrane in the peritoneum. A glucose solution is infused into the peritoneal cavity, and solutes and water from the blood diffuse through this membrane into the sugar solution. The sugar solution is infused and removed at hourly intervals. No direct access to the blood supply is required. The other three applications use a manufactured membrane to achieve the removal, with blood being supplied to the membrane.

Haemodialysis is usually a short-term, intermittent process, of 2-4 h duration, which requires the availability of dialysis trained nursing staff, whereas haemofiltration and haemodiafiltration can be used continuously for up to several days. Haemodialysis involves the removal of plasma water, containing toxins, through the membrane filter, which is usually either a flat plate or hollow fibre configuration.

This filtrate is replaced with a sterile infusion fluid to maintain the patient's fluid status. Where sufficient blood pressure is present, the blood flow can be driven by the patient's own arterial pressure, using either an arteno-venus shunt or an arterial groin line. Where blood pressure is insufficient, a blood pump may be incorporated. Whichever technique is used, the utmost care should be taken to ensure that blood flow is maintained and the filter does not become blocked. Haemodiafiltration uses the same blood circuit, but incorporates a dialysate solution, which is passed through the filter on the permeate side of the membrane, thereby facilitating toxin removal by diffusion. Control of fluid removal may be achieved by adjusting the output of dialysate from the filter in relationship to its input flow.

Conclusion

Ultrafiltration processes have been used for a greater variety of downstream biotechnology applications than any other membrane process. It is used for harvesting and concentration of cells, for product recovery, for product separation and concentration and for salt exchange, diafiltration. Cross flow microfiltration is quite effective in recovering bioproducts from fermentation broths and cell lysates.

They are both liquid phase pressure driven membrane processes which have several similarities, but also essential differences. Both use microporous membranes, with the essential difference lying in the pore size and membrane structure. Microfiltration membranes are porous membranes, which possess pores in the 0.05 to 10 μm range. Ultrafiltration membranes are also porous, however their structure is more

asymmetric compared to microfiltration membranes. Such asymmetric membranes consists of a thin top layer supported by a porous sublayer, with the resistance to mass transfer being almost completely determined by the top layer. As a result characterization of Ultrafiltration membranes involves characterization of the top layer. Pore diameters for Ultrafiltration membranes are generally in the range from 20 – 1000 Angstrom.

Both membrane processes are subjected to concentration polarization with the following consequences:

1) Solute transport may be increased
2) Film resistance to mass transfer in the liquid at the membrane surface mat be significantly increased
3) Flux may be significantly decreased
4) Fouling due to solute-membrane interaction may be increased.

Adequate tangential flow of the solution at the membrane surface can help in controlling concentration polarization in both cases.

Concentration polarization can be quite severe in ultrafiltration, because the flux through the membrane is high, the diffusivity of the macromolecules is rather low and the retention is normally high. This causes the solute concentration at the membrane surface to attain a very high value and a maximum concentration, the gel layer concentration maybe reached for a number of macromolecular solutes for example proteins. The gel layer model is capable of describing the occurrence of a limiting flux which is typical of ultrafiltration processes and to a lesser extent microfiltration. When the limiting flux is reached, further increase in operating pressure does not increase the flux through the membrane. This maximum flux is called the limiting flux and is denoted by J_{∞}. Ultrafiltration processes tend to operate close to the pressure dependent region, where changes in pressure will result in changes in the flux through the membrane.

It should be noted that the above phenomenon is common to both ultrafiltration and microfiltration. In both cases the flux has a pressure-dependent region followed by a pressure independent region, as operation moves from membrane control to gel or cake control.

Rejection in both ultrafiltration and microfiltration is influenced by the solution environment. For example the pH of a protein solution will have a large impact on repulsion between charged protein in solution and charged protein adsorbed on or into the membrane. The addition of salts will diminish the repulsive-charge interactions and thereby decrease protein rejection.

The same fouling phenomena that occur during ultrafiltration also tend to occur during cross flow microfiltration. However, because the cross flow microfiltration membranes are usually of much higher surface porosity, local polarization is minimized. The larger pore size of cross flow microfiltration means that solutes and particulates may be able to enter the membrane and cause internal fouling.

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