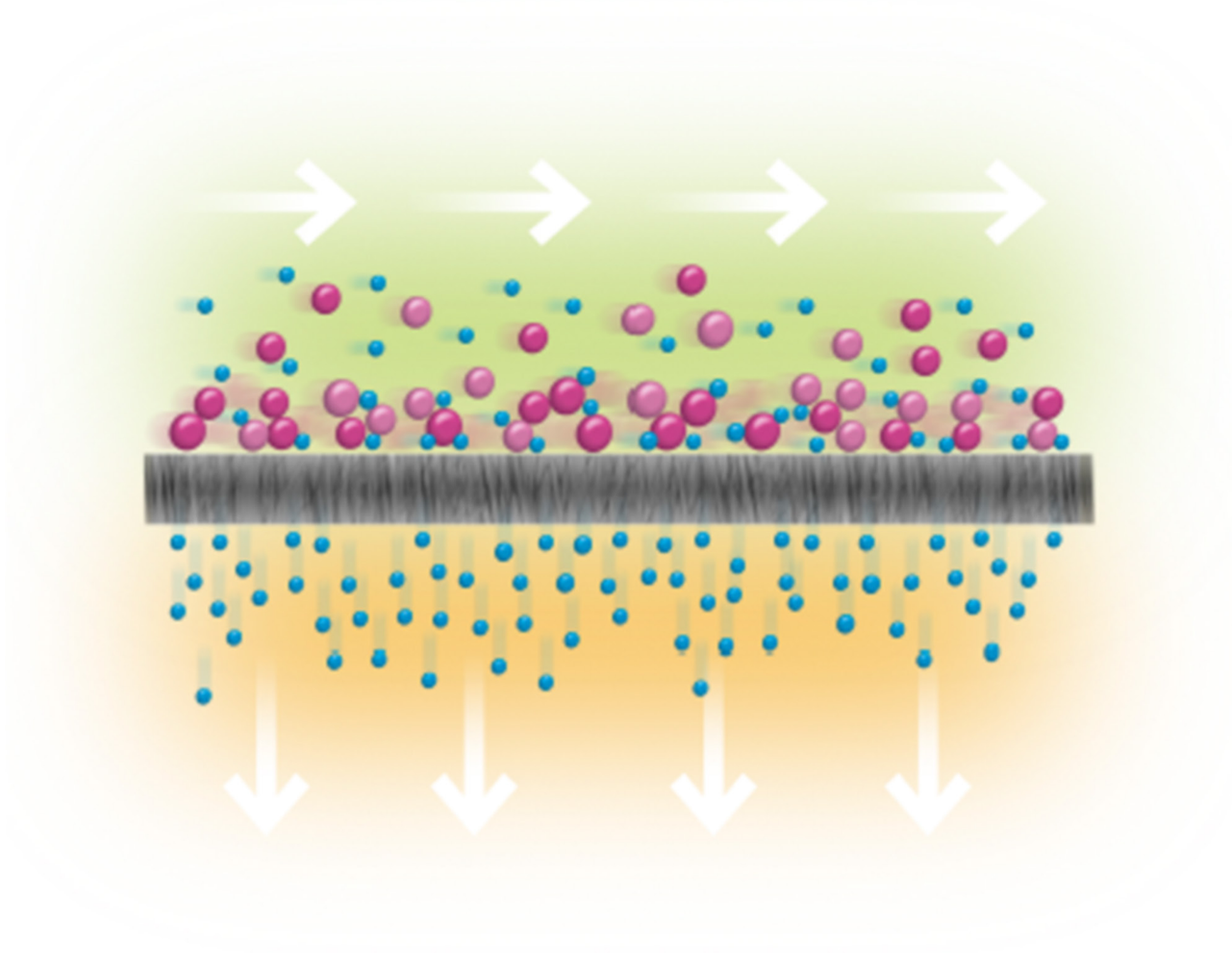
Handbook

Cross flow filtration method



cytiva

Contents

01

Introduction

About this handbook

The Cross flow filtration method handbook gives a general introduction to the principles and applications of cross flow filtration using systems and filters from Cytiva. Detailed instructions for preparing, using, cleaning and storing particular filters are provided with each filter.

About this chapter

This chapter introduces the principles, basic terminology and applications of cross flow filtration.

In this chapter

This chapter contains the following sections:

1.1 What is cross flow filtration?

Cross flow filtration (CFF, also known as **tangential flow filtration** TFF) is a filtration technique in which the starting solution passes tangentially along the surface of the filter. A pressure difference across the filter drives components that are smaller than the pores through the filter. Components larger than the filter pores are retained and pass along the membrane surface, flowing back to the feed reservoir (see Fig 1.1). CFF is simple in concept, but its proper execution requires detailed knowledge and good filtration technique.

Solution that is directed to the membrane surface is called the **feed**. Solution that passes along the membrane surface and back to the feed reservoir is the **retentate**. This solution is usually pumped back to the feed reservoir and recirculated. Solution that passes across the membrane is the **permeate**.

Other terms used in CFF are explained in *Appendix A Abbreviations and glossary,* on page 65 and 66.

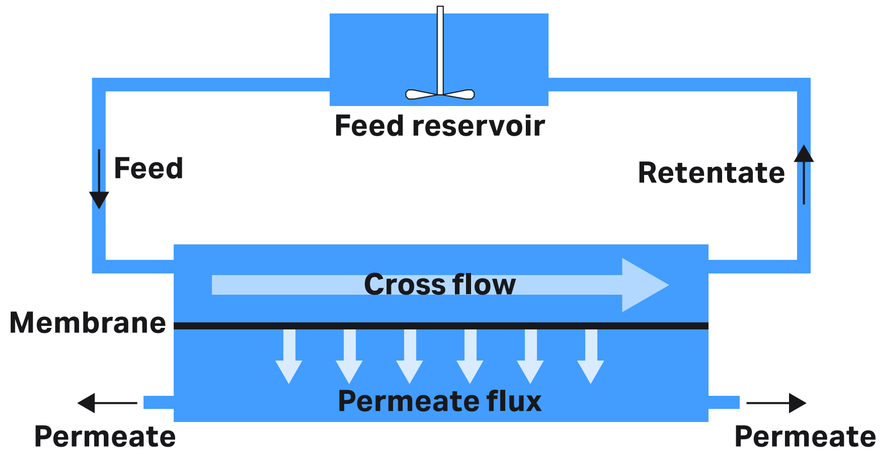


Fig 1.1. Principle of cross flow filtration.

1.2 Key features of CFF

Features

A key feature of CFF is the flow of fluid along the membrane surface that sweeps away the build up of material on the filter surface and reduces fouling of the filter. In addition, retentate solution can easily be recirculated, allowing thorough processing of large volumes of solution.

Differences between CFF and normal flow filtration

CFF differs from normal flow filtration (where the starting material is simply passed through the filters, also known as **dead-end filtration** or **direct flow filtration**) in three main ways:

- CFF filters use membranes exclusively, while conventional filtration may use membranes, paper, or other materials such as glass fiber to separate components in a feed stream
- CFF supports recirculation of the retentate solution. In normal flow filtration, the feed usually passes once through the filter.
- In a CFF system, the retentate remains as a solution and may be recovered directly. Retentate recovery is relatively uncommon in normal flow filtration and requires resuspension of material collected on the filter.

1.3 CFF application areas

CFF is used in research, product development, and production in the biopharmaceutical and medical industries.

Table 1.1 summarizes the characteristics of typical CFF applications.

Table 1.1. Typical uses of cross flow filtration

Application	Comments	Used in
Cell harvesting	Separation of cells from fermentation broth Cells are recovered in the retentate	Upstream processing
Cell or lysate clarification	Separation of target molecules from intact cells, cell debris and molecular aggregates Target molecules are recovered in the permeate	
Product fractionation	Separation of components on the basis of molecular size	Downstream processing
Product concentration	Concentration of product solution prior to further processing by removal of solvent and small molecules Product is recovered in the retentate	Downstream processing Product formulation
Diafiltration	Buffer exchange Product is recovered in the retentate	

Documents describing some examples of CFF applications are available from Cytiva.

02

Cross flow filtration systems

About this chapter

This chapter describes the essential components of CFF systems and introduces systems from Cytiva.

In this chapter

This chapter contains the following sections:

2.1 System configuration

The illustration to the right shows a generalized scheme for the basic configuration of a CFF system.

Pumps and valves

Liquid flow in the system is maintained by one or more pumps in the process lines (only the feed pump is shown in Fig 2.1):

- A **feed pump** maintains the flow of feed into the filter
- A **retentate pump** may be used to maintain and control flow of retentate back into the feed reservoir
- A **permeate pump** may be used to control flow of permeate from the filter. The permeate pump, if used, should not create negative pressure on the permeate side of the filter (i.e., the pump flow rate must be less than the spontaneous permeate flux).
- A **transfer pump** may be used in washing and diafiltration applications to add liquid (usually buffer) to the feed reservoir at a controlled rate

Flow in the various process lines may also be regulated by valves with flow restrictors. Together, the controlled pump rates and valve restriction create the pressure across the membrane that drives the filtration process.

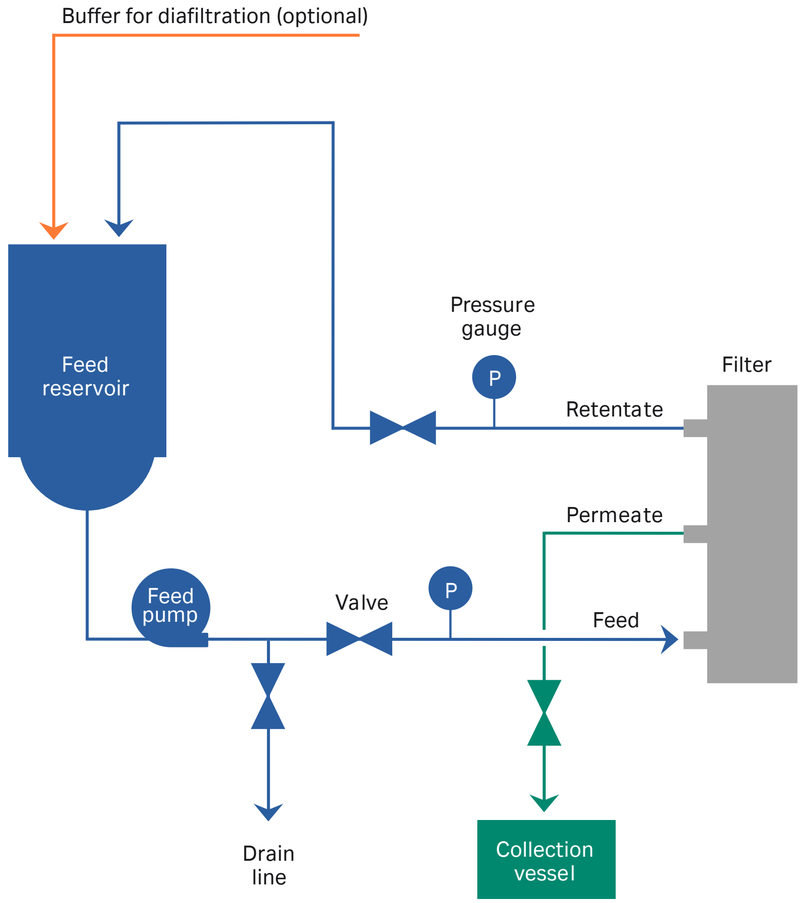


Fig 2.1. Basic configuration of a CFF system.

Pressure sensors

Pressure sensors in the feed and retentate lines are essential for monitoring and controlling the process. A pressure sensor on the permeate side may also be used to monitor permeate pressure.

Flow sensors

Measurement of flow rates for feed solution, retentate and/or permeate and any addition of fluid to the feed reservoir is necessary for monitoring and controlling process conditions during filtration. Flow sensors are placed at strategic points in the system.

Reservoir level sensors

A level sensor in the feed reservoir monitors and controls the amount of liquid in the reservoir.

Air sensors

An air sensor located in the feed stream allows continuous monitoring for air bubbles in the feed prevents the introduction of air into the filter. An air sensor can also be used in the transfer line to detect when the transfer reservoir is empty.

Additional sensors

Temperature, pH, UV absorbance and conductivity sensors may be included in the system according to the requirements of the specific process.

2.2 Filters for cross flow filtration

Filters for CFF may be classified according to filter configuration or filter pore size.

Filter configuration

Two basic filter configurations are generally used (see Fig 2.2):

- In **cartridge filters** (often called **hollow fiber filters**), the membrane forms a set of parallel hollow fibers. The feed stream passes through the lumen of the fibers and the permeate is collected from outside the fibers. Cartridges are characterized in terms of fiber length, lumen diameter and number of fibers, as well as filter pore size. Cartridges from Cytiva have lengths of 30 to 110 cm and lumen diameters of 0.5 to 1.75 mm.
- In **cassette filters**, several flat sheets of membrane are held apart from each other and from the cassette housing by support screens. The feed stream passes into the space between two sheets and permeate is collected from the opposite side of the sheets. Cassettes are characterized in terms of flow path length and channel height, as well as membrane pore size. The channel height is determined by the thickness of the support screen.

Both cartridges and cassettes are constructed from materials chosen for mechanical strength, chemical and physical compatibility, and low levels of extractable and/or toxic compounds.

The total membrane surface area is one of the factors determining how much feed can be handled in a process run. Typical values for the selection of filters by membrane surface area are as follows:

- For microfiltration, 30 to 100 liters of feed per m^2 of membrane surface area
- For ultrafiltration, 100 to 200 liters of feed per m^2 of membrane surface area

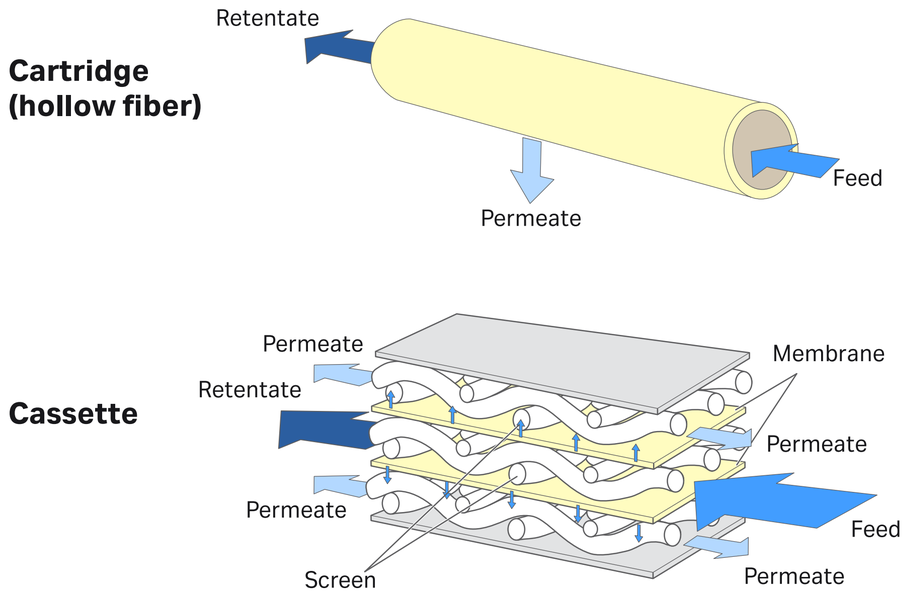


Fig 2.2. Configuration of hollow fiber cartridges and flat sheet cassettes.

Filter pore size

Two classes of filters are distinguished on the basis of filter pore size, which also distinguishes between application areas. In both cases, the pore size is expressed as an average value. The range of actual pore sizes in a given filter determines the selectivity of the filter.

Microfiltration filters

Filters with pore sizes of 0.1 µm and larger are classified as microfilters. In CFF applications the pore size is usually in the range 0.1 to 1 µm. These membranes are used for separation of cultured cells from the growth medium (broth), as well as for removal of particulate material in numerous biopharmaceutical processes.

Ultrafiltration filters

Ultrafiltration membranes have pore sizes in the range 20 to 100 nm, and are generally characterized in terms of the nominal molecular weight cutoff (NMWC), which is the molecular weight of the largest globular protein that can pass through the membrane. NMWC values range from 1 to 100 kD (kiloDalton). These filters are used for concentrating and fractionating protein streams, virus concentration, desalting and buffer exchange. The objective of most ultrafiltration processes is to retain soluble macromolecules such as proteins above a certain size, while allowing smaller molecules such as salts, amino acids, and mono- or disaccharides to pass through the membrane.

Filters from Cytiva

Cytiva supplies cartridges for both microfiltration and ultrafiltration, and cassettes for ultrafiltration. The choice of filter type to use for a given application is made in the first place on the filtration requirements: thus cartridges may be used for all applications, while cassettes are suitable for handling proteins.

See *Section 3.2 Filter selection,* on page 19 for more detailed guidelines concerning filter selection. Additional information is available from Cytiva.

2.3 System volumes and process capacity

Minimum working volume

The minimum working volume of a CFF system represents the amount of feed/retentate fluid required to operate the system at the desired cross flow rate without drawing air into the feed pump. The minimum working volume is determined by the design of the system (feed and retentate tubing volume, reservoir bottom design), the filter hold-up volume, and the cross flow rate. It is important to consider the minimum working volume of a system in the design of a CFF process and in particular to confirm that the final target retentate volume is not less than the system's minimum working volume.

Hold-up volume

The term **hold-up volume** refers to the volume of liquid in the filtration system. For some purposes it is useful to distinguish between the **filter hold-up volume** (the volume in the filter itself) and the **system hold-up volume** (the volume in the system tubing and pumps). The filter and system should be chosen with the smallest hold-up volume that is compatible with other performance requirements in the process.

Process capacity

The system process capacity (the volume of starting material that can be processed in one run) should be chosen in relation to the planned volume of starting material. Process capacity is partly a function of system size and design, but also varies according to the tendency of starting material to foul the filter. Using a high capacity system for a small sample volume will lead to unnecessary loss of material in the system dead volume. For processes that will be scaled up for production, it will be necessary to switch between different systems one or more times during process development (see Table 2.1). CFF processes using systems from Cytiva are scalable, making transitions between systems straightforward.

2.4 Systems from Cytiva

CFF systems from Cytiva cover a full range of filtration capacity from laboratory research to production, and offer different levels of monitoring and control from manual to fully automated systems. Customized systems can also be provided.

Table 2.1 summarizes the characteristics of the available systems. Additional information including system application matrix and selection guides is available from Cytiva.

Table 2.1. Cross flow filtration systems from Cytiva

System	Scale	Applications
ÄKTACrossflow™	Laboratory	Research
		Process development
ÄKTA™ flux	Laboratory to pilot	Research
		Process development
		Small-scale production
UniFlux™	Pilot to production	Manufacturing

03

Process design and operation

About this chapter

This chapter describes design and operation of a CFF process.

In this chapter

This chapter contains the following sections:

3.1 Process considerations

Several factors influence the design of a process. Among the foremost are properties of the starting material (feed) and product, and the total time required for the process. Other factors such as product yield and concentration may also be critical. Sometimes, factors may be mutually exclusive and a compromise must be reached: for example, it may not be possible to combine maximum yield with maximum product concentration.

Feed and product properties

The table to the right lists some of the main properties of feed and product that must be considered.

Time requirements

The time requirements for completing a CFF run will vary widely according to the application and requirements and system characteristics, and may be a crucial factor for the economics of the process as well as the stability of the product. As a general indication, the total time required for a typical run ranges typically from 3 to 8 hours, including system preparation and clean-up.

- Preparing the filter and system for processing (up to 2 h)
- Conducting the filtration process (dependent on application)
- Cleaning and flushing the system and filter for storage (up to 2 h)

Other considerations

Other important factors in designing a CFF process include:

- Product yield — how important is it to maximize yield of the product?
- Product concentration — how important is it to obtain product at a high concentration?
- Selectivity — how important is the purity of the product?
- Long-term filter stability — what is the working life of the filter under process conditions?
- Downstream processing — how will the product be used in the next processing step?

Property	Significance
Feed composition	The composition of the feed affects the tendency for filter fouling (see *Filter pore size,* on page 13), which in turn influences the processing capacity of the system
Feed and product volume	Make sure that the chosen process design can handle the initial feed volume as well as the target volume
Temperature sensitivity	Increased viscosity at lower temperatures may limit the process flow conditions
Sensitivity to shear stress	Shear stress may be considerable at high flow rates, and sensitivity to shear stress may limit the usable flow rates
Solubility	Bear in mind that concentrations close to the membrane may be significantly higher than the feed or target concentrations (see *Concentration gradient and gel layer,* on page 32*)*
Viscosity	Make sure that the process design can handle the viscosity of the feed, retentate and permeate streams, as well as any increase in viscosity resulting from locally increased concentration of material at the membrane surface

3.2 Filter selection

The choice of filter between microfiltration and ultrafiltration and between cartridges and cassettes is governed by the nature of the application.

Application	Separation principle	Technique and filter
Cell harvesting	Cells are separated from soluble molecules	Microfiltration (cartridge)
Cell or lysate clarification	Cells and cell debris are separated from soluble molecules	
Protein fractionation	Macromolecules are separated on the basis of size	Ultrafiltration (cartridge or cassette)
Concentration and diafiltration	Macromolecules are separated from low molecular weight buffer components	

Within that framework, filters are chosen from consideration of selectivity, filter pore size and protein binding.

Filter selectivity

Filter selectivity describes the ability of a filter to separate particles or molecular species on the basis of size. Filters with a narrow pore size distribution will be highly selective, while a broader pore size distribution will give a less selective filter.

Harvesting and cell clarification applications involve separation of relatively large particles (cells and/or cell debris) from macromolecules, so that high selectivity is generally not required. Lysate clarification on the other hand may make more stringent demands, since the lysate will contain a wide range of proteins and other macromolecules. The most important factor is that the target protein can pass freely through the filter so that yields are not compromised.

Filter selectivity is also not critical for diafiltration, where a macromolecular product is separated from buffer components. The controlling factor here is that the target protein is completely retained by the filter, so that product is not lost in the permeate. An ultrafiltration filter with a nominal molecular weight cut-off (NMWC) that is 3× to 5× less than the molecular weight of the target molecule is generally recommended.

Filter pore size

All filters will tend to become fouled (blocked by particulate material accumulating in the filter pores), especially during applications such as lysate clarification involving particulate starting material. Fouling will shorten the time for which a filter can be used before it must be cleaned, and therefore restrict the maximum processing capacity per run. The choice of filter pore size is important for minimizing filter fouling. In general, filters with smaller pores will show less tendency to fouling, because particulate material cannot penetrate and block the pores.

Use the guidelines in the table below for selecting filters for harvesting and clarification applications:

Application	Filter pore size
Mammalian cell harvesting	0.2 to 0.65 µm
Yeast and bacterial cell harvesting	0.1 µm
Cell and lysate clarification	NMWC about 10× target molecule size

Protein binding

The level of protein binding depends upon the material in the filter and the protein characteristics, and increases with increasing hydrophobicity. Protein binding is seen as a reduction in yield that is not accounted for by product remaining in the retentate. Normally, protein binding remains insignificant at the laboratory scale, but for low NMWC ultrafiltration membranes it can be an indicator of a propensity towards filter fouling.

For both clarification and diafiltration applications, protein binding to the filter usually becomes an issue when attempting to process small amounts of protein. In such cases it is important to select a filter with low binding tendency for the target protein, and to choose buffer conditions that minimize binding.

3.3 Filter preparation

Preparation of filters for a process involves rinsing to remove storage solution and conditioning the filter with process buffer. For some processes the filter may need to be sanitized and depyrogenated before use. This section gives a brief overview of preparation procedures. More details are provided in the instructions accompanying each filter.

Sanitization and depyrogenation

Follow the steps below to sanitize and depyrogenate the filter if necessary.

Step	Action
1	Clean and rinse the filter thoroughly
2	Recirculate a solution of 0.1 to 0.5 M NaOH (pH 13) for 30 to 60 minutes at 30°C to 50°C
3	Drain the system thoroughly
4	Rinse the filter with clean water for 30 minutes

Rinsing

New ultrafiltration filters are supplied filled with a glycerol solution which must be removed from the filter before use. Soaking the filter in 25% ethanol or isopropyl alcohol for 1 hour before rinsing will enhance glycerol removal. Complete removal of glycerol is important for filters that will be autoclaved or sterilized by steam-in-place procedures.

Rinsing is also recommended for used filters to remove storage solution. Rinsing steps are outlined below. Detailed instructions are provided with the filters.

Step	Action
1	Fill the feed reservoir with deionized water or water for injection (WFI). Use room temperature or temperatures up to 50°C. Cold water will be less effective. Addition of 100 ppm sodium hypochlorite (NaOCl) to the rinse water will enhance glycerol removal.
2	Start the pump at a low speed and adjust the TMP to • 1 bar (15 psi) for 1000 to 3000 NMWC filters • 0.7 bar (10 psi) for 5000 to 50 000 NMWC filters • 0.3 bar (4 psi) for larger pore sizes
3	To reduce water consumption, adjust the pump speed and retentate back pressure so that the retentate flow is approximately one tenth of the permeate flow
4	Rinse with 50 L water per m^2 membrane surface area, adding more water as required
5	If NaOCl is used, rinse the filter thoroughly before introducing the process solution

Conditioning

Before processing samples, it is recommended to precondition the system with a buffer similar in pH and ionic strength to that of the sample to avoid denaturation and precipitation of proteins. Conditioning the system also helps to remove trapped air.

Conditioning steps are outlined below. Detailed instructions are provided with the filters.

Step	Action
1	Circulate buffer through the system with approximately 0.3 to 1 bar (4 to 15 psi) retentate pressure. Run until no bubbles appear in the permeate stream.
2	To ensure removal of trapped air, increase the retentate flow rate and run for several minutes until no bubbles appear in the retentate stream
3	Circulate the buffer through the retentate and permeate at a feed pressure of 1.6 to 2.8 bar (25 to 40 psi) for four minutes to condition the system for pH and ionic stability
4	Remove the buffer from the feed reservoir. Keep buffer in other parts of the system to prevent air from entering the system.

3.4 Operating parameters

A CFF process may be controlled by an interplay of several operating parameters according to the specific process requirements. The most important parameters are:

- Pressure at various points in the system
- Flow rate at various points in the system
- Process time

Pressure and flow rates

Liquid pressure and flow rates are essential factors for controlling and monitoring a CFF process.

Pressure

Pressure may be monitored in the feed stream, the retentate stream or the permeate stream. Two differential pressure measurements are generally used, ΔP and transmembrane pressure (TMP).

- ΔP is the difference in pressure between the feed and retentate streams, and can be used to control cross flow

  ΔP = Feed pressure – Retentate pressure

- TMP represents the driving force for transfer of material across the filter, and is calculated as shown below.

  TMP can be used to control flux.

$$\text{TMP} = \frac{\text{Feed pressure} + \text{Retentate pressure}}{2} - \text{Permeate pressure}$$

Flow rates

Flow rates may be monitored at various points. The sum of the flow rates out of the filter on the retentate and permeate sides is equal to the flow rate of feed into the filter. The retentate flow rate is also known as the **cross flow rate** or **recirculation rate**. The permeate flow rate (the rate of liquid flow through the filter membrane) is known as the **flux**.

Flux is commonly expressed in units of liters per m^2 of membrane per hour (LMH). This value is scalable, meaning that it can be kept constant when the process is scaled up.

For hollow fiber filters, the cross flow rate through the fiber lumen is often expressed as the **shear rate**[1] in units of s^{-1}, which is a function of the flow rate per fiber and the diameter of the fiber lumen.

Shear rate is calculated as:

$$y = 4q/\pi R^3$$

Equation (1)

Variable	Description
y	shear rate (s^{-1})
q	volume flow through the fiber lumen, cm^3/s per fiber
R	fiber radius (cm)

Expressing cross flow rate as shear rate makes it possible to scale up or down between cartridges. By using a shear reference chart, it is possible to approximate the flow rate that will yield the same shear rate at the new scale.

Shear rates are not commonly used for cassettes since calculation is complicated by the influence of the support screen. Calculation of shear rates for cassettes is beyond the scope of this handbook.

[1] Do not confuse shear rate with shear stress. Shear stress is the force that can disrupt cells or denature proteins under conditions of rapid flow. Shear rate is a liquid flow rate that influences but is not equivalent to shear stress.

Process control modes

CFF systems from Cytiva that run under UNICORN™ software support the process control modes commonly used in microfiltration and ultrafiltration/diafiltration applications, such as TMP control and flux control. These control modes can be combined with selectable feed pump instructions such as feed flow rate, feed pressure, ΔP, retentate flow rate or shear rate.

TMP control mode

In this mode, the TMP is mainly controlled by the retentate control valve, adjusting the valve to maintain a constant TMP. The flux is allowed to vary, usually within specified limits.

TMP control is usually used in ultrafiltration where the system forces retentate through the relatively small pores of the membrane. The TMP control mode is mostly used at constant feed flow, constant retentate flow or constant ΔP.

Flux control mode

In this mode, the flux is maintained at a controlled rate by regulating feed or retentate and permeate flow. The TMP is allowed to vary if necessary, usually within specified limits.

Flux control is usually used in microfiltration where the system limits the permeate flow through the relatively large pores of the membrane. The control mode is used at constant feed flow, constant retentate flow, constant shear rate or constant ΔP. In this mode the TMP value is a function of the permeate flux.

During flux control it is common that the feed pressure is so low that the permeate pump creates a negative pressure in the permeate stream. An automated system should include control procedures to handle this situation, for example by temporarily reducing the retentate flow to increase the retentate and permeate pressure.

Process time

Process time from start to finish (including system preparation and clean-up) may be of crucial importance for the economics of a process, or for the quality of a product that displays limited stability (for example, cell lysates often contain enzymes that may degrade the product, and it is important to work fast in the early separation stages). Practical considerations, such as being able to complete the process in one working day, may also be relevant.

Process time requirements may influence the choice of control mode as well as the choice of system and filter.

3.5 Recovering product

Product is recovered either from the retentate or the permeate according to the type of application. Recovery in the permeate can be maximized at the expense of product concentration by washing the retentate with buffer as a final step in the filtration. Recovery in the retentate is affected by accumulation of material (including product) in a high concentration layer on the membrane surface and by liquid remaining on wetted surfaces of tubing and reservoirs.

Recovering product from the membrane surface

Product may be recovered from the membrane surface without adding buffer or permeate to the system. This enables the most highly concentrated product to be obtained at the expense of some yield loss.

Typically, product recovery from the membrane surface involves the following steps:

Step	Action
1	At the end of the process of harvesting cells or concentrating a protein, close the permeate valve or reduce the feed pressure to 0.3 bar (5 psi)
2	Reduce the cross flow rate to 1/10 of the recommended processing cross flow rate and reduce the mixer speed
3	Circulate the feed for 15 minutes. This will help to recover product that has accumulated on the surface of the membrane.
4	Pump the product to the collection vessel

Flushing out product with buffer

Flushing product out of the filtration system with buffer enables the highest yield to be obtained at the expense of concentration. In this technique, the product is first collected from the system, then a small volume of buffer or permeate is added to the system to flush out the residual product from the feed retentate loop.

This approach can be combined with recovery of product from the membrane surface. Typically, product flushing involves the following steps:

Step	Action
1	As the CFF process nears completion, decrease the pump speed and mixer speed to minimize flow rate, vortexing in the feed tank, and the possibility for product foaming
2	When the slightly over concentrated volume is reached, pump the concentrated product to the collection vessel
3	Add an appropriate volume of buffer to the reservoir. The buffer should be circulated for two to three minutes with the permeate valve closed to help bring the residual product into suspension.
4	Pump the buffer solution from the system into the collection vessel

3.6 Cleaning and testing filters

Filter life cycle

Many laboratory filters are designed for single use, but cleaning and re-use of filters is an important economic consideration at process development, pilot and production scales. Figure 3.1 illustrates the typical life cycle of a CFF filter.

Typically the performance of the filter is checked both before and after use by measuring the rate of water flow through the membrane under controlled conditions. The filter should be replaced when the water flux drops to unacceptable levels. Additionally, air diffusion and/or bubble point tests may be included to ensure filter integrity.

Water flux test

The water flux test measures the flow rate of water through the membrane under controlled conditions. The flow rate provides an indication of the performance capability of the membrane. By tracking the water flux measurements over time, it is possible to determine when a filter reaches the end of its service life. Water flux will normally drop by up to 20% of its starting value the first use and cleaning cycle. The performance level should remain stable from that time forward. Water flux testing is usually carried out when the filter is new and after each use or cleaning cycle.

Details of how to perform and evaluate a water flux test are provided with filters supplied by Cytiva.

Air diffusion and bubble point tests

Air diffusion and bubble point tests are supported in some CFF systems from Cytiva to check filter integrity. Details of these tests are provided in the respective system documentation.

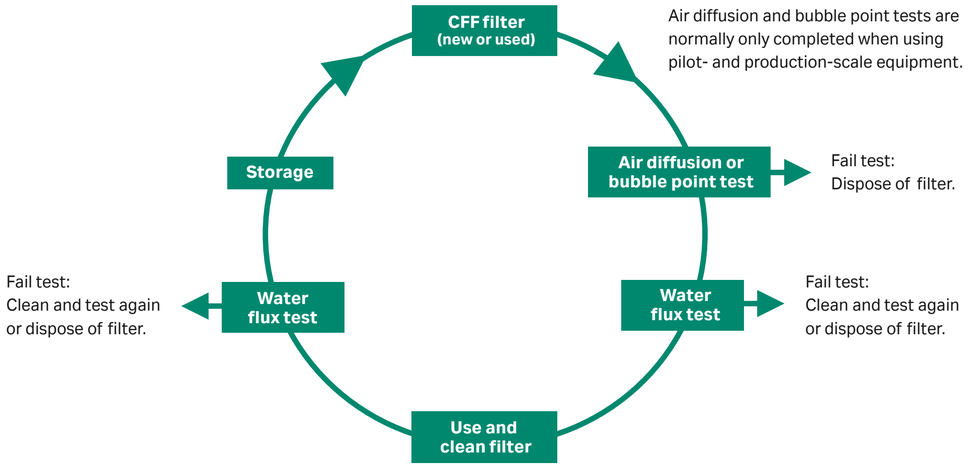


Fig 3.1. Operational cycle of membrane filters.

Cleaning procedures

Recommended cleaning agents for CFF filters are listed in Table 3.1. More detailed information is provided with the filters.

Table 3.1. Recommended cleaning agents for Kvick cassettes

Cleaning agent	Conditions:
1.5% Alconox™ detergent	Contact time 60 minutes Temperature 40°C (104°F)
0.1 to 0.5 M NaOH	Contact time 60 minutes Temperature 40°C (104°F)
200 to 300 ppm sodium hypochlorite in 0.1 to 0.5 M NaOH	Contact time 60 minutes Temperature 20°C (68°F)

Troubleshooting filter performance

The table below lists possible causes and corrective actions for problems when flux deteriorates.

Table 3.2. Troubleshooting cross flow filtration processes

Symptom	Possible cause	Corrective action
Slow gradual decrease of flux during operation to about 90% of starting flux at steady state operation	Normal operation	None
Moderate decrease of flux during operation to about 75% or less of starting flux at steady state operation (acceptable decrease may be greater in concentration applications)	Best pore size for application not being used	Re-evaluate pore size and membrane area selection
	Insufficient cross flow rate, ex-cessive gel layer formation	Increase cross flow rate
	TMP too high, excessive gel layer formation	Lower TMP, reduce permeate flow rate using permeate flow control
	Chemical incompatibility between process fluids and membrane, membrane damaged	Check chemical compatibility between membrane and process fluids
		Replace cartridge or cassette
Water flux after cleaning is less than 60% to 80% of initial water flux	Insufficient cleaning	Increase cleaning temperature
		Increase concentration of cleaning solution
		Increase cleaning circulation time or rate
		Use a cleaning solution with better solubilization properties
	Chemical incompatibility between cleaning agents and membrane, membrane damaged	Check chemical compatibility between membrane and cleaning agent
		Replace cartridge or cassette
Water flux less than 60% of water flux when the cartridge was new; data shows a gradual decrease over many runs	Normal decline in operational efficiency	Replace cartridge or cassette
Water flux less than 60% of water flux when the cartridge was new; data shows decrease was sudden	Chemical incompatibility between cleaning agents and/or process fluids and membrane, membrane damaged	Check chemical compatibility between membrane and cleaning agents and/or process fluids
		Replace cartridge or cassette
	Insufficient cleaning	Increase cleaning temperature
		Increase concentration of cleaning solution
		Increase cleaning circulation time or rate
		Use a cleaning solution with better solubilization properties

3.7 Scaling up processes

The ability to scale a process from the laboratory to manufacturing is a key factor in process development. Normally, the scale-up sequence is completed in multiple steps: lab scale to pilot scale, and pilot scale to production scale. Reasonable scale-up increments are typically 5 to 20 times.

Scaling up a process involves increasing the filter area in order to handle larger volumes of starting material without significantly changing process conditions. The following parameters should be kept constant where possible:

- Ratio of filter area to feed volume
- Fiber or cassette path length
- Channel height (cassettes) or lumen size (hollow fiber cartridges)
- Membrane characteristics (pore size, selectivity, materials)
- Cross flow rate per unit filter area
- TMP
- Temperature
- Feed concentration
- Process steps and sequence

04

Optimizing CFF processes

About this chapter

This chapter considers optimization of CFF processes.

In this chapter

This chapter contains the following sections:

Process parameters may be optimized with respect to several factors, such as capacity, total process time, product yield and purity and so on.

4.1 Optimizing process parameters

Recommendations in this section apply mainly to concentration and diafiltration applications. While similar principles apply to process optimization for other applications, details will differ depending on the starting material and on whether the product is recovered in the permeate or retentate.

Concentration gradient and gel layer

One factor that influences the optimization of process parameters is the tendency to form a concentration gradient of material (and in extreme cases a compact gel-like layer) on the surface of the filter membrane.

During filtration, molecules and particles that do not pass through the membrane accumulate to some extent at the membrane surface, forming a concentration gradient (Fig 4.1A). The concentration gradient layer reduces the flux compared to water or buffer flux. Turbulent liquid flow across the membrane surface helps to wash the concentrated material back into the retentate, reducing but not eliminating the accumulation effect. Decreasing TMP can lower the concentration gradient layer and its effects on flux. Increasing the cross flow rate helps to redistribute concentrated solutes back into the bulk feed stream and maintain flux.

As the concentration gradient becomes more pronounced, the highest concentration of material at the surface of the membrane will tend to form a gel-like layer, markedly impeding permeate flow (Fig 4.1B). Formation of a gel layer is seen as a reduction in the rate of increase of flux as the TMP is increased. The gel layer also has a considerable effect on the filtration process, influencing both filter efficiency and selectivity. To control the filtration process, steps must be taken to minimize the formation of a gel layer.

The following operating conditions reduce the risk of gel layer formation:

- Low TMP
- High cross flow rate
- Low feed concentration

Optimization of a CFF process should include determination of the combination of TMP and cross flow rate that gives the highest flux rate without forming a gel layer.

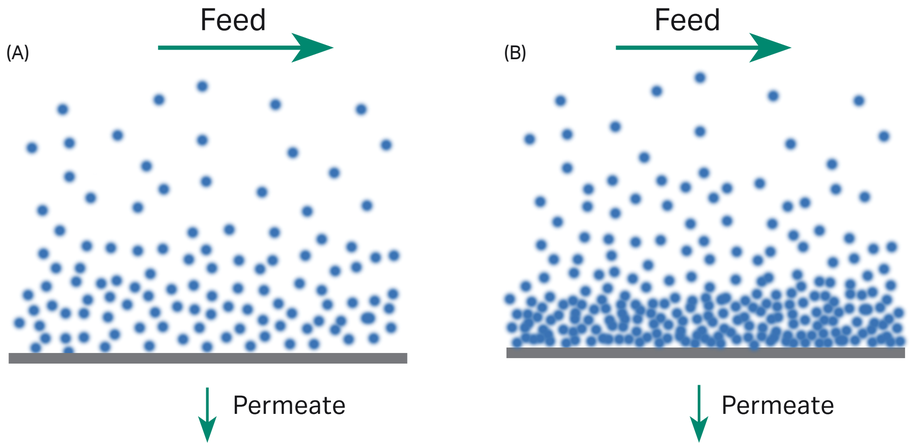


Fig 4.1. During processing, a gradient of increasing concentration of solutes forms between the bulk feed flow and the membrane surface (A). If the permeate flow is too high in relation to the cross flow, the concentration at the membrane surface may become high enough to form a gel-like layer (B).

Flux versus TMP

In CFF, the key optimization parameter is the flux rate as a function of TMP. For a given cross flow rate, TMP controls flux at the beginning of a run. If a gel layer forms, increases in TMP will not result in increases in flux and will provide little if any performance gain. The optimal TMP range for efficient and economic operation is just before the gel layer starts influencing the flux (Fig 4.2).

TMP and cross flow

At a given TMP, increasing the cross flow rate helps to reduce the concentration gradient layer and increase flux. Cross flow rates may be increased until process yield, product quality or process economics are adversely affected through, for example, shear stress effects. Optimization of a CFF process such as protein concentration must include an examination of the interaction of the two most important variables: cross flow and TMP. At the optimum combination of the highest cross flow and TMP, just before gel layer accumulation, the highest flux rate will be achieved (Fig 4.3).

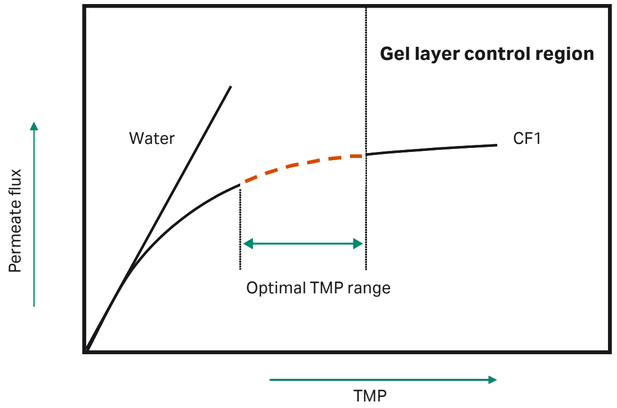


Fig 4.2. Optimal TMP range under a constant cross flow rate.

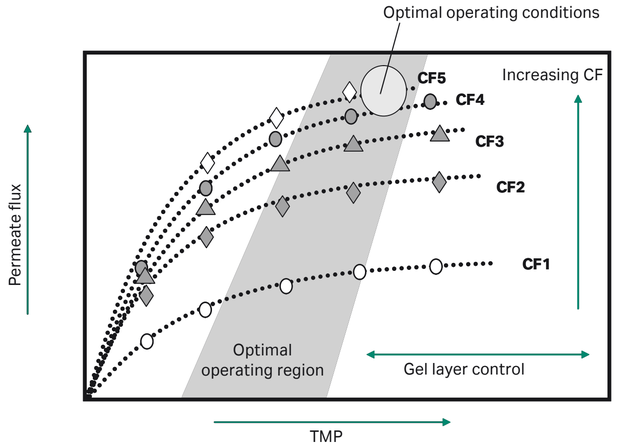


Fig 4.3. Relationship between flux and TMP at different cross flow rates.

TMP scouting

TMP scouting (sometimes known as **TMP excursions**) is an important part of process optimization. Increasing TMP when ultrafiltering pure water results in a proportional increase in flux. With a process fluid that contains solutes the rate of increase in flux drops as the TMP increases and the concentration gradient restricts passage of liquid through the filter. At high TMP values, formation of a gel layer effectively blocks the filter and no further increase in flux is seen. Higher cross flow rates help to prevent the formation of a gel layer, allowing higher flux rates to be achieved before the flux becomes independent of TMP.

TMP scouting involves measuring the interdependence of cross flow rate, TMP and flux in order to determine the optimum conditions for filtration, where flux is high but is still dependent on TMP. The standard procedure is to perform a TMP scouting experiment in which a series of TMP setpoints is measured at different cross flow rates. From these experiments the effect on flux is evaluated, and optimal cross flow and TMP may be identified.

As an example, Figure 4.4 shows the results of TMP scouting for a solution of BSA at a concentration of 30 g/L and 150 g/L (representing the initial and target concentrations respectively). Flux is measured at 6 TMP points and 3 cross flow rates with permeate recycled to the feed reservoir to maintain a steady state.

At the low protein concentration, flux increases with TMP at all cross flow rates and there is no clear optimal setting. At the high protein concentration, however, the curves flatten out at high TMP values, indicating that the formation of a concentration gradient is beginning to restrict flux across the membrane. With this information, it is possible to design a process control scheme that maintains a high flux value for a reasonable process time and stable process conditions.

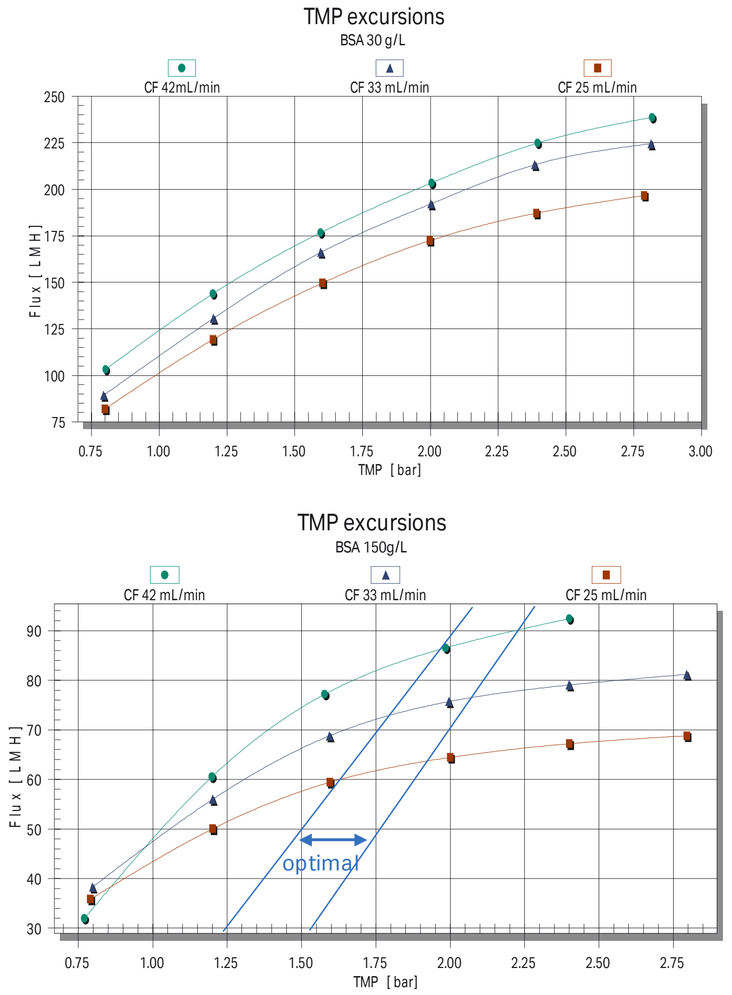


Fig 4.4. TMP scouting results at two concentrations of BSA (top 30 g/L, bottom 150 g/L). Note the difference in flux rate scale at the different protein concentrations.

Process time

In a concentration process, the optimized cross flow and TMP conditions established above can be used to identify the diafiltration point (the point which provides the fastest buffer exchange), and optimal buffer consumption. A typical result file for diafiltration time optimization is shown in Figure 4.5.

Plotting flux*concentration factor against concentration factor enables the optimization of diafiltration time (Fig 4.6). The highest value on the y axis at the highest concentration represents the fastest diafiltration with the lowest buffer consumption. In this example, diafiltration takes the same time if performed at four times or five times concentration, because the decrease in retentate volume at five times concentration is offset by the decrease in flux.

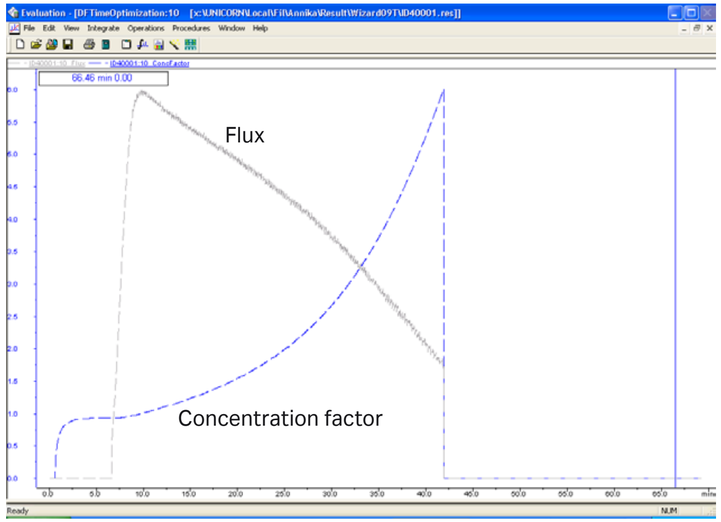


Fig 4.5. Result file for diafiltration time optimization.

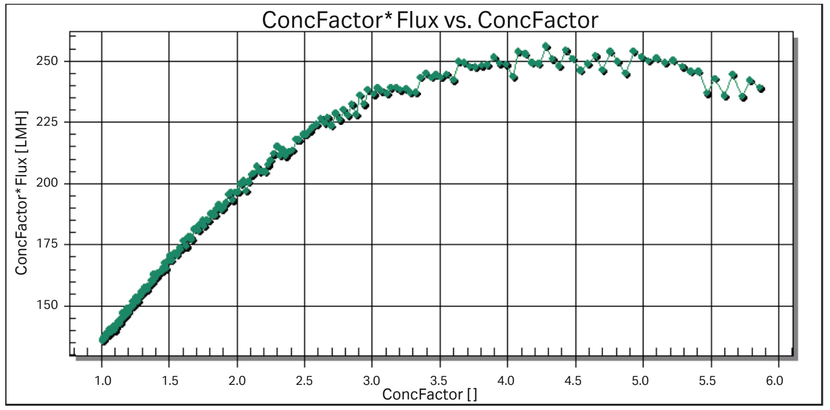


Fig 4.6. Diafiltration time optimization.

4.2 Optimizing yield

Optimizing a laboratory filtration process for product yield ensures efficient use of lab resources as well as accurate projections for scaling to pilot equipment. In production contexts, optimizing yield can have a major impact on process economics.

In addition to filter selection, the key factors that influence product yield are:

- Non-recoverable product
- Product losses through denaturation and degradation

Non-recoverable product

The system design can affect product yield if it impedes recovery of the product. Poorly designed systems include long tubing runs, unnecessarily wide tubing, poor tank drainage, and other non-recoverable volume such as poorly positioned drain valves. Even in well-designed systems, some retentate product remains on the wetted surfaces of the tubing and reservoir unless the system is flushed with buffer. The choice of recovery option depends on the relative importance of total yield and final product concentration:

- Draining the system without flushing leaves some process fluid in the system but recovers product at the highest concentration
- Flushing the retentate side of the system maximizes product recovery at the expense of concentration

Denaturation and degradation

Loss of product through denaturation and/or degradation may occur as a result of excessive shear stress, unsuitable temperature or enzymatic action during the filtration and recovery process.

Shear stress

The sensitivity of biomolecules to shear stress generally increases with molecular size. Most proteins are relatively resistant to denaturation through shear stress. If the shear sensitivity of the product is not known, a quick feasibility study may be performed by circulating the product through the feed-retentate path and monitoring the bioactivity as a function of process time. Where feasible, low pressures and low pump speeds should be used to minimize shear stresses in the flow path.

Temperature

For heat sensitive proteins the process solution temperature can be modified in a number of ways during processing to optimize product yield:

- Precondition the system with cooled buffer before starting
- Lower the feed temperature before beginning the filtration process
- Use chilled buffer during diafiltration
- Use low pressure and low pump speed to reduce heat generation in the flow path
- Use a low ratio of feed volume to filter surface area to shorten process time
- Place the system in a cold room
- Use a heat exchanger or tank jacket

Enzymatic action

Proteolytic enzymes released during cell culture or lysis may follow the target protein through the filtration process and cause degradation and loss of yield. The effect may be more pronounced in ultrafiltration or diafiltration applications, where both enzymes and target proteins may be concentrated together in the concentration layer at the filter surface or in the retentate.

Enzymatic activity may be reduced by:

- Including enzyme inhibitors in the sample
- Using a low ratio of feed volume to filter surface area to shorten process time
- Lowering the temperature of the process
- Adjusting buffer conditions (ionic strength, pH, metal ions, etc.) to minimize enzyme activity

05
Cell harvesting

About this chapter

This chapter considers the use of CFF for harvesting cultured cells.

In this chapter

This chapter contains the following sections:

5.1 Introduction

Cell harvesting is the process of separating cells from the fermentation broth in which the cells are grown. Hollow fiber microfiltration or higher NMWC ultrafiltration cartridges may be used effectively for cell harvesting. The harvested cells are recovered in the retentate.

Successful cell harvesting relies on knowledge of parameters such as:

- Robustness of the cultured cells
- Starting volume and concentration of cells
- Desired finished concentration and volume
- Desired yield and integrity of the cells

5.2 Cell harvesting process

The cell harvesting process involves both concentration and washing of the cells.

Concentration

Cells are concentrated as part of the harvesting process (Fig 5.1).

The concentration factor that can be achieved depends on the starting concentration, and is usually limited by the fluidity of the concentrated cell suspension. Typical concentration factors are shown in Table 5.1.

Table 5.1. Typical concentration factors for different cell types

Cell type	Typical concentration factor
E.coli	5×
Yeast	2×
Mammalian	7 to 10×

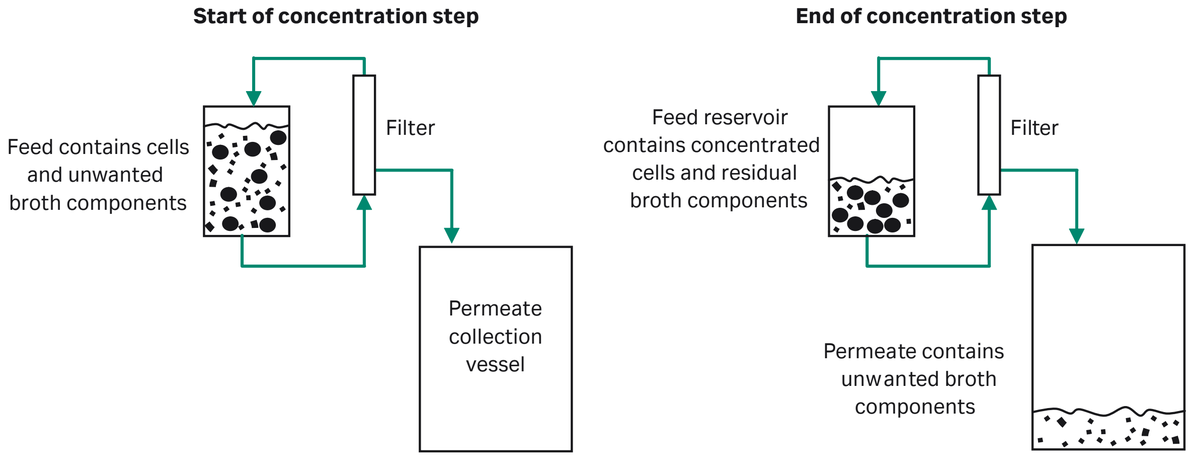


Fig 5.1. Concentration is the first step in cell harvesting.

Washing

Cell harvesting usually includes a washing step to ensure effective removal of broth components from the cells (Fig 5.2).

After washing, the ideal end product would consist of the concentrated cells suspended in the buffer used to wash the cells. However, in practice the harvested cells in buffer can contain varying levels of unwanted elements such as precipitated proteins, enzymes, and cell debris.

The washing process is commonly a constant volume diafiltration process, in which buffer is added to the cell suspension at the same rate as the permeate flows across the membrane. Unlike centrifugal techniques where cells are packed in a dense cake or pellet, washing the cells in a buoyant state enables effective removal of contaminants.

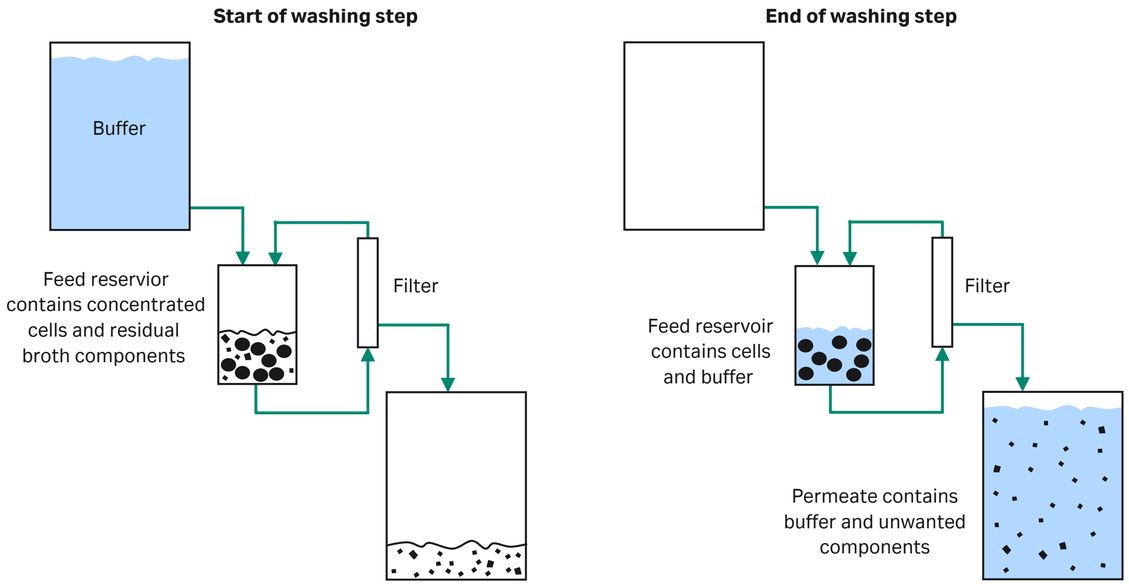


Fig 5.2. Washing harvested cells after concentration.

Typical process steps

Typical steps in a cell harvesting method are outlined in Table 5.2:

Table 5.2. Typical steps in a cell harvesting method

Step	Operation	Purpose
Preparation	Rinsing	Rinse storage solution from filter
	Water flush	Flush cleaning solution from system
	Water flux test	Determine performance of filter before processing
	Buffer conditioning	Condition filter and system components before adding product
Harvesting	Cell harvesting (concentration)	Concentrate cells
	Cell washing (diafiltration)	Remove unwanted components
	Product recovery	Recover harvested cells
Finishing	Buffer flush	Flush residual product from system
	CIP	Recirculate cleaning solution
	Water flush	Flush cleaning solution from system
	Water flux test	Determine the performance of filter after use and cleaning
	Storage	Flush filter with storage solution to prevent bacterial growth in storage

5.3 Membrane and cartridge selection

In cell harvesting, microfiltration membranes will easily retain all cells. The key to membrane selection is based on process optimization rather than retention. For example, smaller pore size membranes often show less tendency to fouling and provide the highest permeate flux once the system is in a steady state (Fig 5.3). The 500 000 NMWC ultrafiltration membrane is often the cartridge of choice for harvesting *E. coli*, even though it has a relative small pore size compared to the size of the cells.

Shorter cartridges allow a low pressure drop for difficult separations using low TMP. Longer cartridges have a similar membrane area, but will require less circulation flow per unit area. Longer cartridges are therefore preferred for applications in which higher TMP does not adversely affect the separation (Table 5.3).

Suitable fiber lumen diameters for cell harvesting are 0.75 to 1.0 mm. Larger diameter fibers should be used for solutions with high suspended solids, high cell densities, and high viscosity.

Table 5.3. Recommended cartridges for cell harvesting

Cell type	Suspended solids/viscosity	Path length (cm)	Fiber lumen diameter (mm)	Nominal pore size/NMWC
E. coli	Moderate	30 or 60	1.0	0.1 µm
				500 kD
				750 kD
Yeast	High	30	1.0	0.1 µm
				0.2 µm
				750 kD
Mammalian	Low to moderate	30 or 60	0.75 or 1.0	0.2 µm
				0.45 µm
				0.65 µm

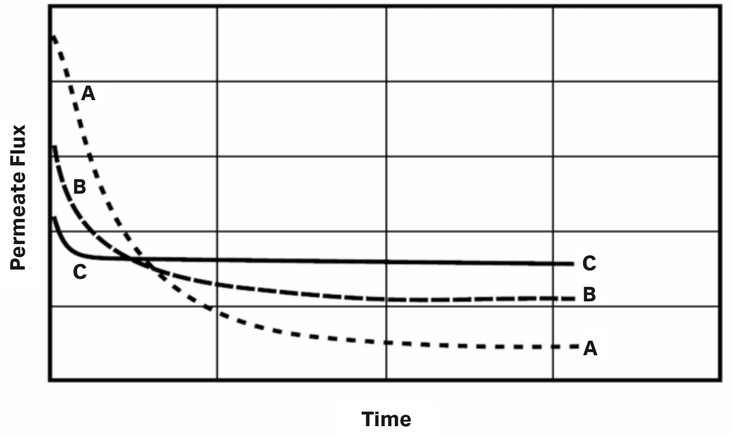


Fig 5.3. Flux of three membranes with all parameters held constant except pore size. Membrane A has a larger pore size than membrane B, which has a larger pore size than membrane C.

5.4 Operating parameters

Permeate flow control

In most cell harvesting applications, the permeate flow is often high even at low TMP, and the process is run under flux control to prevent premature fouling of the membrane. Restricting permeate flow generates back pressure on the permeate side of the filter, which effectively lowers the TMP and reduces fouling (Fig 5.4).

Typical starting conditions

Table 5.4. Recommended starting point for developing process conditions for harvesting applications

Bacterial cells	Mammalian cells	Virus particle concentration
10× concentration followed by 3× diafiltration	10× concentration followed by 3× diafiltration	5× concentration followed by 3× diafiltration
Average flux 25 LMH with high cell density starting material and unrestricted permeate	Permeate flow control at 30 LMH, no retentate back pressure	Low TMP and 6000 s^{-1} shear, 20–50 LMH
This process description is for removal of cells.	This process description is for removal of cells and optimal recovery of expressed target protein only.	This process description is for purification of virus particles with gentle process conditions only.

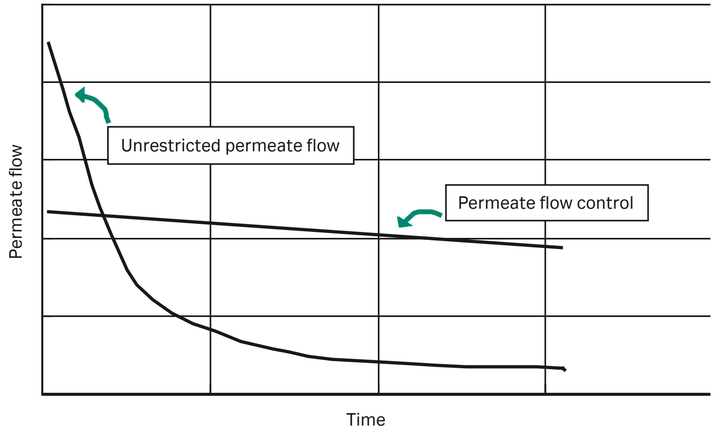


Fig 5.4. Using permeate flow control results in more stable flux.

Operating parameters

The table to the right summarizes the main aspects of process design for cell harvesting.

When working with cells which may still be partially active, rapid methods may be important. This can be achieved by increasing the ratio of membrane area to process volume. Using more membrane area not only allows higher permeate flow rate, but it also reduces the risk of fouling by spreading material over a larger area.

Flux is dependent on the concentration of particles. With the high particle load typical of cell harvesting, low to moderate transmembrane pressures should be used (< 1 bar, 15 psi).

Process temperature

Room temperature is recommended, but only if process components are stable at this temperature. Otherwise operate at 4°C to 12°C but with lower flux.

Table 5.5. The influence of process variables and feed solution on the CFF process

Variable	Selection considerations
Process variables	
Cell harvesting	Use microfiltration cartridges for cell harvesting. Select membrane pore size based on the specific application to achieve a stable flux rate.
Solution variables	
Cell concentration	Determine wet cell percent to anticipate the degree of concentration that may be used. A highly concentrated cell mass may seem efficient but may also result in high inlet pressures: a shorter, less efficient path length (30 cm) may be preferable.
Solids loading	For whole cells, it is not uncommon to reach 70% wet cell weight while maintaining steady state conditions. However, lysates tend to need a lower solids level to promote passage of the target material. Start with solids in the 5% to 10% range and monitor transmission as well as TMP during the concentration phase.
Shear sensitivity	If the feed stream is particularly shear sensitive and the recirculation flow rate is reduced, it may be necessary to lower the permeate flow rate (when using permeate flow control) to optimize throughput.
Volume	When scaling a process, cartridge housing diameter is increased in order to maintain constant volume to area ratio. Estimate the flux rate so that the starting volume is suitable for the target process time.
Temperature	As temperature decreases, the filtration time often increases due to viscosity effects, and larger cartridges might be appropriate. For example, cold-room processing at 4°C can take twice as long as room-temperature processing.
Other variables	
Time constraints	Increased membrane area and larger housing size shorten process time
Heat sterilization	Choose autoclavable or steam-in-place models for processes that will be scaled up for production.

06
Cell and lysate clarification

About this chapter

This chapter considers the use of CFF for clarification of cell cultures and lysates.

In this chapter

This chapter contains the following sections:

6.1 Introduction

Clarification processes are used in two contexts:

- Cell clarification
- Lysate clarification

In both cases the product is recovered in the permeate, while cell debris and larger particles remain in the retentate and are discarded (Fig 6.1). A wash step following the initial clarification is commonly used to maximize product recovery (Fig 6.2). The permeate will usually include unwanted components from the culture medium or cell lysate, and additional downstream steps are needed to purify the product.

Successful clarification of feed streams to recover target proteins requires knowledge of the starting product and the finished product specifications such as:

- Target molecule molecular weight, morphology, and robustness
- Starting volume and concentration of the target protein
- Desired finished protein concentration and volume
- Desired yield and quality (activity) of the protein
- Level of suspended solids

As with cell harvesting, rapid processing times may reduce the exposure of the target protein to shear forces, enzymatic action and elevated temperature.

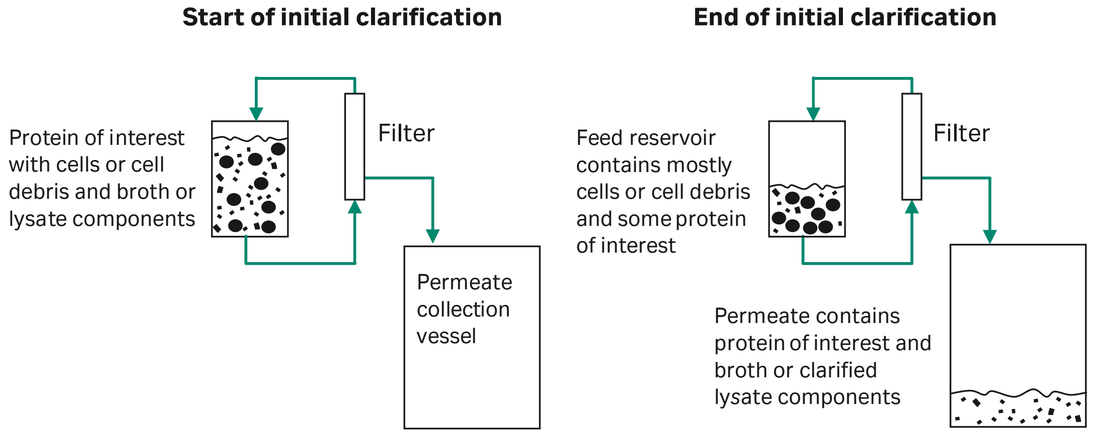


Fig 6.1. The initial clarification step recovers most of the product in the permeate and leaves unwanted particulate material in the retentate.

Cell clarification

Cell clarification is used to recover target protein that is expressed in the culture medium during cell culture. The cells are filtered and remain in the feed/retentate loop, while the permeate contains the protein or molecule of interest. Separating a protein from a cell culture is similar to cell harvesting except that the product of interest is the protein in the permeate. An effective cell clarification process enables the passage of the greatest amount of target molecules. To optimize recovery of the target protein, a wash step is often added to the cell clarification process to help flush the target molecules through the membrane (Fig 6.2).

The cells are concentrated as liquid is removed in the permeate. The level to which cells can be concentrated during cell clarification varies according to cell types. For typical concentration factors, see Table 5.1.

Lysate clarification

Lysate clarification is used after lysis of harvested cells to recover the target molecule from the cell contents. Components in lysates often tend to foul membrane pores. To minimize pore fouling, and to enable the filter to operate under equilibrium conditions, initial lysate clarification trials often include a constant volume wash with little or no concentration. A large wash volume (typically 5× the starting volume of lysate) is used to ensure efficient recovery of the target protein.

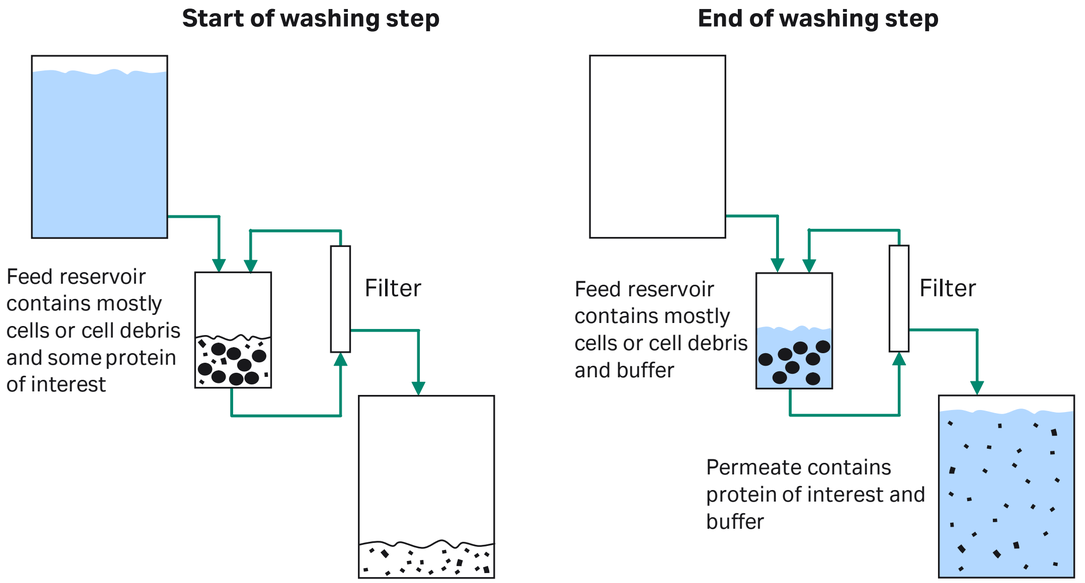


Fig 6.2. A washing step followng the initial clarification maximizes product recovery.

6.2 Membrane and cartridge selection

For cell and lysate clarification, microfiltration membranes will easily retain all cells and cell debris. The key to selection is based on passage of the target molecule rather than retention of unwanted components.

Membrane selection

Smaller pore size filters resist fouling more than filters with larger pore sizes. A general guideline is to select the smallest pore size ratings that is at least 10× larger than the size of the target protein in it largest state or longest dimension.

For clarification of mammalian cell cultures, microfilters (pore size 0.2 to 0.65 µm) are generally suitable. Clarfication of bacterial cultures typically use ultrafilters with NMWC 500 or 750 kD. Both 750 kD ultrafilters and 0.1 µm microfilters have been found suitable for yeast cultures.

When working with lysates, which can contain a wide range of particle sizes and many types of proteins and sticky cell components, choosing a small pore size can help prevent fouling of the membrane pores.

Table 6.1 presents typical cartridge and membrane characteristics for common clarification applications.

Cartridge selection

The presence of particles in the feed stream requires the selection of short path length cartridges (30 to 60 cm) with large lumen diameters (0.75 to 1.0 mm). See Table 6.1 for cartridge specifications.

Table 6.1. Recommended cartridges for cell culture and lysate clarification

Clarification type	Suspended solids/viscosity	Path length (cm)	Fiber lumen diameter (mm)	Nominal pore size/NMWC
Monoclonal antibody from hybridoma cell culture	Low to moderate	30 or 60	1.0	0.2 µm 0.45 µm 0.65 µm
Clarification of adenovirus from 293 cell culture	Low	30	0.75	0.65 µm
Clarification of protein (< 60 kD) from *E. coli* whole cell broth	High	30 or 60	1.0	0.1 µm 500 kD 750 kD
Clarification of 20 kD protein from *E. coli* lysate	Moderate	30 or 60	1.0	750 kD
Clarification of 40 kD protein from *Pichia pastoris*	High	30	1.0	0.1 µm

Table 6.2 summarizes cartridge selection considerations in relation to variables in the application.

Table 6.2. The influence of process variables in selecting a cross flow cartridge

Variable	Selection considerations
Solution variables	
Cell concentration	Determine wet cell percent to anticipate the degree of concentration that may be used. The benefit of a highly concentrated cell mass should be balanced against the possibility of high inlet pressure requirements or the necessity of using less efficient, short (30 cm) path length cartridges.
Solids loading	Harvested cells may reach up to 70% wet cell weight. However, lysates tend to need a lower solids level to prevent fouling. Start with solids in the 5% to 10% range and monitor flux as well as TMP during the concentration phase.
Size of the target material	For separations with large target material it may be best to avoid any concentration but rather perform a constant volume wash from the start. Remember that using a more open membrane may require the use of short cartridges and permeate flow control. Use open UF membranes to clarify small proteins from streams.
Shear sensitivity	If the feed stream is particularly shear sensitive and the recirculation flow rate is reduced, it may be necessary to lower the permeate flow rate (when using permeate flow control) to optimize throughput. Note that fragile cells may be disrupted by shear forces during clarification, resulting in contamination of the target material with intracellular components.
Other variables	
Time constraints	Increased membrane area and larger housing size shorten production time.
Heat sterilization	Choose autoclavable or steam-in-place models for processes that will be scaled-up for production.

6.3 Operating parameters

The key process variables during clarification are permeate flow rate (flux) and TMP. In clarification processes, as in cell harvesting, the flux is often high even at low TMP values and steps should be taken to decrease the flux to prevent premature fouling of the membrane. Refer to *Chapter 5 Cell harvesting,* on page 41 for more details. Table 6.3 describes typical starting conditions for the clarification of different cell types.

Table 6.3. Recommended starting point for developing process conditions for clarification

Bacterial fermentation **Target protein expressed extracellularly**	**Yeast fermentation** **Target protein expressed extracellularly**	**Mammalian cell culture** **Monoclonal antibody expressed extracellularly**	**293 or HeLa cell culture** **Adeno-associated virus clarification**	**Yeast lysate** **Virus-like particles (VLP) expressed intracellularly**
5× concentration followed by a 3 to 5× diafiltration	Partial concentration 1.5 to 2× at best followed by 3 to 5× diafiltration	10× concentration followed by 3× diafiltration	5× concentration followed by 5× wash	No concentration, constant volume wash up to 5×
For large target proteins, use microfiltration membranes with permeate flow control set at 20 to 30 LMH. For smaller molecules, use 750 or 500 kD membranes with unrestricted permeate flow and TMP readings at 1 to 1.5 bar (15 to 22 psi).	Membranes rated at 750 kD and 0.1 µ have worked well with unrestricted permeate flow. If the cell density is quite high, closely monitor the inlet pressure to avoid over concentration.	Use 0.2 or 0.45 µ microfiltration membranes with permeate flow control set at 30 LMH. No retentate back pressure.	Operate at low shear rate using 0.65 µ membranes and permeate flow control set at 20 to 30 LMH.	Operate at high shear rate with permeate flow control set at 20 LMH.

6.4 Clarification strategy examples

The examples in this section illustrate the development of process conditions with mammalian cells, bacterial cells and yeast.

6.4.1 Mammalian cells

Introduction

Many therapeutic proteins are derived from cell culture sources. These are most often grown with mammalian cell lines in highly purified media. Although there are a variety of cells that are suitable and cell densities range from 10^6 to 10^7 cells per mL, the clarification process is similar for each type.

Membrane and cartridge selection

If the protein of interest is an antibody, 0.2, 0.45, or 0.65 µm membranes can be used. Permeate turbidity will be slightly lower if 0.2 µm filters are used. The 0.65 µm rating usually provides the best throughput. These cartridges can be tested with feed solution to determine which rating provides the best overall performance. The above microfiltration membranes are available in only one fiber diameter, and the only remaining variable is the cartridge path length, 30 or 60 cm. Initial testing should begin with the 30 cm path length to help maintain low TMP readings. When the appropriate membrane and operating conditions have been chosen, additional testing using the 60 cm path length may be used to determine if this design is suitable for scale up.

Process conditions and monitoring

The circulation flow rate should be set to a maximum of 4000 s^{-1}. If tests show damage to the cells, the circulation flow rate should be reduced. The permeate flow rate should be set to 30 to 50 LMH. Using these conditions, the initial TMP will begin at approximately 70 mbar (1 psi). Since the cells are completely retained, and the protein will initially pass through the membrane quantitatively, the objective of testing should be to determine the filtration capacity. As a general rule, once the TMP has increased by a factor of 4 to 5 from the initial reading, the membrane is exhausted. In this example, working with permeate flow control set at 30 LMH, if the TMP begins at 70 mbar (1 psi), when the TMP reaches 250 to 350 mbar (3.5 to 5 psi) the cartridge capacity has been reached. Using the change in TMP as an indicator, it is possible to compare a variety of membrane ratings and process controls.

Once a set of standard conditions has been adopted, the filtration efficiency can also be studied as a function of the cell culture process. For example, with low cell viability, membrane throughputs working with a 0.45 µm membrane might be as low as 50 L/m^2. With healthy cells operating under the same conditions, the throughput might be as high as 120 L/m^2. Operating at a higher flux rate usually decreases throughput capacity.

For high yields working with monoclonal antibodies derived from CHO cells, it is normally possible to concentrate the cells 10× and follow with a 3× to 5× wash.

6.4.2 Bacterial cells

Introduction

E. coli and related bacteria have been used for many years for the expression of a wide range of recombinant proteins, vaccines, and enzymes. Fermentation times can range from under a day to a week. Due to the short doubling time of these cells, prolonged fermentation can result in a significant cell mass. Moreover, the nutrient medium is usually much more complex and less purified than the medium used for mammalian cells. As a result, separating these cells from the target protein can be a more complex process.

Membrane and cartridge selection

For relatively small proteins (< 40 kD), the open ultrafiltration membranes rated at 500 kD and 750 kD should be tested first. These membranes will provide a stable flux rate and resist rapid fouling. For large proteins, microfiltration membranes rated at 0.1 or 0.2 µm should be used, but only in conjunction with permeate flow control. In order to make a comparison of any of these selections without a significant contribution from a gel layer, uniform operating conditions with a relatively high shear rate in the circulation flow and low TMP should be used. Initial testing should use the 1 mm lumen fiber design with a 30 cm path length. When the membrane and operating conditions have been chosen, additional testing using the 60 cm path length may be used to determine if this design is suitable for scale up. Several full scale production processes have successfully utilized the 110 cm ultrafiltration cartridge design with *E. coli* fermentation processes.

Process conditions and monitoring

Unlike fragile mammalian cells, bacterial cells can withstand significant shear forces without damage. Test results have shown that high circulation flows with 12 000 to 16 000 s^{-1} shear rates provide better transmission of the target protein and more stable flux rates. Insufficient shear rate or excessive TMP will cause the formation of a gel layer on the membrane surface that acts as an additional filtration layer. Therefore, initial testing will require a reliable assay to establish a stable process with good yields. With feed streams containing a high cell mass and a large target molecule, testing should begin with the permeate flow control set as low as 10 to 20 LMH. When working with ultrafiltration membranes, the TMP should be gradually increased to see if there is a proportionate and stable increase in flux. With microfiltration membranes, the flux rate should be gradually increased while monitoring the TMP. If the TMP increases over time, the flux rate should be adjusted to a lower setting until it remains stable. Even with a relatively high starting cell mass, it is often possible to perform a 5× concentration without sustaining a significant increase in the pressure drop along the cartridge. If the inlet pressure begins to rise abruptly, it is normally not due to cartridge plugging but to the increased viscosity of the feed, and additional concentration is not advised. With initial testing, a 2× increase in the pressure drop should be used as an upper limit. The constant volume wash should be initiated without interrupting the circulation flow. If the flux rate has decreased by more than 4×, it is advised to temporarily open the back pressure valve and shut off the permeate flow. This technique may help to diminish the effects of gel layer formation.

6.4.3 Yeast

Introduction

Pichia pastoris and other types of yeast have been extremely popular for expressing target proteins. Often a successful fermentation will result in a highly viscous material with as much as 50 percent cell mass. This represents a challenge for any of the candidate clarification technologies. However, the target proteins are usually small. Moreover, the hollow fiber technology is linearly scalable and does not require pre-dilution in order to provide good yields.

Membrane and cartridge selection

The most popular membranes to ensure good passage of target proteins as large as 70 kD molecular weight have been the 0.1 µm microfiltration and 750 kD ultrafiltration membranes. Larger proteins have been successfully processed at full scale with the 0.2 µm microfiltration membranes. With such a high initial viscosity, there is even greater need to use the most dense membrane that will effectively pass the target protein. More open membranes working with high inlet pressures will result in cells being trapped on the membrane surface near the cartridge inlet. Inlet pressures will rise and the process will fail. The 1 mm fibers with 30 cm path length are used exclusively with viscous yeast feed streams. Moreover, the shear rate rarely exceeds 4000 to 6000 s^{-1} in order to keep the inlet pressure readings less than 0.7 bar (10 psi). Due to the high viscosity of the feed stream, it is critical that all characterization testing be done in steady state (with permeate recycled to the feed reservoir). Even a slight increase in the solids concentration will result in a significant increase in the pressure drop.

Process conditions and monitoring

When working with the 750 kD ultrafiltration membrane, the circulation flow rate should be kept constant while gradually increasing the TMP to see if there is a proportionate and stable increase in flux. If the increased TMP only provides a partial increase in flux, the TMP should not be increased as this may result in a drop in protein transmission, which will necessitate extensive washing. With either the 0.1 or 0.2 µm microfiltration membranes, the flux rate should be gradually increased while monitoring the TMP. As in the earlier example, if the TMP increases over time, the flux rate should be adjusted to a lower setting until it remains stable. The TMP readings with these microfiltration membranes will probably remain below 0.3 to 0.4 bar (4.5 to 6 psi) throughout the process. Unless the starting cell density is quite low (< 35%), it is unlikely that the process will allow any initial concentration of the feed stock. Instead, it is best to operate under a constant volume wash mode from the beginning. Since as much as 50% of the feed is actually cells, the wash volumes double their effectiveness. High yields are possible using only 2.5× wash volumes. Moreover, because the membrane is operating under equilibrium conditions, flow rates and pressure readings should remain constant.

Initial testing should be directed at selecting the membrane with the best passage of the target protein. This is usually the membrane that also provides the highest flux rate. Optimization of the operating conditions will involve minor adjustments to the pressure readings and/or flux rates. Capacity will be a function of the protein transmission as this will lead to the determination of the required wash volumes. Effective processes will be between 2.5× and 5× wash volumes. Flux rates can range from 15 to 60 LMH. When working with high cell density feed streams, it is possible to achieve throughputs of 80 L/m^2 based on the starting material.

07

Concentration and diafiltration

About this chapter

This chapter considers the use of CFF for product concentration and diafiltration.

In this chapter

This chapter contains the following sections:

7.1 Introduction

Ultrafiltration CFF filters are used for concentration of macromolecules in much the same way as microfilters are used for harvesting cells. In this process, the retentate becomes increasingly concentrated as permeate is removed. If buffer of a different composition is added to the feed reservoir at the same rate as permeate is removed, the process is referred to as **diafiltration**, where new buffer will progressively replace the old in the retentate with no change in product concentration. Figure 7.1 shows a schematic comparison of concentration and diafiltration processes.

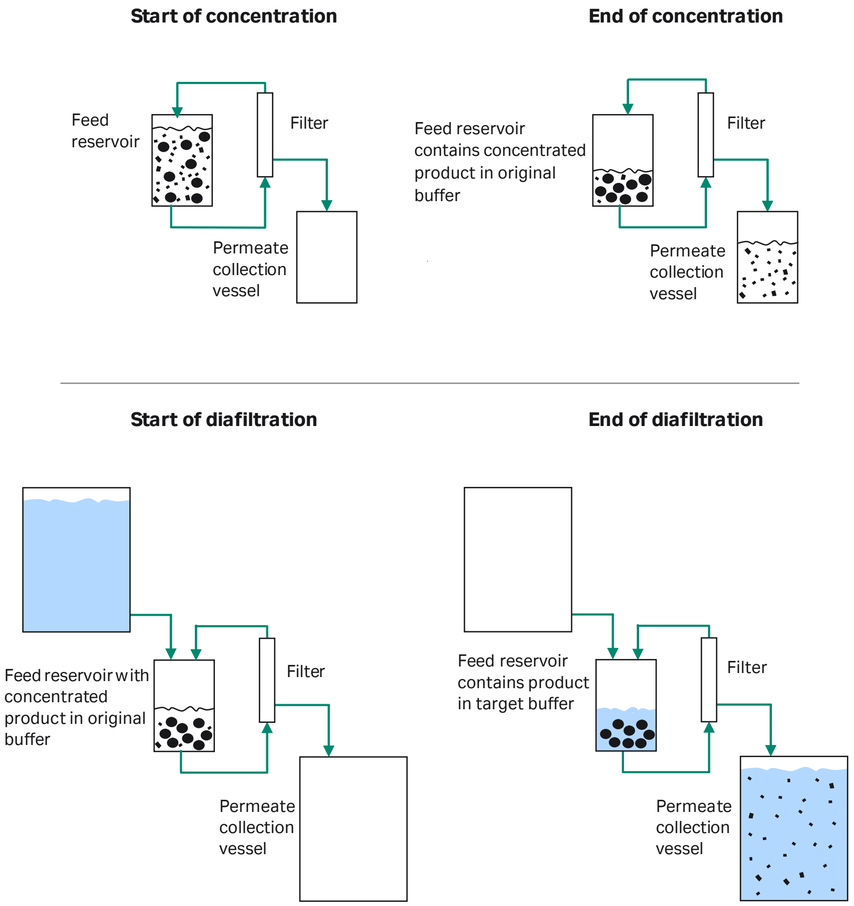


Fig 7.1. Comparison of concentration and diafiltration processes in downstream processing.

Concentration and diafiltration often go hand-in-hand in downstream processing, and may be used at several stages of a purification process. Figure 7.2 illustrates the downstream steps in a purification scheme for IgG.

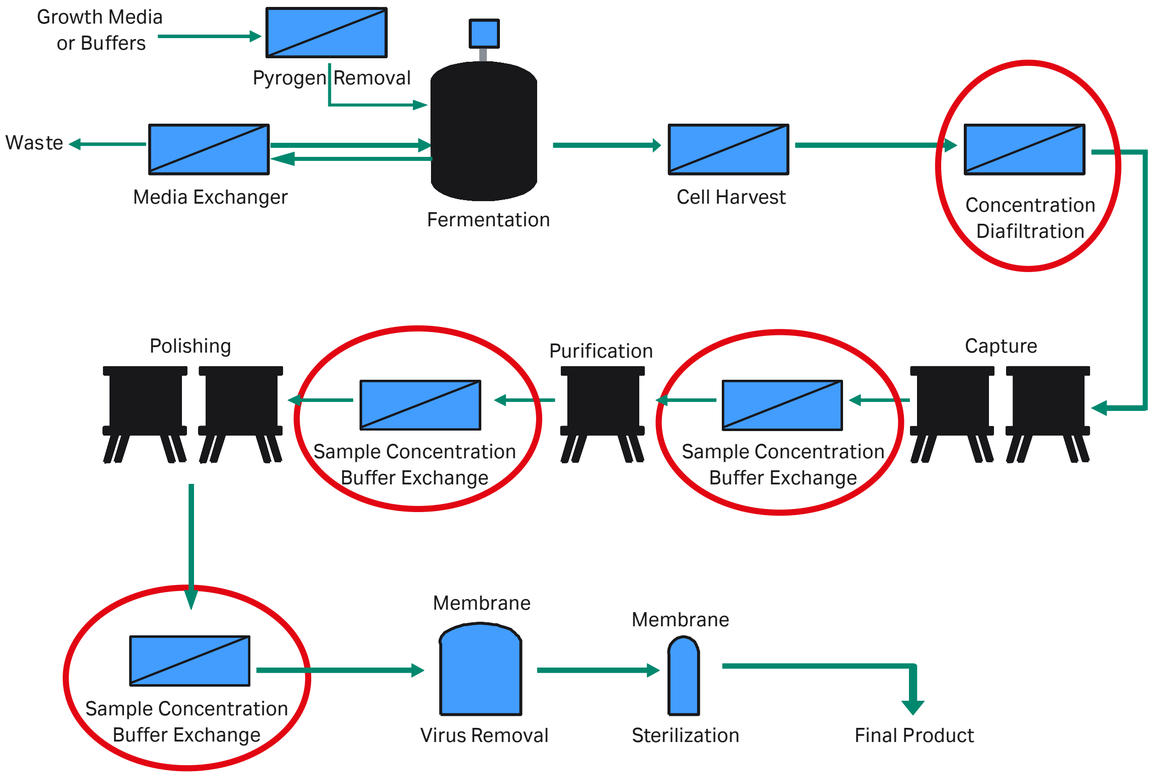


Fig 7.2. Downstream steps in the purification of IgG.

7.2 Product and process considerations

Successful protein concentration and diafiltration using CFF relies on specifying the pre and post-concentration product as follows:

- The characteristics of the target protein (size and shape, solubility, shear sensitivity, temperature sensitivity, ionic condition)
- The characteristics of the starting and target solutions (pH, ionic condition, solubility, compatibility with the next process step)
- The starting volume and concentration of the target protein
- The desired finishing concentration and volume, and the viscosity of the final solution
- Process time and cost
- Diafiltration volumes
- The desired yield and quality (stability) of the target protein

Design of concentration processes

Concentration retains the product in the retentate while removing water and buffer components in the permeate. The choice of filter pore size is crucial for avoiding losses of product in the permeate. The overall process may be limited by the solubility of the product or tendency to aggregate at high concentrations. Viscosity of the concentrated protein solution may also be an important factor. Flux, cross flow rate and TMP need to be adjusted to avoid the formation of a gel-like layer of highly concentrated product on the membrane surface.

The **concentration factor** is the ratio between the final and initial product concentrations. The maximum available concentration factor is limited by the ratio between starting volume and minimum working volume. In addition, over concentration of protein can lead to inefficient diafiltration due to membrane polarization effects and to protein precipitation.

In a process that involves both concentration and diafiltration, the concentration is often performed in two steps separated by the diafiltration step. This allows diafiltration to be done under optimal conditions of low volume and moderate concentration, while processing to the final target concentration is completed in the target buffer.

Design of diafiltration processes

Diafiltration is a buffer exchange process. From an operational perspective the goal is to minimize consumption of the diafiltration buffer and to keep processing time short. The overall process usually consists of concentration to reduce the amount of buffer required to achieve a specific diafiltration factor, followed by continuous diafiltration where the volume of liquid in the feed reservoir is kept constant.

The **diafiltration factor** represents the extent to which original buffer is replaced by new buffer, and may be calculated as:

Diafiltration factor = Buffer Volume/Starting Volume

Equation (2)

Note: Diafiltration never achieves complete buffer exchange since the concentrationof starting buffer decreases exponentially.

A diafiltration process may be designed in several different ways with respect to how new buffer is added to the feed to replace the old buffer. The two commonest approaches are **continuous** and **discontinuous** diafiltration. The effect of continuous and discontinuous dialfiltration methods compared to single bulk dilution (where new buffer is simply added to the sample before it is concentrated) is illustrated in Figure 7.3. **Sequential** diafiltration, with two or more diafiltration steps in direct sequence, may also be used in special cases.

Continuous diafiltration

In continuous diafiltration the volume of liquid in the feed reservoir is kept constant by adding fresh buffer from a transfer reservoir at the same rate as liquid is removed in the permeate. This is the most efficient form of diafiltration in terms of process time required to achieve a given diafiltration factor.

Discontinuous diafiltration

Discontinuous diafiltration is a process where the protein solution is repeatedly concentrated and re-diluted. It is less efficient than continuous diafiltration because a larger volume of finishing buffer is required to achieve the same diafiltration factor.

Sequential diafiltration

Occasionally it may not be possible to exchange the existing buffer with a new buffer directly without damaging effects to the target protein. In sequential diafiltration, several buffer formulations, moving from weaker to stronger chemical solutions are introduced sequentially to the product to achieve the final buffer exchange.

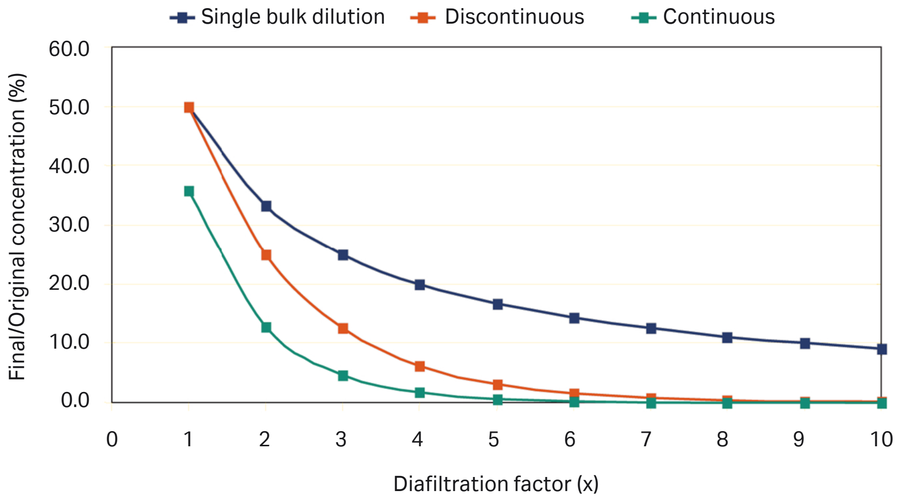


Fig 7.3. Effect of diafiltration methods on the buffer concentration.

7.3 Membrane selection

Selection of the filter pore size is based on the size of the target molecule. A membrane with too large pores will allow the target molecule to pass through and significantly reduce yields. Conversely a membrane with too small pores will reduce flux and lead to longer processing times and oversized systems, with increased capital cost, plant space requirements, working volume and hold-up volume.

A general guideline for selecting a membrane for product concentration is to start with a NMWC that is 3× to 5× smaller than the target molecule. For example, a 50 kD or 30 kD membrane would be a suitable choice to retain IgG (molecular weight 160 kD), and a 30 kD or 10 kD membrane would be a suitable choice for albumin (molecular weight 66 kD).

08
Appendix

Appendix A

Abbreviations and glossary

Abbreviations

Abbreviation	Meaning
CFF	Cross flow filtration
CIP	Cleaning-in-place
ΔP	Pressure differential between retentate and feed
kD	KiloDalton (=1000 Dalton)
LMH	Liter per square meter of membrane surface per hour
NMWC	Nominal molecular weight cut-off
TFF	Tangential flow filtration
TMP	Transmembrane pressure
WFI	Water for injection

Glossary

Air diffusion rate

The rate at which air diffuses through the wetted pores of a membrane at a given differential pressure. Measuring the air diffusion rate is a method used to check the integrity of a membrane filter.

Bubble point

The minimum pressure required to overcome the capillary forces and surface tension of a liquid in a fully wetted membrane filter. This test checks the integrity of a filter.

The bubble point value is determined by observing when bubbles first begin to emerge on the permeate (downstream) side of a fully wetted membrane filter when pressurized with a gas on the feed (upstream) side of the membrane filter. If bubbles appear at a lower pressure than the acceptance criteria the filter integrity is impaired.

Bubble point test

The test procedure for determining the bubble point of a microfiltration membrane.

Cartridge or cartridge filter

The term **cartridge** refers to hollow fiber filter unit, for either microfiltration or ultrafiltration.

Cassette

The term **cassette** refers to a filter unit containing stacked flat sheets of membrane separated by support screens.

Cassettes from Cytiva are exclusively used for ultrafiltration.

Channel height

The height of the path that the feed/retentate solution must pass through for a flat membrane cassette.

Concentrate

Also called retentate. The part of the process solution that does not pass through a cross flow filter.

Cross flow filtration (CFF)

In cross flow filtration, the feed solution flows parallel to the surface of the membrane. Driven by pressure, some of the feed solution passes through the membrane filter. The remainder is circulated back to the feed tank. The movement of the feed solution across the membrane surface helps to prevent the buildup of materials on the surface.

Cross flow rate

Also called retentate flow rate.

The flow rate of feed solution that flows across the surface of the filter and exits the filter as retentate. Higher cross flow rates help "sweep away" material that otherwise accumulates on the surface of the filter. Cross flow rate is most often measured at the retentate outlet.

Cutoff

See Nominal molecular weight cutoff (NMWC).

Dead-end filtration

See normal flow filtration.

ΔP

Pressure differential between the feed and retentate lines. The ΔP equals the feed pressure minus the retentate pressure.

Diafiltration

An operation that uses ultrafiltration filters to remove salts or other microsolutes from a solution. Small molecules pass into the permeate while larger molecules are retained in the retentate.

Microsolutes are generally so easily washed through the filter that for a fully permeated species about three volumes of diafiltration solution will eliminate 95% to 99% of the microsolute.

Diafiltration exchange factor

Diafiltration exchange factor = Diafiltration buffer volume/Sample volume

Differential pressure

See ΔP.

Direct flow filtration

See normal flow filtration.

Effective filtration area

The active area of the membrane exposed to flow.

Glossary

Extractables

Substances that may dissolve or leach from a membrane filter during filtration and contaminate the process solution. For example, these might include wetting agents in the membrane, membrane cleaning solutions or substances from the materials used to encase the membrane.

Feed

Material or solution that is fed into the filter.

Feed pressure

The pressure measured at the inlet port of a cartridge or cassette.

Filter area

The surface area of filter membrane inside a membrane filter.

Filter efficiency

Filter efficiency represents the percentage of a given size particle removed from the fluid by the filter.

Flow path length, nominal flow path length

The total length that a feed solution travels from inlet to outlet. Flow path length is an important parameter to consider when doing any process development, system design or scale-up or scale-down experiments.

The flow path length and other fluid channel geometries such as lumen diameter or channel height can affect the fluid dynamics of the system and will directly affect pump requirements and differential pressure of the filtration step.

Flux

Flux represents the volume of solution flowing through a given membrane area during a given time. Expressed as LMH (liters per square meter per hour).

Fouling

A build up of material on the membrane surface that reduces the filtration rate. Unlike the gel layer, this material is not redispersed in the bulk stream by higher cross flow rates.

Gel layer

During the filtration process, a thin layer of particles or molecules may build up at the membrane surface. Under extreme circumstances this concentrated layer may form a gel, blocking flux across the membrane and potentially resulting in increased TMP.

Operating at a higher cross flow may reduce the thickness of the gel layer.

Hold-up volume

Volume of fluid in the system tubing and filter.

Hollow fiber

A tube of membrane, sealed inside a cross flow cartridge. The feed stream flows into the lumen of one end of the hollow fiber and the retentate (the material that does not permeate through the walls of the hollow fiber) flows out of the other end. The material that passes through the membrane (walls of the hollow fiber) is called the permeate.

Housing

The mechanical structure that surrounds and supports the membrane or filter element. The housing normally has feed, retentate and permeate ports that direct the flow of process fluids into and out of the filter assembly.

Inlet pressure

The pressure of a fluid at the feed port of a membrane filter.

Loading

The volume of solution that will pass through a membrane filter before the permeate output drops to an unacceptable level.

Membrane recovery

The degree to which the original performance of a membrane can be restored by cleaning.

Microfiltration

The process of removing particles from a liquid by passing it through a porous membrane under pressure. Microfiltration usually refers to removing submicron-sized particles.

Microporous membrane

A thin, porous film or hollow fiber having pores ranging from 0.1 to 10 µm. Cross flow microfilters typically have pores ranging from 0.1 to 1.0 µm.

Minimum process volume

Also called minimum operating volume. The least amount of fluid able to be handled effectively by a filtration system.

Glossary

Nominal filter rating

A rating that indicates the percentage of particles of a specific size or molecules of a specific molecular weight that will be removed by a filter. No industry standard exists; hence the ratings from different manufacturers are not always comparable.

Nominal molecular weight cut off (NMWC)

The pore size designation, usually in kiloDaltons (kD), for ultrafiltration membranes. No industry standard exists; hence the NMWC ratings of different manufacturers are not always comparable.

Normal flow filtration

In normal flow filtration, liquid flows perpendicular to the filter media. Material that does not pass through the filter remains on the filter surface

Nominal flow path length

See Flow path length.

Normalized water permeability (NWP)

The water flux at 20°C divided by pressure. Common units LMH/psi, LMH/bar.

Permeate

Also called filtrate. Any components of the feed solution that passes through the membrane.

Porosity

A measurement of the open space in a membrane. The higher the membrane porosity, the more pores there are, and hence a higher flow rate is expected.

Retentate

The portion of the feed solution that does not pass through the filter. Any component that does not pass through the membrane flows out of the filter and back to the feed container.

Retention

The ability of a membrane filter to retain an entity of a given size.

Shear rate

The ratio of velocity and distance expressed in units of s^{-1}. The shear rate for a hollow fiber cartridge is based on the flow rate through the fiber lumen and is used to control cross flow.

Shear stress

Disruptive force that arises in connection with a steep flow gradient. Shear stress can disrupt cells and denature proteins.

Steam-in-place (SIP)

The process of sterilizing a filter unit, such as a hollow fiber cartridge, with steam without removing the unit from the separation system.

Size exclusion

Mechanism for removing particles from a feed stream based strictly on the size of the particles. Retained particles are held back because they are larger than the pore opening.

Tangential flow filtration

See Cross flow filtration.

Transmembrane pressure

The pressure drop across the filtration membrane.

Ultrafiltration

The separation of macrosolutes based on their molecular weight or size.

Upstream

The feed side of a separation process.

Water flux

Measurement of the amount of water that flows through a given membrane surface area in a set time. See also Flux. The water flux test is commonly used to assess cleaning efficacy.

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