CHAPTER TWO

Literature Review

2.1 Introduction to Membrane Processes

Membrane filtration is used for removing solids from a liquid or gas stream via sieving mechanism. A pressure gradient is maintained across the filter to ensure fluid flow through the filtration media. The filtrate or permeate flowing through the filter should be devoid of suspended solids under ideal filtration conditions.

The use of membrane processes has been a relatively recent development in the process industries. Membranes were prepared commercially in the late 1920s for bacteriological laboratory use. These were symmetric microfiltration (MF) membranes. It is inapplicable to reverse osmosis (RO) and ultrafiltration (UF) until asymmetric membranes were prepared. In these membranes the resistance to permeation is concentrated in a very thin layer at the retentate side of the membrane. In 1960s, the asymmetric cellulose acetate membrane by the phase inversion process was synthesized. Simultaneously gas separation membranes were developed from polymer films.

MF membranes used in dead-end mode gain popularity for cleaning a variety of fluid streams and for sterile filtration since mid 1960s. Electrodialysis was the first membrane based separation process to develop industrial applications. While UF did not gain popularity until the end of 1960s, RO process developed rapidly due to demonstration projects in saline water.

The 1970s saw rapid development spearheaded by the diary industry for UF that resulted in many plants for whey protein concentration, as well as for the electro-coat paint industry. Many new polymers were being tested to assess their suitability for making membranes and fundamental work become more intensive. Theoretical developments progressed in both pressure driven and gas separation fields. A high volume of research literature was published and the Journal of Membrane Science was established in 1973.

After some initial problems of poor membrane reliability, manufacturers learnt how to make robust reliable products which were acceptable to industry. The useful life of membrane increased from months to years and modern RO membranes may last over 5 years and UF membranes over 2 years. The beginning of the 1980s saw the announcement of commercial gas separation by Monsanto who had been testing their Prism system on full scale for several years.

2.2 **Membrane Processes in Dairy Products**

The application of membrane filtration is industry specific, consequently the classification of the processes become complex. A number of different unit operations and membrane processes have been investigated in various advanced wastewater treatment applications. In general, the nature of fluid and its constituents determine the application of membrane processes.

In dairy applications, the use of membrane depends on the process. A sequential membrane processing in dairy industry is illustrated in Figure 2.1. In fact, the use of specific membrane will depend on the characteristic and size of the product composition. The size distribution of milk components is shown in Figure 2.2. Lactose and soluble salts pass through UF membranes, while proteins, fats, and some of the insoluble or bound salts are retained.

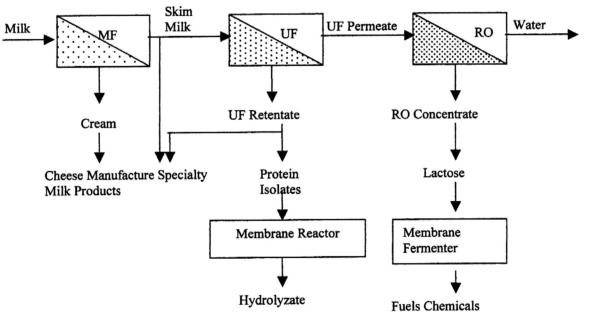

Figure 2.1. Sequential membrane processing in the dairy industry (adapted from Cheryan and Alvarez, 1995).

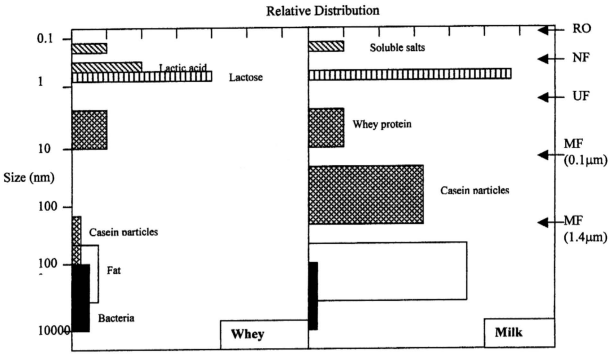

Figure 2.2. Size distribution of components in milk (right) and whey (left), showing possible applications of membrane technology (adapted from Cheryan and Alvarez, 1995).

Raskin et al. (1998) studied the characterization of dairy waste streams, current treatment practices, and the potential for biological nutrient removal. Long term data on wastewater characteristics were obtained from 8 out of the 15 dairy plants (in the Upper Midwest of the United States), and 24 hours composite wastewater samples were analyzed for each plant. Wastewater flow rates and characteristics varied greatly among and within the plant and were not easily predictable even when detailed information on processing operations was available. It is concluded that biological nutrient removal of dairy wastewater should be feasible if it contains relatively high concentration of easily degradable organics, the generally favorable organic matter to total phosphorus ratio, and the very favorable organic matter to nitrogen ratio. It was determined that most of

the on site treatment facilities require renovations and operational changes to comply with current and future discharge regulations, especially with respect to nutrient levels in their waste streams.

2.3 Ultrafiltration in Dairy Products and Waste Treatments

As discussed before, UF systems are pressure-driven membrane operations that utilise porous membranes for the removal of dissolved and colloid materials. UF applications include removal of oil and colour from effluent streams.

2.3.1 General Applications of Ultrafiltrations

UF has emerged as the most economical method for concentrating high quality whey protein from dairy waste (Charles et al., 1989). The use of membrane based processes for separation and purification is attractive because of the high throughput of product, low process cost and ease of scale-up. UF is now widely used for the processing of paints, polymers, therapeutic drugs, enzymes, hormones, vaccines, blood products and antibodies. The major areas of application are:

a) Concentration: removal of solvent from solutions
b) Fractionation of molecules
c) Diafiltration: removal of low molecular weight compounds from solution
d) Removal of cells and cell debris for fermentation broth
e) Virus removal from wastewater or ground water
f) Harvesting of biomass
g) Membranes reactor: separation and processing
h) General or pretreatment of effluent.

2.3.2 Ultrafiltration in Cheese Production

The largest application of membrane technology in diary industry is whey protein concentration by UF. Whey utilization is expected to increase, especially in pharmaceutical and food products.

2.3.2.1 Principle of Cheese Curd Making

Cheese making requires the knowledge of physics, chemistry, biochemistry, and biology amongst other scientific disciplines. Enzymology plays a significant role since enzymes are involved in converting lactose to lactic acid, casien into curds, and proteins, fats and sugars into components which give rise to flavour, texture and taste in the ripened cheese (Scott, 1981). Figure 2.3 shows diagrammatically the formation of raw curd from milk. The Figure 2.3 not only illustrate the formation of either rennet curds or acid coagulated curds but also highlights the ingredients or raw materials used in the process, and the application of heat.

2.3.2.2 Milk as a Raw Material for Cheese

Milk varies in composition depending on its source. The manufacture of cheese, however, does not depend solely on the macro composition but upon the micro constituents of milk. Table 2.1 presents the general information on the macro composition of cow's milk. Similar table can be constructed for other types of milk.

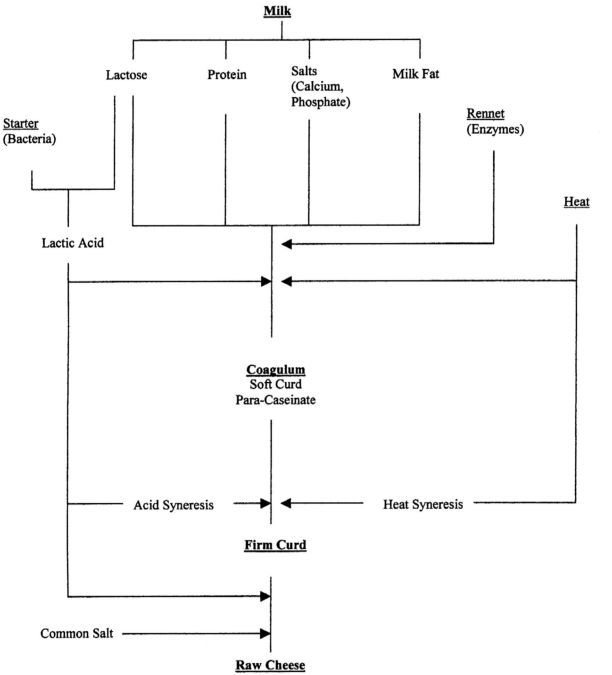

Figure 2.3. Flowchart of the product of raw cheese from milk (Scott, 1981)

Table 2.1: Composition of cow's milk as a raw material for cheese (Kosikowski, 1970)

Macro Component	Approximate % composition	Micro components
Fat	3.75	Some diglycerides but mainly triglycerides (C_4-C_{18},C_{18-1},C_{18-2}, C_{20-2} and C_{20-3})
Lipid	0.05	Lecithin, cephalin, sphingomylin
Proteins	3.38	Caseins, 2.78% α casein, 1.67% β casein, 0.62% γ casein, 0.12% κ casein, 0.37% Whey proteins, 0.60% α lactalbumin, 0.13% β lactoglobulin, 0.35% immunoglobulin, 0.08% serum albumin, 0.04% Traces other nitrogenous Substances
Lactose Salts (minerals)	0.9	Calsium, magnesium, sodium, potassium, phosphates, citrates, chlorides, sulphates, etc. (iron, manganese copper,cobalt,etc.)
Water	87	

	Minor Constituents
Pigments	Carotene, riboflavin, xanthophylls
Enzymes	Lipases, proteases, reductases, phosphatases, lactoper-oxidases, catalase, oxidases, etc.
Vitamins	Fat soluble D,E and K Water soluble C and the B group
Gases	Oxygen, nitrogen, carbon dioxide (as carbonic acid), ammonia, sulphuretted hydrogen, etc.
Volatiles	Extraneous volatiles – petrol, paraffins, etc.
Cellular matter	Epithelial cells, leucocytes
Micro-organisms	Bacteria (normal udder flora), contaminants (ie. Bacteria, yeasts, moulds, etc.)
Contaminants	Result of carelessness during milk production

2.3.2.3 Proteins in Milk

The proteins in milk may be classified into two main groups (Scott, 1981):

a) the casein complex which exists mainly in a colloidal state in milk, and

b) serum proteins (i.e. whey proteins) which are mainly in solution in serum.

The proteins consist of strings of amino acids held together in a formation, often a helix, which gives the protein its character and ability to react. If in the form of helix, the spirals are cross-linked to give stability. Thus, some proteins are elastic and can shrink while others display a more rigid character. When the protein is denatured by heat or acid, these characteristics change and the protein becomes more inert to external influences.

Biochemical reactions in heat treated milks are difficult to forecast (Kosikowski, 1970), especially those assisted or catalyzed by enzymes. The interaction between casein and milk salts is temperature and pH dependent. However, the interaction between lactoglobulin and the casein complex does not take place in normal heat treatment. In disturbed milks, some combination can take place. In the process, denaturation of the whey proteins may also occur. The whey proteins begin to denature at 70°C and progressively denature at higher temperatures. Denatured whey proteins are enclosed in cheese curd. β-lactoglobulin is the most heat sensitive of whey proteins. When substantial amounts are present, heat treatment of milks in the range 74 to 80°C causes harsh curd formation. As a result, the proteins must be treated under controlled conditions.

2.3.2.4 Cheese Whey Ultrafiltration

The cheese made by either traditional or modern methods produces a large quantity of whey, approximately 83% of volume of milk used. It is difficult to divorce whey disposal from cheese technology because the disposal of whey is becoming a major industrial and public health problem. The BOD in whey ranges from 32,000 to 60,000 ppm, which creates a severe disposal problem (Raskin et al., 1998).

Since whey contains valuable nutrients, i.e. proteins, sugar and mineral, it should not be discarded as a waste. Cheese makers however have regarded whey as a waste product for many years. Pig feed has been a traditional method of disposal but the remainder has been dumped in the sea, rivers, quarries, mines and on any convenient land. Disposal on agricultural land as a fertilizer or using it as a pig feed has been discontinued due to changes in farming techniques. Modern and greater appreciation of the inherent nutritional value of the constituents of whey for both humans and livestock has initiated research into new applications. Some indications of the possible disposal methods for whey are given in Figure 2.4.

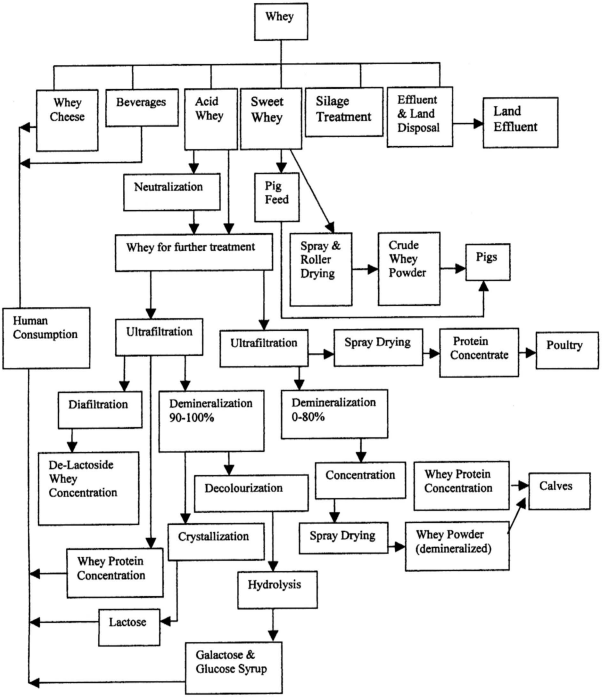

Figure 2.4: Some methods for the disposal of whey. (Charles M. Mohr et al.,1989)

2.3.2.5 Whey Treatment

Whey contains various microrganism and requires a pasteurization process to destroy them, especially lactic bacteria before the subsequent usage. Neutralization of whey by soda or lime produces lactates which, unless removed cause difficulties when used in calf feeds, etc. Neutralization and subsequent demineralization is therefore essential to render the whey suitable (sweet) for further usage (Mahoney and Adamchuk, 1980). Demineralization involved the use of either a two-bed ion exchange resin system or an electrodialysis system, which removes the salts from whey by electrodialysis through suitable membranes. Both systems are expensive and can only be justified where the end products command high prices.

A process which is used in some creameries is based on the principle of UF. The permeate is composed of water and low molecular weight components of the whey, i.e. salts and lactose. Some non-protein nitrogenous compounds may also be present. The type of membrane and its structure determine its permeability towards whey components. Cellulose acetate was the first of the membranes used but synthetic copolymers are now replacing the cellulose membranes on account of their resistance to heat and to detergent or cleaning solutions.

The effluent or retentate contains the high molecular weight components of whey and is called "whey protein concentration". It has high nutritional value and great commercial importance. The permeate in UF of whey contains lactose which can be recovered by RO or NF.

2.3.3 *Protein Concentration and Separation*

Concentration of protein solutions can be accomplished in a number of ways, one such method is concentration by precipitation, followed by re-dissolving in a small volume of liquid. The most direct method for concentrating dilute protein solutions involved simply removing water plus low MW solutes. UF is used to simultaneously fractionate, purify, and concentrate liquid whey for whey protein powder. A typical UF system concentrates whey protein by a factor of 5, and diafiltration must be employed to produce concentrates with up to 80% whey protein. Table 2.2 lists whey constituents in the feed and concentrate of an average UF system designed to concentrate whey protein. Typically, whey concentration using UF is given in Table 2.2 .

Table 2.2 : Whey concentration using ultrafiltration (Matthew, D., 1999)

Constituent	**Feed**	**Concentrate**	**Permeate**
Fat	0.06	0.33	0.00
Whey proteins	0.69	3.70	0.01
Non-Protein Nitrogen	0.18	0.21	0.17
Lactose	5.01	5.74	4.85
Lactic Acid	0.15	0.15	0.15
Soluble Salts	0.65	0.88	0.62
Insoluble salts	0.03	0.16	0.00
Total Solids	6.79	11.16	5.81

- Design and operating parameters

Stages: 2 to 4
Elements per vessel: 3 to 6
Membrane type: polysulfone ultrafiltration
Element size: 96.5mm diameter or up to 1016mm long
Feed flow/vessel: 55 to 66 L/min
Feed temperature: up to 54°C
Permeate flow per element: 1.76 to 2.2 L/min

Daufin et al. (1999) had observed UF modes of operation for the separation of α-lactalbumin from acid casein whey. The two major proteins, β-lactoglobulin and α-lactalbumin, are close to each other with respect to their sizes. The separation of α-

lactalbumin by membrane processes can be made more effective by the appropriate selection of the operation mode. The above-mentioned researchers developed a model, and showed that the performance would depend on initial purity, transmission and the mode of operation. They showed that continuous concentration up to a high volume reduction ratio or a combined continuous concentration-diafiltration would help to obtain a fraction with both enhanced purity and satisfactory yield of α-lactalbumin in the permeate.

2.4 Limitation of Membrane Processes

As mention in Chapter One, concentration polarization refers to the reversible build-up of solutes near the membrane surface. Concentration polarization can lead to irreversible fouling by altering interactions among the solvent, solutes and membrane.

In most operations, the cake layer formation builds on the membrane surface and extends outward into the feed channel (Green and Belfor, 1980). The constituents of the foulant layer may be smaller than the pores of the membrane. A gel layer can result from denaturation of some proteins. Internal pore fouling occurs inside the membrane. The size of the pore is reduced and pore flow is constricted. Internal pore fouling is usually difficult to clean. Fouling can also be understood in terms of the physical and chemical forces involved. Foulants result from attractions between solutes or that between solutes and the membrane. The solvent and ionic environments are also key factors that mediate the solute-solute and membrane-solute interactions.

Zydney (1997) studied the influence of protein-protein interactions on bulk mass transport during UF. He found out that the bulk mass transfer limitations could have a significant effect on the flux and selectivity during membrane UF. He has developed a generalized framework for multicomponent mass transfer that includes both thermodynamic and hydrodynamic interactions. Thermodynamic coefficient was evaluated from osmotic pressure data, while hydrodynamic interaction parameters were determined from permeate flux data obtained in a stirred cell using full retentate membranes. The multicomponent diffusive flux model accurately predicted the large reduction in flux and bovine serum albumin (BSA) transmission upon addition of human immunoglobulin G (IgG). These effects were due to the coupling between BSA and IgG mass transfer caused by protein-protein interactions.

Membrane-protein interactions could cause changes in the structure of the adsorbed molecule; e.g. BSA adsorbed on the surface of a hydrophilic regenerated cellulose membrane had a globular structure very much like it would have in free solution (Zydney and Seksena, 1997). However, on the surface of a polysulfone membrane, the protein appeared long and filamentous, more "open" and denatured.

In cheese whey, proteins are involved in the build up of the polarization layer responsible for reducing the permeate flux (Daufin et al., 1988). Moreover, they adsorb onto the membrane material and increase its hydraulic resistance. Suspended solids contribute as well to irreversible and reversible fouling. Some of these solids are initially present in the fluid and are partly eliminated by centrifugation, whereas others appear during UF, when the residence times of whey constituents are long. Lipids present in sweet whey contribute to a large extent to the polarization layer and

adsorption. The above-mentioned researchers showed that centrifugation and clarification would significantly improve the UF flux. However, an attempt should be made to increase protein rejection rates and simultaneously stabilize insoluble inorganic particles resulting from interactions between proteins and minerals.

The permeability of a solute can be affected by its micro-environmental conditions, such as the pH and ionic strength of the solution. This should not be unexpected since pH and ionic strength would affect the conformation and shape of the solute molecule, and the rejection of the solutes. A higher transmission of proteins was observed with larger MWCO membranes, and it decreased as the pH was increased, probably because proteins acquired a higher negative charge and were repulsed by the negatively charged membranes.

2.5 Gas Sparging in Ultrafiltration

2.5.1 Reduction of Fouling

The reduction in fouling improves the filtration rates and the ease of cleaning. Thus, the reduction of fouling would obviate the need for severe cleaning regimes, and prolong the life of polymeric membranes.

Industry, in seeking flux improvement, generally resorts to very high cross-flow velocities with a consequential increase in power consumption. However, a number of approaches are available to minimize flux decline. The permeate flux depends on the mass transfer coefficient. For a given feed concentration and TMP, the higher the value

of k, the higher the permeate flux. The permeate flux can also be increased by disrupting the concentration polarization layer. Some of the flux enhancement techniques are summarized in Table 2.3.

Table 2.3: Approaches to minimize flux degradation
(summarized from UF and MF Handbook, 1998)

Direct Methods	**Indirect methods**
Use of turbulence promoters	Pretreatment by filtration
Pulse/reversed flow	
Use of abrasive particles	Treatment of membrane surface
Rotating/vibrating membranes	Preparation of more hydrophilic membrane
Rotating blade close to membrane surface	Selection of appropriate operating mode
Cross-flow electrofiltration	Selection of optimum operating conditions
Ultrasonic enhancement	
Periodic hydraulic or chemical cleaning	
Generation of a dynamic membrane layer	
Periodic mechanical cleaning	
Periodic backpulse with permeate or gas	

2.5.2 Enhancement of Gas Sparging on Filtration

Of late, the technique of gas sparging to enhance the filtration processes has been investigated. This technique is not only beneficial in flux enhancement but also on improving the quality of the by products and it is energy saving (Cui et al., 1997). For an easier understanding of air sparging technique, a typical experimental setup is shown in Figure 2.5.

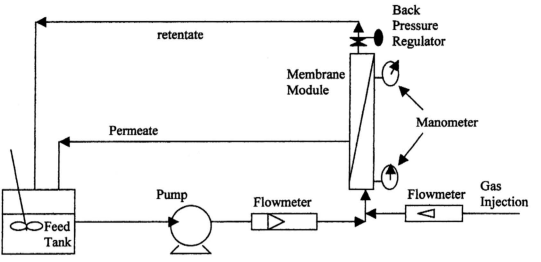

Figure 2.5: Experimental setup for gas sparging (adapted from Elmaleh et al., 2000)

Imasaka et al. (1989) studied the characteristics of cross-flow filtration using liquid single phase, gas-liquid two-phase and gas-liquid-solid three phase systems. One of the findings was the promotion of turbulence in the gas-liquid mixture in a two-phase flow system, where the permeate flux increased satisfactorily with an increase in the feed-gas flow rate.

Cui and Wright (1993) investigated the enhancement of crossflow UF with air sparging. It was found that air injection could greatly suppress the effect of concentration polarization and fouling, while maintaining a high flux rate. The permeate rate was increased by 70 to 250% compared with the single-phase flow, even at a very low gas flowrate. Besides, the rejection ratio of the membrane increased considerably. The rejection ratio also improved with an increase of 5 to 10%, based on a study of BSA and dextran.

Lafforgue-Delorme et al. (1995) pointed out that slug flow augmented the permeate rate by up to 140%, in comparison with the usual filtration processes with no gas injection.

After considering the limitation of this technique, further research was suggested. The effect of gas injection on the permeate flux and membrane sieving coefficient was examined experimentally at different TMPs, feed concentration and gas sparging ratio. The results were encouraging with flux enhancements of 20 to 50% obtained for dextran and 10 to 60% for albumin (Cui, 1996). The sieving coefficient of albumin considerably diminished when gas-liquid two-phase cross-flow was used.

In downwards flow condition, Cui and Wright (1996) examined the effect of operating parameters including gas sparging ratio, TMP and feed concentrations with dextran solutions. Flux increases of up to 320% were achieved with gas sparging and this was profound under the laminar flow regime. However, the enhancement was not very significant when liquid flow was turbulent. The flow pattern proved to be an important parameter and the operation could be optimized to achieve maximum enhancement with minimum gas sparging. A further study on the effect of bubble size and frequency on the permeate flux of gas sparged UF with tubular membranes by Cui et al. (1997) showed that the permeate flux increased with the bubbling frequency in the examined range. The dependence of permeate flux on bubble size had two regions, an increasing region associated with small slugs and a plateau region with large slugs occupying the full cross-sectional area of the membrane tube. It was also found that the gas sparging process would be a low cost process compared to conventional single-phase cross-flow operation, due to saving on capital cost by eliminating the recirculating pump. Hence, this process is particularly attractive to cost-sensitive processes such as waste effluent treatment.

Cabassud et al. (1997) studied the improvement of slug flow in organic hollow fibres UF membrane. They concluded that the wall shear stresses would prevent filtered particles from settling on the membrane surface and enhance the mass transfer.

Further, the mechanism of flux enhancement in UF processes by gas sparging, in case of upward slug flow in tubular membrane module was investigated by Cui and Ghosh (1999). An attempt is made to model gas sparged UF based on three different zones (film, wake and liquid slug zone) of gas slug depended on the nature of flow in the vicinity of the membrane. The mass transfer coefficient for each of these zones was determined for a particular feed solution, and the averaged permeate flux for gas sparged UF was also calculated. The simulated results suggested that gas sparging is more effective at higher TMP, and increasing the liquid flow rate has opposite effects in single-phase flow.

By using the conductance probe technique, Mercier-Bonin et al. (2000) showed that the UF flux improvement was partly due to the increase in the wall shear stress, induced by continuous gas sparging inside the tubular filtration module. They suggested that suppressing the concentration polarization layer will decrease the sieving coefficient and increase the apparent membrane rejection ratio. Hence, the gas sparging technique is more beneficial for operations requiring the concentration of macromolecular solutions.

Besides protein interactions, system hydrodynamics also affect protein fractionation. Therefore, disruption of the concentration polarization layer would help to maintain the native selectivity of the membrane and aid in protein fractionation. Cui and Ghosh (1998) investigated the effect of gas sparging in controlling concentration polarization to enhance the selectivity of separation of BSA and lysozyme. They found that gas sparging enhances protein fractionation under suitable solution conditions, and nearly complete separation of BSA and lysozyme was achieved.

Gas sparging, therefore, improves the hydrodynamic within the membrane module mainly due to bubble induced secondary flow and decrease concentration

polarization and hence increase the permeate flux. The usefulness of gas sparging in protein fractionation was proven with BSA (MW 67,000) and lysozyme (MW 14,100) (Ghosh et al., 1998). However, in whey protein fractionation it is still uncertain if the major proteins can be separated because of the narrow range of molecular weight.

2.6 Mathematical Models

Several mathematical models that attempt to describe the mechanism of transport through membranes are available (Charles et al., 1989; Cui et al., 1996, 1997). Although the operating techniques of MF, UF, NF and RO are similar, the latter two are almost certainly not separation by size alone. In what follows, some of the important models and the possible mechanisms of flow through the membrane are discussed.

In UF, when the wall is porous, solids in the feed are brought to the membrane surface by convection transport, and a portion of the solvent is removed from the fluid. This results in a higher local concentration of the rejected solute at the membrane. This is shown schematically in Figure 2.6. As mention before, this solute build-up is the concentration polarization and it is chiefly responsible for the marked deviation in flux compared to pure water flux.

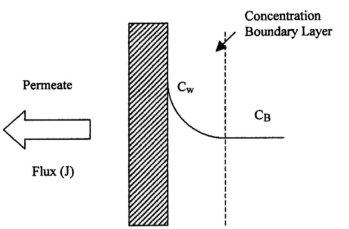

Figure 2.6: Concentration profile during membrane processing of partially or completely rejected solutes. (Bird, R.B. et al., 1960)

There have been several attempts to model permeate flux as a function of the system and operating variables and physical properties. None are wholly satisfactory. The major problem appears to be the inability to precisely model the phenomena occurring near the membrane surface. In an ideal situation, that is with uniformly distributed and evenly sized pores in the membrane, with no fouling, negligible concentration polarization, it is believed that the best description of fluid flow through micro-porous membranes is given by the Hagen-Poiseuille's Law for streamline flow through channels (Bird et al.,1960).

2.6.1 Pore Flow Model

This model, which relates pressure drop, viscosity, density, and channel dimensions (such as diameter of a tube) to flow rate through the channel is usually analyzed by means of a momentum balance using cylindrical coordinates (Bird et al., 1960). One form of the model useful in membrane processing is

$$J = \frac{\varepsilon d_p^2 P_T}{32\mu\Delta x} \qquad (2.1)$$

where,

J - the flow rate through the membrane, i.e., the flux (m/s)
d_p - the channel diameter or the mean pore diameter (m)
P_T - the applied transmembrane pressure (kg/ms^{-2})
μ - the viscosity of the fluid permeating the membrane ($kgms^{-1}$)
Δx - the length of the channel or the membrane thickness (m)
ε - the surface, porosity of the membrane (m^2).

These parameters are shown schematically in Figure 2.7. The net driving force for an ideal membrane process P_T in Eqn. 2.1 should actually be $P_T - \Delta\prod$.

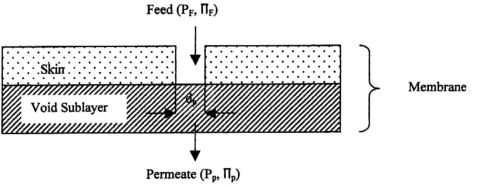

Figure 2.7: Schematic representation of the cross section of typical asymmetric ultrafiltration or microfiltration membrane. (Bird et al., 1960)

Several assumptions have been made in deriving the model shown in Eqn. (2.1):

a) The flow through the pores is laminar; i.e. Re<2100.
b) The density is constant; i.e. the liquid is incompressible.
c) Steady state conditions.
d) The fluid is Newtonian.
e) End-effects are negligible.

According to the model, flux is directly proportional to the applied pressure and inversely proportional to the viscosity. Viscosity is primarily controlled by two factors: solids concentration and temperature. However, this is true only under certain conditions such as (a) low pressure, (b) low feed concentration, and (c) high feed velocity. An asymptotic pressure-flux relationship is due to the effects of the concentration polarization. Under these conditions, the Hagen-Poiseuille model no longer adequately describes the membrane process, and mass transfer limited models must be used.

2.6.2 Concentration Polarization Model

It is observed that the permeate flux in UF process does not increase linearly

with TMP after a certain point or conditions, as illustrarted in Figure 2.8. This is due to the build-up of rejected solute molecules near the membrane surface, i.e. the concentration polarization layer. The concentration profile of solute molecules close to the membrane surface is shown in Figure 2.9. At steady state, a material balance of solute molecules in a control volume within the concentration polarization layer yields the following differential equation:

$$JC - JC_p + D\,(dC/dx) = 0 \tag{2.2}$$

Integrating this with the following boundary conditions

$C=C_w$ at $x=0$;

$C=C_b$ at $x=\delta_b$,

we obtain

$$J = k \ln \left(\frac{C_w - C_p}{C_b - C_p} \right) \tag{2.3}$$

where $k = D/\delta_b$, and Eqn. 2.3 is known as the concentration polarization equation for partially rejected solutes.

For total rejection,

$$J = k \ln (C_w/C_b) \tag{2.4}$$

where $C_p=0$.

When the solute concentration at the membrane surface reaches the saturation concentration for the solute (C_s), the gel concentration (C_g) will not increase, $C_w=C_g=C_s$. Thus,

$$J = k \ln (C_s/C_b) \tag{2.5}$$

This is referred to as the gel polarization equation. In the region of pressure-independent, the permeate flux for a given feed solution is only dependent on the mass transfer coefficient. According to this model, the existence of the limiting flux is a consequence of gelation of the solute at the membrane-solution interface.

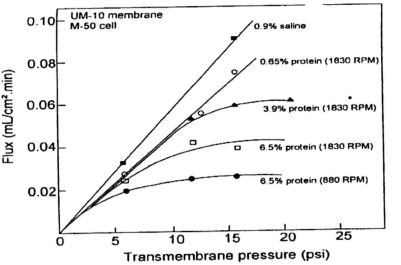

Figure 2.8: The effect of pressure, stirring rate (rpm) and albumin protein concentration on flux in an Amicon stirred cell (adapted from Blatt et al. 1970).

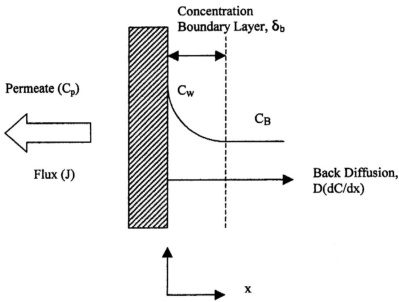

Figure 2.9: Concentration polarization of membrane. (Bird, R.B., 1960)

2.6.3 Mass Transfer (Film Theory) Coefficient

The simplest and widely used theory for modelling flux in pressure-independent, mass transfer-controlled systems is the film theory as discussed in Section 2.6.2. This is expressed mathematically in Figure 2.10. As the solution is ultrafiltered, solute is

brought to the membrane surface by convective transport at a rate J_s defined as

$$J_s = JC_b \tag{2.6}$$

where C_b is bulk concentration of the rejected solute. The resulting concentration gradient causes the solute to be transported back into the bulk of the solution due to diffusional effects. Neglecting axial concentration gradients, the rate of back transport of solute will be given by

$$J_s = D\,(dC/dx) \tag{2.7}$$

where D is the diffusion coeffient and dC/dx is the concentration gradient over a differential element in the boundary layer. At steady state, the two mechanisms will balance each other, and Eqn. 2.6 and 2.7 can be equated and integrated over the boundary layer to give

$$J_s = (D/\delta_b) \ln (C_g/C_b) = k \ln (C_g/C_b) \tag{2.8}$$

In effect, the flux will be controlled by the rate which solute is transferred back from the membrane surface into the bulk fluid. If the physico-chemical properties of the feed are fixed, flux can only be improved by enhancing k as much as possible, such as by reducing the thickness of the boundary layer.

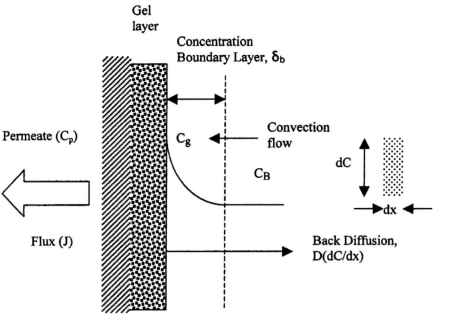

Figure 2.10: Schematic diagram showing the buildup of the polarized (gel) layer and associated boundary layer. (adapted from UF and MF Handbook, 1998)

A number of qualitative relationships correlating the mass transfer coefficient to the physical properties, flow channel dimensions and operating parameters are reported in the literature (UF and MF Handbook, 1998). None of them are wholly satisfactory; many are continuously being refined or modified to suit particular applications and, thus, are not universally applicable. Under these circumstances, when the theoretical approach lacks perfection, dimensionless analysis is a powerful tool. Using the $\prod$ theory or by analogy with heat transfer, one can obtain a general correlation of the form:

$$Sh = A\,(Re)^{\alpha}(Sc)^{\beta} \tag{2.9}$$

where

$$Sh = \text{Sherwood number} = kd/D \tag{2.10}$$

$$Re = \text{Reynolds number} = dU\rho/\mu \tag{2.11}$$

$$Sc = \text{Schmidt number} = \mu/\rho D \tag{2.12}$$

The exponents α and β are constants determined by the state of development of the velocity and concentration profiles along the channel. The Schmidt number dependency (β) is derived from dimensional considerations of the convective diffusion equation under conditions in which the momentum boundary layer is much longer than the diffusion boundary layer, i.e. when Sc=1. For laminar flow systems, if both velocity and concentration profiles are fully developed, both α and β are zero. If velocity profile is fully developed but concentration boundary layer is developing along the entire length of the channel, the Graetz or Leveque solution can be used.

In the case of fully developed laminar flow, the Graetz-Leveque correlation can be used,

$$Sh = 1.62\ Re^{0.33}\ Sc^{0.33}\ (d/L)^{0.33} \tag{2.13}$$

where L is the length of the module.

For turbulent flow (Re> 2000), the Dittus-Boelter correlation can be used,

$$Sh = 0.023\ Re^{0.8}\ Sc^{0.33} \tag{2.14}$$

An alternative correlation for mass transfer coefficient in the case of fully developed laminar flow is given in terms of the shear rate (γ) at the membrane surface:

$$k = 0.816\gamma^{033}D^{067}L^{-0.33} \tag{2.15}$$

where

$\gamma = 8$ U/d for tubes

$\gamma = 6$ U / b for rectangular channels (b = channel depth)

From the correlation mentioned above, it is clear that increasing the Reynolds

number increases the mass transfer coefficient. However, mass transfer coefficient is more sensitive to the Reynolds number in the turbulent region. By increasing the cross-flow velocity within the membrane module, the mass transfer coefficient and the permeate flux can be increased.

Since,

$$Sh = (kd/D), \quad k = ShD/d \tag{2.16}$$

$$J = kln\,(C_g/C_b) = [ShD/d]\,\ln\,(C_g/C_b) \tag{2.17}$$

$$J = 1.62\,Re^{0.33}\,Sc^{0.33}\,(d/L)^{0.33}\,\ln\,(C_g/C_b) \tag{2.18}$$

2.6.4 Development of Dimensionless Numbers at Pressure Dependent Region

Consider the pressure dependent region in figure 2.8, J = k ln (Cw/Cb) and also J = f(TMP), So,

$$\ln\,(Cw/Cb) = f\,(P) \tag{2.19}$$

$$J = f(\,P,\,U_L,\,U_g,\,C_f,\,D,\,\rho,\,\mu,\,d) \tag{2.20}$$

where P= pressure (kg/ms^2)
U= velocity (m/s)
C_f= feed concentration (kgm^{-3})
D= diffusivity (m^2s^{-1})
ρ= density (kgm^{-3})
μ= viscosity ($kgms^{-1}$)
d = the diameter of fibre (m)

To group the dimensionless number, a simple and common dimensional analysis is done. In the case of constant feed concentration and non-diffuse filtration, two parameters (D and C_f) will be ignored from the dimensional analysis.

For $\mu^a\rho^b d^c = M^aL^a\theta^{-a}M^bL^{-3b}L^c$

$$\mu^a\rho^b d^c = M^{a+b}L^{a-3b+c}\theta^{-a} \tag{2.21}$$

To obtain the dimensionless numbers,

$$J/(\mu^a\rho^b d^c) = L\theta^{-1}/(M^{a+b}L^{a-3b+c}\theta^{-a}) \quad (2.22)$$

$a=1; \quad b=-1; \quad c=-3$

$J\rho d^3/\mu$ is a dimensionless number.

$$P/(\mu^a\rho^b d^c) = ML\theta^{-2}/(M^{a+b}L^{a-3b+c}\theta^{-a}) \quad (2.23)$$

$a=2; \quad b=-1; \quad c=-4$

$Pd^4\rho/\mu^2$ is a dimensionless number

$$C_f/(\mu^a\rho^b d^c) = ML^{-3}/(M^{a+b}L^{a-3b+c}\theta^{-a}) \quad (2.24)$$

$a=0; \quad b=1; \quad c=0$

C_f/ρ is a dimensionless number.

From the equations and theories, dimensionless numbers have been developed. They are:

(1) $J\rho d^3/\mu$

(2) $Pd^4\rho/\mu^2$

(3) $Re=U_L\rho d/\mu$

(4) C_f/ρ

(5) $U_g/(U_g+U_L)$

$$\frac{J\rho d^3/\mu}{Pd^4\rho/\mu^2} = \frac{J\mu}{Pd} \quad (2.25)$$

A standard dimensional analysis can yield the following dimensionless groups:

$\frac{J\mu}{Pd}$ become a function of $U_L\rho d/\mu$, $U_g/(U_g+U_L)$ and C_f/ρ

where μ = viscosity (kg/ms)

ρ = density of feed solution (kg/m^3)

d = the diameter of the fibre (m)

$\frac{\rho U_L d}{\mu}$ is the Reynolds number

$\frac{U_g}{U_g+U_L}$ indicates the contribution of the injected gas to the overall flowrate

The dimensionless group, $\frac{J\mu}{Pd}$ can be rearranged as ($\frac{J}{J_w}$ $\frac{J_w \cdot \mu}{Pd}$)

where J_w is the pure water permeability at this TMP. J_w/P indicates the membrane resistance, and J/J_w indicates the reduction in flux due to the resistance of protein molecules. Therefore, $\frac{J\mu}{Pd}$ can be used as an indicator to show the effect of mass transfer on the permeate flux.

$$J\mu/\rho d = A\, Re_L^{a}[U_g/(U_g+U_L)]^{b}(C_f/\rho)^{c} \qquad (2.26)$$

where A,a,b and c need to be determined.

2.6.5 Resistance Model and Osmotic Pressure Model

UF membranes cannot be visualized as having parallel cylindrical pores, a parameter in model equation. Thus, membrane hydraulic resistance R_m is used for calculating the permeate flux. When a pure solvent such as distilled water is filtered through the membrane, the permeation flux J_w is proportional to the applied TMP, ΔP_T:

$$J_w = \frac{\Delta P_T}{\mu R_m} \qquad (2.27)$$

where R_m is the intrinsic hydraulic resistance (m^{-1}) of the membrane, calculated from the experimental measurement of J_w.

Two limiting phenomena occur during UF of a solution such as whey which contain macromolecules and suspended particles: (1) polarization due to a liquid layer of macromolecules concentrating on the membrane, and (2) fouling which may be considered as an additional resistance resulting from adsorption, gel formation, or particle deposition (Daufin, 1988).

Thus, the equation of flux can be written as

$$J = \frac{\Delta P_T - \Delta \Pi}{\mu (R_m + R_f)} = \frac{\Delta P_T}{\mu_p (R_m + R_f)} \qquad (2.28)$$

where R_f is the additional resistance due to fouling which generally increases with time.

Polarization is a reversible phenomenon whereas fouling is mainly irrevesible. In such conditions, if after an ultrafiltration test, the membrane is only rinsed with water, measurement of water permeation flux J_w' under ΔP_T pressure makes it possible to determine the irreversible fouling resistance R_f' from

$$J_w' \frac{\Delta P_T}{\overline{\mu} (R_m + R_f)} \qquad (2.29)$$

From the relationship discussed before,

$$R_f' = R_m (J_w/J_w' - 1) \qquad (2.30)$$

values of J_w' are similar irrespective of the water rinsing time. Here, R_f' is considered as equal to R_f although a small part of fouling might be removed by rinsing.

2.6.6 Dimensionless Numbers

Dimensional analysis of the mass, length and time show that the steady state flux observed for MF or UF through inorganic composite membrane can be expressed using two dimensionless numbers (Elmaleh, 1998). The shear stress number compares the shear stress against the membrane wall to the driving pressure. However, the resistance number compares the convective cross-flow transport through a layer, whose resistance is the sum of all the resistances induced by the different processes which limit the mass transport. Straight lines were plotted whose slopes depended solely on the suspension and the membrane, and not on the solute concentration. A straight line of negative slope followed by a plateau means that an irreversible fouling is superimposed on the reversible fouling.

With gas sparging (Elmaleh, 2000), straight lines were plotted. Their slopes are the decreasing function of the gas-liquid velocity ratio when particle deposition or polarization limited the mass transport. According to Elmaleh, a negative slope and a positive intersection with the generalized shear stress number-axis means that the induced resistance can be completely eliminated with gas sparging. A straight line of negative slope followed by a plateau means that an irreversible fouling is superimposed on the reversible phenomenon. A positive slope means that the flux reaches a plateau when cross-flow is increased. Gas sparging causes the slope to decrease and reach a negative value.

2.7 Protein Analysis

During protein purification, fractionation or concentration procedure, the most important procedure to follow is the recovery of the isolated protein:

- by enzyme activity, if it is an enzyme;
- by bioactivity of non-enzymic protein; or
- by some convenient method of quantifying the desired component, e.g. electrophoretic analysis.

In addition, the total amount of protein present in the fraction is required. From these two measurements, the percentage recovery and the degree of purification can be calculated.

2.7.1 Analytical Gel Electrophoresis

The gel electrophoresis technique allows the investigator to get an idea of the complexity of the sample and, in particular, what the main contaminants are. By using the sieving electrophoresis in the presence of SDS and, the isoelectric focusing, some information on the sample can be obtained. The amount of each component, its molecular weight, the isoelectric point and even the titration curve can be deduced. If an antiserum directed towards the complete mixture is available, through crossed immuno-electrophoresis, one can also observe whether some of the components are immunologically related, and whether they are structurally related (Thaveechai, 1984).

The gel electrophoresis was initially used to describe the behaviour of electrically charged colloidal particles in an electric field. Fundamental to electrophoretical separations is the fact that proteins are electrically charged particles. The charges are derived from amino acids with ionogenic side groups. In addition, the proteins often have associated charge components of non-protein origin, such as lipids or carbohydrates.

When exposed to an electrical field E and a force F, acts upon protein molecules (Thaveechai, 1984). The force F depends on the field strength and the net charge z of the protein, such that F=Ez. The net charge for a particular protein depends on the pH but also on the ionic environment. The association of ions as counter ions or hydrophobic forces such as SDS will modify the charge of the "naked" protein. The migration of a protein in the unit electrical field strength under defined conditions of temperature, buffer, pH, and gel composition is called mobility, and is an intrinsic property of each individual protein.

The gel media have proved outstanding for protein electroresis, polyacrylamide (PAA) and agarose gels (Christer and Ryden, 1989). For the concentrations employed, PAA can be considered as a sieving gel and agarose a non-sieving gel for proteins. The sieving properties are used in the different variants of polyacrylamide gel electrophoresis (PAGE).

2.7.2 Determination of Total Protein

In general, a measure of protein content is obtained upon monitoring the effluent in chromatography by UV-absorption. However, it is not always easy to relate these measurements to the protein content. In fact, the only useful measurement is total amino acid analysis after hydrolysis. Strictly speaking, even this analysis suffers from some shortcomings since tryptophan and cysteine normally have to be analysed separately (Copeland, 1994).

Three main procedures (Copeland, 1994) for protein determination are used routinely: Each of these methods has its advantages and disadvantages:

a) Spectrophotometry at 280nm

This measurement requires the knowledge of the extinction coefficient of the protein(s) to be measured. These vary widely. The values are due to a corresponding variation in the content of the aromatic amino acids tryptophan and to a lesser extend, tyrosine. As a rule of thumb, it is convenient to assume a mean extinction of 1.0 for a 1mg/mL solution and this is often sufficient for practical purposes. When the protein is purified, the extinction coeffient and the wavelength for maximum extinction should however be determined on a solution by spectral and amino acid analysis.

An alternative to this method is the low wavelength measurements at 225nm or below (Copeland, 1994). The absorption is due to the peptide bond, which has a maximum at 192nm but still a considerable absorption is observed at 205 nm and at 220nm at an OD of 11. These measurements are, of course, even more sensitive to contamination and they also require that buffers which are transparent in the low UV regions be used. Great care has to be taken to avoid contaminants and impurities in the buffer salts.

b) Colorimetry by Lowry-Folin-Ciocalteau reagent

c) Dye binding with Coomassie Brilliant Blue G-250

The Lowry method is less problematic but it is not a very sensitive method. Aliquots for analysis should have protein concentrations of 0.1 mg/mL or more. It is often a good alternative in the beginning of purification where direct UV light transmission may be impossible due to turbidity of the sample. The same applies to the use of Coomassie Brilliant Blue. This method is 5 to 10 times more sensitive than the colorimetric method but is more cumbersome to use (Christer & Ryden, 1989).

Both of the latter two methods are destructive, whereas the UV method allows the sample to be recovered.