Filtration for the Bioprocessing Industry
Developed and Written by BPE Subject Matter Experts
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**ABSTRACT:** The use of filtration in the bioprocessing industry is an essential step as a standard unit operation or in conjunction with other unit operations for separation, isolation and purification of the biotherapeutics, be it recombinant proteins, vaccines, gene therapy or cell therapy products. This paper will review the types of filtration mechanisms in both direct flow and tangential flow operations focusing on both liquid and gas handling. It will discuss in detail the operational aspects of these filtration processes.

Key Words: Filtration, direct flow, tangential flow, integrity testing, depth filtration, membrane filtration, spiral wound, cassettes, plate and frame, hollow fiber, monolith, alternating tangential flow, microfiltration, diafiltration, nanofiltration, reverse osmosis

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1.0 Purpose and Scope

The purpose of this document is to familiarize the reader with the concepts of process-scale filtration and the associated equipment. The scope of this document is for production service only.

2.0 Overview

Liquid or gas filtration is used to isolate processes, to purify substances of interest, to concentrate, to exchange buffer/diafiltration, for clarification, particle removal, bioburden reduction, sterilization or viral removal. Filtration may be applied as a unit operation or be adjunct to protecting downstream processes from particulate and/or microbial contaminants.

3.0 Concepts

3.1 Modes

In Direct Flow Filtration (DFF), the process fluid passes through the filtration medium in a single pass. (Figure 1) These filters are typically used for clarification, gas and vent filtration, virus reduction, and process fluid sterilization. This mode of filtration is also known as dead-end filtration, or normal flow filtration.

In Tangential Flow Filtration (TFF), also known as Cross Flow Filtration, the bulk of the process fluid flow is parallel (tangential) to the filter medium surface. (Figure 2) Inlet flow is typically referred to as Feed while the unfiltered outlet flow is referred to as Retentate. The portion of the process fluid that flows through the filter medium is typically referred to as Permeate or Filtrate and is captured as a separate flow stream. TFF filter media are usually classified as Microfiltration, Ultrafiltration, Nanofiltration or Reverse Osmosis based on the apparent size of rejected particles or molecules. TFF is typically used for separation, diafiltration (buffer exchange) and/or concentration of the substances of interest.

3.2 Membrane ratings

**Microfiltration (MF)** - The typical membrane retention size limit is between 0.1 to 10 μm. They can separate microorganisms and particles from dissolved molecules.

**Ultrafiltration (UF)** - The typical membrane retention size limit is between 1 and 1000 nanometers (0.001 to 1 μm). Most membranes are defined by rejection cutoff of molecules at a known molecular weight, i.e. Molecular Weight Cut Off (MWCO). They can separate small molecules from larger molecules or particles.

**Nanofiltration (NF)** - The typical membrane retention size limit is about 1 nm. As with UF membranes, most NF membranes are defined by a MWCO. They are able to separate water and dissolved salts from organic molecules or particles.

**Reverse Osmosis** - Membranes typically without physical pore flowpaths that separate water from dissolved salts and organic molecules or particles by pressure-mediated diffusion.

4.0 Direct Flow Filtration (DFF)
4.1 Configurations

**Pleated or Wrapped Design** – These devices are configured into a cylindrical cartridge containing filtration media that is either pleated or wrapped around a core. The flow path is typically from the outside of the cartridge through the wrap or pleat pack to the core. One end of the cylindrical cartridge can be either sealed (capped) or left open (referred to as “double open end”), with the second end left open to permit flow from the core through to the outlet.

Housing connections typically consist of a double 2-226 O-ring seal; however, flat gasket and various other O-ring configurations are also used. For submicron filtration, single open ended designs are preferred with a secured double O-ring piston connection. (Figures 3, 4)

**Lenticular Design** – These devices comprise of two sheets of filtration media cut in a circular design sandwiched and supported by a flow channel. These formats are usually stacked on top of each other. One end is closed for flow while the other end is connected either by a flat gasket seal or by an O-ring connection. Sealing between the stacked formats is accomplished by either compression or welding. This form is more commonly used with thicker depth filtration media. (Figure 5)

**Hollow Fiber Design** – These devices contain small diameter permeable tubular membranes that are sealed on opposite ends by “resin potting”.

**Bag Filters** – Comprised of Melt-blown or woven fabrics formed into a closed end cylinder or “bag” for separation of high solids feeds (Figure 7a)

**Radial Pleated Design** – The filtration media is pleated in a radial direction against the support tube resulting in extremely high filtration area with large flow rate capabilities (Figure 7b). More configuration range from 20 to 60” in length with 1 to >100 um filtration efficiencies.

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**Figure 3** – (a) Depth Filter, (b) Pleated Depth Filter, and (c) Membrane Filter

**Figure 4** – Cross-section of High-Area Pleated Filter

**Figure 5** – Lenticular Design with cartridge and encapsulated options

**Figure 6** – Hollow Fiber Design

The flow path can be from either direction (from inside to outside or vice versa) of the tubes to either end of the potting. One end of the potting is capped or sealed and the other end is open with a double O-ring piston connection being preferred for submicron filtration.

**Figure 7a** – Bag Filters

**Figure 7b** – Radial Pleated Filter
Monoliths - Ceramic material molded into tubular forms with the distal end closed.

Sheets or Discs – Installed in single or multilayer plate and frame structures

4.2 Categories

4.2.1 Liquid Applications

Pre-Filtration – Use of thick depth or thin membrane filter media (microfilters rated at 0.1 to 10 µm) upstream of finer filters. Prefilters are used to reduce the loading on downstream filters to increase throughput or efficacy of the filtration process.

Clarification – Depth and Membrane microfiltration (0.1 to 10 µm) used in bioprocessing for bulk cell and debris removal or to control maximum particle size in other applications.

Bioburden Reduction – Refers to the removal of microorganisms with high efficiency, but without a claim or supporting validation required to claim effluent sterility. This applies to the viral reduction/removal process and to the maintenance of microbial control for or between other unit operations in a process.

Sterilizing Filtration – Filters for 100% removal of aerosolized microorganisms. Can be met by depth or membrane filters, but membranes are preferred due to their integrity testability. Membranes are typically hydrophobic in nature to minimize risk of wetting by liquid aerosol droplets and resultant blockage to air or gas flow at low pressure differentials.

4.2.2 Air and Gas Applications

Pre-Filtration – Used to protect air filters from particle fouling and to protect off-gas/vent filters from aerosolized droplet fouling.

Coalescing – A prefiltro designed to enhance drainage of entrained aerosolized liquid droplets are reduce liquid fouling.

Sterilizing Filtration – Filters for 100% removal of aerosolized microorganisms. Can be met by depth or membrane filters, but membranes are preferred due to their integrity testability. Membranes are typically hydrophobic in nature to minimize risk of wetting by liquid aerosol droplets and resultant blockage to air or gas flow at low pressure differentials.

Vent Filtration – Sterilizing air or gas filters employed to enable air/gas/steam from entering or exiting a closed vessel while simultaneously preventing release of contained microorganisms and, to prevent ingress of aerosolized microorganisms and particles.

4.3 Housing Types

4.3.1 Multi-Use

Pressure vessels designed to accept and enclose one or multiple filter elements and direct the flow path through the filter element(s) without bypass. An inlet port brings fluid into the housing at the upstream side of the filter element and an outlet port brings filtrate out of the housing from the downstream side of the filter element.

For liquid filtration, a vent port is typically included to allow gas to be removed from the housing during startup and may also be used for integrity testing of membrane filters, a frequently used practice. A drain port can allow drainage of unfiltered fluids from the housing. Housings can be opened to allow for installation and removal of the filtration element(s) and for cleaning. (Figure 8)

4.3.2 Single Use

Encapsulated filters combine a filter element and housing into an integral ready-to-use device. After use, the entire encapsulated filter (housing and element) are typically discarded. Like multi-use housings, encapsulated designs often include vent and drain ports. Some single use filters require a non-process contact holder to support the encapsulated filter design. (Figure 9a and b)

4.3.3 Systems

Figure 8 – Filtration Housings

Figure 9a – DFF Capsule Filters

Figure 9b – Typical Holder for Encapsulated Depth Filters

4.3 Systems

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Direct flow filters are integrally incorporated in many unit operations such as bioreactors, CIP skids, chromatography skids, etc.

For a given filter media and element design, the resistance is proportional to the effective filtration area (EFA) and the fluid viscosity. The pore size and porosity of a filter will also affect the resistance, with smaller pore sizes and lower porosities increasing the filter’s resistance.

At higher flow rates, filter systems (element + housing) will experience turbulent flow, at which point the resistance is not constant and the pressure drop and flow rate are no longer directly proportional. In very high flow rate applications such as air filtration, the resistance through the housing and piping system outside of the filter may be higher than the resistance of the filter element.

4.4.3 Retention Efficiency

The degree to which a filter can remove particles of a given size is determined by direct testing, typically using a challenge of rigid particles or microorganisms. The concentration of the challenge is measured by a method suitable to detect the challenge contaminant – optical particle counting, turbidity measurements, and microbiological analyses are commonly employed. Efficiency is calculated by comparison of the challenge concentration to the concentration in the filtrate and is commonly expressed as a percent retention or a Log Reduction Value (LRV).

5.0 Tangential Flow Filtration (TFF)

5.1 Configurations

Hollow Fiber - Comprised of one or more permeable membrane tubes which are typically sealed into a housing on both ends by potting in a shell and tube design. The “Shell” side of the membranes and the inside of the tubes are generally the two distinct portions of the device as they can communicate only through the membrane of the tube. Hollow fiber elements are typically 0.079 inches (2 mm) or less in inner diameter.

Figure 10 – Typical Direct Flow Filtration P&ID

Figure 11 – Hollow-fiber Devices
arrangement the process fluid is pumped into the inside of the tubes; in this case the Retentate will emerge out the other end of the tube and permeate will be collected on the shell side of the device. In the outside-in arrangement the process fluid is pumped into one end of the shell and the Retentate emerges out the other end; in this case permeate is collected from the inside of the tubes. (Figure 11)

**Tubular** – A tubular membrane is characterized by its discrete tubes and large lumen sizes which are typically larger than 1 mm inner diameter. These tubular membranes can be made from sintered ceramic, metallic or polymeric materials and provide strong structural support for the membranes.

Tubular membranes flow from inside out, with turbulent flow in the retentate stream keeping the membrane surface clean. Permeate flows through the walls of the tubes and is collected on the shell side (outside) of the modules. Devices that are made of ceramic or metal can withstand high temperature and/or aggressive chemicals. Solids levels in excess of 5% by weight are commonly handled by tubular membranes (Figure 12)

![Figure 12 – Tubular TFF Devices](image)

**Cassette** – The framework for the cassette consists of layers of polymeric flat-sheet membranes stacked together and separated by permeate and feed flow channels. Examples of membrane polymers include polysulfone, polyethersulfone, polyvinylidene fluoride and regenerated cellulose. Feed channels can be open, but usually consist of turbulence promoting screens to minimize concentration polarization.

In addition, rigid and porous spacer plates may also be inserted to increase the channel width. The flow channels are made up of two membranes facing each other. The upstream feed side is sealed to separate from the downstream permeate side of the membrane. A silicone or polymeric shell is used to encapsulate the membranes, screens, and spacer plates. (Figure 13)

![Figure 13 – TFF Cassettes](image)

**Plate/Frame** – Plate and Frame, or flat sheet filters, are characterized by an envelope type structure formed out of two pieces of membrane. In this type of device process fluid is passed over the outside of the envelope and passes through the membrane into the inside of the envelope; the remaining material that did not pass through the membrane is the Retentate.

![Figure 14 – Plate and Frame Devices](image)

**Spiral Wound** - Spiral Wound membrane devices are constructed from an envelope of membrane that is wound around a hollow core. The inside of the envelope is connected to the hollow core in such a way as to allow fluid from inside the envelope to pass through the center of the hollow core. The feed material is passed into one end of the device and passes over the outside of the envelope.

The various layers of the membrane are separated by screens on both the feed/retentate side and the permeate side. The screens act as turbulence promoters and serve to minimize concentration polarization. The permeate passes through the membrane into the inside of the envelope and eventually outside the hollow core. The Retentate passes out the opposite end of the spiral device from the feed (Figure 15). The membrane rolls are typically assembled inside stainless steel housings.

![Figure 15 – Spiral-wound Devices](image)

**Other** – Vibrating Disc employs low frequency vibration of filter disks, in addition to the tangential flow, minimizing the fouling of the membrane. These are typically employed with feed streams that contain a relatively high level (>5%) of solids. Permeate control is typically used to control fouling.

### 5.2 Mode of Delivery

#### 5.2.1 Standard
Typically, a feed pump delivers the appropriate cross flow or tangential flow across the membrane to minimize the effects of concentration polarization. In most TFF applications, the ‘retained’ materials are directed into a feed vessel so that the desired separation can be achieved over multiple passes. (Figure 16) The material passing through the membrane is called permeate.

In some TFF systems it is necessary to control the extent of concentration polarization or pre-mature fouling of the membrane to thereby control the passage of the permeating species. The flow rate of the retentate can be controlled using a retentate flowmeter or by using the pressure difference between the feed and retentate pressure sensors ($\Delta P$ control).

Permeate flux is controlled with a pump or a flow control valve on the permeate side to control the flow rate of permeate. In some applications controlling of the transmembrane pressure, or TMP (TMP is the average of feed and retentate pressures minus the permeate pressure) may be necessary.

Level sensors or load cells installed on the feed vessel can help maintain the volume in the feed tank or determine the end point for the process.

In some TFF applications, the necessary separation objective is accomplished in a single pass. (Figure 17).

The necessary cross flow or tangential flow can be achieved using other methods. In an Alternating Tangential Flow system (Figure 18) the feed flows across the membrane surface with the assist of air-actuated diaphragms. The inflation/deflation action of the diaphragms causes the liquid to be moved back and forth across the membrane surface thus maintaining the required sweeping flow rate. By controlling the flow rate of the permeate using a valve or a pump, the fouling of the membrane is further minimized.

In Vibrating Membrane Disc filters, a pump is used to feed the fluid to the membrane. In order to minimize concentration polarization, the surface of the membrane is vibrated at a controlled frequency and amplitude thereby reducing the amount of cross flow or tangential flow needed to achieve the necessary separation. Vibrating membrane filters are able to handle fluids with solids content greater than $5\%$ by weight. Addition of permeate flow control may increase passage of permeating species.

Concentration – Microfiltration and ultrafiltration membranes can be used in the concentration of process streams.

Diafiltration – is used to preferentially exchange one or more solutes in a mixture for another. Diafiltration is applied to perform buffer exchanges to prepare the feed stream for the next unit operation or provide stability to the target molecule. It is also used in cell harvesting step to wash the cells. Diafiltration can be used to enhance product yield when the product is in permeate.

Fractionation – has some applications in protein/protein separation where proteins are separated due to differences in real or apparent size. Charge modified membranes may also be used in these applications to enhance the separation efficiency.

5.4 Equipment

5.4.1 Holder/Housing/Encapsulated

5.3 Categories

5.3.1 Applications

Clarification – Both microfiltration (MF) and ultrafiltration (UF) membranes may be used for cell clarification unit operation. Bioburden reduction may be a potential added benefit.
Housings are designed with different options such as the capacity for handling multiple elements, and various inlet and outlet options. Encapsulated-filtration elements are designed for handling purposes or in place of metallic housings (Figure 19).

Holders are used to stack or hold in place the filter elements and manage or direct the process fluid. The holders are designed to assure proper sealing between the inlet and outlet connections, as well as between the encapsulated elements.

TFF devices and systems need to be designed for effective cleaning in order to prevent environmental contamination, product to product carry-over, or cross-contamination. For TFF, flow rate is an important design parameter for effective cleaning.

5.4.2 Systems – general requirements for these systems.

Designing Skid – The basic components of a tangential flow filtration system, as represented in Figure 20, are:

1. feed,
2. retentate and permeate flow path,
3. process tank for concentration and diafiltration,
4. a well sized pump to handle the needed flow rates,
5. a membrane module which includes the holder for the cassettes and the cassettes themselves,
6. pressure sensors to measure the pressures in the feed,
7. retentate,
8. permeate flow paths and control the transmembrane pressure across the cassettes, and control valves to regulate the pressure.

Flow meters can also be employed on the retentate and permeate lines to measure flow rate and totalize processed volume. The feed vessel can also be equipped with level sensors or load cells in order to maintain the fluid level in the tank and determine processing end point.

In applications that process heat-labile molecules, the temperature of the fluid is maintained using a heat exchanger on the retentate line or by using a jacketed feed vessel.

Recirculation Pump – The recirculation pump installed in the system should be large enough to handle the process flow rates. It should not be operated on the lower or the higher side of the pump curve. The negative pressure should not exceed 5 psi in the suction line to avoid cavitation and collapsed tubing. The maximum positive pressure is usually less than 80 psi.

The ideal pump type should be selected based on the nature of the application and process needs. There are 3 types of pumps used: a rotary lobe pump, 4-piston diaphragm pump or a peristaltic pump.

Finally, the shear sensitive nature of the product also dictates the type of pump selected. It is important to consider flow rates required during processing and cleaning in determining the capacity of the pump.

Process Tanks - are used for recirculation of the feed or in performing a buffer exchange operation. Tank shapes can be different in designs. In biopharmaceutical applications dished ends, cone bottom tanks and tulip shaped tanks are commonly used.

Based on the recirculation volume needs, pressure requirements, need for a jacket installation and costs the...
optimal form needs to be selected. It is important to consider minimum processing volume and cleanability when designing the tank. The process tank (also called feed tank/vessel) can also be equipped with level sensors or external load cells that are used to monitor/control the volume of fluid in the vessel and to determine processing endpoint.

**Membrane module holders** - are usually manually torqued or driven by an automatic hydraulic pump (electro-hydraulic or pneumatic) or a manually operated hydraulic pump. The manually torqued holders need to be checked for constant pressure periodically by the operator. It is critical to ensure that the tie-rods and nuts used to secure the holders in place are free of debris and rust to ensure that the torque applied to the nuts is uniformly transferred to hold the holder plates in place.

Filter holders are available from lab size systems up to production level systems. The filter holder positions and clamps the cassettes in place without a leak so that the filtration operation can be performed.

**Pressure sensors** - are used to measure the transmembrane pressure, pressure differential across the cassettes, clamping force and high pressure on the system. In case of high pressures, it is important to have the feed pump shut-off by an integrated switching module in the pressure sensor.

The switching module has to be hard wired to the frequency drive of the feed pump. There are different kinds of sensors available and selection should be made based on process requirements. Usually inline diaphragm sensors for product wetted areas and other less stringent sensors for non-product contact areas are recommended.

**Flow meters** - The flow rate on the system is measured using in-line flow meters. A mass flow or magnetic-inductive flow meter is used to measure flow on the retentate and permeate side or for feed addition to the process tank. The flow rates are critical to measure and control so that the process and cleaning performance is reproducible across various process scales.

Depending on the need of the process, size and hold-up volume of the system, solutions processed and costs associated, a suitable flow meter needs to be selected.

Magnetic flow meters are usually less expensive than mass flow meters but require the fluid to have some salt content (minimum conductivity) in order to accurately measure fluid flow. In single-use systems, turbine flow meters and ultrasonic flow meters are being increasingly used.

**Temperature, conductivity and UV sensors** – Inline temperature measurements are performed in process pipes and in tanks. A weld-in pocket or a clamp thermometer is used and is selected based on the level of precision required. Both types do not have any contact with the process medium. The cost, accuracy and reproducibility dictate the design needed.

Conductivity is measured to monitor buffer changes and for CIP control. The measuring probes are used in retentate and/or permeate lines to monitor buffer exchange and to measure the conductivity of the rinsing water after cleaning with a cleaning agent.

In certain high concentration protein applications a UV cell can also be installed on the permeate side to monitor protein breakthrough through the membrane. UV sensors may also be installed in the retentate line to determine cut-off for retentate recovery in order to minimize excessive dilution of the final product while maximizing product yield.

**Other design considerations** - Run-dry and overfilling protection are typically employed in the system. Run-dry protection is needed to ensure that the pumps are not run dry which can damage mechanical seals of the pump. A capacitive sensor can be employed just before the recirculation pump to protect the pump from running dry. NP

In the case of overfilling, a sensor could be employed either in the head space of the tank or in the cap of the tank. In either case cleanability and dead-legs need to be monitored. NP

The system should be properly vented and the pipes free of dead spaces and pockets. The piping should be sloped to low points, fully drainable by gravity for proper draining and to prevent accumulation of stagnant pools. The flow velocity through all pipes during CIP should be between 1.5 m/s and 3.5 m/s.

All system components should be designed with corrosion resistant stainless steel, like austenitic stainless steel types UNS 1.4301 or 1.4571 for non-product wetted parts (e.g. skid frame) and low-carbon austenitic stainless steel types UNS 1.4404 or 1.4435 for product wetted parts.

In addition, an easy to clean surface is required. Surfaces with product contact should have a surface roughness of Ra ≤ 0.4 – 0.8 µm or less and need to be electropolished. Surfaces with no product contact can have a higher arithmetic surface roughness of Ra ≤ 1.2 µm and without electropolished surfaces.

### 5.5 Operating Characteristics

#### 5.5.1 Basic Process Overview

The end user should provide a detailed explanation of the separation process that needs to be performed. Information such as the product type (Virus, mAbs, therapeutic protein, etc), system scale (e.g. membrane surface area, process volume), type of purification (e.g. MF or UF), starting and final volumes, starting and final concentrations, diafiltration volumes and major process steps are vital for the success of the system design.
The end user should be able to specify, from the details below, the main steps that are intended to be executed during their process. The sequence may or may not change, depending on the application.

**Filter Preparation**

This step is necessary to remove of residual components or storage agents, for sanitization of the system and for rendering the system closed. Proper filter preparation will ensure that the system is fit and ready for use prior to introduction of product. Steps pertinent to this section consist of the following:

1. Installation of new Filters
2. Pre-Sanitization WFI Flush
   a. Single Pass / Once-Through: This step is usually used to flush a solution (e.g. WFI) through the system to remove residual components and storage agents. Retentate and permeate streams are directed to drain. A solution (WFI, cleaning solution, etc.) is introduced through the filtration device.
   b. Recycle: This step is usually used to recirculate solutions during preparation/sanitization, to equilibrate the system and the device, to recirculate storage solution, etc.
3. Sanitization
   a. Single-Pass/Once-Through: This step is usually used to flush the sanitization solution (e.g. NaOH) through the system to remove sanitize the filtration device and system prior to use. This process could also be used to render a system closed after assembly. Retentate and permeate streams are directed to drain.
   b. Recycle: This step is used to recirculate the sanitization solution through the filtration device and back to the recycle tank. This step transfers solution from the recycle tank through the filtration device and it can direct both retentate and permeate back to the recycle tank, or retentate back to the recycle tank and permeate to drain. Recirculation of the sanitization fluid for the desired length of time minimizes the amount of fluid needed to achieve the desired result.
4. Post-Sanitization WFI Flush
   a. Single Pass / Once-Through: This step is usually used to flush a solution (e.g. WFI) through the device and the system to remove the sanitization fluid. Retentate and permeate streams are directed to drain.
5. Integrity test online/offline - This step is used to perform an air diffusion test on the filtration device prior to process startup and after each post-use cleaning step.
   a. Online: This step is performed on the system with the TFF devices installed. To perform an integrity test (IT) online, it is necessary to fully drain the devices and the piping prior to the start of the test. Then, clean, dry air (process air) is transferred from an external air source to the retentate side of the system through an air flow meter. The air pressure is increased on the retentate side of the device to the recommended test pressure while the air diffuses through the permeate side for a preset amount of time. Online integrity testing can evaluate both device integrity and holder integrity.
   b. Offline: This step is performed on the system using a specific air integrity tester with the TFF devices removed from the system. This only tests the integrity of the filtration devices.
6. Equilibration (buffer conditioning)
   - This step is used to equilibrate the filtration device(s) prior to processing. During this step the equilibration solution is recirculated in total recycle mode until an equilibration condition (e.g. pH or conductivity) has been reached.

**Processing**

This section describes the operational method/mode of the equipment used in order for the equipment to perform the desired separation.

1. Clarification - This step is used to concentrate the product in the (MF) recycle tank by collecting the product through the permeate. During this step, the product is transferred from the MF recycle tank through the filtration device(s) and the permeate is collected as the clarified product.
2. Concentration – This step is used to concentrate the product in the (UF) recycle tank by recovering the product retained in the UF device and removing the solvent(s) that passes through the device. During this step, the product is transferred from the recycle tank through the filtration device(s), directing the permeate to the drain and returning the retentate back to the recycle tank. The batch UF operation usually ends when product concentration, recycle tank, concentration factor, or UF filtrate volume set point is reached. In single-pass TFF applications, the retentate is collected without directing it back to the feed vessel.
3. Diafiltration / Buffer Exchange - This step is used to wash contaminants from the retentate or to exchange the buffer on the final product. This operation pumps solution from the recycle tank through the filtration device, directing the permeate to drain and returning the retentate back to the recycle tank; simultaneously the new buffer is transferred to the recycle tank by maintaining a constant recycle tank level during
the entire diafiltration step (constant volume DF). The diafiltration step ends when dia volumes, filtrate volume or buffer volume transferred set point has been reached. In some product recovery applications (desired product permeating through the membrane), the diafiltration buffer is added at a reduced rate (when compared to the permeate flow rate) to maximize product recovery while minimizing dilution. This is also known as differential diafiltration.

**Product Recovery**

This section details the collection method used to remove the product components/solution(s) from the equipment.

1. **Gravity only / Pumped** - This is a more common product recovery step, and it is used to recover the final product remaining in the recycle tank. The product can be transferred to the final container by gravity only or by using the feed pump. However, some product remains held up in the piping and devices that an extra recovery method is required to increase product recovery.

2. **Buffer Displacement** - This step can be used to recover the maximum amount of concentrated product by using buffer to push the product. This step is done with minimal dilution while the lines still contain the concentrated product. Note: This step is commonly referred to as “a buffer flush” if it is done after the piping has been emptied.

3. **Air Blowdown** - This step is used to recover the maximum amount of concentrated product by using air pressure. The air pressure is introduced at the highest point in the piping and the collection port is at the lowest point. In protein processing applications, care must be exercised during air blowdown to minimize foaming and potential protein denaturation.

4. **Other-any other method or combination of methods as determined by the end user.**

**Post-Processing Filter Treatment**

This section describes the steps incorporated in order to remove residual undesirable components, clean the filter elements or otherwise prepare the filter elements and the system to be used again.

1. None (i.e. Single-Use System)
2. Buffer Flush
3. Air Blowdown
4. Post-Use CIP
5. Storage - This step is used when another product processing run does not immediately follow the cleaning. Then, the storage solution is recirculated in a total recycle mode through the entire system to prevent organism growth. Note: It is the end user’s decision whether the filtration device(s) will be stored along with the system (storage wet) or whether they will be removed and stored separately (system storage dry).
6. Sampling
7. SIP

**System Preparation**

This section details the method to be used in order to remove residual undesirable components, clean the remaining portions of the system or otherwise prepare the other system components (tanks, pumps, piping system, etc) for use.

1. Clean In Place (CIP) Components - All other components – other than the filter element and housing – and sections of the system must be identified as either requiring or not requiring CIP prior to performing another separation. For those component and sections determined to require CIP, the CIP method also must be determined.
2. Sterilization/Steam In Place (SIP) Components – All other components – other than the filter element and housing – and sections of the system must be identified as either requiring or not requiring SIP prior to performing another separation. This information is used by the equipment manufacturer to determine and/or recommend instrument placement and drainage requirements.

The end user must specify the physical attributes, critical attributes, business attributes, desired attributes and critical process attributes for design of a filtration system.

**5.3.2 Process Conditions**

The end user should specify the main process conditions such as: process feed flow rate, process permeate flow rate, Trans Membrane Pressure (TMP), Pressure Drop, feed pressure, retentate pressure, permeate pressure, maximum desired system holdup, CIP/SIP requirements, etc.

**6. Integrity and Installation Testing**

**6.1 Purpose**

After the filtration devices are assembled inside the housings/holders/systems, they are typically tested to ensure that the assembled unit will provide a high degree of assurance that the separation objectives can be achieved. In certain applications, testing may be performed once again after certain intermediate process steps such as cleaning, steaming or autoclaving and yet another time at the end of the filtration process. In applications where the filter is used as a sterilizing or viral removal filter, integrity testing of such filters pre and/or post-use is mandated by the regulatory agencies.

**6.2 Installation Testing**
6.2.1 Leak Detection

Pressure Hold – The hold test pressurizes the system (10-30 psig) and measures the pressure decay in a fixed volume upstream of the membrane over a given time. It is critical to ensure that the temperature of the upstream environment does not fluctuate during the course of the pressure decay measurement. Post-test venting needs to be carefully performed to avoid downstream back-pressure damaging the membrane.

6.2.2 Normalized Water Permeability (NWP) Testing

NWP is used as an indication of membrane permeability, relative to a known baseline. Results of this test are typically used to determine frequency of membrane changeouts. To conduct an NWP test, use purified water/buffer or appropriate solution and measure flow rate through the membrane at a prescribed transmembrane pressure.

For microporous and open ultrafiltration membranes, it may be important to keep the test pressure constant when NWP is measured. In some cases, when high permeate flow rates are achieved, it is important to ensure that parasitic pressure losses in the hardware (piping, cassettes, etc.) are considered during the establishment of the NWP baseline value. During the test, the permeate rate (flux) is measured along with the temperature of the solution.

The Water Permeability is calculated using the following calculation:

\[
\text{Water Permeability} = \frac{\text{Permeate Flux (L/m²-h)}}{\text{TMP (psi or bar)}}
\]

The resulting flow rates are normalized to a standard temperature by applying a temperature correction factor which accounts for the change in viscosity and density. Typically, a baseline measurement of the membranes permeability is obtained at the initial conditioning of the membrane. For all subsequent measurements, the NWP test is performed following the cleaning of the membrane at operating conditions as close as possible to the original test.

6.2.3 Filtration devices containing reverse osmosis membranes

Devices containing certain reverse osmosis membranes may be tested for integrity using a salt rejection test. Depending upon the type of membrane, either a monovalent salt, such as sodium chloride, or a divalent salt, such as magnesium sulfate, is used.

The membrane is challenged with the salt solution at a prescribed transmembrane pressure. Salt concentration or conductivity of the feed and the permeate streams is measured and the percent rejection is calculated. Percent rejection is typically calculated as (feed concentration – permeate concentration)*100/ feed concentration.

Feed and permeate conductivity may also be used in place of feed and permeate concentrations while calculating percent rejection. Devices exceeding the percent rejection specification could be considered integral.

6.3 Integrity Testing

6.3.1 Bubble Point

The bubble point test measures the pressure at which a wetting liquid is removed from the largest set of pores within the filter media. A wetting liquid is held within the pores of a filter media by strong capillary forces, and the gas pressure required to displace the liquid is inversely proportional to the diameter of the pore.

Due to the inverse relationship between bubble point and pore size, the bubble point test can be used to verify that the pores in a filter system are predominantly below a minimum size and therefore can also be correlated to the filter’s retention performance. In the case of a sterilizing filter, the bubble point can be correlated to retention of a specific microorganism under standardized or process-specific conditions. Bubble point pressure is impacted by the surface tension of the fluid in the membrane pores, with lower surface tension fluids typically decreasing the bubble point pressure.

6.3.2 Forward Flow/Diffusion

At applied gas pressures below the bubble point, the flow of gas through a wetted and integral filter system is predominantly due to diffusive flux of the gas through the wetted pores. This diffusive flux is typically lower than the diffusive + convective flux of gas that is obtained above the minimum bubble point of the filter. This is due to the fact that gas must diffuse through the liquid held in the fully wetted pores in order to pass through the filtration media.

Forward flow/diffusion test is impacted by the type of gas that is used and the temperature of the wetting fluid that is used. Both Bubble Point and Forward Flow/Diffusion tests require the filter to be thoroughly wetted in order to provide accurate results. In cases where water doesn’t adequately wet the filter media, lower surface tension fluids such as IPA/water or ethanol/water mixtures can be used to thoroughly wet the filters.

As a post-use test, these tests can also be carried out using product wetted filter media. In such cases, product wetted integrity test specifications need to be developed as they may be different from water or alcohol wetted specifications.

6.3.3 Pressure hold

The pressure hold test first pressurizes the upstream side of a filter system to a test pressure below the filter bubble point and allows time for the pressure and temperature to stabilize. Then, the system is isolated from the pressure source and the pressure on the upstream side of the system is monitored. The pressure decay over time is used to indirectly measure the gas flow through the system (and
A thorough understanding of the bioreactors, chromatography, etc. other unit operations such as virus filtration, protein concentration/diafiltration, single-use processing and integrity testing. He is an active member of various professional/industry organizations such as PDA, ISPE, ASME, ASTM, etc.

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