MILLIPORE

TECHNICAL BRIEF

Protein Concentration and Diafiltration by Tangential Flow Filtration

Table of Contents

An Overview

PURPOSE

Membrane-based Tangential Flow Filtration (TFF) unit operations are used for clarifying, concentrating, and purifying proteins. This technical brief is a practical introduction to protein processing using tangential flow filtration. It is intended to help scientists and engineers achieve their protein processing objectives by discussing how the choice of key components and operating parameters will affect process performance.

What is TFF?

Filtration is a pressure driven separation process that uses membranes to separate components in a liquid solution or suspension based on their size and charge differences. Filtration can be broken down into two different operational modes – Normal Flow Filtration and Tangential Flow Filtration. The difference in fluid flow between these two modes is illustrated in figure 1.

In Normal Flow Filtration (NFF), fluid is convected directly toward the membrane under an applied pressure. Particulates that are too large to pass through the pores of the membrane accumulate at the membrane surface or in the depth of the filtration media, while smaller molecules pass through to the downstream side. This type of process is often called dead-end filtration. However, the term "normal" indicates that the fluid flow occurs in the direction normal to the membrane surface, so NFF is a more descriptive and preferred name. NFF can be used for sterile filtration of clean streams, clarifying prefiltration, and virus/protein separations and will not be discussed in this document.

In Tangential Flow Filtration (TFF), the fluid is pumped tangentially along the surface of the membrane. An applied pressure serves to force a portion of the fluid through the membrane to the filtrate side. As in NFF, particulates and macromolecules that are too large to pass through the membrane pores are retained on the

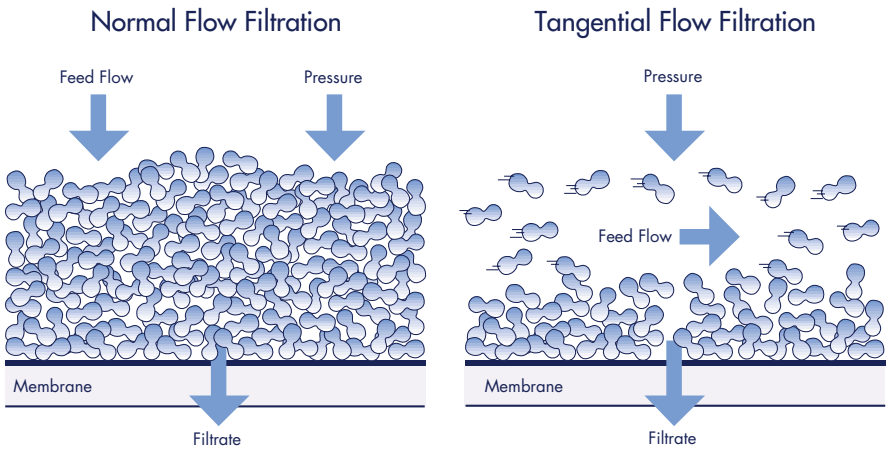


Figure 1. *Comparison of NFF and TFF*

upstream side. However, in this case the retained components do not build up at the surface of the membrane. Instead, they are swept along by the tangential flow. This feature of TFF makes it an ideal process for finer sized-based separations. TFF is also commonly called cross-flow filtration. However, the term "tangential" is descriptive of the direction of fluid flow relative to the membrane, so it is the preferred name.

How is TFF Used in Protein Processing?

TFF can be further subdivided into categories based on the size of components being separated. For protein processing, these can range from the size of intact cells to buffer salts. Figure 2 details typical components that would be retained by a membrane and that would pass through a membrane for each of the subdivisions. In addition, it shows the range of membrane pore size ratings or nominal molecular weight limits (NMWL) that generally fall into each category.

A membrane pore size rating, typically given as a micron value, indicates that particles larger than the rating will be retained by the membrane. A NMWL, on the other hand, is an indication that most dissolved macromolecules with molecular weights higher than the NMWL and some with molecular weights lower than the NMWL will be retained by the membrane. A component's shape, its ability to deform, and its interaction with other components in the solution all affect its retention. Different membrane manufacturers use different criteria to assign the NMWL ratings to a family of membranes. The technical references at the end of this document provide more detail on membrane retention determination as well as additional information on other related topics.

Microfiltration (MF) is usually used upstream in a recovery process to separate intact cells and some cell debris/lysates from the rest of the components in the feed stream. Either the retained cells or the clarified filtrate can be the product stream. Membrane pore size cutoffs used for this type of separation are typically in the range of 0.05 µm to 1.0 µm.

Ultrafiltration (UF) is one of the most widely used forms of TFF and is used to separate proteins from buffer components for buffer exchange, desalting, or concentration. Depending on the protein to be retained, membrane NMWLs in the range of 1 kD to 1000 kD are used.

Two types of UF are Virus filtration (VF) and High Performance tangential flow filtration (HPTFF). For VF, membrane NMWL ratings range from 100 kD to 500 kD, or up to 0.05 µm. This process type is used to separate virus particles from proteins or from smaller media components, as either a virus reduction step or a virus harvest step. HPTFF is a high resolution process where protein-protein separations can be carried out on the basis of both size and charge, resulting in product yields and purification factors similar to chromatography. Membrane NMWLs used for HPTFF are in the range of 10 kD to 300 kD.

Reverse Osmosis (RO) and Nanofiltration (NF) are types of TFF where very tight membranes are used to separate salts and small molecules with molecular masses typically lower than 1500 Daltons from water or other solvents. Membranes with NMWLs of 1 kD and lower are used.

Finally, Diafiltration (DF) is a TFF process that can be performed in combination with any of the other categories of separation to enhance either product yield or purity. During DF, buffer is introduced into the recycle tank while filtrate is removed from the unit operation. In processes where the product is in the retentate, diafiltration washes components out of the product pool into the filtrate, thereby exchanging buffers and reducing the concentration of undesirable species. When the product is in the filtrate, diafiltration washes it through the membrane into a collection vessel.

	Microfiltration	**Virus Filtration**	**High-Performance Filtration**	**Ultrafiltration TFF**	**Nanofiltration/ Reverse Osmosis**
Components retained by membrane	Intact cells Cell debris	Viruses	Proteins	Proteins	Antibiotics Sugars Salts
membrane membrane membrane membrane membrane membrane membrane membrane membrane membrane membrane membrane membrane membrane					
Components passed through membrane	Colloidal material Viruses Proteins Salts	Proteins Salts	Proteins Salts	Small Peptides Salts	(Salts) Water
Approximate membrane cutoff range	0.05 µm – 1 µm	100 kD – 0.05 µm	10 kD – 300 kD	1 kD – 1000 kD	<1 kD

Figure 2. *Subdivisions of tangential flow filtration processes*

The remainder of this document will focus on the development of concentration and diafiltration steps for protein processing.

TFF Basics

In a TFF unit operation, a pump is used to generate flow of the feed stream through the channel between two membrane surfaces. A schematic of a simple TFF system is shown in figure 3. During each pass of fluid over the surface of the membrane, the applied pressure forces a portion of the fluid through the membrane and into the filtrate stream. The result is a gradient in the feedstock concentration from the bulk conditions at the center of the channel to the more concentrated wall conditions at the membrane surface. There is also a concentration gradient along the length of the feed channel from the inlet to the outlet (retentate) as progressively more fluid passes to the filtrate side. Figure 4 illustrates the flows and forces described above with the parameters defined as:

Q_F: feed flow rate [L h^{-1}]

Q_R: retentate flow rate [L h^{-1}]

Q_f: filtrate flow rate [L h^{-1}]

C_b: component concentration in the bulk solution [g L^{-1}]

C_w: component concentration at the membrane surface [g L^{-1}]

C_f: component concentration in the filtrate stream [g L^{-1}]

TMP: applied pressure across the membrane [bar]

The flow of feedstock along the length of the membrane causes a pressure drop from the feed to the retentate end of the channel. The flow on the filtrate side of the membrane is typically low and there is little restriction, so the pressure along the length of the membrane on the filtrate side is approximately constant. A standard pressure profile in a TFF channel is shown in figure 5.

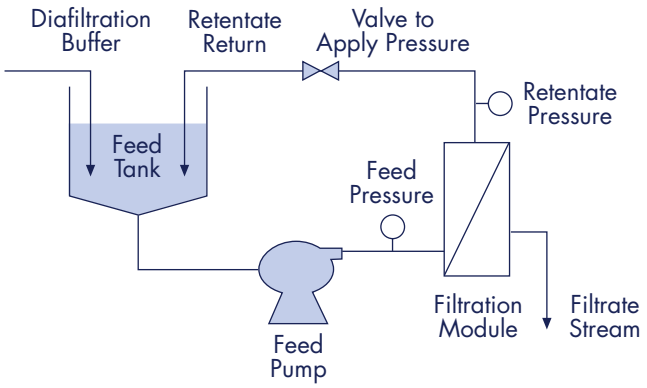


Figure 3. *Schematic of a simple TFF system*

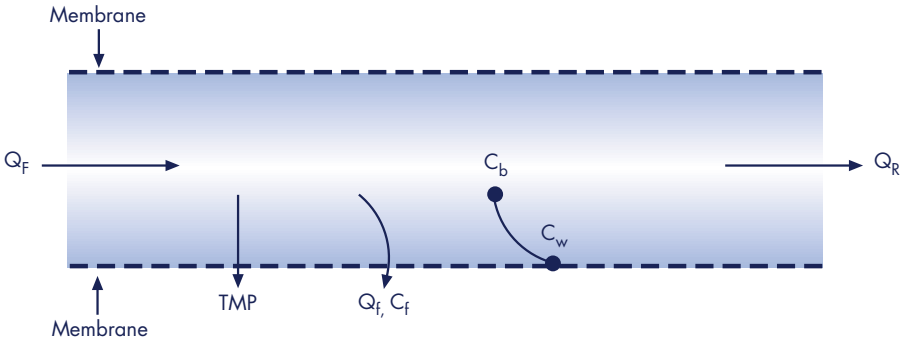


Figure 4. *Flows and forces in a TFF channel*

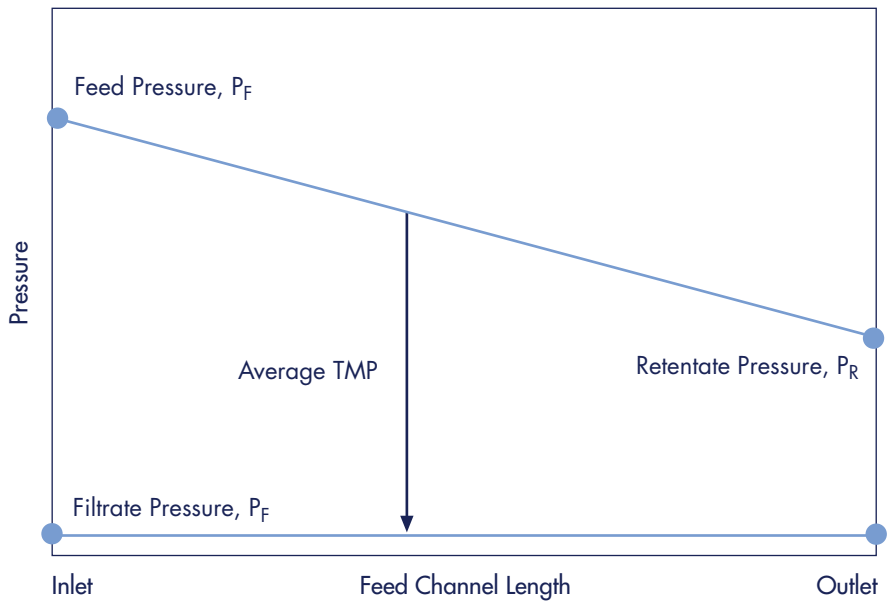


Figure 5. *Pressure profile in a TFF channel*

Definitions

Transmembrane Pressure (TMP) is the average applied pressure from the feed to the filtrate side of the membrane.

$$TMP\ [bar] = [(P_F + P_R)/2] - P_f$$

Pressure Drop (ΔP) is the difference in pressure along the feed channel of the membrane from the inlet to the outlet.

$$\Delta P\ [bar] = P_F - P_R$$

Conversion Ratio (CR) is the fraction of the feed side flow that passes through the membrane to the filtrate.

$$CR\ [-] = Q_f/Q_F$$

Apparent Sieving (S_{app}) is the fraction of a particular protein that passes through the membrane to the filtrate stream based on the measurable protein concentrations in the feed and filtrate streams. A sieving coefficient can be calculated for each protein in a feedstock.

$$S_{app}\ [-] = (\text{concentration in filtrate, } C_f)/(\text{concentration in feed, } C_b)$$

Intrinsic Sieving (S_i) is also the fraction of a particular protein that passes through the membrane to the filtrate stream. However, it is based on the protein concentration at the membrane surface. Although it cannot be directly measured, it gives a better understanding of the membrane's inherent separation characteristics.

$$S_i\ [-] = (\text{concentration in filtrate, } C_f)/(\text{concentration at membrane wall, } C_w)$$

Retention (R) is the fraction of a particular protein that is retained by the membrane. It can also be calculated as either apparent or intrinsic retention. Retention is often also called rejection.

$$R_{app}\ [-] = 1 - S_{app} \text{ or } R_i = 1 - S_i$$

Filtrate Flux (J_f) is the filtrate flow rate normalized for the area of membrane [m^2] through which it is passing.

$$J_f\ [L\ m^{-2}\ h^{-1}] = Q_f/\text{area}$$

Mass Flux (J_m) is the mass flow of protein through the membrane normalized for the area of membrane [m^2] through which it is passing.

$$J_m\ [g\ m^{-2}\ h^{-1}] = Q_f \times C_f/\text{area}$$

Volume Concentration Factor (VCF or X) is the amount that the feed stream has been reduced in *volume* from the initial volume. For instance, if 20 L of feedstock are processed by ultrafiltration until 18 L have passed through to the filtrate and 2 L are left in the retentate, a ten-fold concentration has been performed so the Volume Concentration Factor is 10. In a Fed-Batch concentration process, where the bulk feedstock is held in an external tank and added to the TFF operation continuously as filtrate is removed, VCF should be calculated based only on the volume that has been added to the TFF operation.

$$\text{VCF or X}\ [-] = \text{Total starting feed volume added to the operation/current retentate volume}$$

Concentration Factor (CF) is the amount that the *product* has been concentrated in the feed stream. This depends on both the volume concentration factor and the retention. As with the VCF, for a Fed-Batch concentration process, calculate CF based only on the volume of feedstock added to the TFF operation.

$$CF\ [-] = \text{Final product concentration/Initial product concentration} = VCF(R_{app})$$

A **Diavolume (DV or N)** is a measure of the extent of washing that has been performed during a diafiltration step. It is based on the volume of diafiltration buffer introduced into the unit operation compared to the retentate volume. If a constant-volume diafiltration is being performed, where the retentate volume is held constant and diafiltration buffer enters at the same rate that filtrate leaves, a diavolume is calculated as:

$$\text{DV or N}\ [-] = \text{Total buffer volume introduced to the operation during diafiltration/retentate volume}$$

Define Your Process Goals

The first step of TFF process development is to define what the process must achieve and what goals must be met. A good understanding of these objectives will enable the successful selection of an appropriate unit operation and operating parameters. Important process objectives to define are:

- Final product concentration
- Feed volume reduction
- Extent of buffer exchange
- Contaminant removal specification

Next, identify and quantify any criteria by which the success of the operation will be measured. The primary goals for a successful protein processing are:

- High product yield
- High product quality (or activity)
- High product purity
- Controlled bioburden and endotoxin

In addition, the process should scale up accurately, enable straightforward validation, and be robust during continued use at industrial scale. Finally, the economic objectives for the process must be met and any constraints such as process time, unit operation size, or buffer use must be observed. Discussion on how the process design impacts yield, quality, bioburden, scalability, robustness, and economics begins on page 19.

Choosing the Right Equipment

The primary components of a TFF process are the membrane material and the membrane module format. Choosing the most appropriate components early in the development process, with consideration for the requirements of the process, increases the success and robustness of the final step.

Membranes

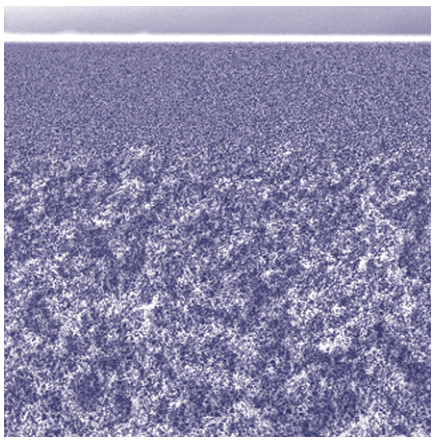
Millipore provides ultrafiltration membranes in several different materials to suit a wide range of applications. The different membrane materials offer alternatives in fouling characteristics and chemical compatibility. Each of the membrane materials is available in a number of NMWLs. Two of the most common materials for ultrafiltration membranes are regenerated cellulose and polyethersulfone.

Millipore's Ultracel® membrane is regenerated cellulose. The Ultracel PL family, standard regenerated cellulose, is available in NMWLs from 1 to 300 kD. The Ultracel PLC composite regenerated cellulose ranges in NMWLs from 5 to 1000 kD. Ultracel PLC membranes are cast on a microporous polyethylene substrate and have superior resistance to reverse pressure versus the PL series.

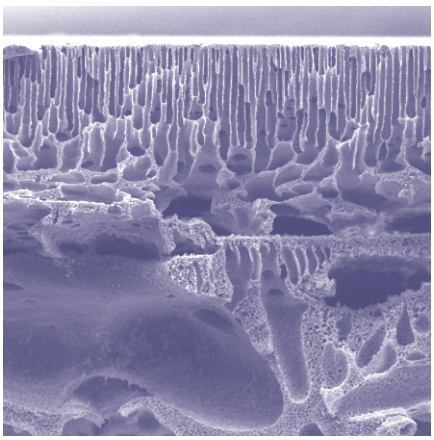
All regenerated cellulose membranes are very hydrophilic, exhibiting low fouling and ultra-low protein adsorption. They are more compatible with organic solvents than are the polyethersulfone-based membranes, but are less tolerant to extreme pH's. ***Ultracel membranes are recommended for use in all applications where harsh pH conditions are not needed and especially when protein loading is low (<20 g/m²) or the feedstock is highly fouling.***

Traditional polyethersulfone membranes tend to adsorb protein as well as other biological components, leading to membrane fouling and lowered flux. Millipore's Biomax® membrane is polyethersulfone-based, but has been hydrophilically modified to be more resistant to fouling. The Biomax membrane line ranges in NMWLs from 5 to 1000 kD. Biomax membranes operate over a wide temperature range and are highly stable at pH's from 1 to 14, but have limited solvent compatibility. ***The use of Biomax membrane is recommended for applications where very harsh pH conditions are required for processing or cleaning.*** In order to minimize adsorption losses maintain moderately high protein loading (>20 g/m²).

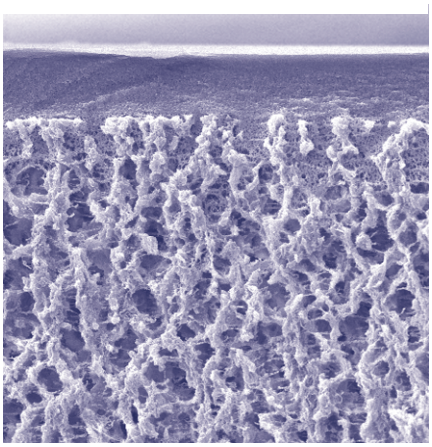
10 kD Biomax®



Traditional PES 10



10 kD Ultracel® PLC



Scanning electron micrograph images of cross sections of different membranes.

Table 1 shows the magnitude of protein losses due to adsorption on several UF membrane materials. These losses were measured at Millipore with model protein feedstocks. In addition, the percentage yield loss due to adsorption is shown for two theoretical processes. The "Low Protein Case" is a process in which 1000 L of 0.1 g/L solution is concentrated to 2 g/L on a 10 m^2 unit. The "High Protein Case" is a process in which 1000 L of 10 g/L solution is concentrated to 200 g/L on a 20 m^2 unit.

The unique construction of both the Biomax and Ultracel PLC product lines makes these membranes free of voids and defects and well-attached to the substrate. The membranes are rugged, have very high integrity, and have excellent retention characteristics. ***A membrane from either the Biomax or Ultracel PLC family should be the first choice when developing a process.***

Since NMWLs for UF membranes do not indicate absolute retention/sieving ratings, some rules of thumb are useful in determining what membrane rating is applicable for a particular process. As a rule, choose a membrane that has a NMWL one-third to one-fifth of the molecular weight of a product that is to be retained. Also, a minimum size difference of approximately five-fold between components that are being separated is optimal.

Highly fouling feedstocks tend to have higher retention of like-sized proteins than cleaner feedstocks. In addition, a process operating at very high TMPs has lower retentions due to an increased protein concentration at the membrane surface. Since each protein feedstock and process is unique, two or more membranes may need to be tested before choosing an optimal one.

Choose a membrane that has sufficiently high retention to meet your yield goal. Product loss to the filtrate due to incomplete retention is cumulative for the concentration and diafiltration sections of a process.

For a batch UF and constant-volume DF process, where retention remains constant throughout the process, this loss is calculated as:

$$\text{Product Loss (\%)} = 100^{*} (1 - e^{(R-1)[\ln VCF + N]})$$

The relationship is plotted in figure 6 for processes in which the product is in the retentate. To illustrate how to use figure 6, consider a process where the goals are to perform a 20-fold volume concentration factor (VCF), a 7 diavolume buffer exchange, and lose less than 7% of the product to the filtrate. For this example, the natural log (ln) of the VCF is 3 and N is 7, so the value of the term (ln VCF + N) is 10. If a membrane is chosen that has a retention of 0.99 for the product, the product loss to the filtrate will be 9.5%, as indicated by point "A" on the graph. Therefore, the yield loss goal is not met. In order to reduce the product loss while still using the same membrane, the amount of diafiltration and/or volume concentration has to be reduced. For example, if the number of diavolumes is reduced to 4.3, the value of the term (ln VCF + N) is now 7.3 and the amount of product lost to the filtrate is 7.0%, as indicated by point "B". However, the extent of buffer exchange is drastically reduced. To reduce the product loss without changing the process, a membrane with higher retention of the product must be chosen. If the retention is increased to 0.999 while the value of (ln VCF + N) remains at 10, product loss to the filtrate drops to only 1.0%, as indicated by point "C". In many cases, product retention is different during the UF and DF sections of a process. It is important to check this for each process. When retention changes, product loss to the filtrate is determined separately for each section by following the appropriate retention curve and summing the two results.

Membrane Material [-]	Protein Adsorption [g m^{-2}]	Low Protein Case [% Yield Loss]	High Protein Case [% Yield Loss]
Polyethersulfone	0.5	5.0	0.10
Biomax (polyethersulfone)	0.2	2.0	0.04
Regenerated cellulose	0.1	1.0	0.02

Table 1. *Typical protein adsorption onto UF membranes*

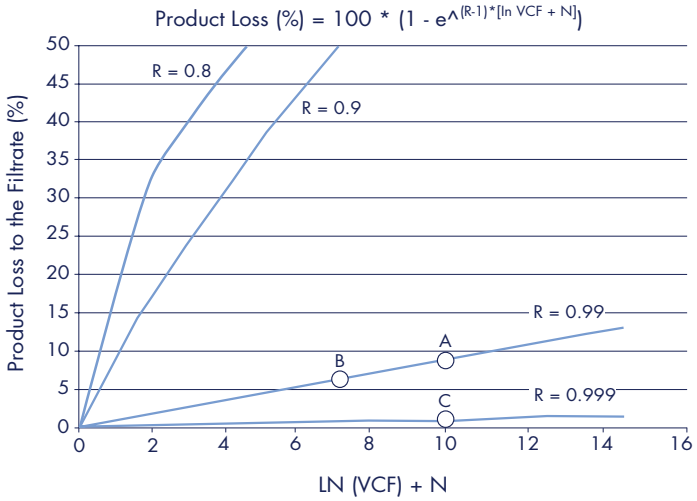


Figure 6. *The effect of product retention on product yield during a batch ultrafiltration/constant-volume diafiltration process where the product is in the retentate and the retention is constant throughout the process.*

Modules

Membranes are fabricated into modules in several formats. The most common formats used for tangential flow filtration are:

- Flat plate
- Spiral wound
- Hollow fiber

The basic flowpaths for each of these modules is shown in figure 7.

Screens are often inserted into the feed and/or filtrate channels in spiral wound and flat plate modules to increase turbulence in the channels and reduce concentration polarization. This is not an option with hollow fiber modules. The turbulence-promoted channels have higher mass transfer coefficients at lower crossflow rates, meaning that higher fluxes are achieved with lower pumping requirements. Turbulence-promoted feed channels are, therefore, more efficient than open channels. Using a suspended screen in a flat plate module gives some of the benefits of both open and turbulence-promoted channels. Figure 8 illustrates the different types of channel configurations.

Flat Plate (Often referred to as Cassettes)

In a flat plate membrane module, layers of membrane either with or without alternating layers of separator screen are stacked together and then sealed into a package. Feed fluid is pumped into alternating channels at one end of the stack and the filtrate passes through the membrane into the filtrate channels. Flat plate modules generally have high packing densities (area of membrane per area of floor space), allow linear scaling, and some offer the choice of open or turbulence promoted channels.

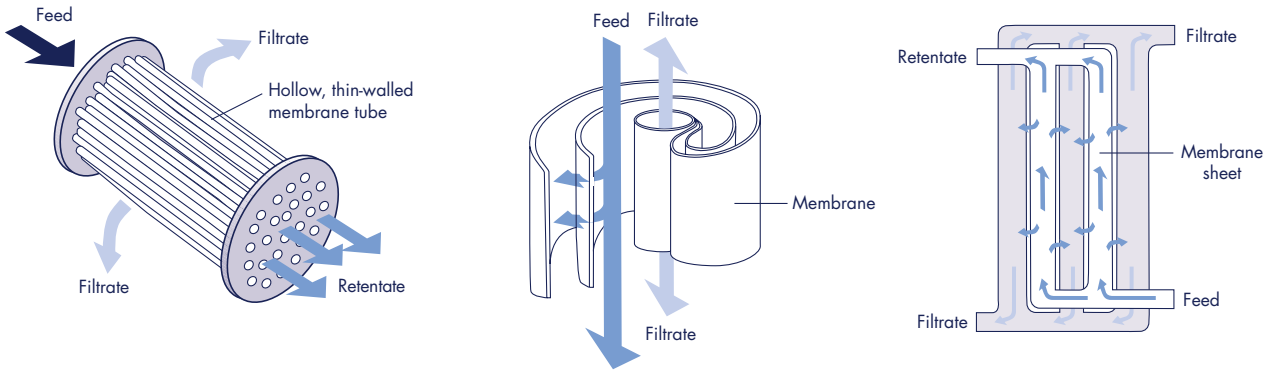


Figure 7. *Fluid flowpaths through different TFF modules*

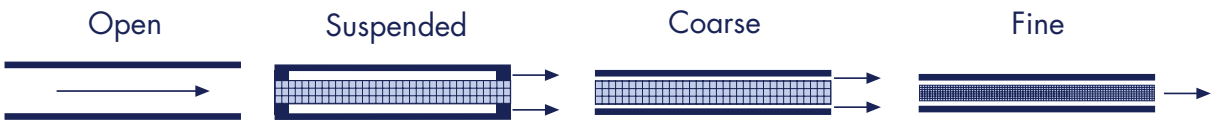


Figure 8. *Open and turbulence-promoted feed channels in TFF module types*

Spiral Wound

In a spiral wound module, alternating layers of membrane and separator screen are wound around a hollow central core. The feed stream is pumped into one end and flows down the axis of the cartridge. Filtrate passes through the membrane and spirals to the core, where it is removed. The separator screens increase turbulence in the flowpath, leading to a higher efficiency module than hollow fibers. One drawback to spiral wound modules is that they are not linearly scaleable because either the feed flowpath length (cartridge length) or the filtrate flowpath length (cartridge width) must be changed within scales. However, spiral wound modules are a good low cost option for very large area unit operations, as required for food and beverage applications.

Hollow Fiber

Hollow fiber modules are comprised of a bundle of membrane tubes with narrow diameters, typically in the range of 0.1 to 2.0 mm. In a hollow fiber module, the feed stream is pumped into the lumen (inside) of the tube and filtrate passes through the membrane to the shell side, where it is removed. Because of the very open feed flowpath, low shear is generated even with moderate crossflow rates. While this may be useful for highly shear-sensitive products, in general it reduces the efficiency of the module by requiring very high pumping capacity to achieve competitive fluxes.

Millipore offers two premier TFF modules for ultrafiltration/diafiltration protein processing. These are the flatplate Pellicon® 2 cassette modules, and the spiral-wound Helicon™ modules. Pellicon cassettes are available in several sizes of linearly scalable modules to process volumes from 20 mL to 10,000 L. These modules incorporate all of the membrane materials discussed above and Millipore offers the choice of several different feed channel screens to optimize the turbulence-promotion for a particular process.

Spiral wound Helicon modules are useful for lower cost processing of very large volumes. Modules with standard polyethersulfone and regenerated cellulose membranes are available. Spiral wound Prep/Scale modules are easy to use for processing smaller volumes when a spiral-wound format is preferred. However, flat plate Pellicon XL modules offer superior low-volume processing.

Prep/Scale filter modules

Pellicon cassettes

Optimization of Key Process Parameters

Before implementing a TFF step for protein processing, the parameters at which the step will operate must be defined. Key parameters to determine are:

- Crossflow rate
- Transmembrane pressure
- Filtrate control
- Membrane area
- Diafiltration design

These parameters are typically arrived at through a combination of rules of thumb, experimentation, and consideration of process requirements and limitations.

Crossflow Rate

The crossflow rate greatly depends on which module and feed channel turbulence promoter are chosen. Millipore provides recommendations for crossflow rates for each feed channel type in the Maintenance Procedures manuals. In general, a higher crossflow rate gives higher flux at equal TMP. It increases the sweeping action across the membrane and reduces the concentration gradient towards the membrane surface. This also tends to slightly increase the observed retention of most components. However, higher crossflow rates cause the product to experience more passes through the pump in a given amount of time. This can lead to degradation of product quality. Also, higher crossflow rates require the use of larger pumps and larger diameter piping, which increase the system holdup volume and could increase product losses due to unrecoverable holdup. Therefore, balance the increase in flux with the increase in pump passes and holdup volume when choosing a crossflow rate.

TMP

In a TFF unit operation, filtrate flux increases with increasing TMP up to a point and then it levels off. The first part of the curve, where the flux increases with pressure, is the pressure dependent regime. Here, the primary factor limiting flux is the fouled membrane resistance. The second, level part of the curve is the pressure independent regime. In this section, the concentration of protein at the membrane surface is high and a significant portion of the applied pressure is working against the protein osmotic pressure. As protein concentration increases or feed flow rate decreases, the TMP at which the flux plateaus decreases. A typical trend of flux with increasing TMP, protein concentration, and feed flow rate is shown in figure 9.

If the process is run with a TMP setpoint in the pressure independent regime, maximum flux is achieved, and this minimizes the required membrane area. However, the protein wall concentration is high and could exceed a solubility limitation, leading to yield losses. On the other hand, if a TMP setpoint is chosen in the pressure dependent regime, fluxes are lower and more membrane area is required. Therefore, for a standard UF/DF process, the optimum TMP at which to run a process is at the knee of the curve, where nearly the highest flux is achieved without exerting excessive pressure or reaching exceedingly high protein wall concentrations. For HPTFF processes, where two similarly-sized components are being separated, the optimum operating point is determined differently.

Filtrate Control

Many TFF applications apply a crossflow and pressure setpoint and the filtrate flows uncontrolled and unrestricted out of the module. This is the simplest type of operation and most concentration and/or diafiltration processes where the target product is in the retentate operate in this manner. For other applications, however, it is helpful to use some type of filtrate control beyond that achieved by simply adjusting the pressure with the retentate valve.

When using very open UF membranes, the membrane permeability is so high that nearly all of the crossflow is converted to filtrate with very little applied TMP. Although this results in high fluxes, it is similar to operating in an NFF mode and the benefits of the tangential flow are lost. Often, very high wall concentrations and high membrane fouling occur, especially during the startup of the process. To reduce the filtrate rate and enable the TMP to be controlled at the low values required for robust TFF operations the filtrate flow must be controlled.

In a controlled flow filtrate operation, a pump or valve on the filtrate line restricts filtrate flow to a set value, as shown in Figure 10. In addition to reducing the filtrate flow to maintain adequate tangential flow, it creates pressure in the filtrate line to reduce the TMP while the feed and retentate pressures remain fixed.

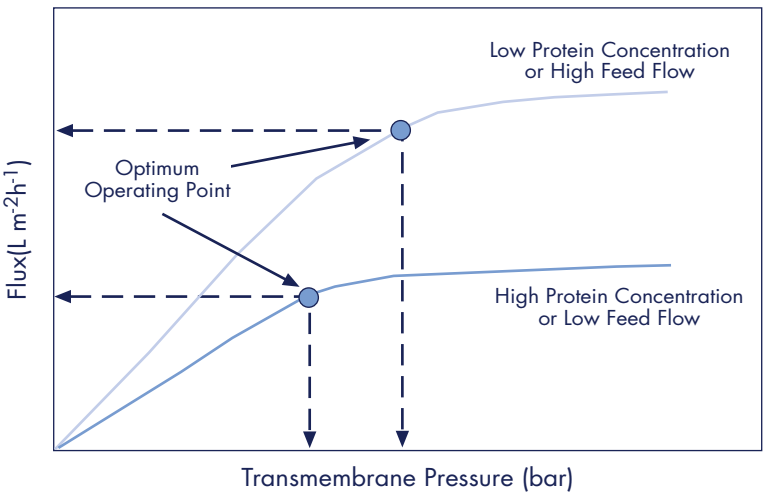


Figure 9. *A typical trend of flux versus transmembrane pressure for a TFF process*

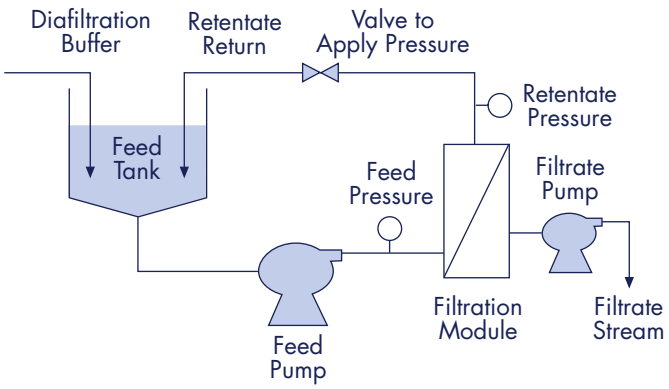


Figure 10. *Schematic of a TFF system using a pump for a filtrate control*

Membrane Area

After determining the process flux and the total volume to be processed, the membrane area required for the final unit operation can be determined. However, since flux is filtrate flow rate divided by both area and time, the membrane area is also a function of the total process time. Choosing a longer process time leads to lower membrane area requirements. This is beneficial because membrane and capital costs are reduced. In addition, unrecoverable holdup volume is lower in smaller unit operations, minimizing yield losses. However, excessively long process times put the product at risk for quality degradation and/or bioburden contamination. Interestingly, product pump passes do not change significantly because a low-area operation has a low crossflow rate but a high process time while a high-area operation has a high crossflow rate and a short process time.

Calculate the membrane area requirements as:

$$\text{Membrane area } [m^2] = \text{Filtrate volume } [L] / \text{Flux } [L\ m^{-2}\ h^{-1}] * \text{Process time } [h]$$

Flux typically drops as protein concentration increases, so choose an average flux for the above calculation. Alternatively, some processes exhibit several distinct sections with different fluxes. For example, a concentration followed by a diafiltration followed by another concentration will generally show a decreasing flux followed by a constant flux during DF followed by another section of decreasing flux. In this case, break out each section as:

$$\text{Membrane area } [m^2] = [\text{Filtrate volume}_1 / \text{Flux}_1 + \text{Filtrate volume}_2 / \text{Flux}_2 + \ldots] / \text{Process time}$$

For a robust scaleup, always incorporate a safety margin into the membrane area requirements to account for lot to lot variability in membrane permeability, feedstock characteristics, and batch volumes. Typically, a safety margin of at least 20% extra membrane area is used, but this could increase or decrease depending on the expected variability in the process.

Diafiltration Design

If the process includes a diafiltration step, first choose the mode of diafiltration control. The two most common modes of diafiltration control are batch and constant-volume. In a batch DF process, a large volume of diafiltration buffer is added to the recycle tank and then the retentate is concentrated. When a certain retentate volume is reached, another volume of buffer is added. This cycle is continued until the desired total volume of DF buffer has been added. The benefit of this mode of diafiltration is that no level control is required. However, the buffer exchange is not as efficient as in a constant volume DF

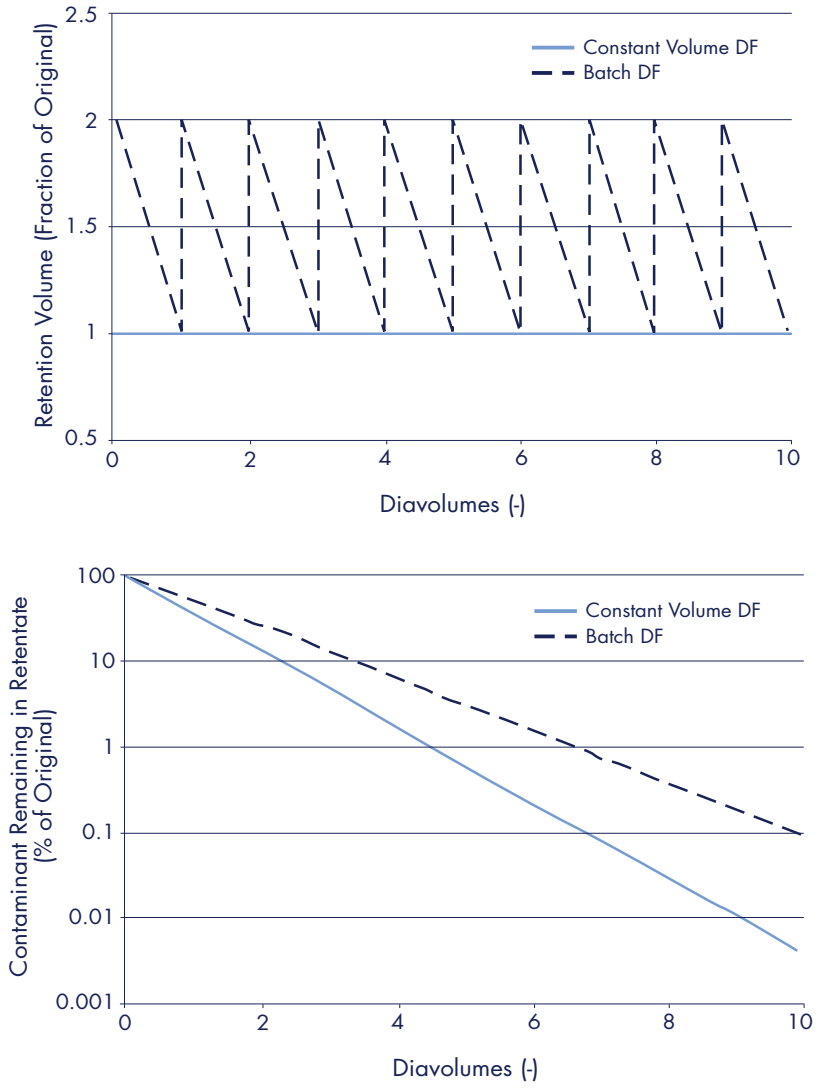


Figure 11. *Retentate volume and buffer exchange during batch and constant-volume diafiltration*

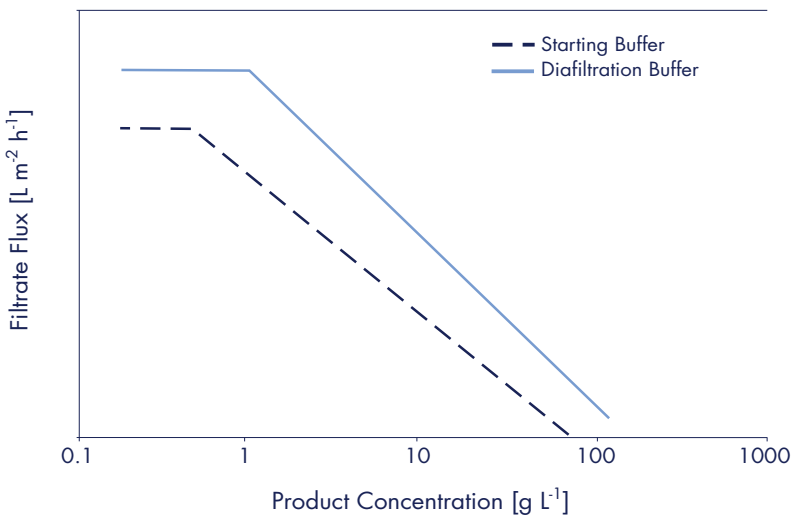


Figure 12. *Typical trend of flux versus protein concentration in different buffers*

process and the product concentration at which diafiltration is performed cannot be optimized because protein concentration changes as the buffer is added and then concentrated.

Constant-volume diafiltration is the more commonly used control mode. To perform a constant-volume DF, buffer is added to the recycle tank at the same rate that filtrate is removed. The total volume of retentate remains constant throughout the process. This mode of operation requires some method of level control that will meter the addition of DF buffer to keep the retentate volume constant. The effect of the two modes of operation on retentate volume and buffer exchange is illustrated in figure 11. The remaining diafiltration discussion and calculations will focus on constant-volume diafiltration processes, since they are more efficient and more commonly used.

A third mode of diafiltration control is known as the optimum diafiltration strategy. It is primarily used when a component that is partially retained is being removed by diafiltration. Here, both the volume and concentration of product are changed along a controlled path throughout the process to simultaneously optimize buffer use, product yield, and buffer exchange. Please contact Millipore Technical Services for more information on this control scheme.

After choosing a control mode, determine the placement of the diafiltration step within the process. For processes where the target protein is retained, flux typically drops as a protein is concentrated. Diafiltration at lower protein concentrations then maximizes flux. However, at low protein concentration, the total volume of product to diafilter is high, increasing the membrane area and buffer volume required. Therefore, there is an optimum protein concentration at which to perform diafiltration where the tradeoff between flux and volume is balanced and the minimum membrane area or process time is needed.

In the past, the optimum concentration for diafiltration has been determined by finding the concentration at which flux drops to zero (historically called c_g) and then dividing this concentration by the constant e (e = 2.718). However, this approach only gives an approximation of the optimum point for processes where the flux decay follows a well-defined standard curve. For standard pressure-controlled UF/DF processes, a more accurate and generally applicable approach for determining the optimum point at which to diafilter is to first plot flux versus the log of protein concentration. It is important to plot this data with the protein in both the initial and final buffers, since flux can often change significantly with different buffers. A typical trend is shown in figure 12.

Next, choose several protein concentrations along each curve that span the range from initial to final concentrations expected in the process and calculate the value of the DF Optimization Parameter at each point using the following equation:

$$\text{DF Optimization Parameter} = C * J_f$$

where:

C = product concentration in feedstock at data point [g L^{-1}]

J_f = filtrate flux at data point [L m^{-2} h^{-1}]

Finally, plot the optimization parameter versus protein concentration for each buffer, as shown in figure 13, to find the product concentration where the value of the optimization parameter is maximized. This is the optimum concentration at which to diafilter to minimize membrane area requirements. If the optimum is very different for the two buffers, it is most conservative to choose the optimum based on the buffer curve that results in the lower value. The actual value will be between the two curves, since throughout the diafiltration the product will be gradually exchanged from the starting to the final buffer.

Although operating at this concentration minimizes the membrane area required, it may not always be practical. Product volume at this concentration may be below the minimum recirculation volume of the unit operation or the product may not be stable at this concentration. In these cases, choose a lower concentration at the expense of using more diafiltration buffer and more membrane area or longer processing time. Alternately, choose a concentration higher than the optimum if the goal is to minimize the volume of diafiltration buffer required at the expense of adding more membrane area or processing time.

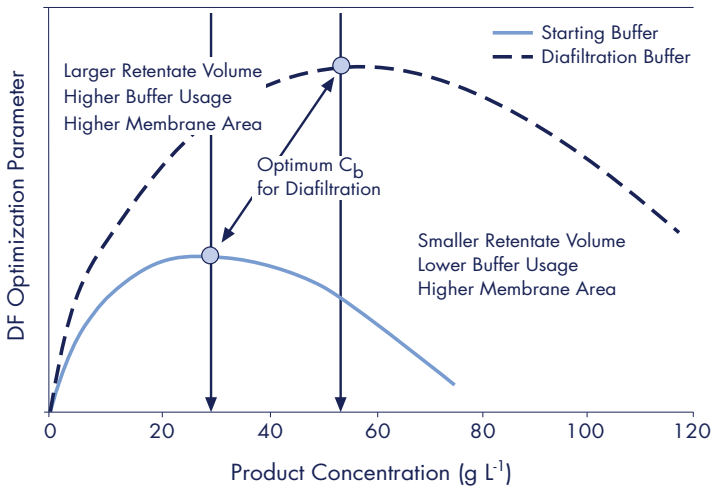


Figure 13. *Determination of the optimum protein concentration for diafiltration for a standard TFF process*

Finally, the goal of a diafiltration step is to reduce buffer or contaminant species from a product in the retentate. Since the number of diavolumes that are performed directly impacts both yield and extent of purification, it must be determined with the goal in mind. Figure 6 illustrates the relationship between product retention and product yield as a function of volume

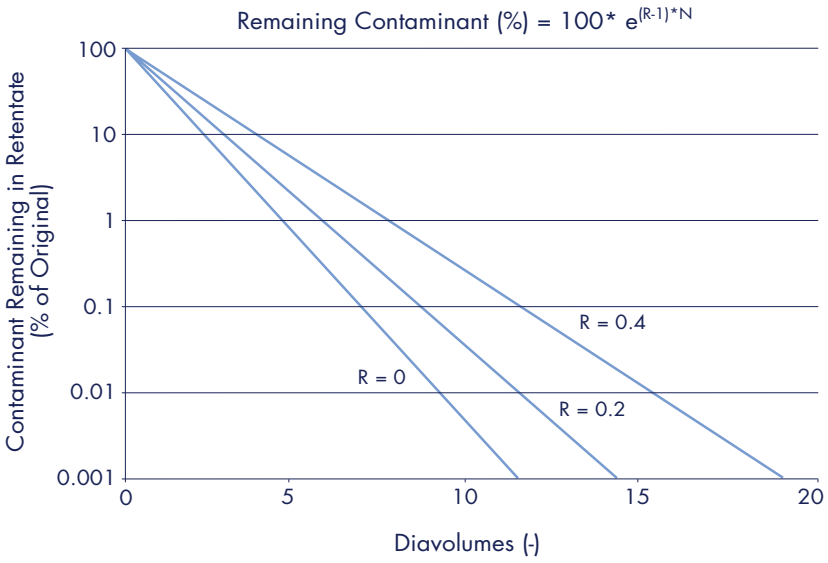


Figure 14. *Removal of a contaminant during a constant-volume diafiltration process where the product is in the retentate and the contaminant is in the filtrate*

concentration factor and diavolumes. Buffer exchange and contaminant removal are easier to view in terms of percent removal versus diavolumes, as shown in figure 14.

There are several common reasons why actual contaminant removal can be lower than the theoretical removal shown in figure 14. For example, retention of the contaminant can change throughout a diafiltration as its concentration and the buffer composition change. The contaminant can bind to the product of interest. The formation of surfactant micelles can change retention or cause partitioning of the contaminant into the micelle. The Donnan effect can increase retention when low ionic strength solutions are used. Finally, deadlegs in the system piping can result in small volumes of solution that are not fully washed throughout the diafiltration. Since contaminants or residuals often must be removed from the product to very low levels, ***incorporate a safety factor of at least two extra diavolumes and test the process to ensure that actual residual levels are acceptable.***

ProFlux® M12 Benchtop TFF system with spiral wound modules

Characterization of Performance

Although the above discussion gives general guidelines on how to choose an appropriate module and operating parameters, the performance of the process must be tested on the actual feedstock. One of the most important experiments for characterizing performance is to generate flux versus TMP curves at several crossflow rates (or pressure drops) and several protein concentrations and to determine product retention at each point. In addition, if the process contains a diafiltration, it is important to generate these flux versus TMP curves in both the starting and final buffers, since flux and retention can change significantly with buffer conditions. If required, the effects of processing at different temperatures can also be incorporated. With a small volume of feedstock and a single day's work, this experiment generates a wealth of information about the process. The experiment will be briefly described here.

Typically, determine TFF performance at approximately three different crossflow rates that span the range of manufacturer recommended rates for the module being used. Likewise, approximately three different protein concentrations should be tested that span the range from initial protein concentration in the feedstock to the highest concentration expected in the process. Investigate at least five transmembrane pressures for each crossflow and protein concentration. TMPs will vary depending on the membrane module and the feedstock, but will typically be in the range of 5 to 50 psid.

Perform the experiment by starting up the module in a total recycle mode, where both the retentate and the filtrate lines are directed back to the recycle tank. Set specific flow, pressure, concentration, and temperature conditions. After the module has equilibrated at the conditions, record the flows and pressures and collect small samples of the feed and filtrate streams for analysis of protein concentration. Then, apply new conditions and repeat the procedure.

The method of startup and the order of conditions tested can impact the results, so take care to always begin with the least fouling conditions and move towards more fouling conditions. During startup of the operation, first slowly ramp the feed rate (and co-flow rate, if applicable) without any applied pressure. When the feed rate setpoint is reached, ramp the applied pressure to its setpoint. Finally, if filtrate control is being used, ramp the filtrate to its setpoint. Shutdown of the operation should occur in reverse order from the startup.

When testing different flow, concentration, and pressure points, conditions that are least fouling are those at low protein concentrations, low TMPs, and high feed rates. A good approach is to start with the highest feed rate and lowest protein concentration and TMP to be tested. At constant feed rate and protein concentration, increase the TMP until it begins to level off. At this point, the membrane is operating in the pressure independent regime (see figure 9) and higher TMPs will cause excessively high protein concentrations within the module without the benefit of increased flux. Maintaining the protein concentration constant, repeat the TMP excursion (low to high TMP) at each feed rate to be tested, moving from high to low feed rates. Then, increase the protein concentration and repeat the entire procedure.

A sample sheet for data collection is illustrated in figure 15. For each test point, calculate flux, TMP, and retention. Then, generate graphs showing flux versus TMP at different crossflow rates and protein concentrations, and retention versus TMP at different crossflow rates and protein concentrations. From the retention data, calculate the predicted yield losses as described by the equation shown in figure 6. The collection of this data enables the choice of successful and robust operating conditions.

Experiment Title: Sample Experiment | **Lab book Reference:** 10739-25 | **Date:** 04/25/99 | **Operator:** JCT
Objective: Determine operating parameters | **Feedstock Product and pool:** Protein Y IEX pool | **Feedstock lot #:** 10739-18
Membrane Material, MWCO: PLCGC | **Membrane Area [m²]:** 0.1 | **Device Holder:** Pellicon-mini | **Device lot #:**

Pre-Use Cleaning and Equilibration

Step	Time [hh:mm]	Feed Rate [L/min]	Pfeed [psig]	Pret [psig]	Pfilt [psig]	Filt. Rate [L/min]	Temp [°C]	DP [psid]	TMP [psid]	Flux [L/m² h]
5 L DI water flush	9:10	0.5	24	10	0	0.17	22	14	17	102
1 L 0.1N NaOH total recycle	9:20	0.5	24	10	0	0.17	22	14	17	102
Normalized Flux testing	9:50	0.5	24	10	0	0.17	22	14	17	102
Integrity testing	10:00		30			0.001				1 L
Equilibration buffer recycle	10:10	0.5	25	10	0	0.15	22	15	17.5	90

Process: Starting Feedstock Volume = 4 L at 2 g/L

Step	Time [hh:mm]	Run Time [min]	Feed Rate [L/min]	Pfeed [psig]	Pret [psig]	Pfilt [psig]	Filt. Rate [L/min]	Filt Vol. [L]	Temp [°C]	DP [psid]	TMP [psid]	Flux [L/m² h]
J v T, Q1, C =2 g/L	10:20		0.7	33	7	0	0.108		22	26	20	65
	10:30		0.7	43	17	0	0.152		22	26	30	91
	10:40		0.7	63	37	0	0.196		22	26	50	118
J v T, Q2, C = 2 g/L	10:50		0.5	17	3	0	0.042		22	14	10	25
	11:00		0.5	27	13	0	0.087		22	14	20	52
	11:10		0.5	37	23	0	0.125		22	14	30	75
	11:20		0.5	47	33	0	0.147		22	14	40	88
	11:30		0.5	57	43	0	0.155		22	14	50	93
J v T, Q3, C = 2 g/L	11:40		0.3	13	7	0	0.032		22	6	10	19
	11:50		0.3	23	17	0	0.065		22	6	20	39
	12:00		0.3	33	27	0	0.092		22	6	30	55
Conc to C = 20 g/L	12:10	0	0.5	37	23	0	0.125	0	22	14	30	75
	12:27	17	0.5	37	23	0	0.110	2	22	14	30	66
	12:43	33	0.5	38	23	0	0.088	3.6	22	15	30.5	53
	12:47	37	0.5	40	23	0	0.073	3.2	22	17	31.5	44
J v T, Q2, C = 20 g/L	12:57		0.5	17	3	0	0.025		22	14	10	15
	1:07		0.5	27	13	0	0.052		22	14	20	31
	1:17		0.5	37	23	0	0.073		22	14	30	44
	1:27		0.5	47	33	0	0.080		22	14	40	48

Post-Use Cleaning

Step	Time [hh:mm]	Feed Rate [L/min]	Pfeed [psig]	Pret [psig]	Pfilt [psig]	Filt. Rate [L/min]	Temp [°C]	DP [psid]	TMP [psid]	Flux [L/m² h]
2 L 0.1N NaOH flush	1:45	0.5	24	10	0	0.16	22	14	17	96
1 L 0.1N NaOH total recycle	1:50	0.5	24	10	0	0.16	22	14	17	96
Normalized Flux testing	2:20	0.5	24	10	0	0.16	22	14	17	96
Integrity testing	2:10		30			0.001				
1 L Storage solution recycle	2:20	0.5	24	10	0	0.16	22	14	17	96

Figure 15. *Example of data collection sheet for a TFF performance characterization experiment*

Test the Process!

After choosing the membrane, module, and all operating parameters, run the entire process to ensure that performance meets all criteria for acceptability. During the process, monitor flows and pressures. Collect samples of all initial and final streams. Calculate process time to ensure that it is within the expected range. Test the quality of the final product with reliable assays, ideally the assays that will be used during actual processing for qualifying product release.

In order to understand where the product is going during a process, it is important to calculate not only yield, but also mass balance. Determine the total protein in each of the retentate, the filtrate, and the unrecoverable holdup volume. Ideally, these amounts sum to the total amount that was put into the unit operation. If they fall short, there were likely some adsorption and/or solubility losses during the process. However, if the amount of protein unaccounted for is a large percent of the total, either the process is not operating correctly or some operating parameters need to be changed to reduce the losses. The yield and mass balance follow the law of conservation of mass where:

$$V_o * C_o = V_r * C_r + V_f * C_f + V_h * C_h$$

The subscripts o, r, f, and h refer to original, retentate, filtrate, and holdup, respectively. The percent yield in any one of the streams can be calculated by dividing the amount protein in that stream by the total amount in the feedstock. For instance, the yield in the retentate is calculated as:

$$\text{Yield [\%]} = 100 * V_r * C_r / V_o * C_o$$

Finally, to understand how robust a process is to feedstock variability and multiple cycles, it is very helpful to run the process several times. Although this is not always possible, especially when feedstock is extremely limited, it can help guard against unexpected performance degradation once the process is in place. In addition, it will help to ensure that the process parameters were not determined based on a best-case run that is not reproducible.

Labscale™ Benchtop TFF System with Pellicon XL module. Complete, linear scalable solution for small-volume processing.

Putting the Process Together

Once a protein processing procedure has been developed, it must be integrated into a complete process. The typical sequence of steps in an ultrafiltration/diafiltration process are outlined in figure 16.

Set Up and Pre-Use Cleaning

Before installation of membranes into a new TFF holder, thoroughly clean and flush all components of the holder and system to remove potential contaminants that were introduced during manufacture and assembly. Scrubbing exposed surfaces with a soap solution and recirculating the solution through all piping with the use of special cleaning gaskets, followed by extensive flushing with high quality water removes residual dirt and oils.

After new membranes have been installed, and before their first use on product, clean, sanitize, depyrogenate, and flush the assembly to remove membrane preservatives and any contaminants introduced during installation. Please refer to the appropriate Millipore Maintenance Procedures for recommended cleaning, sanitization, and depyrogenation solutions, recirculation times, and temperatures.

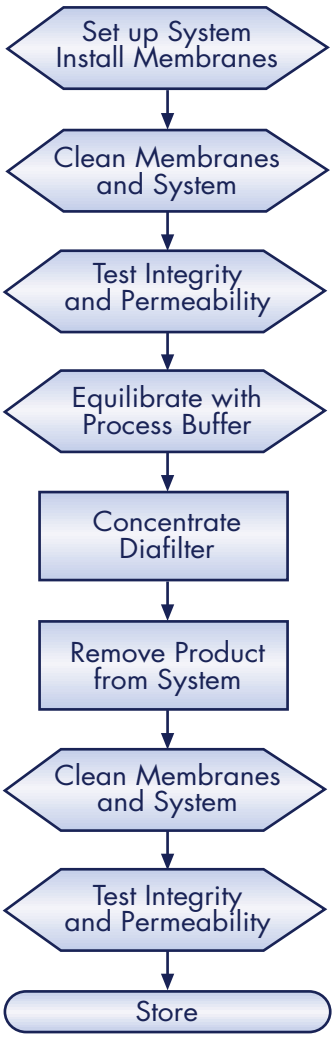


Figure 16. *Typical sequence of steps in a TFF process*

Integrity and Permeability Testing

In order to ensure that the installed membranes have not sustained any damage during storage and handling, Millipore recommends integrity testing all TFF assemblies prior to startup and after each post-use cleaning. An air diffusion test identifies problems such as macroscopic holes in the membrane, cracks in the seals, or improperly seated modules.

When air is applied to the retentate side of the membranes at a controlled pressure, it diffuses through the water in the pores at a predictable rate. If there are defects, the air will be able to flow through them at a much higher rate than the background diffusion, giving a failing test value. To obtain accurate test results, fully wet the membranes with water and then completely drain the modules. In the Maintenance Procedures manuals, Millipore provides instructions on performing integrity tests and lists test pressures and diffusion specifications for all of its membranes.

Prior to the first use on protein and after each post-use cleaning, measure the clean membrane permeability to establish a baseline for flows and pressures. This value, also called the normalized flux, is determined by recording crossflow and filtrate flow rates, feed, retentate, and filtrate pressures, and temperature

Normalized Flux [L m^{-2} h^{-1} $psig^{-1}$] = (flux x temperature correction factor)/TMP

during recirculation of a solution. Then, calculate:

For a given TFF setup, always take the measurement at similar operating conditions, preferably at a low TMP, using the same solution. Refer to the Millipore Maintenance Procedures for specific instructions and temperature correction factors.

Pre-Use Equilibration and Protein Processing

Since the membranes are typically in storage solution just before processing, drain the TFF system and then flush it with high quality water to reduce the level of residual storage components to acceptably low levels. It is good practice to then equilibrate the system in a buffer solution before introduction of protein. This prevents any solubility or product quality changes that could occur if the protein solution was suddenly exposed to very different pH or ionic strength conditions. Also, it minimizes the exposure of the protein to air/liquid interfaces that result from using the protein solution to fill the empty recycle tank or start the recirculation with empty piping. Perform the equilibration step at the same flow and pressure conditions at which the protein will be processed.

Once the piping is filled with buffer and the recycle tank is filled to a level that, at minimum, submerges the retentate return pipe, introduce the protein feedstock into the tank and process according to the determined parameters.

Product Recovery

Product recovery is the process of removing the product from the TFF system into a vessel appropriate for storage or further processing. Devise a procedure to remove the product as completely as possible from the system in order to maximize yield. The bulk of the product, which is typically in the recycle tank, is pumped out using the feed pump. However, some liquid remains held up in the piping and the modules. A well-designed system has minimal deadlegs in the piping and is sloped to a collection port at the lowest point in the piping to improve draining. Beyond simply draining the system, however, one of the following methods can be used to increase product recovery:

- Low-pressure air blowdown
- Buffer displacement
- Buffer flush
- Buffer recirculation

Air Blowdown

Using air pressure to enhance volumetric recovery from a system, introduce the pressurized line at a high point in the piping and collect the product from the lowest point. Take care to avoid bubbling into the product, since this causes

foaming and potential product degradation. In addition, in order to maximize recovery the blowdown should occur gradually, since once an air path through the piping is formed, further blowdown will not increase the volumetric recovery.

Buffer Displacement

Alternatively, use buffer to push out the remaining liquid. If this is done while the piping is still filled with product, it is called a buffer displacement; if it is done after the piping has been emptied, it is called a buffer flush. A buffer displacement can be performed without significantly diluting the final product if the volume of buffer used is only slightly larger than the volume of piping to be cleared. Since the buffer is being used like a plug to push the product through the piping, in theory no buffer should be collected in the product tank until the product has been completely replaced by buffer in the piping. The effectiveness of a displacement step, however, is reduced if a lot of mixing occurs between the buffer displacer and the held up product.

Buffer Flush

During a buffer flush procedure, the buffer rinses the system of residual product. The entire amount of buffer added to the system for the flush is collected along with the product, diluting the final product, so use the minimum amount required for good recovery. This dilution needs to be compensated for during any concentration steps of the process to ensure that the final diluted product concentration meets any specification. Since over-concentrating a product is often impractical because of solubility or minimum volume reasons, a buffer flush is not always feasible.

Buffer Recirculation

A final method for increasing the recovery of product from the membrane modules and piping is a buffer recycle. For this procedure, add buffer to the drained system, recirculate, and then recover it in the same manner that the product was recovered. The buffer dilutes out any residual product in the system. The recirculated buffer is added to the product, so this procedure has the same issues of product dilution as mentioned above. The amount of liquid remaining in different types of filter modules after gravity draining as well as after draining followed by low-pressure air blowdown compared to the volume in the modules when full is shown in table 2. A well-executed buffer flush or recycle will reduce the remaining volumes even further.

Post-Use Cleaning and Testing

After each membrane use and product recovery, clean the TFF assembly using the same cleaning, sanitization, and depyrogenation protocol that was performed before use. In addition, perform integrity and permeability testing. Comparing the post-use cleaned membrane permeability with the original value indicates the effectiveness of the cleaning. The trend of the value with repeated cycles can be used to gauge expected membrane lifetime and to set a limit on maximum number of cycles for one set of membranes.

Storage

If another protein processing run doesn't immediately follow the cleaning, recirculate an appropriate storage solution through the TFF assembly to prevent organism growth. The membrane modules must remain filled with storage solution until the next run to prevent drying of the membranes. At this point, the membranes and holder can be isolated and removed to allow the tank, piping, and instrumentation to be used for other processes.

Module	Liquid Holdup when Full	Liquid Holdup after Gravity Drain	Liquid Holdup after Drain and Blowdown
	[mL m^{-2}]	[mL m^{-2}]	[mL m^{-2}]
Pellicon 2	180	140	10
Helicon	400	20	15

Table 2. *Liquid holdup in UF filter modules*

Item	Sanitary Consideration
Piping, Fittings	Stainless Steel, 316 L or better, 20 Ra ID finish or better
Connections	Tri-Clamp® style
Elastomers	EPDM, silicone
Valves	Diaphragm preferred
Pumps	Circumferential piston displacement, rotary lobe, centrifugal
Instrumentation	Sanitary fluid flow path (materials and design)

Table 3. *Acceptable components and materials for sanitary systems*

System Considerations

To implement a complete TFF process, the piping and equipment associated with the membrane modules must be selected and a method for controlling the process parameters at their setpoints must be chosen.

Equipment Options

In addition to the membrane modules and holder, at minimum a working TFF operation requires a recycle vessel, a feed pump, a retentate control valve, and pressure sensors for the feed and retentate lines. Many systems also include feed and filtrate flow meters, a filtrate pressure sensor, and sensors for temperature, pH, conductivity, or UV absorbance.

Most TFF systems used for protein processing are operated in a sanitary manner. Table 3 lists the primary

sanitary design considerations for different system components.

Finally, consider the process requirements for volume reduction, buffer exchange, and product recovery when choosing equipment design and layout. In a typical ultrafiltration process, the maximum practical VCF is approximately 50 – 100 before the limitation of minimum recirculation volume in a single tank becomes a significant problem, even when more novel tank designs are used. Examples of tank features that can reduce the minimum recirculation volume are a conical bottom, a reduced-diameter lower section, and a low side-entry retentate return port. Likewise, diafiltration of non-retained species is typically limited to a maximum of approximately 14 diavolumes, since beyond this any incomplete mixing or deadlegs in the system will significantly reduce the effectiveness of further buffer exchange or contaminant removal. Postprocessing recovery of a retentate product is enhanced when a system has minimal deadlegs, minimal piping length, and piping that is sloped to a recovery port at the lowest point.

Process Control Options

Throughout a TFF process, as protein is concentrated or exchanged into different buffers, the process parameters need to be adjusted so that they remain at their setpoints. Several methods of process control are used to accomplish this. The tangential flow can be controlled to maintain either

- Constant crossflow rate
- Constant pressure drop

The applied pressure can be controlled to maintain a constant

- Retentate pressure
- TMP
- Flux
- Cwall
- Mixed mode control

Fully automated 80 m² Pellicon system for concentration and diafiltration.

Constant Crossflow Rate

To control the tangential flow based on crossflow rate, install a flow meter on the unit operation downstream of the feed pump and prior to the membrane modules. The benefit of this type of control is that the crossflow rate is known to be constant even if the resistance to flow through the feed channels changes. ***Constant crossflow control is especially useful when processing solutions that experience viscosity changes during processing and to facilitate accurate pump sizing during scale-up.***

Constant Pressure Drop

Alternatively, control the tangential flow by setting a constant feed pressure or pressure drop. A flowmeter is not required for this type of control, only pressure gauges are needed, so the instrumentation is simpler and less expensive. However, pressure drop often changes throughout a process due to changes in solution viscosity or, occasionally, restriction of feed channels by foulants. In addition, variability in membranes and feedstock cause lot to lot pressure drop changes. When choosing a method of tangential flow control, consider the characteristics of the process fluid as well as the precision required to achieve process objectives.

Constant Retentate Pressure

The simplest way to control the applied pressure is to set a constant retentate pressure by adjusting a valve on the retentate line. For unit operations where the tangential flow is controlled based on a crossflow rate, the TMP changes slightly throughout the process. For unit operations where the tangential flow is controlled based on a pressure drop, the TMP remains constant.

Constant TMP

Alternatively, set a constant TMP for crossflow rate controlled operations by changing the retentate pressure setpoint throughout the process as the feed pressure changes. This is slightly more complicated and there is usually no significant benefit.

Constant Flux

Change the retentate pressure throughout a process in response to changes in the filtrate rate to maintain a constant flux setpoint. This type of control is useful for realizing some of the benefits of constant Cwall processing without requiring a fully automated system. A constant flux setpoint can also be achieved through the use of a pump or a control valve placed on the filtrate line, instead of using the retentate control valve. This control scheme is very common on

open UF (>100kD) and MF applications and was described in more detail on page 9.

Constant Cwall

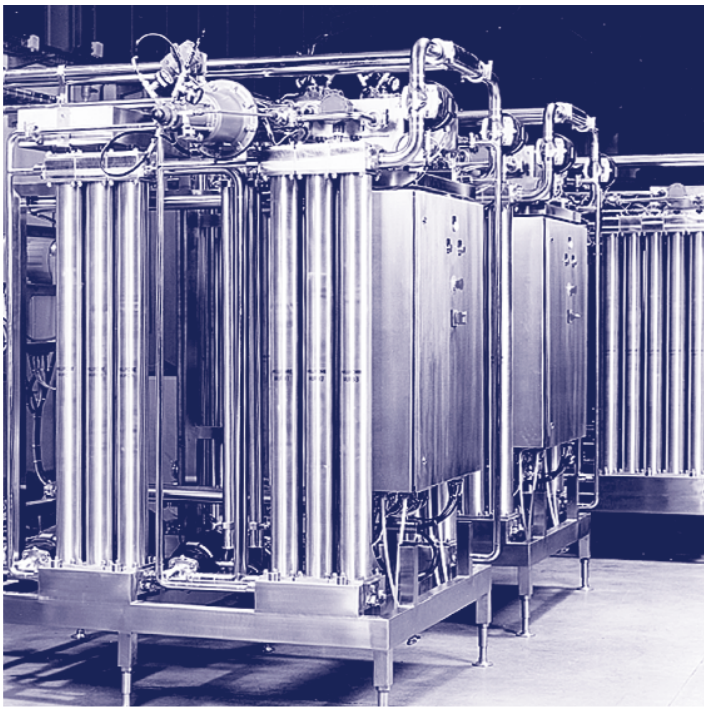
An alternate method of process control, called Cwall process control, maintains a constant protein concentration at the membrane surface throughout a process. The retentate pressure is modified to maintain a flux setpoint that changes according to an algorithm that takes into account both the protein mass transfer coefficient in the specific buffer and the instantaneous protein concentration. One of the benefits of Cwall process control is that it allows operation at the optimum TMP throughout a process even as that TMP changes. Therefore, yield and membrane area are both optimized in the same process. The drawback to this method of process control is that it is more complicated than the other schemes and requires the use of an automated control system.

Mixed Control

To prevent the unit operation from exceeding certain undesirable operating conditions regardless of fluid changes throughout the process, use a mixed mode approach to process control. For example, in addition to a constant filtrate flow setpoint, set a maximum TMP control setpoint that overrides the filtrate control. In many processes, retention changes with TMP, so the overriding TMP setpoint keeps the process from operating at conditions where retention is unacceptable.

Major Process Considerations

As previously noted, it is important to define and prioritize the goals and requirements of a TFF operation during the development phase so that the operating parameters and system options that are chosen will result in a successful process. This section will discuss in more detail the key considerations of product yield, product quality, bioburden control, scalability, robustness, and economics and will define how each is affected by the process design.



Large-scale spiral wound UF/DF system

Product Yield

There are four contributors to product loss during a TFF step:

- Retention losses
- Adsorption losses
- Solubility losses
- Unrecoverable holdup volume losses

In addition, if protein quality is compromised during processing, the yield of usable protein will be reduced. With an optimally designed process, yield loss can be minimized in each of these areas. Table 4 shows the relative magnitudes of product loss that can be attributed to each of the different sources noted above. In addition, the process choices that affect each of the loss mechanisms are listed.

Retention Losses

Choosing a membrane with appropriate retention characteristics is critical to ensuring high product yield. If a product in the retentate is being concentrated or desalted, low retention results in product being lost through the membrane to the filtrate. Even highly retained products can show measurable filtrate loss when they are significantly concentrated or diafiltered. In addition, because of charge effects, retention of a molecule can change if the pH and ionic strength of the solution changes.

Adsorption Losses

Adsorption losses occur when product binds to a membrane and cannot be desorbed in an active form prior to recovery. For applications in which product concentration is high in comparison to the membrane area used to process it, adsorption probably won't be a significant mode of yield loss. However, if product concentrations are very low and/or a very large membrane area is required for processing, this loss mechanism should not be ignored. The membrane material that is chosen will affect how much protein is adsorbed for a given area. In general, hydrophilic membranes will exhibit lower protein binding than membranes that are more hydrophobic. Adsorption losses will also be affected by other components in the feed stream that may interact with both the membrane and the product.

Source of Loss	Retention	Adsorption	Hold-Up	Solubility/Quality
Magnitude of Loss [%]	0.4 – 10	0.02 – 2	0.2 – 10	0.1 – 20
Choices affecting loss	Membrane NMWL Operating parameters Buffer selection	Membrane material System materials	System design Recovery method	Operating parameters System components Protein susceptibility Buffer selection

Table 4. *Typical product yield losses during a TFF process*

Solubility Losses

Solubility losses are a third mechanism of product loss during protein processing. The final bulk concentration of a product may not be beyond its solubility limit. However, polarization at the membrane surface and feed stream volume reduction along the channel result in areas of higher concentration throughout the TFF unit. In addition, inadequate mixing will increase concentration differences. Product concentration in these areas could potentially exceed a solubility limitation. Since higher fluxes lead to higher localized concentrations, reducing flux is one way to minimize solubility losses if they are significant in a particular process. Alternatively, using a process control scheme where the concentration of product at the membrane surface is controlled can maximize flux without exceeding solubility limitations.

Holdup Volume Losses

Finally, unrecoverable holdup volume in a unit operation leads to product loss. After processing, a certain amount of liquid remains in both the filter modules and the system piping. In cases where the final product concentration is high and/or when the final product volume is small, these losses could be significant. Careful design of the piping, optimization of total membrane area, and development of an efficient product recovery step will help to minimize the product loss incurred due to unrecoverable holdup.

Product Quality

During the course of a TFF process, the quality of a protein could be compromised due to aggregation or denaturation caused by

- Micro-cavitation
- Air/liquid interfaces
- High protein concentrations
- Temperature effects

The potential for this damage depends, in part, on the susceptibility of the particular protein being processed. However, even for delicate products, damage can be minimized or eliminated by designing a robust process and system.

Micro-Cavitation

To prevent cavitation, which occurs when a pump pulls a vacuum at its inlet and fluid subsequently degasses, the feed pump should always be run with a minimum inlet pressure equal to the manufacturer's recommendation. However, micro-cavitation will still occur to some extent when the protein feedstock makes multiple passes through the feed pump and/or through a partially closed pressure control valve. The selection of appropriate pumps and valves can help prevent the protein denaturation that microcavitation can cause.

Air/Liquid Interfaces

Other air/liquid interfaces can occur in several places throughout a TFF system. In the recycle tank, the retentate stream should always be returned below the liquid surface to prevent foaming, and vortex formation should be avoided by using an off center drain or baffles in the tank. Finally, filling the system with buffer before introducing protein solution will minimize any air entrainment during startup.

High Protein Concentration

As described in the previous section, there is the potential for highly concentrated areas to exist within the TFF unit. Since protein aggregation is a result of protein-protein interactions that are concentration-dependent, higher concentrations could result in more aggregated protein. The same considerations for process control should be made as mentioned above.

Temperature Effects

Lowering the process temperature at which a TFF process is run is a method often used to attempt to minimize product quality degradation. However, it can exacerbate any solubility problems, since proteins are typically less soluble at lower temperatures. In addition, filtrate flux is reduced at lower temperatures because of the corresponding viscosity increase and mass transfer coefficient decrease. Flux decreases approximately 2 – 3% per degree Celsius of temperature reduction. Therefore, for equal membrane area, process time will be longer at a lower temperature. Since protein degradation can be a kinetic phenomenon, a longer process time may eliminate the benefit of the lower temperature. Alternatively, if the membrane area was increased to compensate for the flux decrease, a higher crossflow rate would be needed, which would expose the protein to more passes through the pump.

Bioburden Control

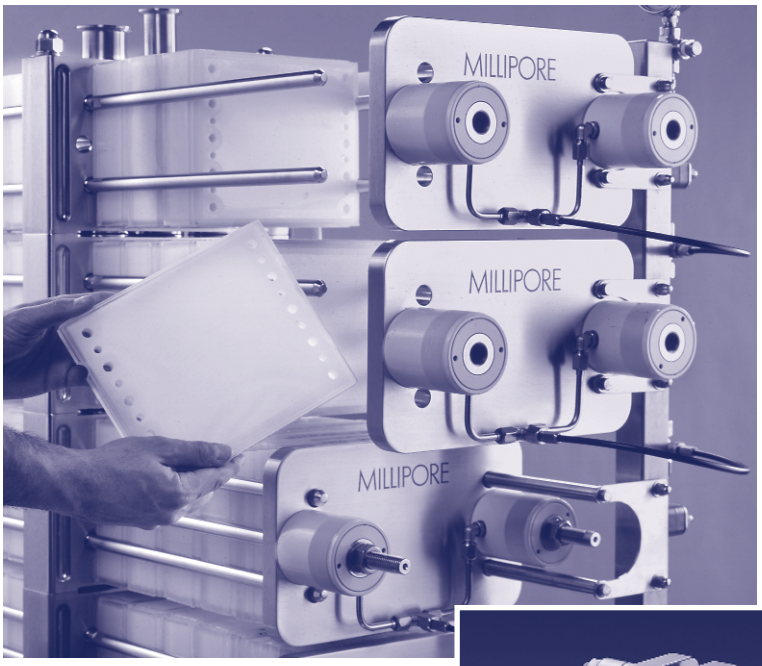
The ability to reliably control bioburden levels in a process stream is very important in protein processing. An outbreak of bioburden in an upstream process step may raise quality concerns but be tolerable, while in a downstream or final process step it may cause failure of the entire batch. While the risk of bioburden contamination is not necessarily greater in a TFF step than in other processing steps, there are ways that the risk can be minimized during TFF processing.

The method used to clean and sanitize a TFF unit operation between uses is obviously important in controlling bioburden (as well as ensuring removal of endotoxin). The chemicals that are chosen must be not only effective for cleaning and sanitization, but also compatible with the membrane and piping materials and able to be rinsed out to acceptably low levels before further processing. Typical chemicals used to clean TFF systems include sodium hydroxide and sodium hypochlorite. The Millipore Maintenance Procedures provide appropriate cleaning concentrations, times, and temperatures for specific membrane systems.

The total cycle time for a TFF process can affect bioburden loads. Since TFF processes are typically run under sanitized but non-sterile conditions, extended processing times increase the potential for higher bioburden loads.

Scaleup and Scaledown

Once a process has been developed at lab bench scale, it must be translated to industrial scale and validated, and this can present unanticipated surprises and challenges. Often, there is little opportunity to perform intermediate-scale runs due to time and material constraints. In addition, material from the initial industrial-scale runs is usually required for time-sensitive clinical or marketed supply. Therefore, accurate and dependable scaleup is critical for the success of a process.



Pellicon 2 Industrial Scale

The simplest way to ensure accurate and predictable translation of product yield and purity from bench to industrial scale is to use linear scale techniques. To linearly scale a TFF process, all fluid dynamic and membrane module parameters must be kept constant between scales of operation. Fluid dynamic parameters, which are set by the user, include the ratio of feed volume to membrane area (at constant feed concentration), feed rate per membrane area, filtrate flux, and retentate and filtrate pressures. Membrane module parameters are inherent in the specific filters that are chosen, and include membrane material and pore size, turbulence promoter, channel height, channel length, and feed and filtrate flow geometries.

Linear scaling is also used to reduce the scale of a process. This can be very useful for process validation, where it is sometimes not economically feasible to perform all of the required validation at full operating scale. Validation performed at small scale will only be acceptable if it can be shown to produce results that are equivalent to industrial scale. In addition, linear scaledown is used to troubleshoot or develop improvements to a process once it has been implemented into a manufacturing line.

Pellicon 2 Mini Holder

Robustness

Once implemented at large scale, a process must be robust if it is to be successful. A robust TFF process will perform well within the lot-to-lot feedstock and membrane variations that it encounters. While developing a process, it can be very useful to test performance at the extremes of these variations, if possible. For instance, determining flux of the most fouling lot of feedstock using membranes having the lowest acceptable permeability

would allow the membrane area to be sized such that the maximum process time was not unacceptable. Alternatively, determining retention of the product in the least fouling lot of feedstock using membranes having the highest acceptable permeability would ensure that the chosen membrane cutoff would not lead to significant product loss. A robust TFF unit operation should also be able to withstand some variations in operation without catastrophic failure. Consideration of membrane and module characteristics such as susceptibility to reverse transmembrane pressure, chemical compatibility, or stability at high pressures help to guide the choice of a unit operation that will operate robustly.

Process Economics

The economics driving the success or failure of TFF process steps are case specific, since different applications have very different economic goals. For some very high value products, the product cost is relatively insensitive to the economics of a single process step, while for other products every cost must be minimized for the product to be competitive. The costs associated with TFF steps break down into four categories – capital, materials, labor, and overhead.

Typical capital costs include membrane holders, plant floor space requirements, utilities, pumps, valves, instrumentation, piping, tanks, and process automation. A unit operation with the smallest acceptable membrane area minimizes the cost associated with the holders and piping. An efficient unit operation will have a high packing density of membranes, requiring minimal plant floor space for a large membrane area. Manually operated systems have lower instrumentation and automation costs associated with them than fully automated systems. Optimizing the diafiltration step and minimizing the number of different buffer or cleaning solutions will minimize tank costs. Finally, a system that processes multiple products will be able to split capital expenses over a larger profit base.

Typical materials costs include membrane modules, buffer and water usage, cleaning chemical usage, power consumption, and product loss. As with the capital costs, consumable costs are minimized if as low a membrane area as acceptable is installed. Choosing membranes that are easy to clean helps to reduce the water and chemicals used after each run. Labor costs are reduced by using an automated unit operation, but this will increase capital expenditures.

The number of times a set of membranes will be used before installing new membranes affects the labor and materials costs. For single use membranes, the membrane expense is high. However, labor, power consumption, and water/chemical costs associated with cleaning are minimized. In addition, the cost for validating the acceptability of reuse is avoided. The labor required for installing a new set of membranes before each run could be high, depending on the membrane area and module type. On the other hand, using membranes for multiple runs lowers the per-run membrane cost at the expense of higher power, water, chemical, and validations costs. However, if a single set of membranes is used for an excessive number of runs, the value gained by not installing a new set of membranes is negated if membrane performance begins to degrade. This approach also greatly increases both validation costs and risk to the product.

Glossary of Terms

C_b: Component concentration in the bulk solution [g L^{-1}]

C_f: Component concentration in the filtrate stream [g L^{-1}]

CF: Protein concentration factor [-]

CR: Conversion ratio [-]

Crossflow: The flow of fluid through the feed channels of the membrane modules created by a pump

C_w: Component concentration at the membrane surface [g L^{-1}]

DF: Diafiltration

DV: Diavolume [-]

Feed: The fluid that flows from the recycle tank into the feed channels of the membrane modules

Filtrate: The fluid that passes through the membranes, also commonly called permeate

HPTFF: High performance tangential flow filtration

J_f: Filtrate flux [L m^{-2} h^{-1}]

J_m: Mass flux [g m^{-2} h^{-1}]

kD: Kilodalton

Mass balance: The amount of the target product in all pools compared to the total amount put into the unit operation [%]

MF: Microfiltration

NFF: Normal flow filtration

NMWL: nominal molecular weight limit

P_F: Feed pressure [bar]

P_f: Filtrate pressure [bar]

Pool: A general term denoting a total volume of fluid

P_R: Retentate pressure [bar]

Q_F: Feed flow rate [L h^{-1}]

Q_f: Filtrate flow rate [L h^{-1}]

Q_R: Retentate flow rate [L h^{-1}]

R_{app}: Apparent or observed retention [-]

Recirculation: The flow of fluid through the channels of the membrane modules created by a pump

Retentate: The fluid that flows out of the feed channels of the membrane modules back into the recycle tank

R_i: Intrinsic retention [-]

RO: Reverse osmosis

S_{app}: Apparent or observed sieving [-]

S_i: Intrinsic sieving [-]

TFF: Tangential flow filtration

TMP: Transmembrane pressure [bar]

UF: Ultrafiltration

VCF: Volume concentration factor [-]

VF: Virus filtration

Yield: The amount of the target product in a pool compared to the total amount put into the unit operation [%]

ΔP: Pressure drop [bar]

Technical References

Cheryan, M. 1986. ***Ultrafiltration Handbook.*** Technomic Publishing Co., Inc., Pennsylvania.

Koros, W.J.; Ma, Y.H.; Shimidzu, T. 1996. Terminology for Membranes and Membrane Processes (IUPAC Recommendations 1996). ***Pure & Appl. Chem.*** **68**: 1479 – 1489.

Ng, P.; Lundblad, J.; Mitra, G. 1976. Optimization of Solute Separation by Diafiltration. ***Separation Science*** **2**: 499 – 502.

Tkacik, G.; Michaels, S. 1991. A Rejection Profile Test for Ultrafiltration Membranes and Devices. ***Bio/Technol.*** **9**: 941 – 946.

van Reis, R.; Gadam, S.; Frautschy, L.N.; Orlando, S.; Goodrich, E.M.; Saksena, S.; Kuriyel, R.; Simpson, C.M.; Pearl, S.; Zydney, A.L. 1997. High Performance Tangential Flow Filtration. ***Biotech. Bioeng.*** **56**: 71 – 82.

van Reis, R.; Goodrich, E.M.; Yson, C.L.; Frautschy, L.N.; Dzegeleski, S.; Lutz, H. 1997. Linear Scale Ultrafiltration. ***Biotechnol. Bioeng.*** **55**: 737 – 746.

van Reis, R.; Goodrich, E.M.; Yson, C.L.; Frautschy, L.N.; Whiteley, R.; Zydney, A.L. 1997. Constant Cwall Ultrafiltration Process Control. ***J. Membrane Sci.*** **130**: 123 – 140.

van Reis, R.; Zydney, A.L. 1999. Protein Ultrafiltration. In M.C. Flikinger, S.W. Drew (eds.) Encyclopedia of ***Bioprocess Technology.*** John Wiley and Sons, Inc. New York.

Zeman, L.J.; Zydney, A.L. 1996. ***Microfiltration and Ultrafiltration: Principles and Applications.*** Marcel Dekker, New York.

For More Information

If you would like to learn more about Tangential Flow Filtration processing, as well as other protein purification operations, Millipore offers a variety of information and assistance.

Training

Millipore offers several classes on TFF processing for customers. These classes blend lecture time with hands on time in the lab to help you become proficient in both the theory and operation of tangential flow filtration. Please visit our website at **www.millipore.com** for information on the variety of training courses available.

Expert Help

Millipore's applications specialists have extensive knowledge of the biotechnology and pharmaceutical fields. The Access℠ Services group works with you to optimize the use of TFF in your facility, and get your process up and running quickly and easily.

Discover the More in Millipore™

In every application, every step and every scale, count on Millipore to be everywhere for you—from monoclonals to vaccines, from clinical through pilot to full-scale manufacturing. Our technologies are used by most of the world's major biopharmaceutical companies. But we deliver more than advanced separation, purification, sterilization and quality control products. With Millipore, you get services to optimize and validate your processes, comprehensive resources to streamline and enhance your operation, unmatched know how forged from nearly 50 years' experience—and solutions that integrate it all. For higher yields, improved process economics and faster speed to market, discover the more in Millipore.

To Place an Order or Receive Technical Assistance

For additional information call your nearest Millipore office:
In the U.S. and Canada, call toll-free **1-800-MILLIPORE (1-800-645-5476)**
In the U.S., Canada and Puerto Rico, fax orders to **1-800-MILLIFX (1-800-645-5439)**
Outside of North America contact your local office. To find the office nearest you visit **www.millipore.com/offices.**
Internet: **www.millipore.com**
Technical Service: **www.millipore.com/techservice**

MILLIPORE

Lit. No. TB032 Rev. C 06/03 03-117